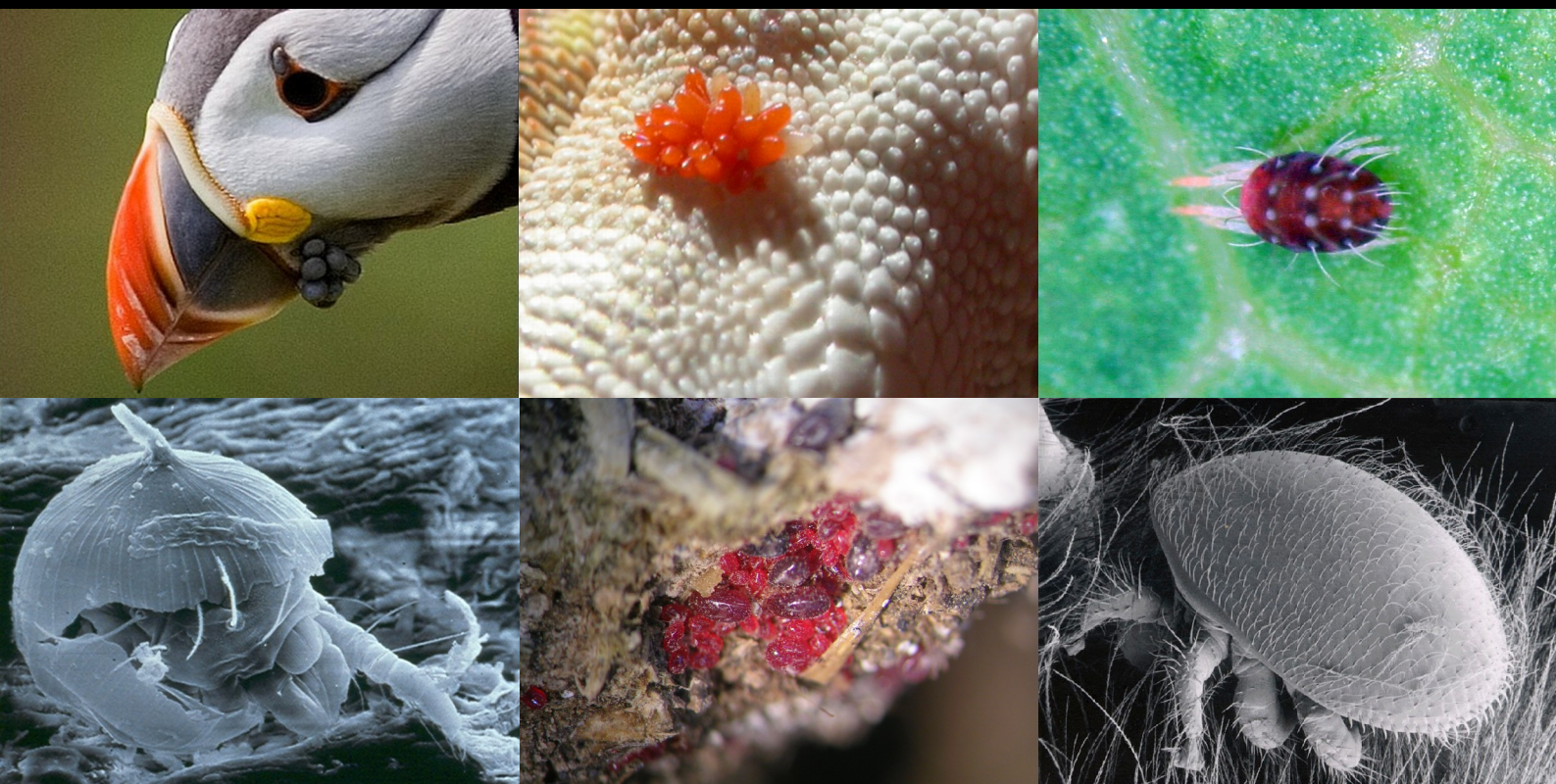
INTEGRATIVE ACAROLOGY

Proceedings of the sixth Congress of the European Association of Acarologists

Editors:
M. Bertrand, S. Kreiter, K.D. McCoy, A. Migeon, M. Navajas, M.-S. Tixier, L. Vial



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EDITORIAL NOTICE

It has been a challenge to publish the Proceedings of the Sixth European Congress of Acarology before the actual meeting. Indeed, we all have the unfortunate habit of being over-booked and therefore of not always being able to meet our deadlines. We therefore thank the participants for their punctuality and their assistance with the production of these Proceedings. It is together that we have overcome this general failing of our profession; each of you made the effort to send your manuscripts on time and to work quickly and efficiently on the revisions. Thank you!

Of course, something done so quickly can never be perfect. You may therefore note some differences in form among the contributions. This was a choice on our side. As we are all united here in Montpellier to exchange information, we decided it was more important to favour scientific communication than to take the time to completely homogenise the form of the manuscripts. We therefore ask for your understanding regarding these imperfections that, despite our good intentions and hard work, may be found in this document. We hope that you find these contributions as enriching and informative as we did while reviewing and preparing them for the Proceedings. They reflect the diversity of work presented during this congress and are the result of each person's contribution to our collective knowledge.

We hope this format will help with the distribution of the contributions included in these Proceedings and, more generally, will promote the dynamism of European Acarology.

The Editors

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PRESIDENTIAL ADDRESS:

WHY INTEGRATIVE ACAROLOGY?

Michel Bertrand

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Dear colleagues and eminent Acarologists,

Beginning the preface by observations on the diversity of Acari, highlighting the number of species of these minute organisms, or on the ecological diversity and the plasticity of this group might be considered as a platitude, a « lieu commun ». Comments on the diversity of ways of life, the diversity of the habitats, of the cycles, of adaptations exhibited by mites and ticks, and uppermost on heterogeneity of this group could be considered as "breaking down an opened door". However we can consider now that the era of our famous ancestors (Berlese, Fain, Grandjean, Hoogstraal, Koch, Karg, Oudemans...), major specialists in the knowledge of many families, seems closed by the specialization of current research lead by scientists working on mites and ticks. Studies on Acari of economic, agricultural, veterinary and medical importance need specialized research, the use of sophisticated tools, and , as a consequence, is becoming more and more expensive. Since the last decades of the XIXth century, Acarological Science has followed the same path as biology in general: a new science for the Third Millenium.

What is the interest of a congress devoted to mite and tick studies nowadays? It is an opportunity for many scientists working on "similar" material to meet together, to exchange experiences and to constitute a community among biologists. Because of the necessary specialization of each scientist, these exchanges are more and more useful, and allow us to share experience on systematic, physiology, ecology...

Why the Euraac congress in France?: 34 years ago, French speaking Acarologists (Société de Acarologues de langue française) initiated the International Course of Acarology, the first edition being in Louvain La Neuve Belgium in 1974. Via this course, the teachers (Lebrun, Fain, Coineau, Travé, Aeschlimann, Athias-Henriot, ...), transmitted to "younger" students the state of the Science in Acarology. These teachers promoted the discipline of (and enthusiasm for) Acarology, that was then transmitted through the following editions, and by annual scientific seminars. Some years ago this course disappeared, notably because the demand changed: young scientists needed more specialized practices, and sophisticated technical tools, to perform competitive research on Acari in the general context of the times, and under the Furculae Caudinae of Impact Factors. By consequence, the only place where acarologists can now exchange information are during different congresses like this one. We therefore hope this congress will play this role, renewing links between Acarologists coming from different countries and continents.

What results can be expected? Congresses serve to expose to each participant of the general scientific community, to explain the current state of the art of research carried out in different domains, and all this in a more convivial ambiance than in front

of a computer screen or through articles in specialized journals. Via these roles, congresses are ideal occasions for boosting acarological science.

Why in Montpellier? The present congress was built up by different Acarologists working in Montpellier (France), from the different institutions (CNRS, INRA, IRD, Supagro, University), and operating in diverse domains, from the most fundamental to the most applied specialities, from the ecology and biogeography of mites in natural environments, to ticks of medical or veterinary importance.

Why "INTEGRATIVE ACAROLOGY":

1 Modern scientists need to be more and more competitive within each specialized domain and need to master a wide knowledge base in neighbouring "specialities" in order to best apprehend and transmit the significance of current research themes.

2 Acarology needs specific tools, but the traditional tools are limited in the context of the competitive environment of the international community of biologists. Several innovative tools are now available, and complete the Acarologist's arsenal of weapons: recent advances in technology can shed new light on more traditional studies and tackle questions that till now have been unreachable. And, finally,

3 We can expect from the use of these tools new important improvements in agriculture, medicine, protection against transmitted disease and systematics. Indeed, by integrating the most modern improvements and combining them with essential morphology and genetic data, we can make spectacular advances in these areas of societal interest.

For these reasons, if "Acarology" cannot be considered as a speciality in the modern world, Mites and Ticks can certainly be counted among the most useful organisms for advancing modern biology.

The VIth Congress organized by European Association of Acarologists in Montpellier hopes to contribute to our current knowledge, and to prepare, through exchanges between persons and institutes, the Acarology of the future. We hope the congress will be profitable to everyone. We hope too that the scientific potential of the Acari to address a wide range of problems will attract more and more young scientists, which will promote these arachnids as first choice tools for studies on all the aspects of modern science.

The communications proposed are organized in 9 Sessions: This diversity reflects the large range of interests of acarologists, and the necessary use of complementary approaches. Furthermore, in each session several themes are represented, and the most up to date tools are used. I would like to translate the feeling shared by the organizing committee in thanking everyone who has participated more or less to the organization of this meeting, to Euraac who gave the opportunity, to the scientific committee, and to the participants for coming to Montpellier to share the findings of their work.

Integrative Acarology
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PLENARY LECTURES

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European Association of Acarolgists, 2008

WHOLE GENOME SEQUENCING OF TETRANYCHUS URTICAE: NOVEL GENOMIC TOOLS IN ACAROLOGICAL RESEARCH

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Abstract

Genetic model systems, such as *C. elegans*, fruitfly, zebrafish and mouse have rapidly advanced our understanding of genetics, development, population biology and evolution. These species became model organisms in part because of several common characteristics: rapid development, relatively small genomes and easy laboratory maintenance. To date, the development of a chelicerate model system has been hampered by their complex ontogeny, long development time and large genomes. Thus, a challenge for the future progress for many aspects of chelicerate biology is the development of a model organism for this group. Toward this end, we are developing a chelicerate genetic model: the two spotted spider mite *Tetranychus urticae*. As representatives of this basal taxon of arthropods, spider mites are of special importance to several areas of science including phylogenetics, developmental biology, evolution, ecology and genomics. In addition, spider mites are major agricultural pests and are therefore of substantial economic importance and significance for the biotechnology of pest control and energy conservation. *T. urticae* has one of the smallest genomes in arthropods determined so far (75 Mbp, 60% of the size of the *Drosophila* genome), undergoes rapid development and is easy to maintain in the lab. These features make *T. urticae* an excellent candidate for developing into a chelicerate model system.
The whole genome sequencing project currently underway (USA Department of Energy, Joint Genome Institute http://www.jgi.doe.gov/sequencing/why/CSP2007/spidermite.html) will produce 8X sequence-coverage of the genome of *T. urticae* London strain and 1X sequence-coverage of the Montpellier strain. In addition to whole genome sequencing JGI will perform 75,000 EST sequences to aid in genome annotation. The whole genome sequence of *T. urticae* will be annotated, defining the total gene number and regulatory and transcribed regions. Final annotation of transcribed regions will be used for the design of a spider mite whole genome expression microarray. Together with already developed protocols for antibody and in situ detection of proteins and RNA distribution and RNAi reverse genetic gene silencing these new tools will open new perspectives and approaches in acarology ranging from comparative and functional genomics to genetics, population biology, plant-herbivore interactions, ecology and pest control.

Introduction

The Chelicerata are the second largest group of predominantly terrestrial animals. Chelicerates (horseshoe crabs, scorpions, spiders, ticks and mites) are at the root of the arthropod phylum (Boore et al. 1995: Friedrich and Tautz 1995), representing the primitive state of the taxon. Among the most economically significant chelicerates, there are spider mites and ticks, which both belong to the order Acari. Spider mites represent major pests in agriculture, while ticks are vectors of human diseases, including Lyme disease and haemorrhagic fever. Unfortunately, the developmental genetics of chelicerates is poorly understood and a major obstacle for future progress in many aspects of chelicerate biology is the lack of a model organism in this group.

T. urticae *development*

Unlike spiders and scorpions, which have long development times, *T. urticae* has a short generation time, completing its embryonic development in only 39 hours and full transition from egg to adult in less than seven days (Rao et al. 1996).

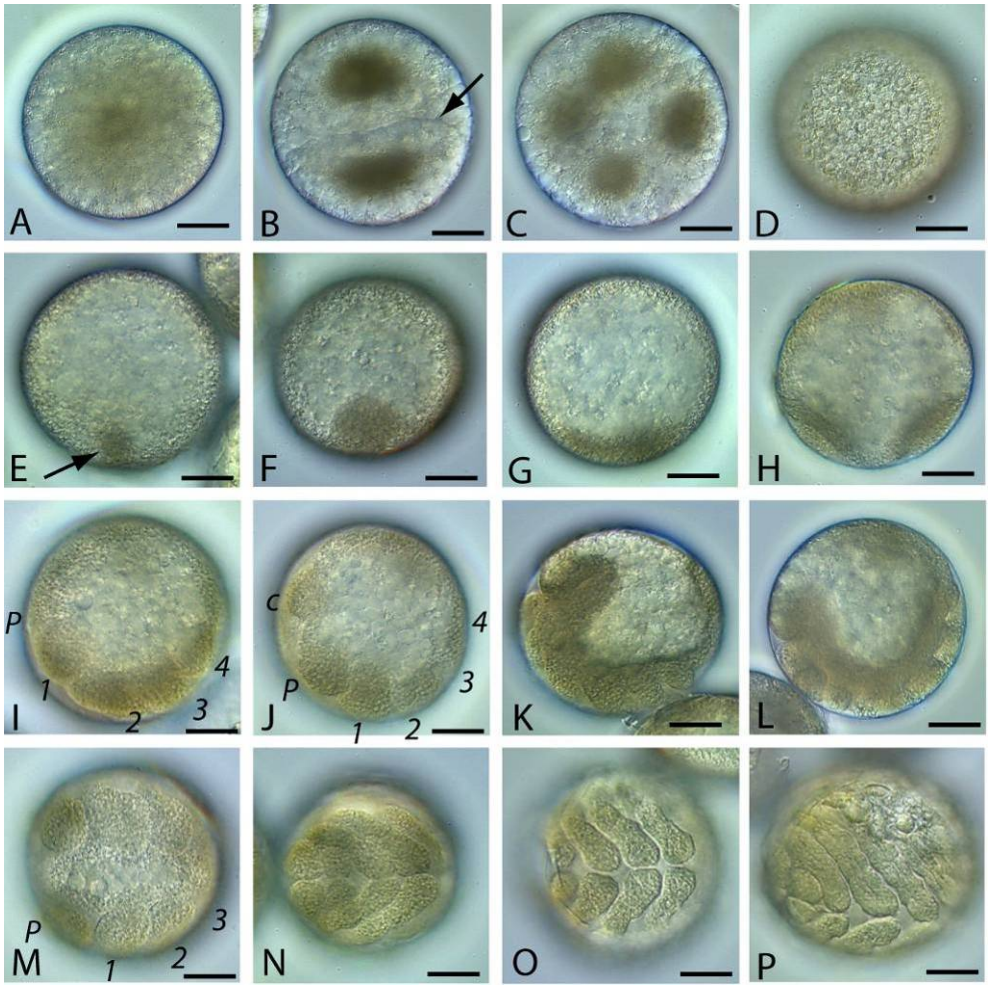

Figure 1. Embryogenesis in *T. urticae.* A-D) Early cleavages. A) Uncleaved egg. The nucleus is just starting to become visible in the center of the egg. B) First division, the egg nucleus (dark) has divided. A clear membrane between the two blastomeres is visible (arrow). C) Second Division. D) Blastoderm stage embryo. Nine divisions have taken place forming a cellular blastoderm.. E-F) Formation of the 'germ disc', and the germ band. E) At 20-22 hours AEL a thickened portion of the germ band is visible (arrow), probably in ventral regions, which we interpret to be the germ disc. The germ disc starts as an ovoid swelling (E and F), and then flattens (G). Flattening of the germ disc is quickly followed by the appearance of leg primordia on both sides of the ventral midline (Viewed from the anterior in H). I-L) Formation of the germ band, limb primordia, and limb outgrowth (dorsal view). All embryos are viewed with dorsal up and anterior to the left. I) The initial limb primordia form by 23 hours AEL. P= pedipalps, 1-4= walking legs. Primordia for the chelicera bearing segment and the opisthosoma germ band become visible soon after (J) C= chelicerae. K and L) Limb buds grow and become jointed. M-P) Formation of the germ band, limb primordia, and limb growth (ventral view). All embryos are viewed from the ventral side, with anterior to the left. Embryos are of the same stage as those in I-L. Scale bars represent 50 µm.

This rapid generation time and simple rearing protocol makes *T. urticae* a great laboratory organism for genetic studies. *Tetranychus urticae* also has small eggs (150 µm) that are surrounded by a transparent chorion. In this species sex determination is haplo-diploid, by arrhenotokous reproduction system. Finally, *T. urticae* has only 3 chromosomes (Oliver, 1971) and possesses the smallest genome determined thus far within the arthopods (75 Mbp, 0.08 pg/ haploid genome, 60% of the *Drosophila* genome size) (Dearden et al. 2002).

Spider mite females lay a spherical, 150 μm egg with little internal morphology (Fig. 1 A) (Dearden et al 2002). Over the course of the first hour after egg laying (AEL), a central nucleus becomes visible. The egg then undergoes nine divisions, approximately one per hour, creating a blastoderm with a layer of cells surrounding a yolk filled center (Fig. 1 B-D). From the first division, cell membranes are visible between the nuclei. The blastoderm remains static for 12-14 hours with no changes in morphology. A small swelling of blastoderm cells then appears, internally, on one side of the egg, which we take to be the 'germ-disc' described for other mite embryos (Fig. 1 E). The germ disc starts as an ovoid swelling (Fig. 1E, F), and then flattens (Fig. 1G). Flattening of the germ disc is quickly followed by the appearance of leg primordia on both sides of the ventral midline (viewed from the anterior in 1H). Leg buds and the prosoma region of the germ band appear rapidly and simultaneously (Fig. 1 I-P) approximately two hours after the appearance of the germ-disc. The two halves of the germ band are separated by a small ventral sulcus, which quickly closes (Fig. 1 M). Limb buds in the chelicera bearing segment and the germ band in the opisthosoma appear 3-4 hours after formation of the germ-band. Eyes become coloured and limbs grow on the chelicera, pedipalp and first three walking leg segments, becoming jointed and hirsute by 30 AEL. The fourth walking leg does not extend in embryonic stages. Hexapod larvae hatch approximately 39 hours AEL.

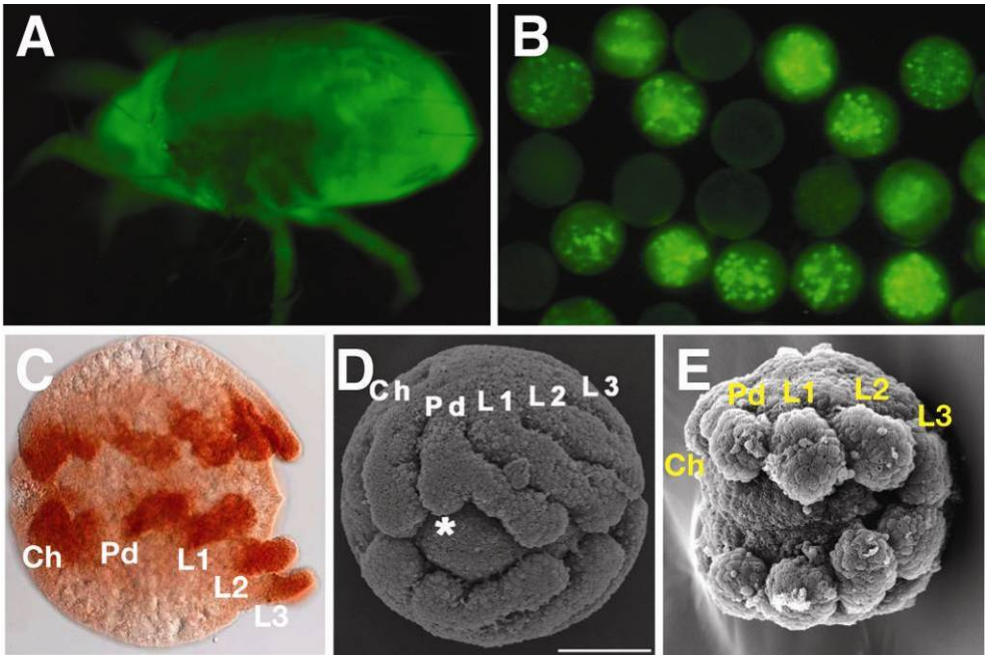

Figure 2. Gene silencing using RNAi in *T. urticae*. A) Female *T. urticae* injected with fluorescently labeled dsRNA. Note that fluorescence spreads throughout the body. B) Eggs of female mite injected with fluorescently labeled dsRNA show various incorporation of the fluorescent label. C) Dll protein pattern (brown) in mite embryonic appendages. D) Wt segmented embryo. E) Embryo of female injected with ds *Dll* RNA shows truncation of appendages. Pp: pedipalpes, Ch: chelicere, L1-L3: walking legs, asterix: pedipalpal lobe.

A major advantage of *T. urticae* for developmental studies is that the transparent eggs can be easily recovered from plants where mites have oviposited. This is accomplished by washing plants in a detergent solution and by recovering eggs from the wash by sifting through various size sifts. This quick procedure separates eggs from plant debris and adult mites, and thousands of embryos can be easily and quickly recovered from only several plants (Dearden et al. 2002, Dearden et al. 2003). Chemical treatment with heptane plus a brief sonication step can be then used to liberate embryos from the impenetrable viteline membrane. Once embryos are permeabilized, standard protocols for immunocytochemistry can be applied for both antibody and *in situ* immunocythochemistry. *Tetranychus urticae* embryos are transparent and small, which makes them ideal for Confocal microscopy. Fluorescent antibody and *in situ* fluorescence staining allow

detection/localization of mRNA and gene products in *T. urticae* embryos at the cellular level. In addition, the transparency of spider mite chorion allow laser ablation of individual cells (Grbic et al. 2007) in similar manner as in worm *C. elegans*.

RNAi mediated gene silencing has also become an important part of the reverse genetic toolkit for functional studies in diverse species. The current model suggests that RNA-induced silencing complex (RISC), which incorporates a single strand from siRNA, binds to cognate mRNA and induces its silencing by cleavage or translational repression. Crucial component of RISC complex is Argonaute (Ago) protein. Ago binds single stranded siRNAs and it is proposed that it can direct both cleavage and translational repression of mRNA (20). In order to facilitate the functional analysis in *T. urticae,* we developed a parental RNAi protocol focusing on *Distal-less* (*Dll*), a conserved gene involved in appendage specification in metazoans. This gene is likely to cause conserved, easy to score developmental phenotypes if knocked out, serving as starting point for development of RNAi in *T. urticae*. Injection of fluorescently labeled dsRNA and siRNA into spider mite female abdomen distributes throughout the abdominal cavity (Fig. 2A) and these females lay eggs that contain fluorescently marked RNA (Fig. 2B) (Khila and Grbic´ 2007), suggesting that dsRNA can be systemically distributed in spider mites. Injection of longer dsRNA, as well as short interfering siRNA, induced canonical limb truncation phenotypes (Khila and Grbic´ 2007) (Fig. 2C-E). Thus, *T. urticae* is another species where reverse genetic approach based on parental RNAi can be used to generate loss-of-function phenotypes.

Why use the spider mite as an arthropod pest model?

In addition to its location at an important phylogenetic node, *T. urticae* also represents an important agricultural pest. The global insecticide market represented 30 billion dollars in 2004, with roughly 3 to 4% of the total insecticide tonnage corresponding to the use of acaricides. The spider mite, *T. urticae,* represents one of the major pests in agricultural crops. It feeds on more then 1000 plant species (about 150 of which are of economic value). It is one of the major pests of greenhouse crops (e.g. tomatoes, aubergine, pepper), *Cucurbitaceae* (e.g. cucumbers, courgettes) and greenhouse ornamentals (e.g. roses, chrysanthemum, carnations). It is also a major pest in annual field crops such as maize, soybean and sugar beet, and in perennial cultures like alfalfa, strawberries, grapes and plums. *Tetranychus urticae* is also a major pest for tropical crops including cotton, okra and papaya (Bolland *et al.* 1998; Jeepson *et al.* 1975). Conveniently, *T. urticae* develops on the model plant *Arabidopsis*, allowing utilization of the plethora of genomic tools available in this plant model species to dissect plant-pest interactions.

Spider mites are pests of dry and hot climates. Computer modeling studies suggest that with intensifying global warming the significance of pests will dramatically increase. Indeed, pest damage has increased in the EU, as exemplified by the damage of Belgian sugar beets in the 1990's (Legrand, 1996) and a general expansion of outbreaks in Europe. Species from the Tetranyichidae family are also invasive species and the recent introduction of the spider mite *T. evansi* from South America is creating novel problems in EU agriculture (Escudero and Ferragut, 2005; Migeon, 2005; EPPO: http://photos.eppo.org/index.php/image/1168-img-6161).

Chemical pesticides are the predominant method of controlling spider mites, but due to their short generation time and high fecundity, mites have rapidly evolved resistance to all major pesticide groups (Croft and Van de Baan, 1988). In addition, *Bacillus thuringiensis* (*Bt*) toxin expressed by transgenic crops has no negative effect on *T. urticae* (Lozzia *et al.* 2000). In fact, it was shown that *T. urticae* prefer to feed on transgenic plants expressing *Bt* toxin over the control, and mite females laid significantly more eggs on Bt-leaves than on control plants (Rovenska *et al.* 2005). In addition to simple resistance to individual compounds, spider mites have developed cross-resistance to several different insecticide groups (ffrench-Constant *et al.* 2004). Control of multi-resistant mites has become increasingly difficult as the genetic basis of such resistance remains poorly understood.

The spider mite is a perfect example of an insect herbivore that can be easily managed in the laboratory. *Tetranychus urticae* feeds by penetrating the plant tissue with their mouthparts and ingesting the plant leaf sap. As a consequence, the leaf mesophyll tissue collapses and chlorotic spots forms at each feeding site. Mite feeding reduces photosynthetic rate, total chlorophyll content, and quality of the leaf (Park and Lee 2002). Often, mite infestation may cause complete plant defoliation.

Currently, there is no well-defined system for dissecting the genetics of the plant-pest interaction. Although *Arabidopsis* makes an

excellent genetic model for such studies (vanPoecke and Dicke 2004), a genetically defined plant-eating arthropod is lacking. *Tetranychus urticae* feeds on *Arabidopsis* plants causing significant damage (Kappers et al. 2005) and special care is required to exclude it from *Arabidopsis* growth chambers. A genetic dissection of mite-plant interactions would thus provide insights into the signalling and transcriptional basis of plant defences used against herbivores (Kappers et al. 2005). In addition, genome-wide sequences of pest organisms lend themselves to analysis of the transcriptome's response to host plant defensive compounds, infection by viruses and microbial pathogens, and any number of phenomena affecting the pest status of arthropods (Reymond et al. 2004). Therefore, the development of a genetic model herbivore representing a major plant pest will be important for plant science.

The new genomic tools in *T. urticae* have a potential to develop a systems biology approach to pest management by combining the existing genomic tools in plants with genomic tools under development in the target pest. The choice of species, *T. urticae,* with its important pest status, small genome, fast development and novel genomic resources opens opportunities for rapid advances of knowledge in this area and direct application in agricultural practice. Dissecting plant-herbivore interactions on the genomic level will open new opportunities for pest control. First, the whole genome sequence of a major agricultural pest will reveal so far unprecedented number of targets for pest control. Second, understanding changes in transcriptome profiles for both the plant and the herbivorous pest upon their interaction will establish the gene regulatory cascades involved in plant defence and pest response. Screening for natural variation of plant resistance will uncover existent plant genes for breeding/biotechnological modification of crop plants for pest resistance. Utilization of naturally-occurring reverse genetics mechanisms, such as gene silencing, will open an opportunity for the development of plants as delivery systems for silencing genes in pest chelicerates, generating a non-chemical approach to pest control. Finally, tools we will develop will greatly increase out current knowledge of pest genomics. For example, spider mite whole genome expression microarrays will allow the precise dissection of the mode of action of different agents in pest control and the rapid screening of methods for pest management.

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THE FIGHT AGAINST *VARROA DESTRUCTOR* - HONEY BEE PARASITE: INTEGRATED *VARROA* MANAGEMENT

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Abstract

Since *Varroa destructor* invaded *Apis mellifera* populations in Europe in the seventies, this parasitism has been extensively studied, from control management to molecular approaches of the host-parasite relationships. Chemical control of the mite was followed by classical events of pest management: resistance of the mite to acaricides and pesticide residues in the wax. These events induced the set-up of biological and technical control measures of the mite. In parallel, the equilibrated parasitism of *Apis cerana*, a honeybee from East Asia, by *Varroa destructor* has been extensively studied and compared to the interaction with *Apis mellifera* in an attempt to understand the mechanisms underlying this host-parasite equilibrium. The knowledge of these mechanisms should have helped sort out selection criteria for *Apis mellifera* breeding programmes. However, despite efforts to breed *Varroa* resistant bees, little progress had been made. The basic biology of the mite, including chemical ecology, behavioural studies, population dynamics and genetics, as well as molecular responses of the honey bees, have been investigated for more than 25 years producing an impressive amount of scientific literature, making the Varroa - honey bee interaction one of the most extensively studied host - parasite model systems. More recently, some natural honey bee populations have been shown to survive to mite infestations, opening the way to the concept of integrated Varroa management. These bee populations will be particularly useful for determining the mechanisms of mite resistance in *Apis mellifera*. In addition, the recent sequencing of the honey bee genome should help us learn more about the molecular responses of the bees to mite parasitism. Comparative studies between resistant and susceptible bees are being developed and should be helpful to identify the genes involved in resistance.

Key-words

Varroa destructor, *Apis mellifera*, integrated pest managment, chemical ecology, breeding and genetics

The acarian *Varroa destructor* is a real threat for the honey bee on which the mite feeds and reproduces. The pest first invaded *Apis mellifera* populations in Europe in the seventies. Since, this parasitism has been extensively studied, from varroas control management to molecular approaches, and constitutes one of the best models for host-parasite relationships studies. Different tools have been set up from field and basic research to control the mite, and varroa resistant bees start to appear in different places, which make possible the development of integrated varroa management limiting the use of acaricides.

The first varroa invasion in Europe leaded to important honey bee colony mortalities linked to specific honey bee symptoms. Chemical control of the mite was rapidly set up using different acaricides as fluvalinate, amitraze or coumaphos. After ten years, classical problems of pest management appeared: resistance of the mite to acaricides and pesticide residues in the wax and

bee products. Molecular evidence of this resistance was found and high amounts of acaricide residues could be detected in different parts of the colony. To prevent those effects, different biological and technical methods were established to control the mite, but they were usually time consuming and not very efficient, and so, not attractive to be used by professional beekeepers.

In parallel, as little was known on the biology of the mite, basic research was done on that field, and many data were then published on the biological cycle, physiology and reproduction of the mite. Host-parasite interactions were studied intensively. Chemical communication between the host and the parasite was also studied to find out how the parasite detects its host: different kairomonal compounds were found but to date they are not yet used to control the mite populations. Detection of the parasite by adult bees is the basis of a major behavioural resistance of the bees, but the chemical communication mechanisms of this resistance are not yet known.

Varroa destructor originated from *Apis cerana,* a bee from South Eat Asia. There, the mite performs an equilibrated parasitism with its host. The interactions between V*arroa destructor* and *Apis cerana* has been extensively studied and compared to *Apis mellifera* in an attempt to understand the mechanisms underlying host-parasite equilibrium. A few behavioural traits were found to be involved in this resistance and to be potentially interesting for breeding varroa resistant bees.

Varroa destructor population genetic studies revealed the presence of two different haplotypes in Europe and in the Americas, the Korean and the Japanese. The Korean haplotype is spread in Europe and has a clonal population structure.

Population dynamic studies of the host and the parasite have shown that mite populations increase very quickly and can kill a bee colony in one or two years. But the mite also triggers the multiplication of honey bee virus. It has been shown that the mite modify the honey bee immune system, so that the virus can multiply.

The knowledge of honey bee resistance mechanisms should have helped to sort out selection criteria for *Apis mellifera* breeding programs. However, despite efforts to breed *Varroa* resistant bees, little progress had been made. Many scientists have try to select resistant bees against the mite. Few traits have been selected but never gave real resistant strains of bees. In the meantime, some honey bees wild populations reappeared in few places. In America, Africanized bee was the first example of bees surviving to the mite without any control. Another example was discovered in a few places in France in 2000-2007 with bees surviving more than 7 years without any varroa control. Those bee populations are particularly useful to determine the possible mechanisms of mite resistance in *Apis mellifera*.

Recently, the whole honey bee genome has been sequenced and micro-arrays containing the whole genome are available. This tool is used to find out the effects of the parasitism on honey bee gene expression. It should help us to learn more about the molecular responses of the bees to mite parasitism and can also be used as a tool to select resistant bees: comparative gene expression studies between varroas resistant and susceptible bees are done to identify genes involved in the resistance. This approach should reveal specific pattern of resistant bees be used in honey bee selection and breeding. Preliminary data are available.

Finally, the basic biology of the mite, including chemical ecology, behavioural studies, population dynamics and genetics, as well as molecular responses of the honey bees, have been investigated for more than 25 years producing an impressive amount of scientific literature, making of the varroa - honey bee interactions one of the most extensively studied host - parasite model system. More recently, some natural honey bee populations have been shown to survive to mite infestations. Including different tools available to control the mite, those resistant honey bee colonies are opening the way to the concept of integrated Varroa management strategies for beekeeping.

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THE SCHLERN/SCILIAR MASSIF (SOUTHERN ALPS, ITALY) – A BIODIVERSITY HOTSPOT FOR ORIBATID MITES (ACARI, ORIBATIDA)

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Abstract

Results on the oribatid mite fauna from a large-scale project on the Schlern/Sciliar mountains are presented. More than 250 species were recorded, among them 21 new records for Italy, and at least 3 species new for science. The oribatid fauna from the Schlern massif is compared with adjacent areas in the Alps as well as with other mountains in Italy. The proportion of “southern species” (species with preference for xerothermic habitats of South Europe and the Mediterranean) is remarkable. Other species seem to have survived the last glaciation period on nunataks.

Key-words

Distribution, biogeography, faunistics, Europe

Introduction

The Schlern massif in the Southern Alps has been a favoured destination for natural scientists since a long time (e.g. Gredler 1863). Several botanical and entomological investigations were carried out. Studies on mites are scarce yet, but the Tierser and Tschamin Tal (Val di Tires and Valle di Ciamin) in the southern part of the massif are "notorious" spots for chigger mites (*Neotrombicula autumnalis* and *N. desaleri*, Schmölzer & Hellrigl 1996). Prior to the present study no oribatid mites were known from the Schlern massif itself, apart from first results from recent "biodiversity days" (Schatz 2005b, 2006, Fischer & Schatz 2007). Few species were recorded from the adjacent Langkofel (Sasso Lungo) (Janetschek 1957) as well as from the surrounding Dolomite mountains (Marcuzzi 1956, 2003).

Until ten years ago only about a hundred oribatid species were known from South Tyrol (Schmölzer & Hellrigl 1996). Due to intensive investigations, mainly in the course of the "biodiversity days" (Schatz 2005b, 2005c, 2006, Fischer & Schatz 2007) as well as during a study in riverine forests along the river Adige (Schatz 2004, 2005a) this number could be more than doubled. Nevertheless, the oribatid fauna of South Tyrol and of the Southern Alps can be considered to be still poorly known. Almost every larger collection contains species new for the region.

The aim of this study was a survey of oribatid species in representative habitats of the Schlern as part of a large-scale project to investigate the flora and fauna in the Schlern/Sciliar region ("The Schlern/Sciliar Habitat"), initiated by the Museum of Nature South Tyrol, the Agency for Nature Parks of the Provincial Department for Nature and Landscape, and the Provincial Department for Forestry.

Investigation area

The Schlern massif in the Southern Alps is part of the Dolomites. These mountains consist mainly of sedimentary limestone with typical stratification. The Schlern was formed as a reef-basin in the Tethys and uplifted during the Late Trias. The compact formations were broken by intruding magma. These volcanic embeddings weathered to a greater extend than the surrounding rocks forming jagged peaks and ridges. During the last glaciation (highest level about 20.000-18.000 yrs bp) the Schlern massif was covered with ice to a level of about 2200 m a.s.l. (Van Husen 1987, Keim 2008).The highest peaks protruded as unglaciated mountains from the surrounding ice forming exposed nunataks. After the deglaciation (about 11.500 yrs ago) large rockfalls and moraine deposits changed the landscape. The highest elevation of the Schlern massif is Mount Petz (2563 m a.s.l.). The characteristic shape of the Schlern with its lateral peaks, the "Santner" and "Euringer", are regarded as an emblem of South Tyrol. The adjacent Seiser Alm (Alpe di Siusi) is the largest mountain pasture at high altitude in Europe. The mixture of different bedrocks causes a high diversity of habitats, from the forested montane and subalpine zone up to the alpine zone.

Material and methods

The oribatid mites were collected in 16 different sites within typical habitats of the nature park Schlern–Rosengarten (Sciliar–Catinaccio) (table 1) by extraction of soil and litter samples, sieving, pitfall traps and hand collecting. Material from previous studies in the area (biodiversity days in St. Konstantin, Schatz 2005b, Tiers-Rosengarten, Schatz 2006, Plattkofel, Fischer & Schatz 2007) was included into this study. More than 30.000 adult specimens were determined. Juvenile instars were not considered in this study.

Table 1. Oribatid mites on the Sciliar massif (South Tyrol, Italy) – investigation sites

Altitudinal belt	Investigation site	Site #	Altitude (m a.s.l.)
alpine	alpine pasture (high plateau of Schlern)	1	2450
	bog in alpine meadow (high plateau of Schlern)	2	2400
	calcareous scree around summit of Mount Petz	4	2550-2560
	alpine meadow and cushion plants on volcanic rocks (high plateau of Schlern)	5	2250
subalpine	dwarf-shrub and cushion plants on dolomitic rocks (scarp north of high plateau)	3	2200-2220
	Pinus mugo shrubs with *Erica*, *Rhododendron* (scarp north of high plateau)	6	2170
	meadows mixed with arid grassland and bog (Seiser Alm)	7	1820-1870
	Pine forest (*Pinus sylvestris* and *P. mugo*) with undergrowth of *Rhododendron* and grass	10	1500
	Pinus mugo shrubs and grass on dolomitic scarp	13	1600
montane	arid meadow with larch (*Larix decidua*) (Tierser Tal)	8	1250
	Pine forest with arid grass, (burnt 1997) (Tierser Tal)	11	1180
	dry fir forest (*Picea abies*) (Bad Ratzes)	9	1270-1300
	fir and spruce forest (*Picea abies, Abies alba*) with boulders (Bad Ratzes)	12	1220-1270
	riparian forest (*Alnus incana, Picea abies*) on stream (Bad Ratzes)	15	1220
	bog in pine forest near Völser Weiher (lake Fiè)	14	1020-1050
	reed swamp with *Salix* at edge of Völser Weiher (lake Fiè)	16	1050

Results

A total of 263 species belonging to 58 families were encountered in the Schlern area. Table 2 lists remarkable findings. A complete species list with details of distribution on the Schlern massif is given in Schatz (2008). Among them, 74 species are new records for South Tyrol and 21 new for Italy (see Bernini *et al*. 1995, updated). The circumtropical species *Galumna flabellifera* Hammer, 1958 was recorded for Europe for the first time, at least three species are new for science and will be

described in the near future (*Tectocepheus* sp., *Trichoribates* sp., cf. *Trichoribates* sp., Bayartogtokh & Schatz submitted).

About half of the species belong to 10 families (Brachychthoniidae – 14 spp., Camisiidae – 11, Carabodidae – 10, Ceratozetidae – 18, Damaeidae – 16, Galumnidae – 12, Mycobatidae – 9, Oppiidae – 14, Phthiracaridae – 10, Suctobelbidae – 15 spp.). Some species are very frequent and occur in almost all investigated sites (most frequent species are *Atropacarus striculus, Chamobates voigtsi, Dissorhina ornata, Hemileius initialis, Oppiella subpectinata, Oribatula interrupta, O. tibialis, Phthiracarus laevigatus, Tectocepheus sarekensis, T. velatus, Trichoribates trimaculatus*). On the other hand, 97 species were recorded only in a single or in two sites, and 49 species are represented only by 1 or 2 specimens. The high number of single specimens indicates a probable occurrence of additional species in the area.

Table 2. Oribatid mites on the Schlern massif (South Tyrol, Italy) – list of remarkable findings, species new for the Italian (It) or North Italian (NIt) fauna, species with a distribution restricted to the Alps (alp end), or to Central and South Europe, and species with ecological preference to xerothermic habitats ("southern species" Ssp).
Nomenclature according to Weigmann (2006). Investigation sites on Schlern see table 1. Remarks: General habitat preference (according to Schatz 1983a, Weigmann 2006, updated: alpine al, aquatic-limnic aq, arboricolous ar, euryoecious eu, hygrophilous hy, lichenicolous li, mesohygrophilous mh, muscicolous mu, praticolous pr, silvicolous si, tyrphophilous ty, xerophilous xe. General distribution: alpine endemite alp end, Central Europe ceur, Central and South Europe cseur, Central and North Europe cneur, (semi)cosmopolitan cos, Europe eur, Holarctic region hol, Palaearctic region pal, South Europe seur.

Species	Sites on Schlern	Remarks
Allosuctobelba grandis (Paoli, 1908)	9	mu si / hol
Allosuctobelba ornithorhyncha (Willmann, 1953)	9 12	si / alp end / It
Amerobelba decedens Berlese, 1908	9	si xe / cseur / Ssp
Anachipteria alpina (Schweizer, 1922)	4	al li mu xe / cseur / Ssp
Arthrodamaeus reticulatus (Berlese, 1910)	4 5 13	xe / cseur / Ssp
Belba bartosi Winkler, 1955	9 10 15	si / cseur / Ssp
Camisia invenusta (Michael, 1888)	3 5	li mu / pal
Carabodes schatzi Bernini, 1976	4 6 11	al / alp end
Carabodes subarcticus Trägårdh, 1902	1 5	hy si ty / pal, arctic (cneur) / It
Carabodes tenuis Forsslund, 1953	15	si / pal / NIt
Centroribates mucronatus (G. & R. Canestrini, 1882)	11	mu / seur / Ssp
Cepheus tuberculosus Strenzke, 1951	9	si xe / cseur / Ssp
cf. *Trichoribates* sp.	3 6	? / alp end / It
Ctenobelba pectinigera (Berlese, 1908)	3 5 9 10 11 13	si xe / cseur-pal / Ssp
Eobrachychthonius longisetosus Csiszar, 1961	16	? / ceur / It
Epidamaeus berlesei (Michael, 1898)	6 8 9 10 11 12 13 15	eu / cseur / Ssp
Eueremaeus valkanovi (Kunst, 1957)	1 3 4 5 6 7 8 9 11 13	ar mu xe / cseur-pal / Ssp
Eupelops subuliger (Berlese, 1916)	3 6 9 12 15	si / cseur / Ssp
Eupelops variatus (Mihelčič, 1957)		? / s eur / Ssp
Fosseremus laciniatus Berlese, 1905	13	xe / hol-cos / Ssp
Furcoribula furcillata (Nordenskjöld, 1901)	11	si / hol / It
Fuscozetes intermedius Caroli & Maffia, 1934	1 2 4 5 7	al? / cseur / Ssp
Galumna flabellifera Hammer, 1958	10	? / hol-cos / Ssp / It
Galumna obvia (Berlese, 1915)	14 16	hy pr si / pal / Ssp?
Globozetes longipilus Sellnick, 1929	8 9 11 13 15	si xe / cseur / Ssp

Table 2 (continued)

Species	Sites on Schlern	Remarks
Gymnodamaeus bicostatus (C.L. Koch, 1836)	9	ar li mu si xe / hol / Ssp
Hemileius? sp.	5	? / pal? / It
Hermanniella septentrionalis Berlese, 1910	10 13 15	mh mu si / hol / Ssp?
Heterochthonius gibbus Berlese, 1910	9	li mu / pal / Ssp
Hungarobelba visnyai (Balogh, 1938)	12	? / pal / Ssp
Kunstidamaeus diversipilis (Willmann, 1951)	2 4 6	al li mu pr / alp end
Lagenobates lagenula (Berlese, 1904)	9	ty / pal
Licneremaeus licnophorus (Michael, 1882)	8 9 12 13 15	ar mu si xe / pal / Ssp
Licnodamaeus pulcherrimus (Paoli, 1908)	9 11	si xe / cseur-pal / Ssp
Liebstadia longior (Berlese, 1908)	8 13	ar li mu / pal / Ssp
Liebstadia pannonica (Willmann, 1951)	8 12 13	pr xe / pal / Ssp
Liebstadia willmanni Miko & Weigmann, 1996	1 3 4 6 10 13 15	hy pr si / ceur / It
Limnozetes ciliatus (Schrank, 1803)	2	aq hy ty / eur / It
Liochthonius hystricinus Forsslund, 1942	9 12	eu / hol / NIt
Liochthonius leptaleus Moritz, 1976	13	si / pal / Ssp
Microzetorchestes emeryi (Coggi, 1898)	8	mu si xe / pal / Ssp
Mucronothrus nasalis (Willmann, 1929)	7	aq hy mu ty / hol-cos
Multioppia glabra (Mihelčič, 1955)	3 5 8 9 11 13	hy si ty / pal / Ssp
Mycobates alpinus (Willmann, 1951)	2 3 4 6	al ar mu pr xe / ceur
Mycobates bicornis (Strenzke, 1954)	2 15	al mu / cseur-pal / Ssp
Mycobates carli (Schweizer, 1922)	1 2 4 6 7 9	li mu / cseur / Ssp
Nanhermannia sellnicki Forsslund, 1958	5 12 14 16	hy si / pal / It
Neonothrus humicolus (Forsslund, 1955)	2	si ty / hol / It
Ommatocepheus ocellatus (Michael, 1882)	12	ar li / cseur-pal / Ssp
Oppiella (Oppiella) uliginosa (Willmann, 1919)	6 8 9 10 11 12 14 16	ar mu si / cseur
Oribatella hungarica Balogh, 1943	13	si xe(?) / cseur / Ssp
Oribatella longispina Berlese, 1915	1 3 4 5 6 9 10 11 12 13 15	al pr mu si / cseur / Ssp
Oribatella sexdentata Berlese, 1916	8 13	si / hol / NIt
Oribatella superbula (Berlese, 1904)	5	mu xe / pal / Ssp
Oribatula amblyptera Berlese, 1916	5 7 9 11 12 14 15	xe / cseur / Ssp
Oromurcia sudetica Willmann, 1939	2 4 7	al hy pr ty / cseur
Pantelozetes paolii (Oudemans, 1913)	9 12 14	pr xe / hol / Ssp
Passalozetes africanus Grandjean, 1932	3 5 11	pr xe / hol / Ssp
Passalozetes intermedius Mihelčič, 1954	5 7 8	pr xe / pal / Ssp
Passalozetes perforatus (Berlese, 1910)	1 2 3 4 5	pr xe / pal / NIt
Pergalumna myrmophila (Berlese, 1914)	13	mh si? / cseur / Ssp

Table 2 (continued)

Species	Sites on Schlern	Remarks
Phauloppia nemoralis (Berlese, 1916)	8 9	li mu xe / cseur / Ssp
Phthiracarus boresetosus Jacot, 1930	14 15 16	si xe / hol-cos / It
Phthiracarus clavatus Parry, 1979	12	mh mu si / hol / NIt
Pilogalumna crassiclava (Berlese, 1914)	11 13 15	si xe / pal / Ssp
Platyliodes scaliger (C.L. Koch, 1839)	4 5 9 11 13	mu pr xe / hol / Ssp
Poecilochthonius italicus (Berlese, 1910)	11 13 15	pr xe / hol / Ssp
Poroliodes farinosus (C.L. Koch, 1840)	8	ar li mu xe / pal / Ssp
Protoribotritia oligotricha Märkel, 1963	12	? / hol / It
Provertex kuehnelti Mihelčič, 1959	5	li mu xe / ceur
Quadroppia galaica (Minguez *et al*., 1985)	4 5 6	mh / cseur / Ssp?
Quadroppia hammerae Minguez *et al*., 1985	5 6 11 13 15	si / pal-cos
Quadroppia longisetosa Minguez *et al*., 1985	4 5 9	si? / cseur / Ssp
Quadroppia monstruosa Hammer, 1979	6 9 10 11 12 13 14 15	si / hol / Ssp?
Scheloribates (Topobates) circumcarinatus Weigmann & Miko, 1998	14 16	hy pr ty / ceur
Scheloribates ascendens Weigmann & Wunderle, 1990	16	ar / ceur
Scutovertex sculptus Michael, 1879	11	ar mu pr xe / cseur-pal
Steganacarus vernaculus Niedbala, 1982	4 6 9 10 11 12 13 14 15	? / ceur / It
Suctobelba altvateri Moritz, 1970	3 4 5 6 9 10 11 12 13 14 15 16	hy mu si / cseur / It
Suctobelba secta Moritz, 1970	12 15	si / eur / It
Suctobelba sp.	6	?
Suctobelbella cf. *similis* (Forsslund, 1941)	9	si / pal
Tectocepheus knullei Vanek, 1960	14 16	si xe / pal / It
Tectocepheus sp.n.	4	al / alp end / It
Tricheremaeus abnobensis Weigmann & Miko, 2006	5	? / ceur / It
Trichoribates monticola (Trägårdh, 1902)	3 4 6	al / eur / It
Trichoribates sp.n.	1 2 3 4 5 6	al / alp end / It
Tritegeus bisulcatus Grandjean, 1953	3 5 9 10 12 14 15 16	si / eur / Ssp?
Unduloribates undulatus (Berlese, 1914)	3 4 6	al pr / pal / Ssp
Xenillus tegeocranus (Hermann, 1804)	1 3 4 5 6 13 14 16	eu / pal / Ssp?
Zetorchestes falzonii Coggi, 1898	8 11	li mu si xe / cseur-pal / Ssp
Zetorchestes flabrarius Grandjean, 1951	4 10 11 13 15	mu si xe / cseur / Ssp

Discussion

General distribution

The majority of the species on the Schlern show a wide distribution in Europe and beyond. Species with a marked distribution center in Central and South Europe constitute about 12% of the particular species spectrum in each site. Additional species with a preference for xerothermic habitats and a wider general distribution (e.g. whole mediterranean, southern palaearctic or holarctic region, "southern species") augment this proportion to about 20% (fig. 1).

In the wet sites around the Völser Weiher (Lake Fiè) the proportion of southern species is lowest. Mostly cold adapted, "northern" species occur here. The highest proportion of southern species was found in the arid sites around Tiers as well as in the calcareous scree around Mount Petz.

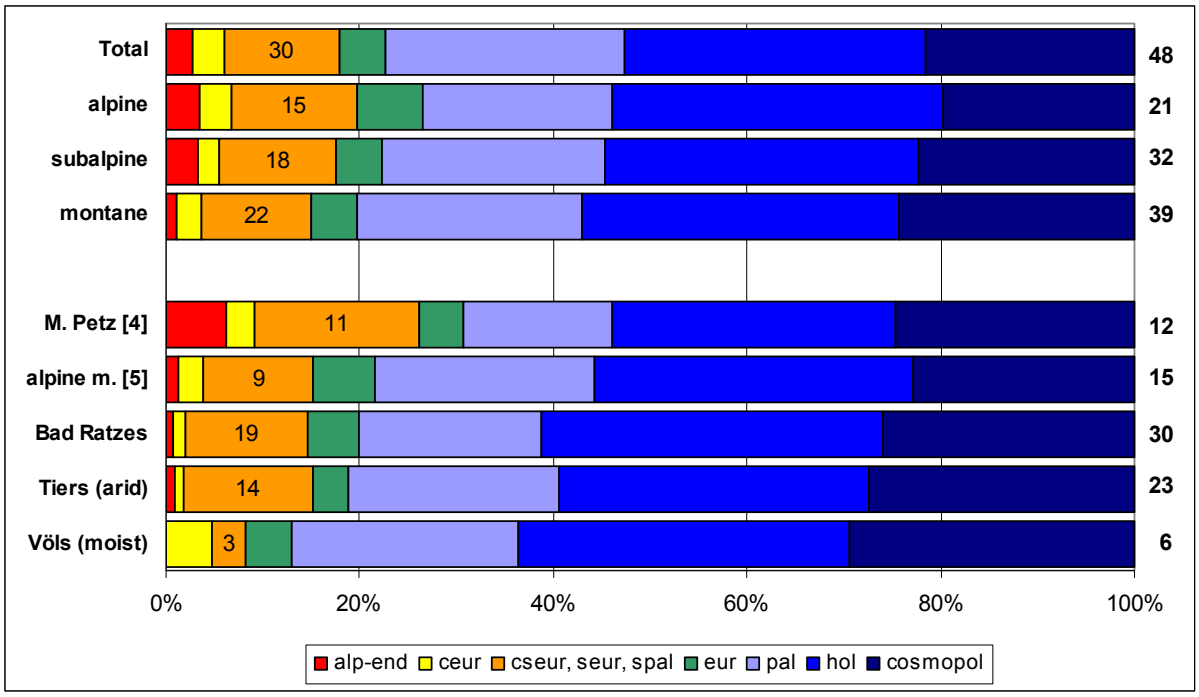

Figure 1. Oribatid mites on the Schlern massif (South Tyrol, Italy) – General distribution of species. Allocation to distribution types see table 2. Values at end of bars: number of "southern species" (species with preference for xerothermic habitats of South Europe and the Mediterranean). Grouping: Total (all sites 1-16, see table 1), alpine (sites 1 2 4 5, 2250-2560 m a.s.l.), subalpine (sites 3 6 7 10 13, 1600-2220 m a.s.l.), montane (sites 8 9 11 12 14 15 16, 1050-1300 m a.s.l.), Mount Petz (alpine meadow and calcareous scree, site 4, 2550 m a.s.l.), alpine meadow on volcanic rocks (alpine m., site 5, 2250 m a.s.l.), Bad Ratzes (montane forests, sites 9 12 15, 1220-1300 m a.s.l.), Tiers (arid habitats, sites 8 11, 1180-1250 m a.s.l.), Völs (moist sites, bog, swamp around Völser Weiher, lake Fiè, sites 14 16, 1050 m a.s.l.).

In the course of the postglacial recolonization the arch of the Alps acted as a barrier for most of the "southern species" (in the sense of Schuster 1959 and Tarman 1977). These species intruded into the inneralpine areas along suitable passages and became established only in xerothermic habitats. At the edge of the Alps, these "southern species" are more frequent (e.g. in Eastern Austria: Hundsheim mountains, Schatz & Fischer 2007), in the northern part of Central Europe these species are restricted to disjunct, climatically favoured habitats (e.g. Kaiserstuhl, Kyffhäuser in Germany, Weigmann 2006).

The proportion of alpine-endemic species (including the undescribed species) is generally low and increases slightly with altitude. On the summit of Mount Petz four alpine endemites (*Carabodes schatzi*, *Kunstidamaeus diversipilis*, *Tectocepheus* sp.n., *Trichoribates* sp.n.) contribute more than 6% to the total species number. *Allosuctobelba ornithorhyncha* (found in montane forests of the Schlern massif) is also a known alpine endemite.

The Schlern as a nunatak – possible preglacial relict species among oribatid mites

A continuous colonization throughout the glacial period in the Alps was only possible in certain habitats and retreats such as massifs de refuge along the margins of the Alps, inner alpine

nunataks or subterranean habitats (caves, ground water below the ice and in crevices), or the surface of the ice itself (Janetschek 1956, Thaler 1976). The highest peaks of the Schlern massif surmounted the glaciers. The necessary minimum area for populations of minute animals such as mites (Schatz & Schatz 1991) would be available and preglacial relicts were able to survive the pleistocene period in the same place where they occur at present. Adaptations of oribatid mites to the extreme conditions on high mountains with short vegetation periods include cold hardiness (Schatz & Sømme 1981) and prolongation of life cycle (e.g. Schatz 1983b, 1985, Grishina 1997).

Some oribatid species encountered on the Schlern occur mainly at high altitudes, as the alpine endemites *Trichoribates* sp.n., cf. *Trichoribates* sp., *Anachipteria alpina*, *Kunstidamaeus diversipilis*, *Unduloribates undulatus*, as well as *Belba compta*, the latter with an arctoalpine distribution. Another group of species expanded during the postglacial period from their main occurrence in the alpine zone and colonized also lower altitudinal levels (e.g. *Fuscozetes intermedius*, *Trichoribates monticola*).

Some species show a disjunct distribution at present, suggesting a wider geographical extension previous to the last glaciation and great geologic age (Schmölzer 1999). The species *Hemileius*? sp. may belong to this group. The single specimen from the alpine grassland on volcanic rocks (2250 m a.s.l.) shows strong similarity to an undescribed species from the eastern palaearctic region (Mongolia, B. Bayartogtokh, personal communication). If the population from the Schlern massif is conspecific, it is probably a relict species which survived the glaciation on this nunatak. The cosmopolitan but disjunct distribution of *Mucronothrus nasalis* which also occurs on the Schlern was discussed previously (Hammer 1965, Norton *et al*. 1988).

Comparison with the oribatid fauna of Massiccio del Pollino (South Italy)

The oribatid fauna of the Monte Pollino massif in the southern Apennines (Parco Nazionale del Pollino, Calabria / Basilicata) is well known (Bernini *et al*. 1986). Geology and vegetation

of this mountain range shows similarities to the Schlern. Biogeographically, the Monte Pollino represents an important refuge for the fauna of high altitudes as discussed by Bernini *et al*. (1986): during the pleistocene glaciation a cold-adapted flora and fauna was able to expand towards the southern tip of the Apennine peninsula and to colonize its mountains. Furthermore, tectonic upliftings occurred during the postglacial period. The massif of Monte Pollino was elevated 150-200 m since the end of the last glaciation (Ghisetti 1981). Today alpine species coexist with a mediterranean fauna on Monte Pollino. The same phenomenon can be observed on the Schlern massif.

In the Massiccio del Pollino a total of 299 taxa of oribatids were recorded, belonging to 243 known species (Bernini *et al*. 1986). The coefficient of species similarity to the Schlern massif constitutes more than 50 % (index of Sørensen), with 127 species in common. Among these, 27 species show an ecological preference for xerothermic habitats ("southern species", among them *Amerobelba decedens*, *Centroribates mucronatus*, *Eupelops variatus* [at M. Pollino as cf. *variatus*], *Heterochthonius gibbus*, *Hungarobelba visnyai*, *Phauloppia nemoralis*, *Poecilochthonius italicus*). Another group is representative of temperate or cooler habitats ("fauna fredda" according to Bernini *et al*. 1986, among them *Carabodes rugosior*, *Liochthonius sellnicki*, *L. strenzkei*, *Platynothrus thori*).

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LOCAL DISTRIBUTION AND GENETIC STRUCTURE OF TICK-BORNE PATHOGENS: AN EXAMPLE INVOLVING THE MARINE CYCLE OF LYME DISEASE

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Abstract

Despite the potential importance of the local structure of micropathogen populations for the epidemiology of vector-borne diseases, the spatial and temporal heterogeneity of these populations is often neglected. This variability may have strong effects on micropathogen transmission, and therefore needs to be considered more explicitly to understand disease dynamics. Here, we examine the effects of time (years) and space (cliffs) on the local distribution and genetic structure of Lyme disease bacteria *Borrelia burgdorferi* sensu lato in the marine system involving seabirds and the tick *Ixodes uriae*. We tested for the presence of *Borrelia* spp. in 351 ticks collected from Black-legged kittiwakes (*Rissa tridactyla*) within a large seabird colony by amplification of the *flaB* gene. Overall, the prevalence was 11% (±2%) and varied among sub-colonies (i.e., cliffs), but not among years. Direct sequencing of the amplified products revealed the presence of three species of *Borrelia burgdorferi* s.l.: *B. garinii*, *B. burgdorferi* sensu stricto and *B. afzelii*. This is the first record of *B. afzelii* in the marine system. Isolates of *B. garinii,* the most abundant bacterial species, were genetically structured among cliffs, but did not change significantly over time. Our findings indicate that LB spirochetes circulating in the marine cycle are highly diverse, even at a local scale, and that the spatial structure revealed by our data should be considered more widely in this system and in epidemiological studies of Lyme disease in general. For example, adequate sampling designs to reliably estimate parameters such as local prevalence, abundance and diversity will need to take this heterogeneity into account.

Key-words:

Infectious disease, micropathogen population structure, colonial seabirds, *Ixodes uriae, Borrelia burgdorferi* sensu lato

Introduction

In epidemiological studies pathogen populations are often treated as being unstructured at the scale of the host population. However, micropathogen populations may vary both spatially and temporally, even at relatively small spatial scales (e.g., Wood *et al.* 2007) and this structure can have important implications for local transmission dynamics, the evolution of these pathogens, and our perception of disease risk. Indeed, due to their ability to trigger sudden epidemics and their potential for rapid evolution, parasites and emerging infectious diseases have become a major focus in ecology and evolution and the importance of integrating their dynamics into epidemiological modelling is becoming increasing apparent (Levin *et al*. 1999).

The spatial environment of parasites is structured at two ecologically different spatial levels: the host (biotic environment) and the habitat of the host

(abiotic and biotic environments) (Thomas *et al*. 2002). Both host heterogeneity (inter and intraspecific) and the spatial variability of the host's habitat (i.e., ecosystem) may represent strong constraints on parasite ecology. These factors, and the resulting infection dynamics, can also vary over time. For example, periodic changes in climate or resource availability may modify interactions between hosts and parasites (i.e. encounter rates, virulence, resistance) and thus modify the probability of transmission (Altizer *et al.* 2006). As most pathogens have short generation times, large population sizes and are under relatively strong selection pressures, these types of ecological changes can accelerate micropathogen evolution (Ferguson *et al*. 2003). The spatial and temporal variation of the parasitic environment may be especially important in complex disease cycles that involve numerous host types, as is the case for vector-borne parasites. These systems are frequent in nature and often of great medical and economic interest. Indeed, about a third of micropathogens responsible for emerging infectious diseases are transmitted to humans via vectors (Jones *et al*. 2008).

Despite the recognized need for the integration of spatio-temporal dynamics to better understand the epidemiology of vector-borne disease, few field studies explicitly consider these factors and partially so at local spatial scales (but see Burdon & Thrall 1999; Bensch & Akesson 2003; Wood *et al*. 2007). This may be due in part to the fact that the integration of these aspects is not always easy. Such systems are complex by definition (e.g., several possible host types within the same habitat), sampling can be difficult and basic knowledge of the species involved may be missing. Here, we consider a biological system involving the tick, *Ixodes uriae*, and one of its colonial seabird hosts, the Black-legged kittiwake *Rissa tridactyla*. Along with other pathogens, this ectoparasite and its seabird hosts carry bacteria responsible for human Lyme disease. This system has the advantage that the populations of the host and vector are spatial discrete and seasonally predictable. In addition, much is already known about the basic biology of the organisms involved (see below). This system is therefore highly suitable for studying the spatial and temporal factors shaping local pathogen dynamics.

Lyme disease is the most commonly reported vector-borne human disease in temperate regions of the Northern hemisphere and has significant medical and economic impacts (Zhang *et al*. 2006). The causative agents are spirochetes belonging to the *Borrelia burgdorferi* sensu lato complex. This complex contains at least 13 species, among which four are currently described as pathogenic for humans: *Borrelia burgdorferi* sensu stricto, *B. garinii*, *B. afzelii* and *B. spielmanii*. The terrestrial cycle of this disease, involving ticks of the *Ixodes ricinus* complex and a wide variety of vertebrate hosts (mammals, birds, reptiles), is relatively well studied. However, comparable informations on the general importance of the marine cycle and the role of seabirds in the global epidemiology of Lyme disease are lacking.

Initially, *Borrelia garinii* was the only species described circulating in the marine system (Olsen *et al.* 1993; 1995; Gylfe *et al.* 1999). However recently, *B. burgdorferi* sensu stricto and *B. lusitaniae* have also been discovered in seabird ticks (Duneau *et al*. 2008). Although described as a seabird generalist parasite, recent genetic work has shown that *Ixodes uriae* has in fact formed distinct seabird species-specific host races and that the evolution of these races is on-going and recurrent (McCoy *et al*. 2001; McCoy *et al*. 2005). This divergence could lead to cascading host-associated genetic differentiation in *Borrelia* (McCoy *et al*. 2008). Microhabitat heterogeneity (cliff topography, temperature, hygrometry, etc), inter-individual differences in bird immunity and a limited capacity for active dispersal can also greatly affect tick ecology and potentially lead to within-colony structure of these populations (e.g., Needham & Teel 1991; Boulinier *et al*. 1996; Gylfe *et al*. 1999; McCoy *et al*. 1999; McCoy *et al*. 2003; Staszewski *et al*. 2008), and consequently of the parasites they carry. We can therefore expect variability in LB spirochetes at this spatial scale. Similarly, temporal variation in environmental factors such as temperature or resource availability can greatly affect the dynamics of seabirds (Sandvik *et al*. 2005) and possibly of their ticks (Ogden *et al*. 2006; Rosa *et al*. 2007). However, the degree to which this variability affects the transmission of pathogens like *Borrelia* spp. is unclear. In this sense, it would be interesting to monitor changes in prevalence and genetic variability of LB spirochetes present in ticks over time.

To improve our understanding of the importance of local factors that may affect pathogen transmission patterns in vector-borne systems, we analyzed the effect of time (years) and space (cliffs) on the prevalence and genetic structure of LB spirochetes within a large seabird colony. We focused our investigations on ticks sampled from the most numerous seabird host in the studied area, the Black-legged kittiwake (*Rissa tridactyla*). We discuss the ecological factors that may lead to

the observed patterns and the consequences of these results for the transmission, dynamics, and evolution of Lyme disease bacteria.

Materials and methods

1. Tick sampling

Ticks of different stages (nymphs, adult males and females) were sampled directly from kittiwakes (*Rissa tridactyla*) in a large colony on Hornøya, an island in Northern Norway (70°22'N, 31°10'E) (Figure 1).

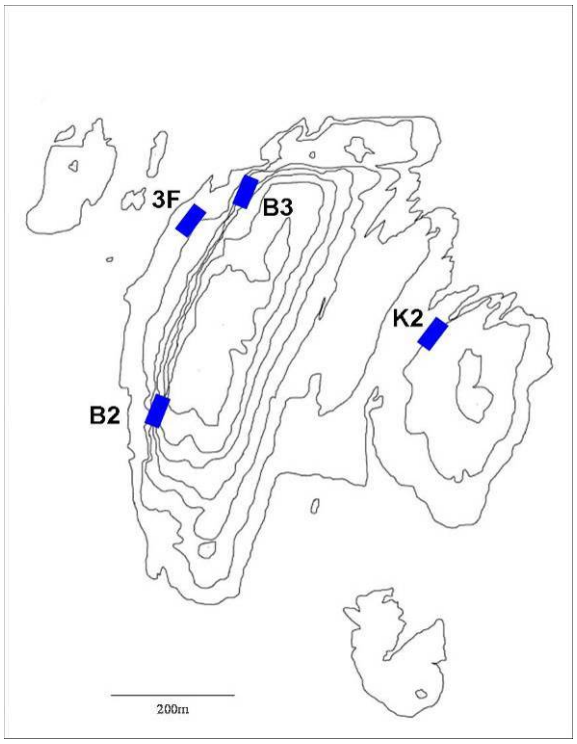

Figure 1. Location of the kittiwake cliffs sampled for Ixodes uriae on Hornøya in 1998 (70°22'N, 31°10'E). Approximately 15 000 pairs of kittiwakes breed in small cliffs spread across the island (Sandvik et al. 2005).

This colony is spatially sub-divided into discrete breeding cliffs where breeding pairs nest in groups on the vertical parts of the cliff face. Ticks can readily be collected from nestlings during the chick-rearing period (McCoy *et al.* 1999). To investigate the effect of year, we used ticks collected in 1998, 2005 and 2006 from across the island. To test the effect of cliff, we analysed kittiwake ticks collected in 1998 from birds found in different cliffs (K2, B2, B3 and 3F; Figure 1). An effort was made to collect at least 40 ticks for each cliff (K2, B2, B3 and 3F) and each year of the study (1998, 2005 and 2006). Within each cliff, the majority of ticks were collected in different nests. After collection, ticks were stored in 70-90% ethanol until DNA extraction.

2. DNA extractions, nested PCR and sequencing

DNA extractions were performed using a DNAeasy Tissue Kit (Qiagen, Valencia, CA). Conserved ticks were cut in half so as to include part of the gut and the salivary glands. A steel bead was then added in a 1.5ml tube and ticks were frozen using liquid nitrogen and ground with a mixer mill 301 (Retsch, Germany). Extractions were then performed following the kit procedures. DNA was eluted in 100µl of AE buffer and was subsequently diluted on the basis of a spectrophotometric analysis to standardize the amount used in PCRs.

To determine if a tick was infected by LB spirochetes, we used a nested PCR procedure for the amplification of the *flaB* gene using primers designed to amplify all species of *B. burgdorferi* sensu lato (Johnson *et al*. 1992). This gene is located on the linear chromosome of the bacterium and encodes a 41kDa flagellin protein. During the first PCR, a 611pb portion of the gene was amplified with primers outer1 and outer2 (Johnson *et al*. 1992). A small amount (0.5µl) of the amplicon from the first PCR was then used as the template in the second PCR. The second PCR used primers inner1 and inner2 and enabled the amplification of a 390pb sequence of the polymorphic region of this gene (Gassman *et al.* 1989). Each 25ml reaction mixture was composed of 2.5ml 10x buffer (Tris-HCl, pH 9.0, KCl, Triton1 X-100), 2ml MgCl2 (25 mM), 2ml dNTP (2.5mM), 0.5ml forward primer (20mM), 0.5ml reverse primer (20mM), 1.25U Taq polymerase (Promega), 20-50ng DNA and sterile, distilled water. The PCR conditions consisted of an initial denaturation step at 95°C for 1min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 52°C for 40s, and extension at 72°C for 1min, with a final extension at 72°C for 5min. The second PCR followed the same program, but the annealing temperature was 55°C. All PCRs were run with positive (DNA from cultured *B. garinii*-20047) and negative (distilled water) controls. A 390pb band on a 2% agarose gel was used as an indication of the presence of LB spirochetes in the sample. All positive amplifications were re-amplified and sent for direct sequencing (Genome Express, Meylan France). To increase the quality of sequences, both DNA strands (forward and reverse) were sequenced. All negative samples were tested at least twice.

3. Analyses of diversity and spatial and temporal structure

In order to describe the diversity and relationship of the detected LB spirochetes strains, we carried out a phylogenetic analysis. The freeware BIOEDIT (Hall 1999) was used to verify the complementarity of the *flaB* sequences obtained. We aligned our sequences with 24 reference sequences obtained from Genbank. Reference sequences included two divergent sequences from each of 13 currently described species of the *B. burgdorferi* s.l. complex, if available, and a *B. hermsii* sequence (relapsing fever *Borrelia*) used as the outgroup (e.g., Fukunaga *et al*. 1996) (Table 1).

Table 1. Reference sequences used in the phylogenetic analyses.

Species	Isolate	Genbank accesion no.
Borrelia afzelii	Iper 3	AY342020
	ACA1	AB035613
Borrelia andersonii	21038	D83763
	19857	D83762
Borrelia bissettii	CA128	DQ393343
Borrelia burgdorferi sensu stricto	B31	X15661
	GeHo	X15660
Borrelia californiensis	CA443	DQ393348
	CA404	DQ393346
Borrelia garinii	Ip90	L42885
	20047	D82846
Borrelia japonica	NT112	D82853
	HO14	D82852
Borrelia lusitaniae	47ZLIM	DQ788619
	D23-04	DQ016623
Borrelia sinica	CMN1a	AB022138
	CMN3	AB022138
Borrelia spielmanii	PC-Eq2/1	AY450560
Borrelia tanukii	Hk501	D82847
	OR1eR	D85070
Borrelia turdi	Kt501	D82851
Borrelia valaisiana	OS66/01	AB091715
	CMN1b	AB022134
Borrelia hermsii (outgroup)	YOR	AY597806

We used MODELTEST 3.7 to search for the best-fit model of nucleotide substitution for our sequence data (Posada & Crandall 1998). The selected model was then applied in a maximum likelihood phylogenetic analysis using PHYML (Guindon & Gascuel 2003) and the resulting tree was drawn using TREEVIEW (Page 1998). Bootstrap analysis (1 000 repetitions) was performed to evaluate the robustness of branches.

Differences in prevalence of *Borrelia* positive ticks among years and cliffs were tested using Fisher's exact tests with the program STRUC of the software GENEPOP (Raymond & Rousset 1995). We then tested whether these isolates showed spatial or temporal genetic structure. AMOVA analyses were performed among years and among cliffs using ARLEQUIN 3.1 (Excoffier *et al*. 2005). The significance of the estimated variance components was tested by a permutation procedure.

Results

Among the 351 ticks tested, we found 38 positive for *B. burgdorferi* s.l. The bacterium was found in ticks of the 3 years and in the 4 cliffs considered (Table 2).

1. Phylogenetic analyses

Among the 38 positive samples, 29 clear sequences were obtained and we found 14 different haplotypes. For the phylogenetic analysis, we therefore used the 14 sequences from our samples and the 24 references sequences from Genbank. A given sequence was only included once in tree construction to avoid giving disproportionate weight to certain mutational events. After alignment, a 367pb region of the *flaB* gene was used for the analysis. The sequences from the present study clustered into three well-supported groups (Figure 2). The majority of our samples (72%) clustered with the *B. garinii* reference sequences (Group 1). Seven sequences (24%) grouped close to *B. burgdorferi* s.s (Group 2), and one sequence (4%) was most closely related to *B. afzelii* (Group 3).

2. Prevalence

The overall prevalence across years and cliffs was 11% (± 2%). There was no difference in the prevalence of infected ticks among the three years (1998, 2005 and 2006; Fisher's exact test, p=0.360; Figure 3a). However, the time step between years 2005 and 2006 may not represent independent samples given that a single tick generation takes approximately 4 years to complete. We therefore also grouped these two years and tested for a difference between 1998 and 2005/2006: no significant difference was found in this time period (Fisher's exact test, p=0.280). However, prevalence did vary significantly among cliffs (Fisher's exact test, $p<0.05$). The prevalence from ticks collected

in cliffs B2, B3 and 3F was similar and significantly higher than that of cliff K2 (Figure 3b).

Table 2. Prevalence of LB spirochetes in kittiwake *Ixodes uriae* ticks from Hornøya.

	1998	2005	2006	Total
No. ticks (N, AM, AF)	233 (110, 0, 123)	57 (0, 35, 22)	61 (2, 0, 59)	351 (112, 35, 204)
No. positive ticks (N, AM, AF)	22 (15, -, 7)	9 (-, 9, 0)	7 (0, -, 7)	38 (15, 9, 14)
Prevalence (%) (N, AM, AF)	9 (14, -, 6)	15 (-, 26, 0)	11 (0, -, 12)	**11 (13, 26, 7)**

* N : nymph; MA : adult male; AF : adult female

3. Genetic structure

Given that *B. garinii* was the most common species found in ticks (72%), we excluded *B. burgdorferi* s.s and *B. afzelii* from the following analysis to avoid bias induced by the presence of highly divergent *Borrelia* species. We did not performed independent analysis for this species because of their low prevalence. We found that *B. garinii* isolates were not genetically structured in time (1998, 2005, 2006; AMOVA, F_{st}=-0.322, p=0.936 and 1998 vs. 2005/2006; AMOVA, F_{st}=-0.308, p=0.935). For the spatial analysis, we excluded cliff K2 because only one sequence was available for this cliff. We found significant spatial genetic structure among cliffs. *B. garinii* isolates from cliff 3F were genetically different from isolates of the two other cliffs (AMOVA, $\Phi_{ST(3F,B2)}$=0.628, p<0.001; $\Phi_{ST(3F,B3)}$=0.626, p<0.001).

Discussion

Despite almost two decades of research focused on understanding the global epidemiology of Lyme disease, we still lack key elements on the local structure of host, vector and micropathogen populations and how these structures may alter transmission patterns. We also are missing information on the relative importance of alternative transmission cycles, such as the role of seabirds in the maintenance and transmission of LB spirochetes. Here, we focused on the structure of the micropathogen population at a fine spatial scale. We analyzed the prevalence and genetic structure of LB spirochetes in kittiwake ticks to examine the factors that may affect local transmission dynamics and to consider the potential importance of such variability for epidemiological studies. We found a global prevalence of 11% (± 2%) and detected the presence of three species of LB spirochetes, one of which had never been previously detected in the marine cycle.

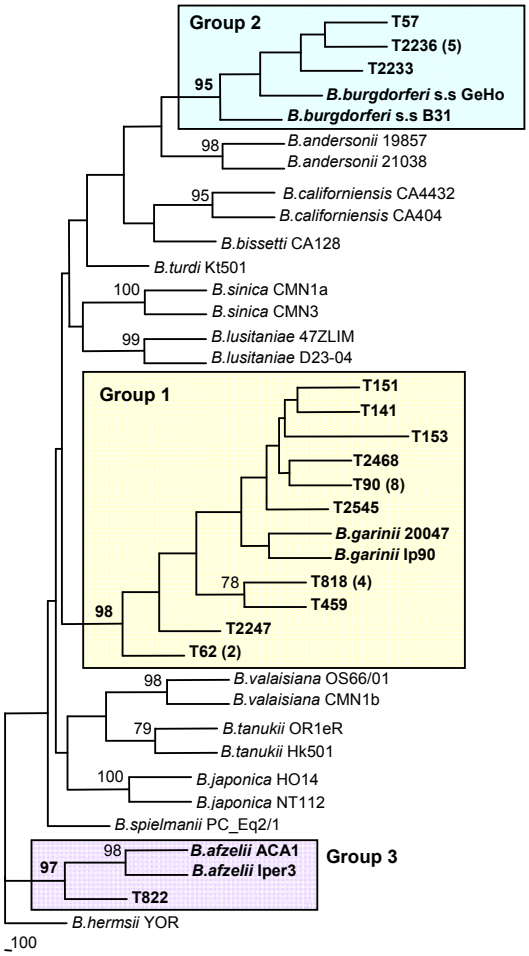

Figure 2. Maximum likelihood phylogenetic tree of the *Borrelia flaB* sequences. Only bootstrap values greater than 75% are indicated on the tree. The sequences from this study are referred to by a ''T'' followed by a number. Several copies of a given sequence were sometimes found; the total number is indicated in brackets. Sequences clustered in three well-supported groups: group 1 is closely related to ***B. garinii***, group 2 clustered with ***B. burgdorferi* s.s** and group 3 is related to ***B. afzelii***.

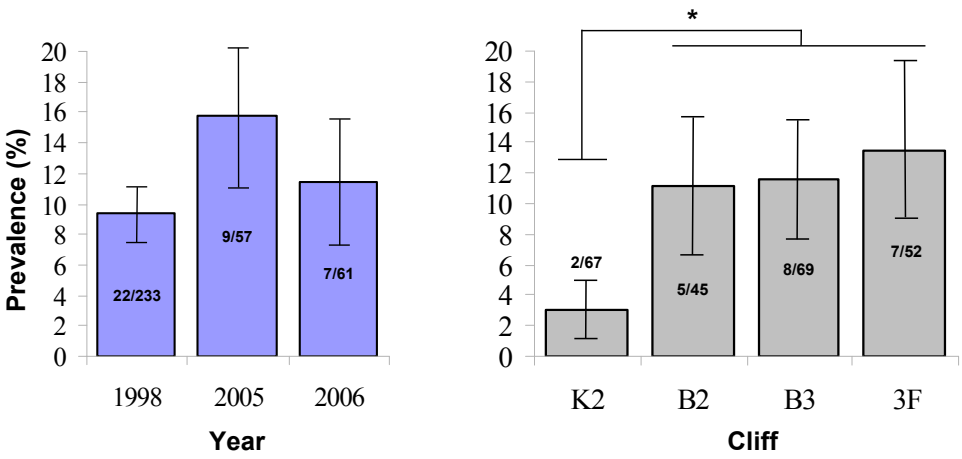

Figure 3. Prevalence of *B. burgdorferi* s.l. in the tick *Ixodes uriae*. (a left) Among kittiwake ticks collected in different years (b right) Among kittiwake ticks collected in different cliffs within the same year (1998); the symbol (*) means p<0,05. The confidence interval of the prevalence was computed by calculating the standard deviation of binomial law $\sqrt{((p(1-p))/n}$ where *p* is the prevalence and *n* the total number of analyzed ticks. The number of the positive ticks over the number tested is indicated on the histogram.

Diversity of LB spirochetes in the marine cycle

In this study, we identified three species of LB spirochetes belonging to the *Borrelia burgdorferi* s.l complex. The most abundant species, *B. garinii* was previously described in seabird ticks of both hemispheres (Olsen *et al*. 1993; 1995). This bacterial species seems to be the dominant species in the marine system and shows relatively high polymorphism (Lagal *et al*. 2002; Duneau *et al*. 2008). We also detected *B. burgdorferi* s.s, a species which has been detected once before in the same colony in a tick sampled from a common guillemot (*Uria aalge*) (Duneau *et al*. 2008). It should be noted that we have also found this bacterial species in further recent analyses of puffin ticks on Hornøya (data not shown). In the terrestrial cycle, *Borrelia burgdorferi* s.s is considered to be a generalist in terms of the vertebrate host that it can infect (Hanincova *et al*. 2006). Our results from kittiwake ticks support the presence of *B. burgdorferi* s.s in the marine cycle. Interestingly, we also found *B. afzelli*, a species which has never been previously described in seabird tick. We have also found this bacterial species in puffin and guillemot ticks on Hornøya (data not shown). The question of a link between terrestrial and marine cycles therefore requires further consideration as several bacterial species seem to be shared between these supposedly independent cycles.

In this study, we did not detect *B. lusitaniae*, another species recently found in *Ixodes uriae* in Iceland (Duneau *et al*. 2008). As the prevalence of *B. garinii* isolates varies between geographic sites in the North Atlantic (Duneau *et al*. 2008), and *B. burgdorferi* s.s and *B. afzelli* have not yet been found in ticks from colonies other than Hornøya, this could suggest that the circulation of some strains is constrained geographically. This observation is in agreement with the genetic structure found in the tick vectors of this pathogen, where populations in Northern Norway were found to be strongly differentiated from other North Atlantic colonies (McCoy *et al*. 2005). It should nevertheless be noted that a full picture of the circulation of the bacterial strains in the marine cycle will require further sampling and analyses in other large colonies before any generalization can be made. Nevertheless, we can say our results support the hypothesis that seabirds are avian reservoirs of LB spirochetes and that the dispersal abilities of birds (seabirds, migratory passerines) could be an important element in the transmission of these pathogens at large spatial scales (Olsen *et al*. 1993; Olsen *et al*. 1995; Larsson *et al*. 2007; Duneau *et al*. 2008).

Temporal dynamics

A consideration of temporal infection dynamics is required to understand the interactions among the different species implicated in vector systems. Periods of high micropathogen transmission can alternate with periods of low transmission, sometimes leading to the local extinction of micropathogen populations (e.g., Craig *et al*. 2004). For instance, in the case of marine cycle of Lyme disease, birds may be submitted to greater stress

and spirochetemia may be higher (Gylfe *et al*. 2000) in years when resources are scarce, thereby increasing the infection rate of feeding ticks. In years when resources are abundant, tick infestation and LB spirochete transmission could decrease because the immune response of birds is more effective at reducing circulating bacteria. In the same way, annual changes in climatic conditions (temperature, humidity, etc) can affect the number of ticks likely to transmit LB spirochetes (Ogden *et al.* 2006). By limiting the population size of the vector, such fluctuations can modify the genetic diversity of micropathogens and lead to rapid genetic drift (Ferguson *et al*. 2003).

In our study, prevalence did not vary in time and *B. garinii* isolates were not genetically structured between years. This suggests that the epidemiological cycle of LB spirochetes at the study site is relatively stable over time. However, these results should be treated with caution with respect to the time scale considered here. A time step of eight years may not be long enough to detect molecular divergence in isolates, and particularly so with the conserved gene we considered here. Likewise, the number of years analyzed may not be enough to observe a difference in prevalence. Nevertheless, different hypotheses could explain these preliminary results. First, the environmental conditions may be constant enough in time (e.g. climate, fish resources for birds) to stabilise transmission rates. Recent work has shown an interannual repeatability in seabird immunity against LB spirochetes (Staszewski *et al*. 2007). This could be due to repeated exposure to the bacteria or to the persistence of LB spirochetes in infected birds. Indeed, *Borrelia burgdorferi* s.l can persist in birds over several months (Isogai *et al*. 1994; Olsen *et al*. 1996) and parasitemia can be reactivated in periods of stress (Gylfe *et al.* 1999). The temporal patterns observed may also depend on when tick infection takes place. If infections in birds are reactivated after migration to the breeding site, effects such as resource availability at the breeding site and adult body condition may not come into play in infection dynamics (i.e., vectors feed on birds before these effects are seen).

Spatial structure

Habitat heterogeneity has been studied in detail in the terrestrial cycle of Lyme disease (e.g., Van Buskirk & Ostfeld 1998; Medlock *et al.* 2008). Van Buskirk & Ostfeld (1998) have shown, for example, that spatial variability may have an important effect on the distribution of ticks and the prevalence of LB spirochetes. In the marine cycle, the distribution of *Ixodes uriae* among hosts has been found to vary at different hierarchical scales (Bouliner *et al*. 1996). In the Southern hemisphere, among different parts of a King penguin (*Aptenodytes patagonicus*) colony, ticks were found to be more abundant in dry habitats with shelter rocks compared to more humid areas of the colony (Gauthier-Clerc *et al.* 1998, 1999). Therefore, habitat structure is likely an important factor shaping the local distribution of ticks and thus the transmission probability of LB spirochetes.

In our study, prevalence varied at a small spatial scale; LB spirochetes were more abundant in ticks from cliffs on the west side of the island (cliffs B2, B3 and 3F) compared to the east side (cliff K2). Moreover, we observed genetic structure of *B. garinii* isolates among cliffs. These results could be explained by both abiotic and biotic factors. For example, cliff topography may play a role in the ability of ticks to disperse locally (McCoy *et al*. 2003). Given that seabirds are highly faithful to their nest sites among years (Danchin *et al*. 1998), ticks may be more or less isolated within a cliff depending on the local density of nests. Likewise, a recent study has shown that the specific immunity of seabirds against LB spirochetes can also vary between cliffs (Staszewski *et al.* 2008). Combined with the spatial stability of these birds and their nest parasites, the resulting patterns of among cliff variability in *Borrelia* spp. may be linked to differences in individual susceptibility to infection by the bacteria.

Conclusion and perspectives

Like other vector-borne disease systems, the marine cycle of Lyme disease is a complex and dynamic system (Kurtenbach *et al*. 2006); hosts, vectors and micropathogens can vary at local scales and this variability can alter the transmission patterns and, as a consequence, the distribution of disease risk. Together with previous studies on this system, our results underline the fact that the three elements implicated in vector systems have to be treated together to better understand disease epidemiology. More specifically, spatial and temporal variability in micropathogen populations will need to be considered explicitly in empirical studies of Lyme disease. For example, samples may need to be obtained from throughout a given population in order to obtain reasonable estimates of diversity and prevalence.

Here we found that *Borrelia* spp. infection in *I. uriae* ticks varies among different cliffs within a single colony. The next step will now be to link our

results with other ecological and epidemiological parameters (microhabitat conditions, tick infestation rates, bird immunocompetence and philopatry) to improve our understanding of the spatial and temporal dynamics of LB spirochetes in marine birds. Moreover, the same approach in ticks from other seabird species (e.g., puffins, guillemots) and in other colonies will be necessary to verify the general nature of our results. Finally, further genetic analyses would help us to determine how close the bacterial isolates found in this study are to those in the terrestrial cycle (see Duneau *et al*. 2008). Given that LB spirochetes in birds often show higher polymorphism than those circulating in mammals (Ras et al. 1997; Wang et al. 1999), seabirds could represent an important source a novel strains of LB spirochetes. According to the degree of interaction between terrestrial and marine Lyme disease cycles, and given the high dispersal capacity of birds, the involvement of the marine system may greater alter our current perception of Lyme disease epidemiology.

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PHYLOGENY AND SPECIATION

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AN INTERESTING CASE OF VICARIANCE IN THE ENDEMIC MITE GENUS *ACROSEIUS* IN EASTERN AUSTRALIA (ACARI: UROPODINA: TRACHYTIDAE)

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Abstract

A new species in the Australian endemic genus *Acroseius* (Acari: Trachytidae) was recently found in rainforest leaf litter in north Queensland. The three known species of *Acroseius* occur in different geographic areas, and each occurs in one of the three major forest types in eastern Australia. This is an interesting case of vicariance in mites.

Key words

Acroseius, Trachytidae, rainforest, Australia, vicariance

Introduction

The Australian continent is characterized by unique fauna and flora. Owing to its isolation, Australian Uropodid fauna is very interesting from zoogeographical and phylogenetical points of view, as it has been proven by Błoszyk and Hallida (1995, 2000). The presence of several distinct taxa in this region should be expected and would give much more information about the evolution and the origin of this group of mites. The genus *Acroseius* (Błoszyk *et al*. 2005) can be considered one example.

During a study on mites belonging to the suborder Uropodina in Australia, we described the observed different species from the endemic Australian genus *Acroseius* and also found a new species never identified before. This new species was collected in rainforest leaf litter, in Eungella National Park, north Queensland. This is the third species identified in the genus; the others are *Acroseius tuberculatus* (Womersley 1961) and *Acroseius womersley*i Błoszyk *et al*. (2005).

Morphological characteristics of species of the genus *Acroseius* in Australia

The three species of *Acroseius, Acroseius tuberculatus* and *Acroseius womersley*i that have been already described and the third one that has been found during our last investigations, are morphologically close related, but can be easily distinguished from each other (Fig. 1).

Long, flat setae on the rear part of the body, as well as wide shields on legs I, are the features that enable to differentiate the new species from the remaining two. Female epigynium does not have the characteristic notch, as in the case for *A. tuberculatus,* and the ventral part is less ornamented.

A full description of the new species, along with an analysis of morphological variability of all three species, will be published in a separate paper.

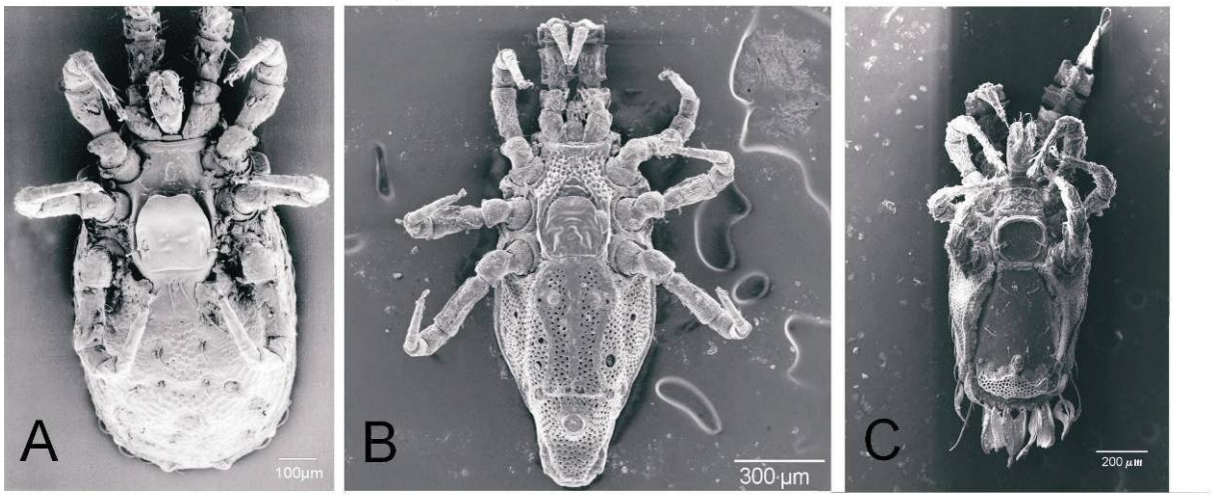

Figure1. A - *Acroseius tuberculatus*, B - *Acroseius womersleyi*, C - *Acroseius* sp. nov.

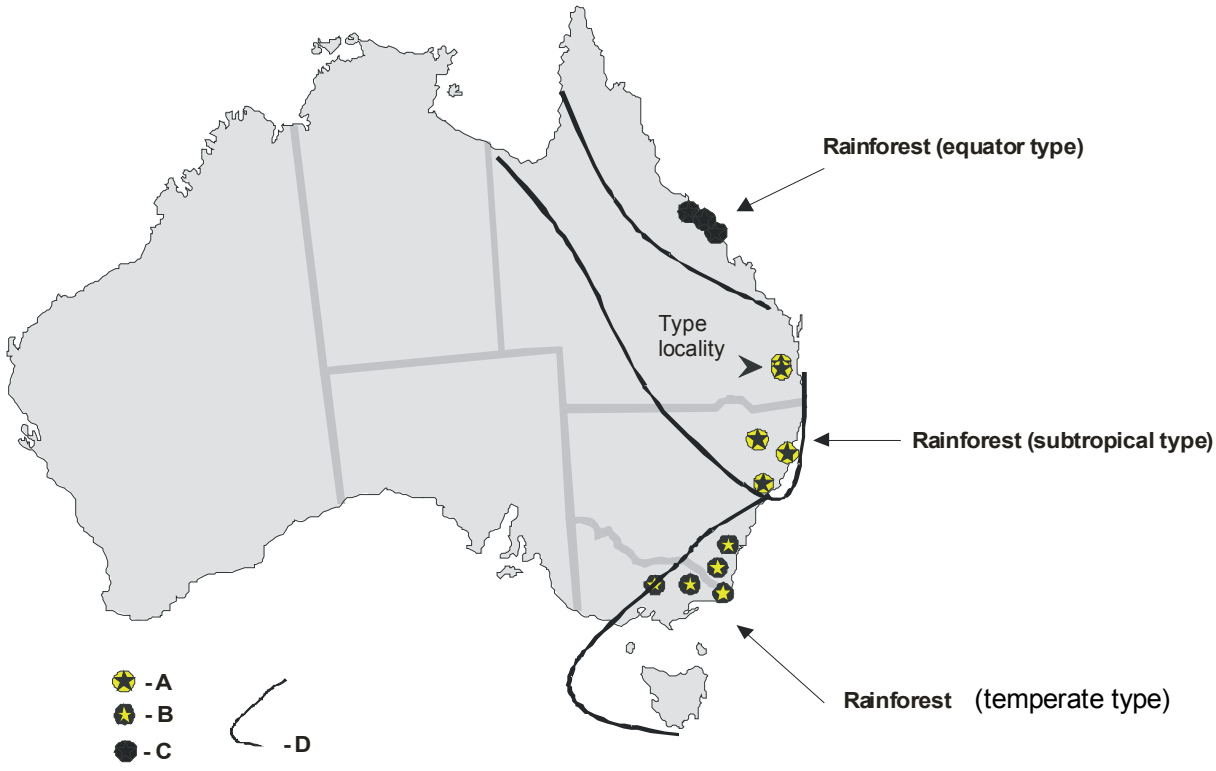

Figure 2. The distribution of Acroseius in eastern Australia. A - *A. tuberculatus*, B – *A. womersleyi*, C – *Acroceius* sp.nov., D – range of distribution of particular types of forests.

Distribution of species of the genus *Acroseius* in Australia

Geographic distribution is an important but little-used feature to differenciate species, which can also allow drawing conclusions about their relationships.

The distribution of the genus *Acroseius* in eastern Australia is shown in Figure 2. The three species occur in separate non-overlapping areas. The species with the widest range is *A. tuberculatus*, which occurs in New South Wales and southern Queensland. The southern limit of the genus is occupied by *A. womersleyi* while the northern limit is strictly colonized by the new species.

These different distribution ranges actually correspond to distinct habitats. *A. womersleyi* occurs in *Eucalyptus* forests in the state of Victoria and in southern New South Wales. *A. tuberculatus* occurs in subtropical forest in New South Wales and southern Queensland. The newly-discovered undescribed species occurs in tropical rainforest in northern Queensland.

Discussion and conclusion

It appears that the distribution of the three endemic Australian **species of the genus *Acroseius*** coincides with different types of forests in eastern Australia. It might be an interesting case of vicariance in mites from the suborder Uropodina. *A. womersleyi* occurs in temperate *Eucalyptus* forest, *A. tuberculatus* in temperate to subtropical rainforest, and the new species in tropical rainforest.

It is also interesting that no species of the genus has yet been found in Tasmania. Such a situation might be caused by colder climate or different environmental conditions characterizing this area.

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DISCOVERY OF A NEW SPECIES OF GENUS *POLLUX* (ERYTHRAEIDAE) FROM PAKISTAN

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Abstract

The genus *Pollux* is known from Australia and South India. A species belonging to this genus *Pollux* n. sp. was collected from a weed plant (*Sorghum halepense*) from district Okara, Punjab, Pakistan.

Key Words

Erythraeidae, *Pollux*, Larvae, Punjab, Pakistan.

Introduction

The genus *Pollux* belongs to the subfamily Balaustiinae Southcott 1957, Erythreoidea Robineau-Desvoidy, 1828 (Acarina), and was created by Southcott in 1961. (type species: *P. workandae* Southcott 1961.

The species of the genus *Pollux* are predatory in nature, live freely on plants and leaf debris and are only known by the larvae. Three species have been described, two are from Australia i.e *P.cristatus* Womersley, 1934 and *P. workandae* Southcott, 1961.The third species *P. kovalamicus* Haitlinger, 2002 was described from India.

In this paper the authors present new data, having identified a new species for the science collected from Punjab, Pakistan. Key to all species of this genus in the world is also given in present paper. Terminology and abbreviations are adapted from Haitlinger & Saboori (1996). All measurements are given in micrometers (μm).

Genus *Pollux* Southcott

Balaustium Womersley, 1934,:251.

Pollux Southcott, 1961:558;

Haitlinger, 2002:173.

Type species: *Pollux workandae* Southcott, by original designation.

Diagnosis (larva)

Dorsal: One eye on each side between the bases of anterior and posterior sensillae. Scutum present, long, narrow, lightly chitinized, with distinct crista metopica. The edge of dorsal scutum is very softly chitinized. Scutal setae: *AL* and *PL* scutalae present, placed anterolaterally upon the scutum at the edge. Crista metopica with anterior and posterior sensillae. Sensillae lightly ciliated. Crista is divided anteriorly and enclose the triangular anterior sensillary area. Posteriorly the crista is entire, and runs between the

posterior sensillae. Legs, ventral side: Pedal coxalae 1, 2: 1:1 ; trochanteralae 2, 3, 3. Lateral tarsal claws are dissimilar: anterior claw is falciform, strong, and simple; median (empodium) is long, slender and falciform; posterior claw is pulvilliform, without rod or claw element, and has the form of a brush of branching ciliations. Chelicerae bases are narrow. Galeala is present, simple, and thickened. Coxalae lacking. Palpal supercoxala present.

KEY TO SPECIES OF GENUS *POLLUX* SOUTHCOTT

1: (a) Dorsal body setae relatively short (20-30μm), Australia ..... *Pollux cristatus* Womersely

1: (b) Dorsal body setae rather long (30-60μm) 2

2: (a) more then 30 pairs of dorsal setae, 5 setae on palp genu 3

2: (b) 25 pairs of dorsal setae, 4 setae on palp genu, India ..... *Pollux kovalamicus* Haitinger

3: (a) AW>PW, PL>AL,ISD >70μm; palp with 2 setae on trochanter, Australia ..... *Pollux workandae* Southcott

3: (b) AW=PW, PL=AL ISD >50μm; palp trochanter with a single,..... *Pollux* n.sp. from Punjab Province, Pakistan

The Pakistani species: *Pollux* n.sp

(This species will be named in separate publication with complete description)

(Fig. 1A-F)

Description and diagnostic characters of the larva:

Dorsum**:** Idiosoma oval in shape, 510 μm long, 370 μm wide. Total length of body from tip of cheliceral fang to posterior pole of idiosoma 600 μm. Crista present anteriodorsal with anterior and posterior sensillary areas, and surrounded by upon inconpicous dorsal scutum. Scutum narrow, finely punctate, widen anteriorly, 85 μm long, 43 μm wide at the level of AL scutalae. Scutum convex forward and posteriorly blunt ended and carries linear, rode shaped crista, 2.50 μm across. The rod divides anteriorly in Y shape and the V part of the Y shape of crista bears the anterior sensillary areas. These areas areas somewhat triangular and caries a pair of slender and slightly ciliated anterior sensillae (ASE) , 39 μm long , SBa 10 μm. Posterior sensillae slender, slightly ciliated, present on posterior part of crista, 71 μm long, SBp 15 μm, ISD 55um. Dorsal scutum with two pairs of slightly plumose (ciliated), pointed tipped scutalae (AL and PL) as shown in figure 14A . AL present at the same level of ASE bases, 27μm long , PL 27 μm long , AW 37 μm , PW 37 μm , AP 21 μm. One eye present slightly behind the middle of scutum, on lateral sides, 11 μm across. Dorsal setae on idiosoma, 35 pairs with pointed tips, very finely barbed or setose and 27-50 μm long. Posterior idiosomal setae are larger than the remaining dorsal setae. Dorsal setae are fairly numerous as figured and arranged in irregular rows. fD=70 (Fig. 1A).

Venter**:** enter with slender, pointed tipped and very slightly barbed (setulose) setae. Sternalae 1a, 58 μm long present between coxae I, sternalae 2a, 39 μm, present between coxae II, 9 pairs of setae present between intercoxal fields of coxae II and III, 17 pairs of setae present behind the coxae III. These setae increase in length toward posterior pole of venter. FV=52; NDV=70+52=122 (Fig.1B).

Gnathosoma**:** Gnathosoma somewhat slender, with small, pointed tipped nude (simple) galealae 8μm and hypostomalae finely barbed, 16μm long. Accessory claw present along with palp tibial claw (Fig.1C).

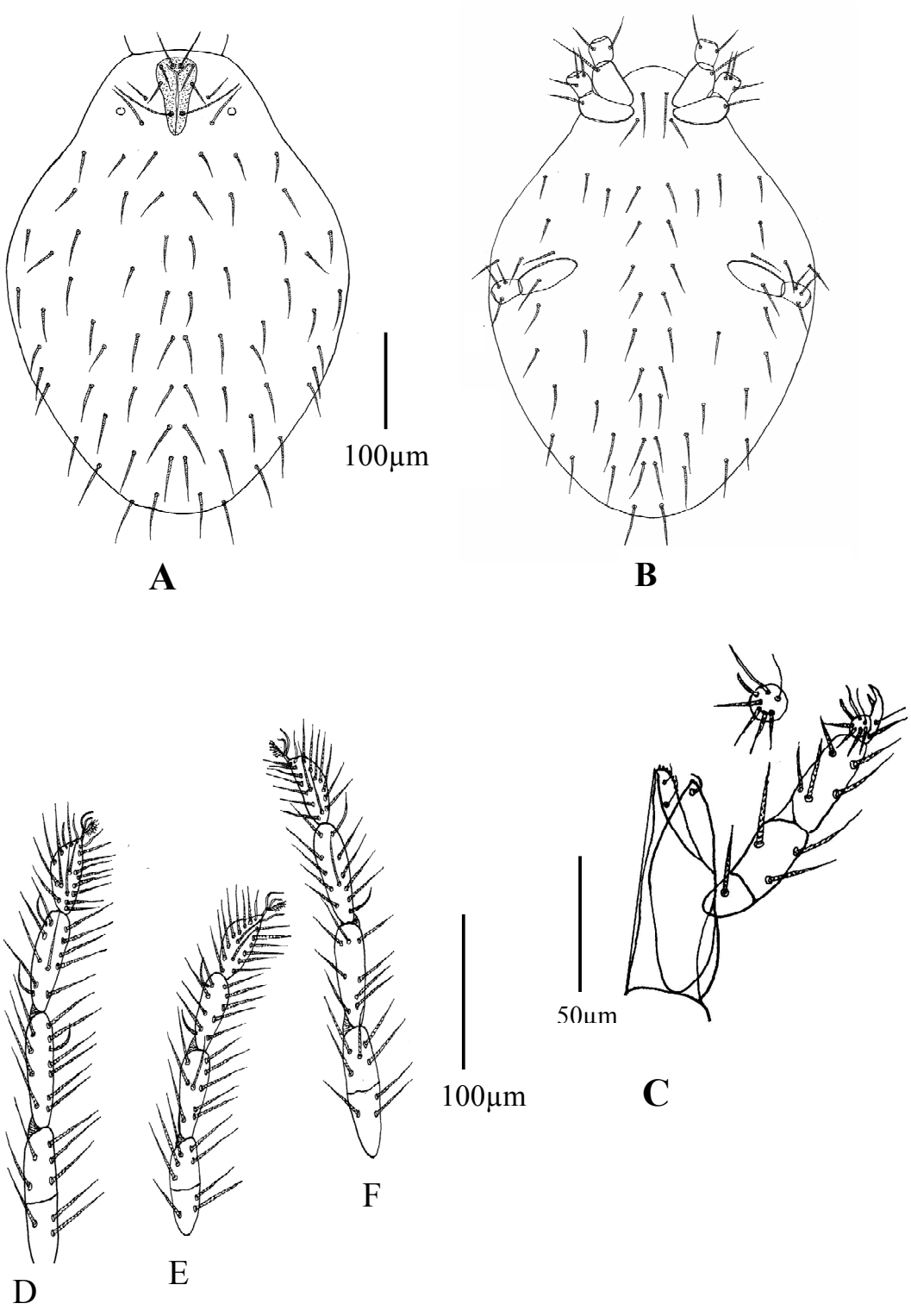

Figure 1. *Pollux* n sp: A, Dorsum ; B, Venter ; C, Palp ; D, LegI (femur –tarsus); E, LegII; F, LegIII.

Palp setal formula:
B–BBB–BBBBB–NB–NBBBBω ζ

Legs: three pairs. Leg I the longest one. Legs I-II-III, 391 μm , 309 μm and 360 μm long respectively including coxae (Fig.1D-F).
IP = 391+309+360 =1060

Table 1. Leg setal formula.

	Tarsus	Tibia	Genu	Femur	Trochanter	Coxa
Leg I	-1ω , 2ζ, 16B	-2φ ,10B	-1σ ,10B ; Tfe –5B	Bfe – 3B	2B	1B.
Leg II	1ω , 2ζ ,15B	-2φ , 9B	–9B	Tfe –5B BFe-3B	- 3B	-1B.
Leg III	-1ζ, 16B	– 1φ 11B	–8B	TFe – 5B BFe – 3B	- 3B	- 2B

Table2. Larva of *Pollux* n. s, metric data.

Character	Holotype	Character	Holotype	Character	Holotype
IL	510	2a	39	Ta II (H)	22
1W	370	1b	63	Ti II	50
L	85	2b	41	Ge II	55
W	43	3b	43	Tf II	32
AW	37	GL	88	Bf II	28
PW	37	PaScFed	37	Tr II	26
SBa	10	PaScFev	33	CX II	64
SBp	15	Ta I (L)	63	legII	309
ISD	55	Ta I (H)	25	Ta III (L)	58
AP	21	Ti I	67	Ta III (H)	20
AL	27	Ge I	75	Ti III	65
PL	27	Tf I	45	Ge III	65
ASE	39	Bf I	45	Tf III	43
PSE	71	Tr I	31	Bf III	40
DS	27-50	CX I	65	Tr III	30
PDS	45-50	LegI	391	CX III	69
1a	58	Ta II (L)	62	legIII	360

Etymology**:** This species will be named after the name of locality from where holotype larva was collected.

Material for types: The larval holotype was collected from chak No. 7/4L 5km South of district Okara on 15 -08-2005 (Muhammad Kamran rec.) from weed plant baru (*Sorghum halepense)*. Paratypes: 15 larvae, locality and host data is follows.

Number of larvae	**Locality**	**Host**	**Collection date**
4	Qamar Pull (D. G. Khan)	Sorghum	10-09-05
5	Kot Addu(Muzaffar Garh)	Baru	10-10-05
6	283/ TDA (Layyah)	Swank grass and Lucerne (Alfalfa)	06-05-06

Remarks: differential diagnoses: This new species *Pollux* differs from Australian *P. workandae* Southcott by following characters.

Palp trochanter with one setae in this species *P. workandae* palp trochantor with 2 setae. AL=PL; AW=PW in this species, in *P. workandae* PL>AL;PW>AW.

ISD=55 in this species but in *P. workandae*, ISD>70.

Number of setae and solenidia on leg segments femur, genu, tibia and tarsus different.

Scutum convex anteriorly in this species scutum anteriorly flat in *P. workandae*.

It also differs from the Indian species *P. kovalamicus* Haitlinger by:

1. Palp trochanter with one setae in this species but in *P. kovalamicus*, palp trochanter with 2 setae.

2. Dorsal body setae 35 pairs in this new

species but in *P. kovalamicus*, dorsal body setae 25 pairs.

3. Three setae on palp femur and five setae on palp genu in this species but in *P. kovalamicus,* two setae on palp femur and three setae on palp genu.

4. AL=PL;AW=PW in this species but in *P. kovalamicus*, *PL>AL; PW>AW.*

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PHYLOGENY AND BIOGEOGRAPHY OF THE GENUS *PHYTOSEIULUS* EVANS (ACARI: PHYTOSEIIDAE)

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Abstract

The genus *Phytoseiulus* included in the sub-family Amblyseiinae is quite particular among the Phytoseiidae because of morphological and biological characteristics. The biogeographic and phylogenetic relationships of the species of *Phytoseiulus* were studied in this paper. *Phytoseiulus macropilis* and *P. persimilis* are phylogenetically close to each others, sharing a large number of synapomorphies. These two species are then grouped with *P. fragariae* and then with *P. longipes*, which seems to have the most plesiomorphic character states. Except for the East Palaearctic region, the genus *Phytoseiulus* is found in all the other biogeographic regions. However, it is poorly reported from the Oriental, Nearctic and Australasian areas. As containing the highest diversity, the Neotropical region could be hypothetised as the area of origin of this taxon, suggesting at least a Gondwanan origin of the genus *Phytoseiulus.*

Key words

Phytoseiulus, phylogeny, parsimony, biogeography, endemism

Introduction

The species of the family Phytoseiidae Berlese, are well known as predators of phytophagous mites (Kostiainen & Hoy 1996; McMurtry & Croft 1997). This family is constituted of about 2,000 species and includes 3 sub-families: Amblyseiinae, Phytoseiinae and Typhlodrominae (about 1,100 Amblyseiinae, 200 Phytoseiinae and 700 Typhlodrominae) (Chant & McMurtry 2007). The present study focuses on the phylogenetic and biogeographic relationships between the species of one genus of the family Phytoseiidae, belonging to the sub-family Amblyseiinae: *Phytoseiulus* Evans 1952. Until 2006, according to the last world catalog of the family Phytoseiidae (Moraes *et al.* 2004), this genus included 5 species: *Phytoseiulus macropilis* (Banks), *P. persimilis* Athias-Henriot, *P. longipes* Evans, *P. fragariae* Denmark & Schicha and *P. robertsi* (Baker). In 2006, Chant & McMurtry re-defined this genus, which contains now 4 species, removing *P. robertsi* in a new genus *Afroseiulus* (Chant & McMurtry 2006). The genus *Phytoseiulus* presents a particular interest in plant protection as it includes one species, *P. persimilis*, widely used in biological control and also the most widely studied (biological and ecological aspects). The species of this genus are reported as specialist predators greatly linked to their preys, especially to the phytophagous mites belonging to the genus *Tetranychus* (Tetranychidae) (McMurtry & Croft 1997). They have been essentially reported from the tropical and sub-tropical regions (Takahashi & Chant 1993a). At last, the species of the genus *Phytoseiulus* also present particular morphological characteristics, as large body size and particularly

long setae on the dorsal shield. Many studies have been performed on the biology of *P. persimilis* and in a less extent of *P. macropilis*. However, few works deal with *P. longipes* and *P. fragariae*. In the same way, a little number of studies concerns the systematic of this genus. Takahashi and Chant (1992, 1993a,b,c,d and 1994) achieved several papers on biology, taxonomy and geographic distribution of the species of *Phytoseiulus*. However, the phylogenetic methodologies used in these papers were poorly adapted to this kind of analysis. The present paper aims to carry out a phylogenetic analysis based on parsimony in order to determine the evolutionary relationships between the different species of the genus *Phytoseiulus* and to assess the monophyly of the genus. This analysis is associated to a biogeographic study, as the biogeographic distribution is linked to historical and ecological factors (Brown & Lomolino 1998; Humphries & Parenti 1999), and could thus support the discussion on the phylogenetic hypotheses.

Material and methods

Genus* Phytoseiulus *Evans, 1952: 397

The setal nomenclature of Rowell *et al.* (1978) is followed in this paper. The four species of *Phytoseiulus* have particular morphological characters that allow to distinguish them easily from the other genera of Phytoseiidae.

In the Phytoseiidae, there are two variations of chaetotactic formulae for the tibia I: 2-2/1, 2/1-2 with only one anteroventral seta (av), and 2-2/2, 2/1-2 with two anteroventral setae (av1, av2). The four species (in the contrary to the other Phytoseiidae) present the second type of chaetotactic formulae. Among all the other species of Phytoseiidae, only *Metaseiulus* (*Metaseiulus*) *smithi* (Schuster, 1957) (sub-family Phytoseiinae) has this character (Chant & Yoshida-Shaul 1984; Takahashi & Chant 1993c). However, *M. smithi* could be easily distinguished from the species of *Phytoseiulus*, having dorsal seta Z1 absent, and J2, z3, s6 and JV3 present (Takahashi & Chant 1993c).

More than 82 % of the Phytoseiidae species have dorsal shield setae J2, S2 and S4 present. These setae are absent in the genus *Phytoseiulus* (Chant & Yoshida-Shaul 1989; Takahashi & Chant 1993c; Chant & McMurtry 2007).

Caudoventral setae JV3 & ZV3 are absent in all the species of the genus *Phytoseiulus* (Takahashi & Chant 1993c).

All the legs are much longer than the dorsal shield, whereas more than 75 % of the Phytoseiidae have the legs equal or shorter than the dorsal shield length (Takahashi & Chant 1993c).

Detailed morphological characteristics of the four species are presented in the table 1 in which measurements are expressed in micrometers (µm).

Biogeographical analysis

Data on the geographic distribution of the four species of *Phytoseiulus* were obtained from the two world catalogs on the family Phytoseiidae (Moraes *et al*. 1986, 2004), from the paper of Takahashi & Chant (1993a) and from all the publications concerning the study of the species of *Phytoseiulus*. These data constitute an exhaustive compilation of all available information concerning the geographical distribution of the four species of *Phytoseiulus* and their host plants. Zoogeographic areas used in this analysis are those defined by Wallace (1876): Nearctic (North America without Florida), Neotropical (South and Central America, Caribbean Islands and Florida), West Palaearctic (West Europe extending to Ural, northern India and North Africa), East Palaearctic (from Ural to Japan, without the South of China and Okinawa, Japan), Oriental (Hong Kong, India, Indonesia, Japan, Malaysia, Okinawa, Philippines, South of China, South Korea Taiwan, Thailand), Australasian (Australia, New Caledonia, New Zealand, Pacific islands, Papua New Guinea), and Ethiopian (Africa including Madagascar but not North Africa). The Wallace Line was used to separate Oriental and Australasian regions. A PAE (Parsimony Analysis of Endemicity, Rosen 1988) was carried out to determine the relationships between the different biogeographic regions according to the species shared by these regions. This analysis is based on the hypothesis that the taxa considered is monophyletic (Cracraft, 1991). Jaccard similarity indexes were also calculated between the different regions as follows: I = C/(N1+N2-C); C = number of species shared in the two regions, N1 = total number of species existing in the first region, N2 = total number of species existing in the second region (Brown & Lomolino 1998).

Morphological Analysis

A phylogenetic analysis based on morphological characters was carried out using PAUP (Phylogenetic Analysis Using Parsimony; version 4.0, Swofford 1998). This analysis uses a heuristic research procedure, repeated at least 100 times, with the randomised addition of taxa and branch-swapping algorithm (TBR). The 24 characters and states of characters selected are presented in the appendix 1. The analysis comprises the four

species presently included in the genus *Phytoseiulus* and four other species considered as out-groups, to root the tree and to clarify plesio- and apo-morphies. Two of these out-groups belong to the same sub-family (Amblyseiinae): *Afroseiulus robertsi* Chant & McMurtry and *Neoseiulus cucumeris* (Oudemans), and two others species to another sub-family (Typhlodrominae): *Typhlodromus* (*Anthoseius*) *rhenanoides* Athias-Henriot and *Neoseiulella tiliarum* (Oudemans). The data matrix is shown in the Table 2. The characters used in this analysis have usually been taken from the observation of the slides of the species present in the mite collection of the laboratory of acarology (Montpellier SupAgro, France), from Takahashi and Chant (1993c) for the four species of *Phytoseiulus* and/ or from the original descriptions and redescriptions [Baker (1990) for *Afroseiulus robertsi*; Athias-Henriot (1960), Charlet & McMurtry (1977) and Denmark & Welbourn (2002) for *Typhlodromus (Anthoseius) rhenanoides*; Oudemans (1930), Athias-Henriot (1960), Chant & Hansell (1971) and Schuster & Gonzalez (1963) for *Neoseiulus cucumeris* and Oudemans (1930), Chant & Yoshida-Shaul (1989) and Kolodochka (1986) for *Neoseiulella tiliarum*]. To reduce misleading effect of homoplasious characters, *a posteriori* reweighting was applied according to the rescaled consistency index (RC) after each tree search, until the number of trees stabilized (Farris 1969, 1989).

Results and discussion

Biogeographical analysis

The species of *Phytoseiulus* were reported from all the biogeographic areas, except in the East Palaearctic region (Figure 1). This taxon is also poorly reported from the Oriental and Nearctic areas. *Phytoseiulus macropilis* and *P. persimilis* are the two most frequent species and are present in nearly all the biogeographic regions (Table 3). This ubiquity is certainly mainly due to the commercialisation and to the introduction of these two species for biological control purposes to regulate phytophagous mites. *Phytoseiulus longipes* and *P. fragariae* are poorly reported and their presence is only localized to some biogeographic areas (Table 3). *Phytoseiulus longipes*, observed only 9 times, has been mainly found in the Ethiopian region and two times in the Neotropical region. *Phytoseiulus fragariae* has been only reported from the Neotropical region. Endemism levels are quite low (Table 3), essentially because of the broad distribution of *P. macropilis* and *P. persimilis*. The Neotropical region presents both the highest number of reports and the highest specific diversity. In the Ethiopian region, three of the four species have been recorded. The PAE consensus tree confirms these observations (Figure 2). Three major groups were observed: Neotropical, Ethiopian, and all the other regions. The presence of both *P. macropilis* and *P. persimilis* grouped the Australasian, Nearctic, Oriental and Palaearctic areas. However, the Oriental and East Palaearctic areas could be differentiated because of the low occurrence of *Phytoseiulus* species in these two regions. The Neotropical and Ethiopian areas are separated from the others by a high diversity of species of *Phytoseiulus*, and between them by the presence of an endemic species (*P. fragariae*) in the Neotropical area. Jaccard similarity indexes, varying between 0 and 1, were quite high because of the ubiquity of *P. macropilis* and *P. persimilis* (Table 4). We can also observe the great link between the Neotropical and Ethiopian regions (0,75). According to the "center-of-origin" concept of the phylogenetic biogeographers Pielou (1979); and Wiley (1981), all these results suggest that the genus *Phytoseiulus* could originate in the Neotropical area, or more broadly from the West Gondwana part. This hypothesis is in accordance with Takahashi and Chant (1993a). The species of the genus *Phytoseiulus* were found on a high diversity of host plants (Table 5): 170 plant species belonging to 57 families ranging from annual grasses, flowers and ornamentals to perennial shrubs and trees (fruit and forest). *Phytoseiulus macropilis* is the species reported from the highest number of plant species (123 host plants belonging to 50 families). *Phytoseiulus persimilis* was found on 71 plant species (27 families). *Phytoseiulus longipes* was found on 9 plant species (8 families). Finally, *P. fragariae* was found on 8 plant species (6 families). The highest diversity of host plants of *Phytoseiulus* was found in the Neotropical area (65 host plant species belonging to 29 families) followed by the Australasian and the West Palaearctic areas. However, Rosaceae, Solanaceae and Fabaceae were the most frequent families on which the four species of *Phytoseiulus* were found (Table 5). The host plant diversity for each of the four species clearly reflects the biogeographic distribution and the number of collection records. The Neotropical area presents the highest diversity of *Phytoseiulus* species as well as of host plants.

Table 1. Detailed morphological characters of the four species of the genus *Phytoseiulus* Evans (adult female) after Takahashi & Chant (1993b) (M: seta smooth; S: seta serrated; P: seta present; A: seta absent).

Phytoseiulus Species	Dorsal shield length	Dorsal shield width	Nbre pairs setae/ DS	j1 nature	j1 length	j3 nature	j3 length	j4 nature	j4 length	j5 presence	j5 nature	j5 length	j6 nature	j6 length	z2 position	z2 nature	z2 length	z4 nature	z4 length	z5 nature	z5 length	s4 nature	s4 length	r3 nature	r3 length	J5 nature	J5 length
P. macropilis	300-323	208-230	16	M	20-26	S	37-48	S	40-50	P	S	55-73	S	123-135	j3-j4	M	15-20	S	45-62	M	8-12	S	145-170	M	21-24	M	4-6
P. persimilis	314-330	215-232	16	S	25-32	S	38-46	S	48-52	P	S	65-74	S	145-160	j3-j4	M	10-13	S	57-65	M	8-12	S	158-172	M	20-26	M	5-6
P. longipes	325-343	210-217	14	M	11-20	S	85-93	M	13-22	A	-	-	S	95-107	j1-j3	M	19-30	S	92-107	M	4-8	S	125-136	M	32-40	M	4
P. fragariae	320-330	225-240	16	M	17-28	M	26-40	M	14-20	P	M	13-24	M	67-87	j3-j4	M	7-12	S	57-70	M	8-12	S	155-185	M	17-22	M	6-10

	Z1 nature	Z1 length	Z4 nature	Z4 length	Z5 nature	Z5 length	S5 presence	S5 nature	S5 length	R1 nature	R1 length	JV1 length	JV2 presence	JV2 nature	JV2 length	Jv4 presence	JV4 nature	JV4 length	JV5 nature	JV5 length	ZV1 length	ZV2 length	ST1 length	ST2 length	ST3 length	ST4 length
P. macropilis	S	95-101	S	105-123	S	105-125	P	S	23-44	M	22-30	35-40	P	S	25-36	A	-	-	M	33-40	35-40	23-30	50-60	50-65	55-67	55-61
P. persimilis	S	105-115	S	131-138	S	120-132	P	S	25-38	M	25-32	38-46	A	L	40-52	A	-	-	M	35-44	45-50	30-38	60-68	60-70	60-72	60-68
P. longipes	S	95-120	S	100-115	S	100-110	A	-	-	S	72-80	56-62	A	L	48-52	P	L	22-25	S	60-74	45-56	37-44	52-74	64-74	65-76	45-64
P. fragariae	S	120-145	S	133-150	S	125-140	P	M	24-30	M	26-40	40-44	P	L	40-47	P	L	13-20	M	50-60	45-51	34-40	55-65	55-65	55-61	55-64

	Peritremal sheild length	Peritreme level	Peritreme length	Nbre setae on sternale sheild	Cervix length (spermatheca)	Fixed digit length	Nbre teeth/ FD	Movable digit length	Nbre teeth/ MD	Leg I length	Leg II length	Leg III length	Leg IV length	Nature of setae/genu & tibia I to III	MS/ge IV presence	MS/ge IV nature	MS/ge IV length	MS/ti IV presence	MS/ti IV nature	MS/ti IV length	MS/Sti IV presence	MS/Sti IV nature	MS/Sti IV length
P. macropilis	260-280	j1	115-140	3 paires	26-32	20-26	7-8	24-26	3	455-498	346-377	370-400	520-585	S	P	S	65-80	P	S	30-40	P	S	90-116
P. persimilis	275-295	j1	160-175	3 paires	26-32	23-25	7-8	24-28	3	492-540	370-406	400-437	570-610	S	P	S	80-91	P	S	40-48	P	S	110-135
P. longipes	195-230	j1-j3	132-157	2 paires	24-26	21-23	3	20-22	3	430-470	355-380	360-390	490-525	M	A	-	-	A	-	-	P	M	105-117
P. fragariae	275-285	j1	135-160	3 paires	18-22	22-25	7-10	24-28	3	485-535	380-442	355-405	540-610	M	P	M	50-64	P	M	36-44	P	M	77-91

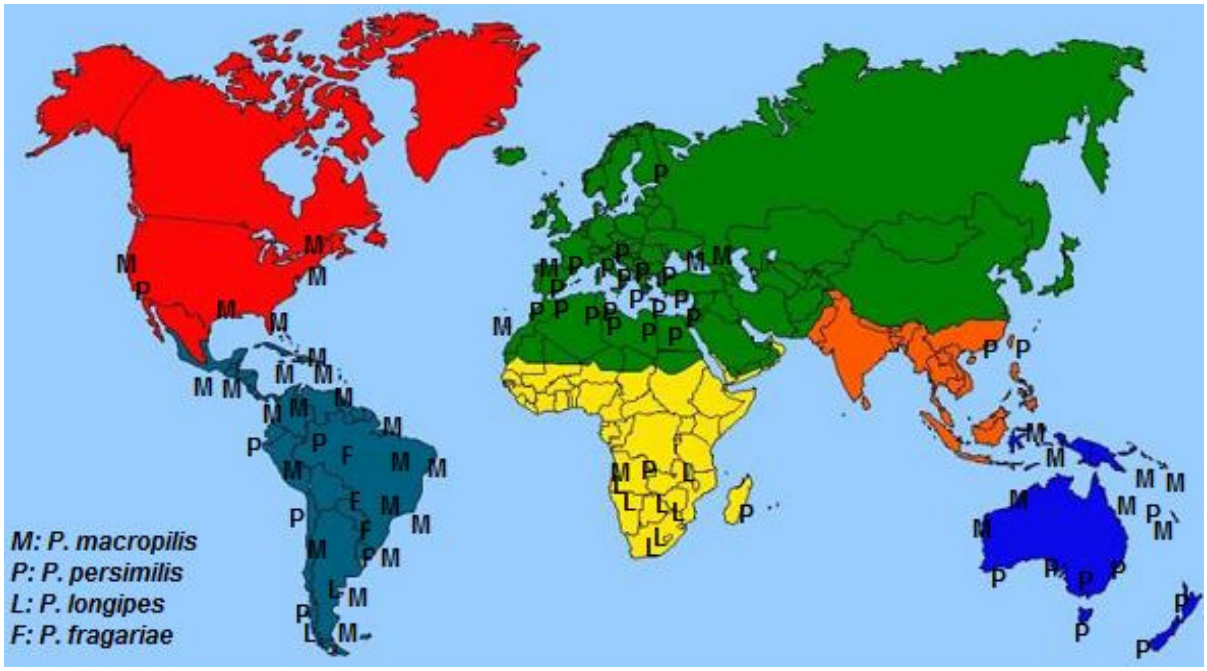

Figure 1. Bioeographic distribution of the four species of *Phytoseiulus* after the two world catalogs of Phytoseiidae (Moraes *et al*. 1986, 2004).

Morphological Analysis

The consensus tree is shown on the figure 3. The out-groups are well separated from the species of *Phytoseiulus*, suggesting that the species of this genus constitute a monophyletic group. This monophyly is sustained by several characters as the length of j6, z4 and Z5; the presence of z3, s6, J2 and JV3. This confirms thus the hypothesis of Chant & McMurtry (2007) that excluded *A. robersti* from the genus *Phytoseiulus.* This differentiation is especially supported by the characters presence/ absence of S2, Z1 and ZV1. The genus *Phytoseiulus* would thus be constituted of 4 species.

Inside the genus *Phytoseiulus*, *P. macropilis* and *P. persimilis* are grouped together by the characters j3, j4, j6 and R1 length; JV4 absence. Only two morphological characters separate *P. macropilis* from *P. persimilis*: length of z2 and position of JV2 (outside/ inside ventrianal shield). *Phytoseiulus macropilis* and *P. persimilis* were then grouped with *P. fragariae* constituting a sub-group supported by a bootstrap value of 77 related to the characters: presence of setae j5, S5 and macrosetae on genu and tibia of the leg IV; position of ST3 (on/ outside sternal shield); length of z4 and R1; number of teeth on the fixed digit of chelicerae. This later sub-group was then related to *P. longipes* which seems to have the most plesiomorphic characters (missing of j5, S5 and macrosetae on genu and tibia IV; ST3 on metasternal shield; Length of j3, j4, z4 and R1). Molecular analyses (Tixier *et al.*; unpublished data) support the morphological phylogeny presented in this paper. *Phytoseiulus macropilis* and *P. persimilis* are morphogically and phylogenetically very close. They are also the two more frequent species and are reported from the same areas, and very frequently observed on the same host plants. According to these similarities and the questionable status of the length of z2 and position of JV2 for species diagnostic, we can wonder if these two entities correspond to different species. Recent molecular analyses seem to show that these two species are relevant ones (Tixier *et al.*; unpublished data).

Table 2. Data matrix used for phylogenetic analysis.

N° character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Phytoseiulus macropilis	3	3	1	5	2	3	1	2	1	1	0	0	2	1	1	1	5	0	1	1	0	0	0	0
P. persimilis	3	3	1	5	1	3	1	2	1	0	0	0	2	1	1	1	5	0	1	1	0	0	0	0
P. longipes	5	2	0	5	2	5	0	4	0	0	1	0	1	0	0	1	5	0	1	1	0	0	0	0
P. fragariae	2	1	1	4	1	3	1	2	1	1	1	1	2	1	1	1	5	0	1	1	0	0	0	0
Afroseiulus robertsi	4	1	0	1	1	4	0	2	1	1	0	0	1	1	1	0	4	1	0	0	0	0	0	0
Neoseiulus cucumeris	2	2	1	2	2	1	1	2	1	1	1	1	1	1	1	1	4	1	1	1	1	0	0	0
Typhlodromus (*Anthoseius*) *rhenanoides*	2	2	1	2	2	1	1	2	1	1	1	1	?	0	1	0	3	1	1	?	1	1	1	1
Neoseiulella tiliarum	2	2	1	2	2	2	1	2	1	1	1	1	1	0	0	1	3	1	1	1	1	1	1	1

Table 3. Number of records of the 4 species of genus *Phytoseiulus* in the 7 biogeographic regions and endemism levels.

Biogeographic Region / Species, records and endemicity	Neotropical	Ethiopian	Australasian	Oriental	East Palaearctic	West Palaearctic	Nearctic	Total Number
P. macropilis	68	1	16	0	0	8	11	104
P. persimilis	7	4	7	2	0	36	1	57
P. longipes	2	7	0	0	0	0	0	9
P. fragariae	4	0	0	0	0	0	0	4
Total Number of records	81	12	23	2	0	44	12	174
Number of endemic species	1	0	0	0	0	0	0	
% Endemism	0.25	0	0	0	0	0	0	

Table 4. Indexes of similarity (Jaccard) between the biogeographic regions according to the distribution of the 4 species of genus *Phytoseiulus*.

Areas	Neotropical	Ethiopian	Australasian	Oriental	East Palaearctic	West Palaearctic	Nearctic
Neotropical	-						
Ethiopian	0.75	-					
Australasian	0.50	0.67	-				
Oriental	0.25	0.33	0.50	-			
East Palearctic	0	0	0	0	-		
West Palearctic	0.50	0.67	1	0.50	0	-	
Nearctic	0.50	0.67	1	0.50	0		-

Table 5. Number of reports of the 4 species of *Phytoseiulus* on different families of plant substrate through out the world (*After* Takahashi & Chant 1993a; Moraes *et al.* 1986, 2004).

	Acanthaceae	*Adoxaceae*	*Alliaceae*	*Amaranthaceae*	*Anacardiaceae*	*Apocynaceae*	*Araceae*	*Asteraceae*	*Betulaceae*	*Bignoniaceae*	*Boraginaceae*	*Brassicaceae*	*Bromeliaceae*	*Caricaceae*	*Cecropiaceae*	*Chenopodiaceae*	*Cleomaceae*	*Commelinaceae*	*Convolvulaceae*	*Cucurbitaceae*	*Cupressaceae*	*Ericaceae*	*Euphorbiaceae*	*Fabaceae*	*Fagaceae*	*Poaceae*	*Hydrangeaceae*	*Lamiaceae*	*Lauraceae*	*Liliaceae*
P. macropilis		1	1	2	1	2	5	5		1	1	1	1	1	1		1	1	10	2	1	1	9	12	1		1	1	1	1
P. persimilis	1	1	1	3				4	1			1		1					3	3			2	10		1		1		
P. fragariae				2				1																1						
P. longipes																1			1					1			1	1		

	Magnoliaceae	Malvaceae	*Meliaceae*	*Menispermaceae*	*Moraceae*	*Musaceae*	*Myrtaceae*	*Oleaceae*	*Orchidaceae*	*Passifloraceae*	*Pinaceae*	*Piperaceae*	*Pittosporaceae*	*Plantaginaceae*	*Poaceae*	*Polygonaceae*	*Pontederiaceae*	*Rosaceae*	*Rutaceae*	*Sapindaceae*	*Solanaceae*	*Thymelaceae*	*Ulmaceae*	*Urticaceae*	*Verbenaceae*	*Violaceae*	*Vitaceae*
P. macropilis	1	6	2		2	1	1	1		3	1	1	1	1	3		2	6	3	2	13	1	2	1	1	2	1
P. persimilis		4	1		2	2			1							2	1	10	2		10		1		1	1	
P. fragariae				1														1			2						
P. longipes														2				1			1						

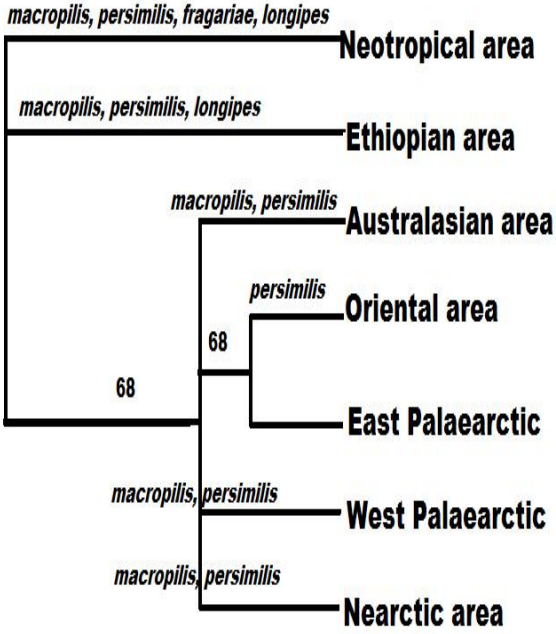

Figure 2. Cladogram representing the consensus tree obtained from the PAE analysis carried out with the four species of the genus *Phytoseiulus*: CI = 1,0, RI = 1,0. The values at the nodes correspond to bootstrap values.

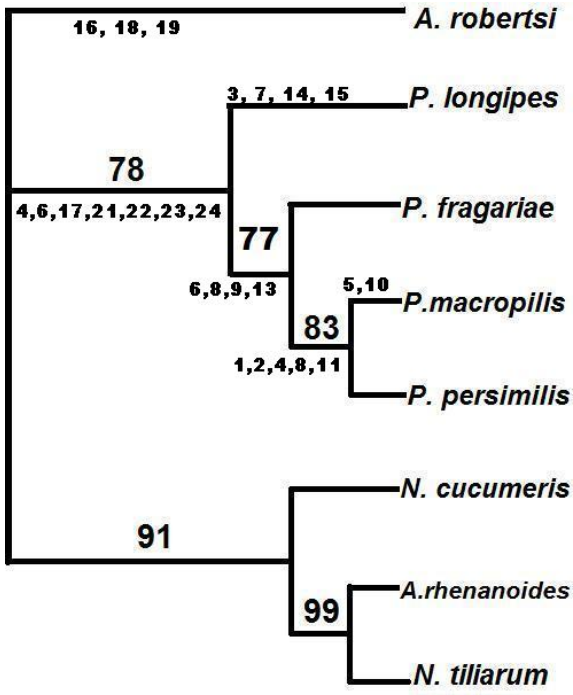

Figure 3. Cladogram representing the consensus tree obtained with 24 morphological characters, carried out on the species of the genus *Phytoseiulus* and on the four species "out-groups" Consistency index (CI) = 0.79; Retention index (RI) = 0.70. The values at the nodes correspond to bootstrap values. The numbers on branches correspond to the characters supporting these branches.

Conclusion

Both phylogenetic and biogeographic data seem to show that the genus *Phytoseiulus* originated from the western part of the Gondwana (Ethiopian and Neotropical regions), where *P. longipes*, presenting the most ancestral characters is present. Then, after the Gondwana break, especially the break between the Ethiopian and the Neotropical regions, differentiation of *P. fragariae* could be hypothetised, as this species is endemic from the Neotropical region. Afterwards, the differentiation of *P. macropilis* and *P. persimilis* could have occurred. However, evolutive historical hypothesis are difficult to develop for these two latter species, as they are nowadays spread out all around the world. Thus, it is very difficult to determine their biogeographic origin. However, as *P. persimilis* was more frequently reported from mediterranean regions, some authors hypothesized a mediterranean origin of this later species. Indeed, *P. persimilis* was abundantly found in the West Palaearctic region especially from the mediterranean regions. Among the 36 records of this later species in the West Palaearctic area (Table 3), it was observed 10 times before 1970 in this part of the world and 15 times between 1970 and 1980. This could indicate the early and widely presence of *P. persimilis* in the West Palaearctic area before the massive introductions of this species for biological control purposes in other parts of the World. Concerning *Phytoseiulus macropilis,* the distribution pattern is totally different. It has been very poorly reported from the West Palaearctic region (Table 3), but is mostly found in the Neotropical area. Before 1970, this species was essentially reported from the Neotropical and Nearctic regions, and from Hawai.

To explain this distribution, several historical evolutive scenari could be hypothesized: (1) Both *P. macropilis* and *P. persimilis* originated from the Neotropical region. *Phytoseiulus macropilis* could have stood in the Neotropical area, and poorly dispersed because of favourable conditions for its development in this area. On the other hand, *P. persimilis* spread through the Neartic (Bering bridge) to colonise the West Palaearctic region, where it would have found particularly good conditions for its development. (2) As the hypothetised origin of *Phytoseiulus* is in the Neotropical-Ethiopian region, the ancestor of these two species could also have appeared in this part, before the separation between Africa and South America. *P. macropilis* could have appeared in the Neotropical region after this separation, whereas

the differentiation of *P. persimilis* would have appeared after the dispersal of the common ancestor from the Ethiopian to the West Palaearctic region through Gibraltar. This latter hypothesis would suppose the extinction of *P. fragariae* in the Ethiopian part or/ and a non-report of this species despite its presence in this region.

The historical evolution of the four species of *Phytoseiulus* could be the subject to many speculations. Other studies are thus required to test the different hypothetical scenari presently proposed. The evolutionary convergence and the biogeographical mechanisms (the dispersal and the sequence of vicariance events) might be distinguished for better-understanding of the differentiation of these four species. Genetic studies would be required for example, especially to apply molecular clock to relate the divergence time of the species with biogeographic events.. At last, it is also planned to carry out this same kind of studies on other genera phylogenetically close to the genus *Phytoseiulus*.

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Appendix

Morphological characters used in the analysis of relationships of the four species of *Phytoseiulus* Evans; For each character a number and a coding have been attributed; The characters of absence/presence of setae follow the code: 0: seta absent, 1: seta present; Length of dorsal setae follows the code: 1: 0-15 µ, 2: 15-30 µ, 3: 30-45 µ, 4: 45-60 µ, 5: > 60 µ.

(1). j3 length; (2). j4 length; (3). j5 Presence; (4). j6 length; (5). z2 length; (6). z4 length; (7). S5 Presence; (8). R1 length; (9). ST3 Position: 0: On metasternal shield, 1: On sternal shield; (10). JV2 Position: 0: Outside ventrianal shield, 1: Inside ventrianal shield; (11). JV4 Presence; (12). ZV2 Presence; (13). Number of teeth on fix digit of the chelicerae: 1: > 5 teeth, 2: 5 teeth at minimum; (14). Presence of macrosetae on genu IV; (15). Presence of macrosetae on tibia IV; (16). Z1 Presence; (17). Z5 length; (18). S2 Presence; (19). ZV1 Presence; (20). Number of teeth on movable digit of the chelicerae: 0: 0 teeth, 1: 1 teeth at minimum; (21). J2 Presence; (22). JV3 Presence; (23). z3 Presence; (24). s6 Presence.

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MOLECULAR BIOLOGY FOR PHYTOSEIIDAE IDENTIFICATION: PRELIMINARY RESULTS

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Abstract

The Phytoseiidae has stirred a great interest since the 1950's as the role of a few efficient species in the biological control of tetranychid mites has been emphasized. Whereas only less than 30 species were known in 1951, more than 2,000 have been described up to now. Species description is only based on a morphological species definition. The characters commonly used are the length of idiosomal setae, the extension level of peritreme, the dentition of chelicerae, the shape of the insemination apparatus or "spermatheca" and of the ventrianal shield. However, intermediate morphological patterns between two species could exist. Furthermore, differences could be sometimes very tiny, could concern only one character, and nothing is known about the reliability of these morphological characters in Phytoseiidae diagnostic. Finally, mites are microscopic organisms and very few characters are available to distinguish between species. The aims of this study are (1) to determine how molecular tools could be useful for diagnostic of Phytoseiidae at species level, and thus (2) to assess the reliability of the morphological differences usually used to discriminate species entities. This work is developed in the framework of recent widely proposals developed to use the sequences of mitochondrial DNA fragments (Cytochrome Oxydase 1) to identify specimens across all the Tree of life and branded ''DNA barcoding''. This process requires to show a very low frequency of divergence within a species, but a significant divergence at higher taxonomic levels. In this work, two mitochondrial DNA fragments, COI and 12S, were sequenced for three species of Phytoseiidae (*Neoseiulus californicus, Kampimodromus aberrans* and *Euseius stipulatus*) and relatives. The aim is to evaluate intraspecific and interspecific genetic distances for separating sympatric and morphogically close species. The results presented here are preliminary and show on one hand the difficulty to obtain COI sequences and on the other hand the good reliability of the 12S sequences for the diagnostic of the species considered.

Key-words

Barcoding, taxonomy, diagnostic, Phytoseiidae, Neoseiulus californicus, Kampimodromus aberrans, Euseius stipulatus

Introduction

The family Phytoseiidae belongs to the order Mesostigmata. It is widespread all over the world and includes more than 90 genera and more than 2,000 species (Chant 1993; Chant & McMurtry 2003a, b; Moraes *et al.* 2003; Ragusa 2003; Chant & McMurtry 2004a, b; Moraes *et al.* 2004; Chant & McMurtry 2005a, b, c; 2006a, b; Kreiter & Tixier 2006). Many species are considered as main predators of phytophagous mites in various crops worldwide (Kostiainen & Hoy 1996; McMurtry & Croft 1997). Specific diagnostic within this family is based on several characters such as dorsal setal length, leg chaetotaxy, spermatheca shape and cheliceral dentition (see Chant & McMurtry 1994, 2006a, b; 2007). However, intermediate morphological patterns between two species could exist. Furthermore, differences could be sometimes very tiny and/or could concern only

one character. Finally, mites are microscopic organisms and very few characters are available to distinguish between species. The present study focuses on the diagnostic of three species of Phytoseiidae: *Neoseiulus californicus* (McGregor), *Kampimodromus aberrans* (Oudemans) and *Euseius stipulatus* (Athias-Henriot). These three species have an agronomic interest as they are known and used to control mite pests in several crops all over the world.

Neoseiulus californicus naturally occurs in agrosystems but is also commercialised to control mite pests particularly in greenhouses and in orchards. It is reported from 17 countries in Europe, North and South America, North Africa and Asia (Moraes *et al.* 2004). The genus *Neoseiulus* contains 335 species and is the largest genus within the family (Moraes *et al*. 2004; Chant & McMurtry 2007). Distinction between the different specific entities could be quite difficult because, of an important intraspecific morphological variability of the characters used for diagnostic particularly the seta lengths (Tixier *et al*. in press).

Kampimodromus aberrans is a very common phytoseiid mite species in Europe on both crops (apple orchards and vineyards) and wild plants (Duso 1992; Ragusa *et al*. 1995; Ragusa & Tsolakis 1996; Schausberger 1997; Kreiter *et al*. 2000; Tixier *et al.* 2000; Kreiter *et al.* 2002). It has been found feeding on several prey species, particularly *Eotetranychus carpini* (Oudemans) in vineyards in Southern Europe accurately France and Italy (Duso 1992; Kreiter *et al.* 2002). The genus *Kampimodromus* contains 15 species (Moraes *et al.* 2004; Chant & McMurtry 2007) and some doubts have been emphasised on the specific status of some species (Tixier *et al.* 2008). As well, some studies have shown the great intraspecific variability at the seta lengths for the species *K. aberrans* (Tixier *et al.* 2003, 2008).

The genus *Euseius* is one of the most diverse (187 species described), within the sub-family Amblyseiinae (Moraes *et al*. 2004; Chant & McMurtry 2007). *Euseius stipulatus* is widely used in biological control, especially in citrus orchards (Ferragut *et al*. 1992, 1997). It is mainly found in the West Palaearctic region, where it is sympatric with *Euseius finlandicus* (Oudemans) and *E. scutalis* (Athias-Henriot). The identification of *Euseius* species is quite difficult because of low variation in seta lengths among the 187 species described. Chant & McMurtry (2007) noted for instance, that the precise identity of 23 species is uncertain. These authors also emphasized that spermatheca is highly variable and without marked discontinuities between the various forms.

Accurate identification of these three species is of first importance for biological control success and development. However, the poor knowledge on the weight of particular morphological characters for species status makes this task quite difficult even for taxonomists. In this context, molecular biology could be of great help. DNA sequences have been currently used for delineating and identifying species, particularly in the framework of barcoding of life (www.barcodinglife.com). DNA barcoding consists in sequencing a short standardized DNA sequence, usually the mitochondrial cytochrome *C* oxidase subunit I (COI) gene, and in relating the genetic distances to taxonomic rank (intra or interspecific ones) (Monaghan *et al.* 2005). The main purposes of this approach are to (1) identify and assign unknown specimens to species that have been previously described, (2) enhance the discovery of new species using a threshold of sequence divergence (Hebert *et al.* 2003; Moritz & Cicero 2004; Hebert *et al.* 2004). Up to now, COI was the most used DNA fragment, but molecular identification may be based on one or several mitochondrial as well as nuclear DNA regions, depending on the organisms studied and on the suitability of the marker considered. The present study aims to determine how molecular sequencing could be useful for Phytoseiidae species diagnostic and thus to determine the gap values between intra- and interspecific molecular variability, with the examples of three species important for biological control: *N. californicus*, *K. aberrans* and *E. stipulatus*.

Material and methods

Species and populations studied

Neoseiulus californicus. The three populations considered were collected from Chile and Italy (Sicily, Tuscany). The two species chosen to determine the interspecific distance (control) are both sympatric to *N. californicus* and quite morphologically close. *Neoseiulus picanus* (Ragusa) was collected in Argentina (unpub. data) and Chile on Solanaceae. For *Neoseiulus fallacis* (Garman), the sequences were taken from the Genbank database (accession number: AY099364) (Table 1).

Kampimodromus aberrans. The two populations of *K. aberrans* considered were collected from France and Austria (Table 1). As previously, the species chosen to determine the interspecific distances (control), are sympatric to *K. aberrans* and quite

morphologically close: *Kampimodromus ericinus* (Ragusa) and *Kampimodromus corylosus* (Kolodochka). These two species were collected in France: for *K. corylosus* in Burgundy on *Corylus avellana* L. and for *K. ericinus* near Montpellier (Villeneuvette, Hérault) on *Cistus monspeliensis* L. (Table 1).

Euseius stipulatus. The two populations studied were collected from France and Spain on *Citrus* sp. The species chosen to determine the interspecific distances (control) was *E. finlandicus.* This species was collected on *Malus* sp. in Burgundy, France (Table 1).

Table 1. Origin of the species and strains of Phytoseiidae studied. Number of sequences (individuals) analysed for each species and strains, for both 12S rDNA and COI mtDNA.

Species	Country	Locality	Host plant	Collection date	12S	COI
N.californicus	Chile	La Cruz	*Phaseolus vulgaris L.*	2000	7	-
N.californicus	Italy Sicily	Palermo	*Fragariae vesca L.*	2004&2008	18	-
N.californicus	Italy Tuscany	Firenze	*Fragariae vesca L.*	2004	10	-
N.picanus	Chile	-	Unknow host plant	2008	1	-
N.picanus	Argentina	-	*Solanacea*	2006	14	-
N.fallacis	USA	Biostactics Riverside	Unknow host plant	2002	1	-
K.aberrans	France	Montpellier	*Celtis australis L.*	2007	3	3
K.aberrans	Austria	Vienna	*Malus sp.*	2007	3	2
K.ericinus	France	Villeneuvette	*Cistus monspelliensis L.*	2004	-	2
K.corylosus	USA	Oregon	*Corylus avellana L.*	2004	-	1
K.corylosus	France	Burgundy	*Corylus avellana L.*	2004	1	1
E.stipulatus	France	Montpellier	*Citrus sp.*	2007	3	4
E.stipulatus	Spain	Valencia	*Citrus sp.*	2007	2	2
E.finlandicus	France	Burgundy	*Malus sp.*	2007	3	3

Molecular markers used. Mitochondrial DNA (mtDNA) was widely used in taxonomic and population studies for insects (Simon *et al.* 1994; Roehrdanz & Degrugillier 1998) and for some mites (Cruickshank 2002; Evans & Lopez 2002; Jeyaprakash & Hoy 2002, 2007; Navajas *et al.* 1996; Navajas & Fenton 2000; Otto & Wilson 2001; Warrit *et al.*, 2005). The markers chosen in the present study were the COI and the 12S DNA fragments. These two markers have been successfully used for assessing relationships among recently diverged species for insects (Simon *et al.* 1994;), and for mites, as ticks (Murrel *et al.* 2005), mesostigmatids (Anderson & Morgan 2007) and for Phytoseiidae mite diagnostic (Jeyaprakash & Hoy 2002; Tixier *et al.* 2006, 2007).

Molecular experiments

Total DNA was extracted from a single individual using the DNeasy Tissue Kit (Qiagen, Hilden). The three species and their relatives were not all studied with the two markers considered. The number of sequences obtained for each marker and for each strain considered as well as the sequence taken from the Genbank database are shown in the table 1.

The primers used to amplify the 12S rDNA fragment, were those proposed by Jeyaprakash & Hoy (2002) for Phytoseiidae: 5'-3'TACTATGTTACGACTTAT and 3'-5' AAACTAGGATTAGATACCC. The primers used to amplify the part of the mitochondrial COI gene were those proposed by Navajas *et al.* (1994) 5'-3' TGATTTTTTGGTCACCCAGAAG and 3'-5' TACAGCTCCTATAGATAAAAC. PCR was done in a total volume of 25 µl containing 2 µl of mite DNA, 1 µl of DNTP (2.5 Mm for each nucleotide), 2.5 µl of Taq buffer, 1 µl of each primer (100 µM), 0.5 µl of Taq (Qiagen, 5 U / µl) and 18.9 µl of water. Thermal cycling conditions were as follows: 95 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 40 °C for 30 s and 72 °C for 1 min for the 12S rDNA marker and 95 °C for 5 min, followed by 35 cycles of 92 °C for 1 mn, 45 °C for 30 s and 72 °C for 1 min for the COI mtDNA marker. An additional 5 min at 72 °C was added for final strand elongation.

Electrophoresis was carried out on 1.5 % agarose gel in 0.5 X TBE buffer during 30 min at 100 volts.

PCR products were sequenced using the dynamic ET terminator cycle sequencing kit. Purification of DNA was carried out with Exosap-IT (Amersham). The sequencer used was the Megabase 1,000 apparatus. All DNA fragments were sequenced along both strands. Sequences were analysed, checked and read manually using Mega3.1® (2005). They were aligned then using ClustalW® (1997) (Higgins *et al*. 1994) and analysed with Mega3.1® (2005).

Data analysis

Molecular analyses were conducted using PAUP*4.0 b10 (Swofford 1999). The distance matrix was constructed using the Jukes & Kantor model as the rate of transition / reversion is 1.

Results

The genetic distances are reported in histograms where the pairwise differences between the sequences of different strains within a species and between two species are represented in percent of nucleotidic divergence.

Neoseiulus californicus

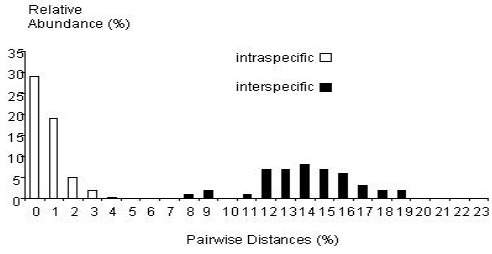

Figure1. Pairwise genetic distance frequences within strains of *Neoseiulus californicus* and *N. picanus* (intraspecific) and between *N. californicus*, *N. picanus* and *N. fallacis* (interspecific) for the 12S rDNA fragment considered.

There is not overlapping between the intra- and interspecific distances for the *Neoseiulus* specimens considered. The intraspecific distances range from 0 to 4 % (weighted mean=0.62, σ=0.17). The majority of the pairwise distances has a value of 0 (28 %) or 1 % (20 %). For a very low number of pairwise distances (both between populations and within populations), the genetic distances are higher. The highest intraspecific genetic distances (4 %) are observed within the population of *N. picanus* collected from Argentina.

The interspecific genetic distances range between 8 and 23 % (weighted mean=14.09, σ=1.20). The lowest value (8 %) is observed between some specimens[number of sequences of specimens used for this study] of *N. californicus* (collected in Italy Tuscany [5], Italy Sicily [6], Chile [1]) and *N. fallacis*. Even if a great variation range is observed at the interspecific level, the majority of interspecific pairwise distances varies from 12 to 16 % (427 pairwise sequences).

The highest interspecific nucleotidic divergence was observed between *N. fallacis* and *N. picanus* from Argentina, ranging from 16 to 23 % (72 pairwise sequences).

Kampimodromus aberrans

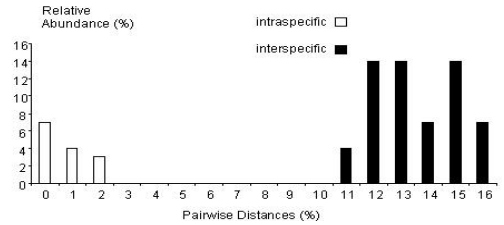

Figure 2. Paiwise genetic distance frequences within strains of Kampimodromus aberrans (intraspecific) and between K. aberrans, K. ericinus and K. corylosus (interspecific) for the COI mtDNA fragment considered.

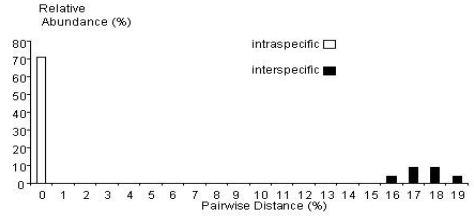

Figure 3. Paiwise genetic distance frequences within strains of *Kampimodromus aberrans* (intraspecific) and between *K. aberrans and K. corylosus* (interspecific) for the 12S rDNA fragment considered.

For the two DNA fragments (12S rDNA and COI mt DNA), there is not overlapping between the intra- and interspecific distances for the *Kampimodromus* specimens considered.

For the COI mtDNA fragment, the intraspecific genetic distances range from 0 to 2 % (weighted mean=1.90, σ=0.64). The genetic distances have a value of 0 % within each population of *K. aberrans* (France and Austria) and have a value of 1 % between the two populations of *K. corylosus* (France and USA: each population is represented by one sequence). Between the two populations of *K. aberrans*, this distance value is of 2 %. The

interspecific nucleotidic divergences range from 11 to 16 % (weighted mean=14.00, σ=8.30), the highest value being observed between *K. corylosus* from USA and *K. ericinus,* and the lowest between *K. aberrans* from Austria and *K. corylosus* from France.

For the 12S rDNA fragment, the intraspecific genetic distances range between 0 and 1 % (weighted mean=0.32, σ=0.35) within each population of *K. aberrans* (France and Austria). Between *K. aberrans* and *K. corylosus* the interspecific genetic distances range from 16 to 19 % (weighted mean=9.85, σ=1.40). Both the highest and the lowest values are observed between specimens from the population of *K. aberrans* from Austria and *K. corylosus* (France).

Euseius stipulatus

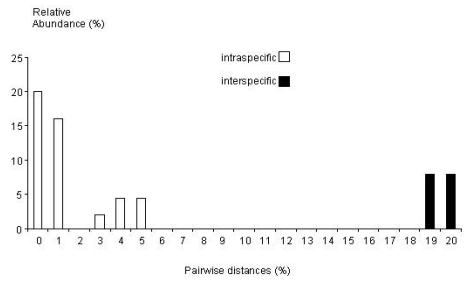

Figure 4. Pairwise genetic distance frequences within strains of *Euseius stipulatus* (intraspecific) and between *E. stipulatus* and *E. finlandicus* (interspecific) for the COI mtDNA fragment considered.

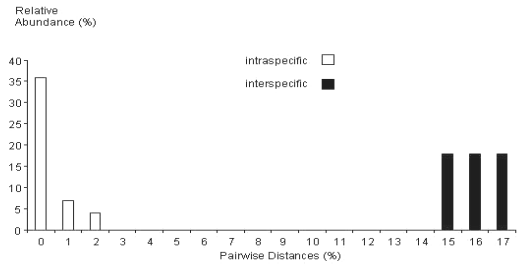

Figure 5. Pairwise genetic distance frequences within strains of *Euseius stipulatus* (intraspecific) and between *E. stipulatus* and *E. finlandicus* (interspecific) for the 12S rDNA fragment considered.

For the 12S rDNA and COI mtDNA fragments considered, there is not overlapping between the intra and interspecific distances for the *Euseius* specimens considered.

For the COI mtDNA fragment, the intraspecific genetic distances range from 0 to 5 % (weighted mean=1.46, σ=2.70). The pairwise distances has a value of 0 % within each population of *E.stipulatus* (France and Spain) and also between some specimens (n=2) from these two strains. The genetic distances usually observed between the two populations of *E. stipulatus* has a value of 1 %. A higher intraspecific genetic distance range is observed for the specimens of *E. finlandicus*. Except for one pairwise distance, these values range from 3 to 5 %.

The interspecific genetic distances between *E. stipulatus* and *E. finlandicus* range from 19 and 20 % (weighted mean=19.00, σ=0.53). The lowest interspecific genetic distance (19 %) is observed between *E. finlandicus* and *E. stipulatus* from France and the highest between *E. finlandicus* and *E. stipulatus* from Spain.

For the 12S rDNA fragment, the intraspecific genetic distances range from 0 to 2 % (weighted mean=0.58, σ=0.65). The pairwise distances have a value of 0 % within each population of *E.stipulatus* (France and Spain) and also between these two populations. The intraspecific genetic distances range from 1 to 2 % for the species *E. finlandicus.* The interspecific genetic distances range from 15 to 17% (weighted mean=16.00, σ=0.27). Similarly to what has been observed previously with the COI mtDNA fragment, the lowest interspecific genetic distance (15 %) is observed between *E. finlandicus* and *E. stipulatus* from France and the hightest (17 %) between *E. finlandicus* and *E. stipulatus* from Spain.

Discussion

Molecular markers have proved their utility in systematic and evolutionary acarology. The use of molecular data in taxonomy and population genetics starts to become a standard. This enormous progress occurred in less than 15 years: in 1993 only 49 DNA sequences originating from the Acari were reported in the GenBank database, whereas at the beginning of 2006 there were almost 93,000 sequences (Dabert 2006). However, for the family Phytoseiidae, only 54 sequences are reported up to now in the genBank database. These sequences mainly concern species belonging to the genus *Neoseiulus.* Sixteen partial sequences of mitochondrial DNA are available for Phytoseiidae (8 for 12S and 8 for COI mtDNA fragments).The genetic distances obtained, both at intra- and interspecific levels, are quite similar for the two DNA fragments tested. These two genes seem thus to have are very close evolution rate.

The results of the present study emphasize that the genetic distances observed between and within species, for both DNA fragments considered (12S rDNA and COI mtDNA) clearly allow a specific delineation. No overlapping was observed

between the intra- and interspecific distances. Intraspecific distances range from 0 to 5 % for all the species considered and for both genes, whereas the interspecific ranges from 8 % to 23 %. A great gap was thus observed between the two taxonomic levels. For the genus *Neoseiulus* the value of gap between the intra and interspecific distances is of 4 % for the 12S rDNA fragment. For the genus *Kampimodromus* this value is of 9% for the COI mDNA fragment and 16 % for the 12S rDNA fragment. At last, for the genus *Euseius* this value is of 14 % for the COI mtDNA fragment and 13 % for 12S rDNA fragment of 12S.The highest and the lowest gap values are observed for the genera *Kampimodromus* and *Neoseiulus*, respectively. These observations are quite different to some other results obtained with the COI mtDNA fragment, that showed difficulties in the determination of molecular threshold to assist species delimitation (Meier *et al*. 2006). If we compare our results with those obtained in other studies also using the COI mtDNA fragment, the overall intraspecific variability presently observed is low. In a study conducted on Diptera, where 127 species and 1,011 sequences were considered, the intraspecific variability ranged from 0 to 7 % and the interspecific one from 1 to 15 %. In this case, overlapping between intra and interspecific distances was observed, avoiding thus the use of such molecular tools for alpha-taxonomy (Meier *et al.* 2006). However, in the present study, only three species of Phytoseiidae and a low number of populations have been studied. To confirm the good reliability of these two molecular markers in species diagnostic, molecular analysis of other strains and and other morphological close species have to be conducted. Up to now unpublished results on the genus *Phytoseiulus* (including important species for biological control), seem also to show the good reliability of these molecular markers to separate the four species included in this genus.

Furthermore, it is also planed to test the utility of other DNA fragments as the ITS nDNA, that has been very used for mites (i.e. Hans *et al.* 2007; Klimov & O'Connor 2008) and accurately to differentiate two species of Phytoseiidae, *Typhlodromus exhilaratus* Ragusa and *Typhlodromus phialatus* Athias-Henriot, only differing in the insemination apparatus shape (Tixier *et al*. 2006).

Conclusion

The intraspecific and interspecific distances emphasized with the two DNA fragments tested (12S rDNA and COI mtDBA) seem to be different enough, to encourage the development of further molecular tools for Phytoseiidae specific diagnostic. However, problems occurred especially for the amplification of the COI mtDNA fragment. Conversely to the 12S rDNA fragment, obtaining amplified fragments of the COI mtDNA considered was unsuccessfull for more than 50% of the samples tested. Even if interesting, it seems impossible to develop molecular diagnostic routine tools using the COI mtDNA fragment. To confirm these results, further experiments are planed, testing other species and populations to get an adequate sample unit, testing other markers such as ITS, cytb, and NAD5 and other primers for succeeding in a good amplification of the COI mtDNA.

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NEW ERIOPHYOID MITES (ACARI: ERIOPHYOIDEA) OCCURRING ON PERENNIAL PLANTS IN POLAND

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Abstract

This paper presents information about four mite species: *Aceria ajugae* (Nalepa, 1892); *Aceria eupatorii* Roivainen, 1953; *Epitrimerus liroi* Roivainen, 1947 and *Neoleipothrix* n. sp. (this species will be described later) found for the first time on perennial plants in Poland.

Key–words

Aceria ajugae, *Aceria eupatorii*, *Epitrimerus liroi*, perennial plants

Introduction

Until now over 90 species of eriophyoid mites have been found on perennial plants in Poland (Skoracka et al. 2004). These mites live on the leaf surface or inside buds. Some of them do not cause significant damage to their hosts but others induce various kind of galls which they inhabit or they cause discolouration of affected foliage.

This paper presents the data on the occurrence of eriohyoid mites living on perennial plants in botanical gardens and ornamental nurseries located in different parts of Poland.

Material and Methods

In years 2000–2006 the observations were carried out on above 150 species of perennial plants in nurseries and botanical gardens located in different parts of Poland. The infested shoots and leaves were sampled from May to September. Mites were mounted in Heinz media and studied under a phase–contrast microscope. All measurements are given in micrometers and were made at 1000 magnification. Type materials are deposited at the Research Institute of Pomology and Floriculture in Skierniewice.

Results and Discussion

Four new species of eriophyoid mites to polish fauna were found in collected samples. Three of them were recorded for the first time in Poland: *Aceria ajugae* (Nalepa, 1892); *A. eupatorii* Roivainen, 1953; *Epitrimerus liroi* (Roivainen, 1947). The fourth one - *Neoleipothrix* n. sp. is new to science.

***Aceria ajugae* (Nalepa, 1891) [Fig. 1]**

Until now this mite was recorded on *A. reptans* and *A. genevensis* (Amrine & Stasny 1996). In Poland this species was found for the first time in 2000 on *A. reptans* (Soika et al. 2004). *A. ajugae* was described from France by Nalepa (1910) on *Ajuga genevensis* and *A. reptans.* Recently, it was recorded also in Serbia by Petanović & Stanković (1999) who observed flower deformation induced by mites. Previous description of this species by Nalepa (1910) is incomplete. Detailed morphological description is given below.

Female: (n= 5). Body spindleform, whitish coloured. Body length 187,5-237,5; width 62-72. Gnathosoma 20 long, dorsal pedipalp genual setae 4-5 long; cheliceral stylets almost straight, 14-15 long. Prodorsal shield rhomboidal, without frontal lobe over gnathosoma, 41-42 long, 44-50 wide; Sculpture of prodorsal shield, median line present on rear half of shield, admedian lines from anterior margin diverging to rear, slightly concave in the middle, submedian lines in front part continuous and in back intermittent, dashed lines in front of conical of scapular setae present. Numerous, short lines present on surface rear part of shield. Tubercles of scapular setae ahead from rear margin of shield, 20-23 apart; scapular setae 15-17, projecting to rear.

Leg I 35-37 long; tibia 7-8 long with paraxial tibial seta 6-7. Tarsus 8 long; tarsal solenidion 10 long unknobbed, tarsal empodium simple 6-7 long, 4-rayed. Leg II 30-32 long; tibia 6-7 long. Tarsus 7-8 long; tarsal solenidion 9-10 long unknobbed; tarsal empodium 6-7 long.

Coxae with a pattern; on coxae I numerous short dashes disposed irregularly, on coxae II smooth surface. Sternal line clear, forked on both ends, 4-5 long. Anterolateral setae on coxisternum I 12-14 apart, 8-10 long, proximal setae on coxisternum I 8-10 apart, 17-20 long; proximal setae on coxisternum II 21-27 apart, 40-48 long.

Opisthosoma with 70-80 microtuberculate annuli. Microtubercles numerous, set along annuli margins. Lateral setae 15-17 long located on 12-14th ventral annulus; 1st ventral setae 40-45 long located on 26-28th annulus; 2nd ventral setae 8-12 long located on 44-47 ventral annulus; 3rd ventral setae 8-12 long on 6-7th annulus from the rear. Accessory setae 4-long.

Genital parts 18-22 long, 23-26 wide, genital coverflap with 8-12 ribs distally and dashed small transverse lines proximally; genital setae 10-15 long, 16-19 apart.

Male: (n=2); Body spindleform. Body length 197,5-202,5; width 60-65. Gnathosoma 20 long; chelicerae 15 long.

Prodosal shield 40-41 long, 45-47 wide. Shape and sculpture similar to that of female. Tubercles of scapular setae ahead rear margin of shield, setae 16-17 long, 23-24 apart; projecting to rear.

Opisthosoma with 68-69 microtuberculate annuli, Microtubercles numerous, set along annuli margins. Lateral setae 16-20 long located on 13th ventral annulus; 1st ventral setae 40 long located on 23-26th ventral annulus, 2nd ventral setae 8-12 long located on 44-47th ventral annulus; 3rd ventral setae 10-15 long on 7th annulus from the rear. Accessory setae 3-4-long.

Leg I 30-32; tibia 7 long, seta 7 long, tarsus 6-7 long; tarsal solenidion 9-10, tarsal empodium 6, 4-rayed simple. Leg II 32; tibia 5-6; tarsus 7; tarsal solenidion 10.

tarsal empodium 6, 4-rayed.

Genital parts 23-24 wide, genital setae 15-17 long, tubercles 16-17 apart. similar to that of female.

Locality and date: Kraków - Botanical Garden on August 27, 2000, Warszawa - Botanical Garden on July 10, 2000, Rzeszów-commercial nursery on June 26, 2000 (Soika et al. 2004).

Host plant: *Ajuga reptans* L. (Lamiaceae)

Relation to host plant: mites living as vagrants on lower surface of the youngest leaves and causing discolouration and leaf edge rolling.

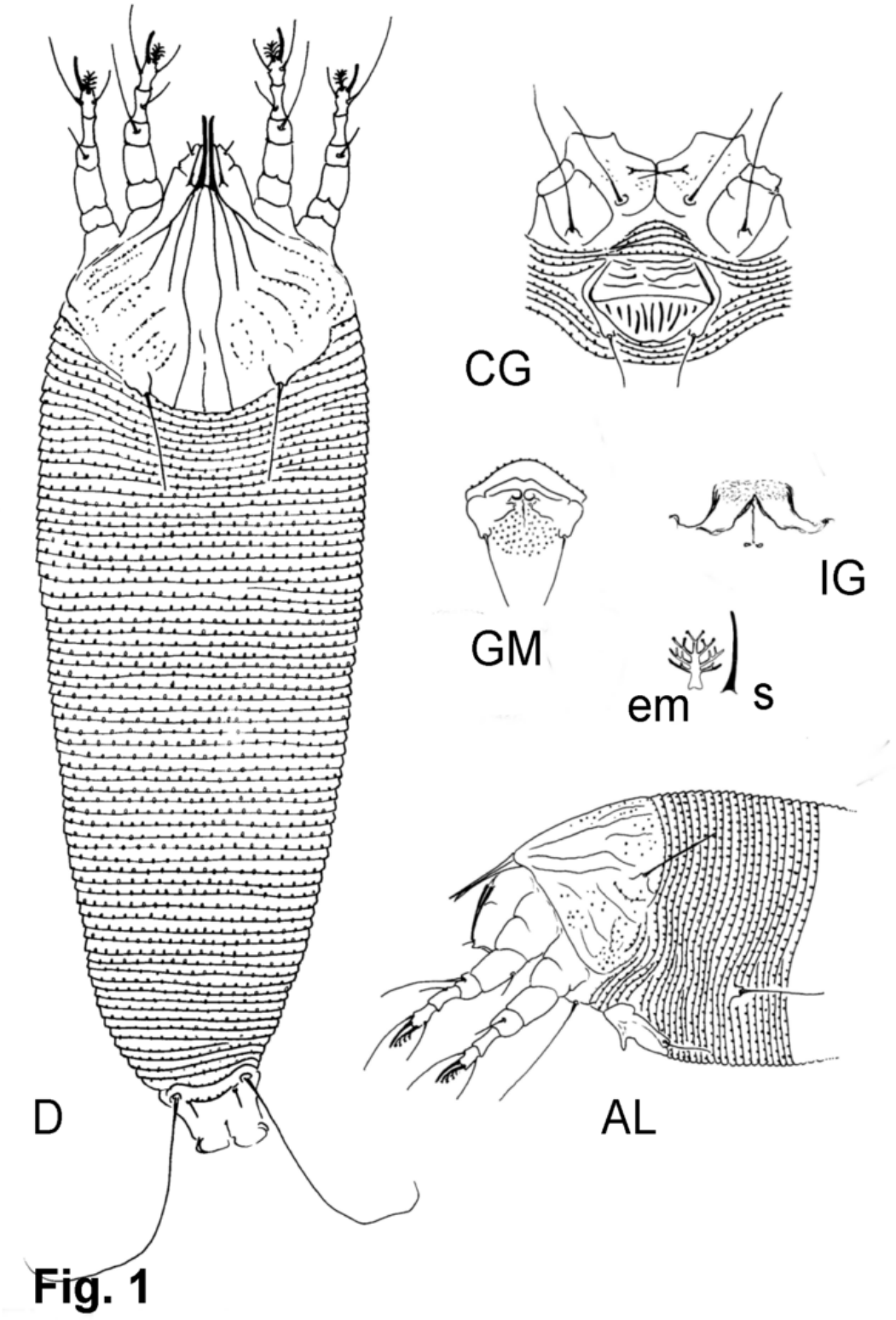

Figure 1. *Aceria ajugae*, female: D – dorsal aspect, IG – internal genitalia, CG – coxigenital region, GM – genital male, AL – anterior lateral body region, em – empodium, s-solenidion.

***Aceria eupatorii* Roivainen, 1953 [Fig. 2]**

Until now this mite was noted in Spain on *Eupatorium cannabinum* L. by Roivainen (1953). This mite lives on apical leaves and causes rolling margins of leaves, creating dense hair on leaf surface. It was also found on *E. cannabinum* by Petanović and Stanković (1999) in Montenegro.

The original description of the species is quite broad (Roivainen 1953). However, individuals collected from *E. purpureum* L. differed in some characters in comparision to those collected from *E. cannabinum* in Spain. Below it was showed the detail description including characters, which were not took into account in the original description.

Because Roivainen (1953) did not describe males, these data are supplemented in the description.

We found also deutogyne females. They were differed from protogyne females by some characters, which were listed in table 1. Moreover, they differed in the appearance of microtubercles on annuli and pattern on dorsal shield. These characters of females protogyne are more distinguish in comparison to deutogyne ones

Female protogyne: (n=3). Body vermiform, whitish coloured. Body length 202,5-230; width 65-70.

Gnathosoma 22 -23 long, dorsal pedipalp genual setae 6-7 long; cheliceral stylets almost straight, 18 long. Prodorsal shield semicircular, without frontal lobe over gnathosoma, 32-34 long, 40 wide; Sculpture of prodorsal shield, median line present, running from anterior margin to rear one. Admedian lines complete, submedian lines short and subparallel to median line. Numerous broken lines and granules present in shield side. Tubercles of scapular setae on rear margin, 25 apart; setae 50-52, projecting to rear.

Leg I 33-35 long; tibia 8 long with paraxial tibial seta 7. Tarsus 8-9 long; tarsal solenidion 8-9 long unknobbed, tarsal empodium simple 6-7 long, 5-rayed. Leg II 27-30 long; tibia 6-7 long. Tarsus 7 long; tarsal solenidion 10 long unknobbed; tarsal empodium 6-7 long.

Coxae with a pattern; both on coxae I and on coxae II numerous short lines. Sternal line clear, forked on both ends, 9-10 long. Anterolateral setae on coxisternum I 10-11 apart, 9-10 long, proximal setae on coxisternum I 9-10 apart, 23-25 long; proximal setae on coxisternum II 25 apart 50-52 long.

Opisthosoma with 66-72 microtuberculate annuli. Microtubercles numerous, set along annuli margins. Lateral setae 20-23 long located on 12-13th ventral annulus; 1st ventral setae 53-60 long located on 27th annulus; 2nd ventral setae 14-15 long located on 44-45th ventral annulus; 3rd ventral setae 23-25 long on 5-6th annulus from the rear. Accessory setae 6-8 long.

Genital parts 18 long, 23-24 wide, genital coverflap with 14-16 ribs; genital setae 15-17 long, 18-19 apart.

Male: (n=1); Body spindleform. Body length 187,5; width 60. Gnathosoma 21 long; chelicerae 16 long.

Prodorsal shield 33 long, 35 wide. Shape and sculpture similar to that of female. Tubercles of scapular setae on rear margin of shield, scapular setae *35* long, 25 apart; projecting to rear.

Opisthosoma with 68 annuli, Annuli with numerous microtubercles, set along annuli margins. Lateral setae 20 long located on 12th ventral annulus; 1st ventral setae 40 long located on 21th annulus; 2nd ventral setae 15 long located on 36th ventral annulus; 3rd ventral setae 20 long on 7th annulus from the rear. Accessory setae 5 long.

Leg I 37; tibia 8 long, paraxial tibial seta 5 long, tarsus 8 long; tarsal solenidion 9, tarsal empodium 6, 5-rayed simple. Leg II 30; tibia 6; tarsus 7; tarsal solenidion 11; tarsal empodium 6, 5-rayed

Genital parts 22 wide, genital setae 12 long, tubercles 17 apart.

We found also deutogyne females. They were differed from protogyne females by some characters, which were listed in table 1. Moreover, they differed in the appearance of microtubercles on annuli and pattern on dorsal shield. These characters of females protogyne are more distinguish in comparison to deutogyne ones.

Locality and date: Skierniewice, September 16, 1999; Warszawa – University Botanic Garden, July 12, 2001.

Host plant: *Eupatorium purpureum* L.*; E. cannabinum* L. (Asteraceae)

Relation to host plant: Mites living on the youngest leaves of *E. purpureum* and causing considerable distortion of affected foliage

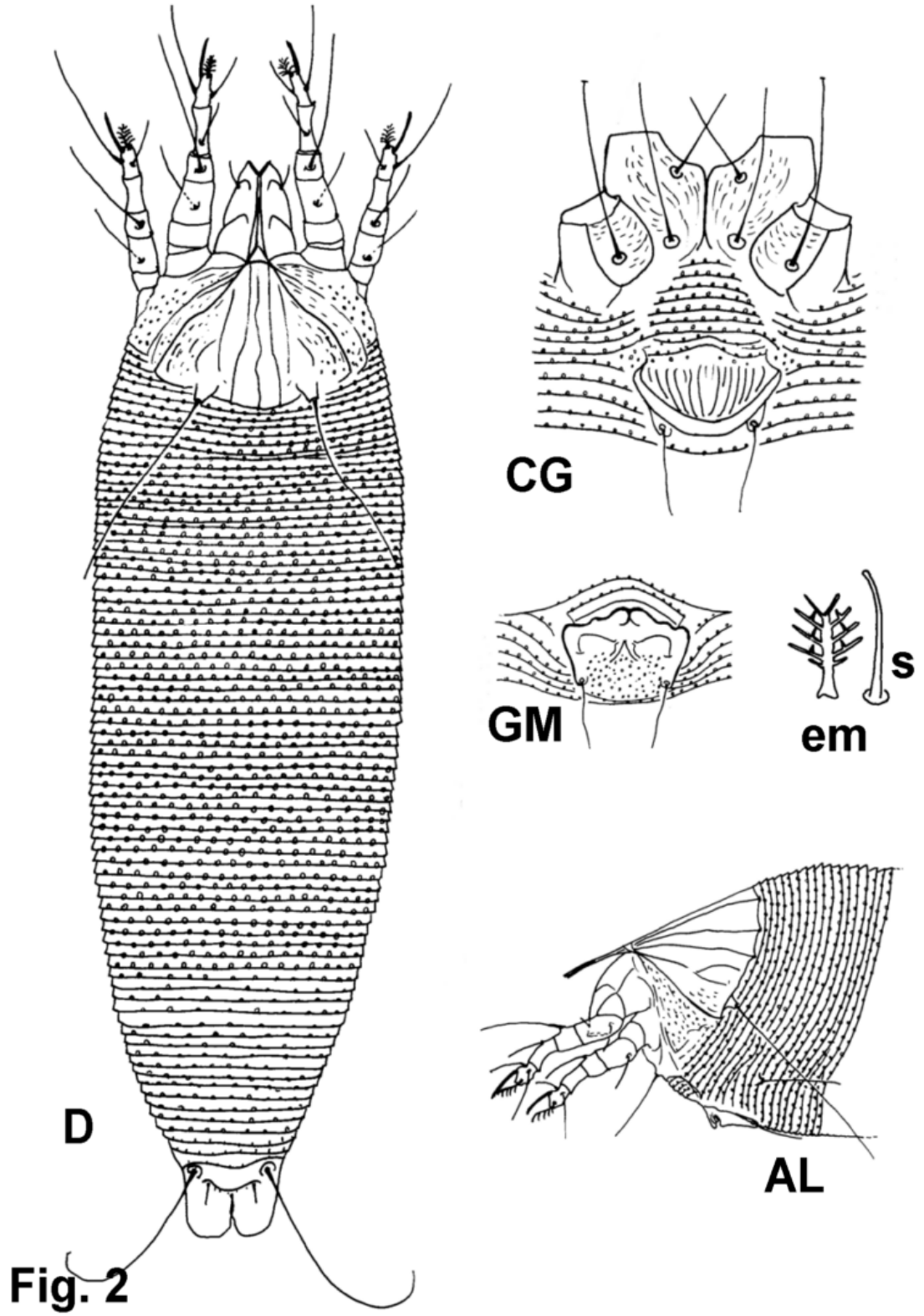

Figure 2. *Aceria eupatorii* .-,protogyne female: D – dorsal aspect, CG – coxigenital region, AL – anterior lateral body region, GM – genital male, em – empodium, s - solenidion

Table 1. Comparison in characters of *Aceria eupatorii* protogyne and deutogyne females collected in Poland

Characters	protogyne	deutogyne
Length of body	202,5-230	187,5-200
Length of scapular setae	50-52	60-65
No of annuli	66-70	64-66
Location of 1st ventral setae	27	22-24
Length of 2nd ventral setae	14-15	15-18
Location of 2nd ventral setae	44-45	35-38
Length of 3rd ventral setae	23-25	20-27
Length of leg I	33-35	38-40
Length of tibia I	8	8-10
Length of tarsus I	7-8	9
Length of leg II	27-30	35
Length of tibia II	6-7	8-9
Length of anterolateral setae on coxisternum I	9-10	10-12
Length of proximal setae on coxisternum II	50-52	43-55
No. of ribs on genital coverflap	14-16	12-14

***Epitrimerus liroi* Roivainen, 1947 [Fig. 3]**

This mite was described by Roivainen (1947) from *Primula veris* L. in Finland and probably these were deutogyne females. Previous description of this species was incomplete and therefore the morphological characteristic is given below. Males not seen.

Female deutogyne: (n= 10). Body spindleform, amber coloured. Body length 230-272,5; width 79-84.

Gnathosoma 23-25 long, dorsal pedipalp genual setae simple 15-16 long; cheliceral stylets almost straight, 16-18 long. Prodorsal shield subrhomboidal, with frontal lobe over gnathosoma, 55-59 long, 67-70 wide; Sculpture of prodorsal shield: median line lack, admedian lines from anterior lobe base diverging to centre of shield and slightly concave on rear half one Tubercles of scapular setae situated ahead of rear shield margin, 18-20 apart; scapular setae 4-6, projecting to up centrally.

Leg I 40-41 long; tibia 9-11 long with paraxial tibial seta 3-5 long. Tarsus 7-8 long; tarsal solenidion 7 long knobbed, tarsal empodium simple 6-7 long, 4-rayed. Leg II 31-35 long; tibia 8-9 long. Tarsus 7-8 long; tarsal solenidion *7* long knobbed; tarsal empodium 6-7 long.

Coxae with numerous short lines. Sternal line, forked anteriorly, 10-13 long. anterolateral setae on coxisternum I 16-18 apart, 10-12 long, proximal setae on coxisternum I 8-9 apart, 25-35 long; proximal setae on coxisternum II 27-35 apart, 35-45 long.

Opisthosoma with 45-47 smooth dorsal annuli and 67-71 microtuberculate ventral annuli. Microtubercles numerous, set along annuli margins. Lateral setae 20-25 long located on 12-13th ventral annulus; 1st ventral setae 34-40 long located on 24-27th annulus; 2nd ventral setae 19-23 long located on 43-47th ventral annulus; 3rd ventral setae 26-38 long on 5-6th annulus from the rear. Accessory setae 3-5-long.

Genital parts 19-21 long, 23-25 wide, genital coverflap with numerous dashed line on surface; genital setae 17-18 long, 13-15 apart.

Locality and date: Rogów – Arboretum on June 20, 2006 and April 25, 2008.

Host plant: *Primula veris* L. 'Cabrillo' (Primulaceae)

Relation to host plant: mites live as vagrants on lower surface of leaves and cause browning.

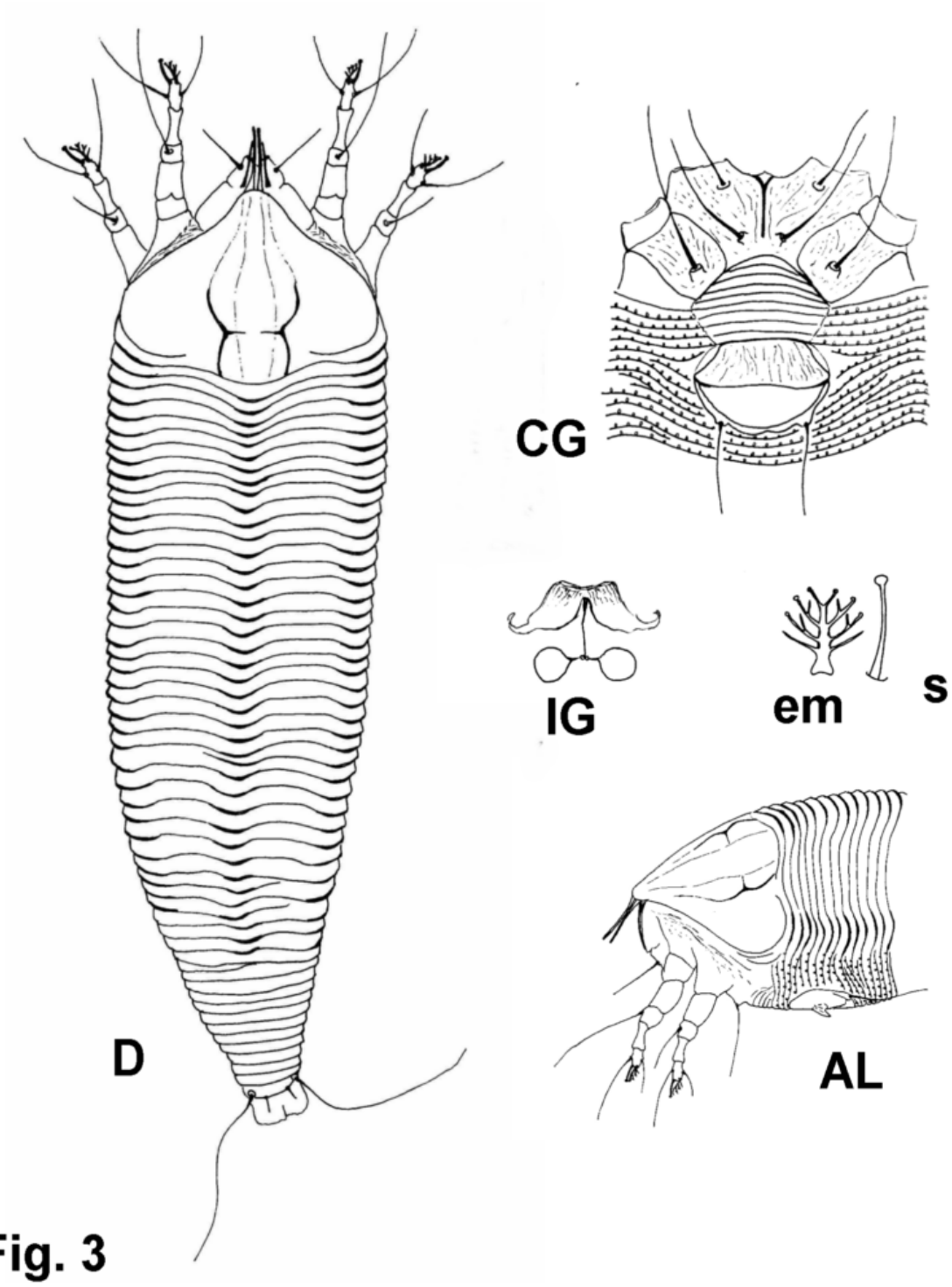

Figure 3. *Epitrimerus liroi*., female: D – dorsal aspect, IG – internal genitalia, CG– coxigenital region, AL – anterior lateral body region, em – empodium, s - solenidion.

***Neipothrix* n. sp. [fig.4]**

This species will be described later. We give here diagnosis characters.

Until now only one species of eriophyoid mite, *Aceria macrotuberculatus* Nalepa, 1895 was recorded on *Valeriana officinalis* L. (Valerianaceae) in Europe (Amrine & Stasny 1996). In Poland the second species *N. valerianae* n. sp. is noted for the first time as living also on *V. officinalis.*

Below it was presented the preliminary description of this species. The detailed information concerning holotype and paratypes will be published in future.

Female protogyne:(n= 10). Body spindelform, cream coloured. Body length 202,5-237,5; width 76-83.

Gnathosoma 22-23 long, dorsal pedipalp genual setae simple 15-17 long; cheliceral stylets almost straight 12-13 long. Prodorsal shield almost triangular, with frontal lobe over gnathosoma 55-58 long, 65-70)wide; Sculpture of prodorsal shield, median line very short, present only in shield side. Admedian lines complete, curved, submedian lines running back on outer side of dorsal tubercles from about ½ on shield. Individual short curved line present in shield side. Tubercles of scapular setae ahead of shield margin, 15-19 apart; scapular setae 5-7, directed up and centrally.

Leg I 37-40 long; tibia 8-9 long with paraxial tibial seta 3-5. Tarsus 6-7 long; tarsal solenidion 6-7 long knobbed, tarsal empodium simple 6 long, 4-rayed. Leg II 32-34 long; tibia 7-8 long. Tarsus 6-7 long; tarsal solenidion 6-7 long knobbed; tarsal empodium 6-7 long.

Coxae with a pattern; both on coxae I and on coxae II present some dashes lines. Sternal line clear, forked on both ends, 10 long. Anterolateral setae on coxisternum I 18-21 apart, 10-11 long, proximal setae on coxisternum I 7-11 apart, 17-27 long; proximal setae on coxisternum II 28-33 apart 35-45 long.

Opisthosoma with 43-49 smooth dorsal annuli and 65-71 microtuberculate ventral annuli. Microtubercles numerous, set along annuli margins. Lateral setae 18-21 long located on 9-12th ventral annulus; 1st ventral setae 27-30 long located on 22-27th annulus; 2nd ventral setae 15 long located on 42-50th ventral annulus; 3rd ventral setae 25-30 long on 5-6th annulus from the rear. Accessory setae 3-4 long.

Genital parts 20-22 long, 23-25 wide, genital coverflap with longitudinal lines and granular; genital setae 15-17 long, 13-16) apart.

Male: (n=1); Body spindleform. Body length 165; width 59. Gnathosoma 21 long; chelicerae 12 long.

Prodorsal shield 51 long, 52 wide. Shape and sculpture similar to that of female. Tubercles of setae ahead on rear margin of shield, scapular setae *5* long, 13 apart; projecting up and centrally.

Opisthosoma with 44 dorsal annuli and 63 microtuberculate ventral annuli. Annuli with numerous microtubercles, set along annuli margins. Lateral setae 14 long located on 11th ventral annulus; 1st ventral setae 22 long located on 23th; 2nd ventral setae 13 long located on 40th ventral annulus; 3rd ventral setae 22 long on 5th annulus from the rear. Accessory setae 3 long.

Leg I 34; tibia 9 long, paraxial tibial seta 6 long, tarsus 6 long; tarsal solenidion 6 knobbed, tarsal empodium 6, 4-rayed simple. Leg II 30; tibia 7; tarsus 6; tarsal solenidion 7; tarsal empodium 6, 4-rayed

Genital parts 18 wide, genital setae 12 long, tubercles 11 apart.

Some of characters of deutogyne females are given in table 2. These females were different in body length from protogyne females, length of prodorsal shield and lobe, length of tibia I and forecoxal setae I and forecoxal setae II. Moreover, they differed in the appearance of microtubercule on sternal annuli and pattern on the prodorsal shield.

Table 2. Characteristics of protogyne and deutogyne females of *Neoleipothrix* n. sp collected in Poland.

Characters	protogyne	deutogyne
Length of body	225-237,5	217,5-262,5
Length of prodorsal shield	55-58	63-70
Length of lobe	9-11	13-15
Dorsal tubercle apart	15-17	16-18
Length of tibia I	8-9	10-11
Length of antrolateral setae on coxisternum I	10-11	11-15
Length of proximal setae on coxisternum II	35-45	22-30

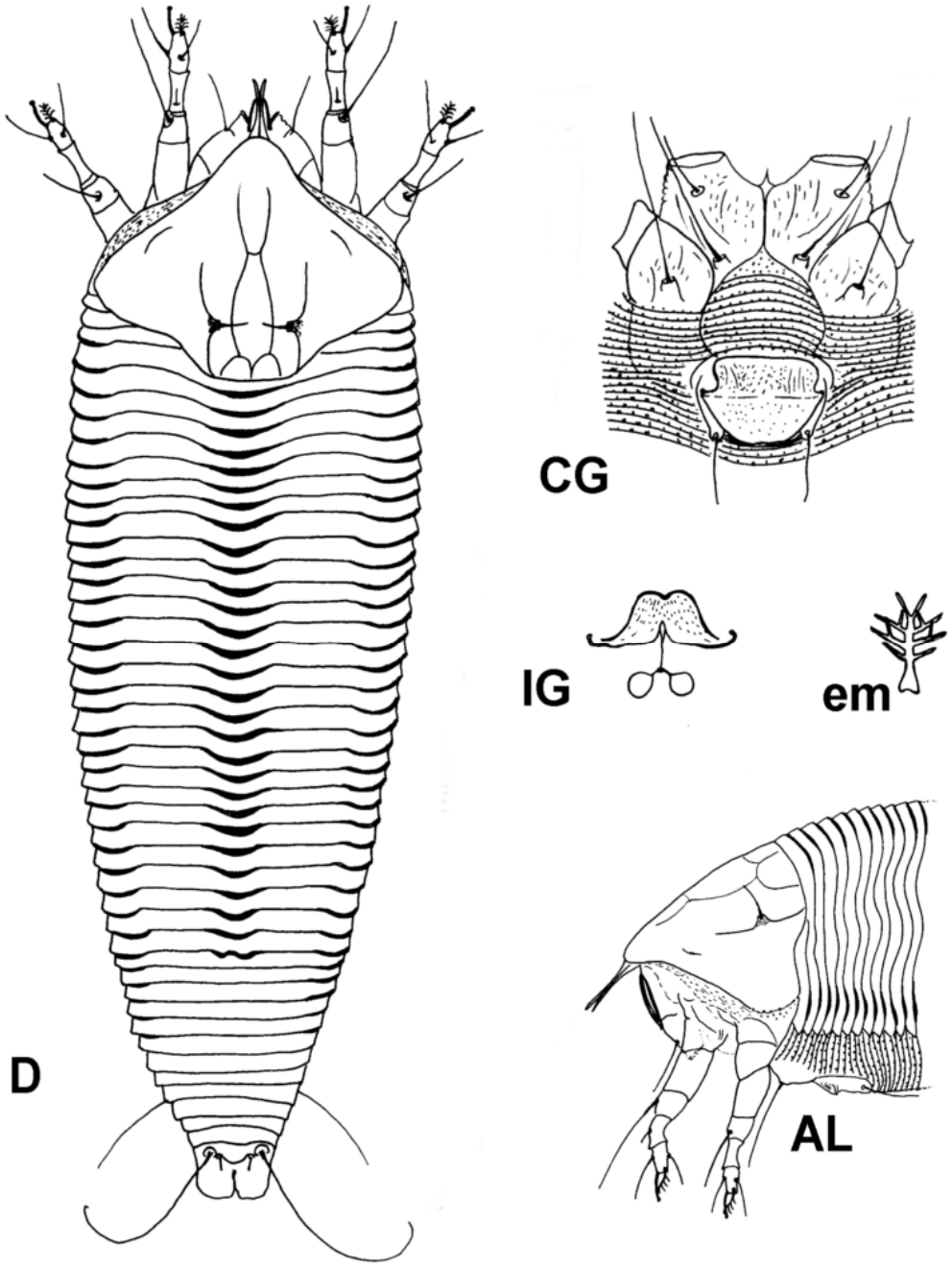

Fig. 4

Figure 4. *Neoleipothrix* n. sp., female: D – dorsal aspect, IG – internal genitalia, CG– coxigenital region, AL – anterior lateral body region, em – empodium.

Table 3. Comparison of *Neoleipothrix* n. sp. found in Poland and *N. eupatorii* (Boczek & Petanović, 1994)=*Epitrimerus eupatorii* protogyne females.

	Neoleipothrix n.sp. (n=10)	*N. eupatorii* (n=10)
Length of body	225-237,5	240 (177-268)
Width of body	79-83	75
Length of antapical setae	15-17	18
Length of p. shield	55-58	66 (64-69)
Length of lobe	9-11	10
Length of dorsal shield	5-7	5
Dorsal tubercles apart	15-17	16
No of dorsal annuli	42-49	42 (41-44)
No of central annuli	65-72	85
Length of sternum	10	9
Length of lateral s.	19-25	20
Location of lateral setae	9-12	21
Length of 1st ventral setae	27-30	32
Location of 1st ventral setae	22-30	21
Length of 3rd ventral setae	25-30	30
Location of 3rd ventral setae	5-6th from rear	6th from rear
Length of leg I	37-40	38
Length of tibia I	8-9	9
Length of tarsus I	6-7	6
Length of tarsal solenidion I	6-7	5
Length of tarsal empodium I	6	6
No. of rays of empodium .	4	4
Length of leg II	33-35	31
Length of tibia II	7-8	9
Length of tarsus II	6-7	6
Length of solenidion II	6-7	6
Tubercles of anterolateral setae on coxisternum I apart,	18-21	16
Length of anterolateral setae on coxisternum I	10-11	11
Tubercles of proximal setae on coxisternum I apart	7-11	7
Length of proximal setae on coxisternum I	35-45	20
Tubercles of proximal setae on coxisternum II apart	28-33	28
Length of proximal setae on coxisternum II	35-45	30
Length of genitalia	18-22	22
Width of genitalia	21-25	21
Length of genital setae	13-17	20
Genital tubercles apart	15-16	11

Locality and date: Warszawa – University Botanic Garden, July 10, 2000, Warszawa Botanical Garden, May 24, 1999, Nowy Dwór n/Skierniewice, August 10, 1999.

Host plant: *Valeriana officinalis (Valerianaceae)*

Relation to host plant: These mites live on lower

side of leaves causing no visible damage. The protogyne female of this new species is close to *N. eupatorii* (Boczek & Petanović 1994), but it can be distinguished by the appearance of prodorsal shield, body width, prodorsal shield length, number of ventral annuli, length of solenidion I and legs II, proximal setae on coxisternum I, proximal setae on coxisternum II, genital setae and genital tubercles apart (tab. 3).

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ON THE MORPHOLOGICAL ANOMALIES IN TYDEOIDEA (ACTINEDIDA)

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Abstract

Some morphological anomalies and phenomena in the morphology of Tydeoidea are presented and discussed (abnormal palpal tarsus, abnormal legs, single tarsal claws, Peruvian over-sized species from Machu Picchu).

Key words

Acari, Tydeoidea, morphology, anomalies.

Introduction

Up to now the morphololological anomalies in Tydeoidea sensu André et Fain 2000 have been noted only sporadicaly and incidentaly. Usually, these anomalies concerned genital chaetotaxy: the doubing or lack of some setae *ge* and *ag*, and thus some asymmetry (Kaźmierski 1989, 1990, Momen & Lundqvist 1995, 1996, Momen & Solhøy 1996). A single case of abnormally developed legs and palps has also been reported (Kaźmierski 1998).

In the present papers, we are describing four separate phenomena.

Material and methods

The phenomena mentioned above were observed on sixteen specimens of twelve species, deriving from four continents (Europe, Asia, Africa and South America) and six countries (Poland, Bulgaria, Spain, India, Kenya and Peru). Specimens examined are as follows:

Lorryia globulipalpa Kaźmierski,1998 – a single female (holotype) from Poland, Kielce province. Slide signed as HCM 8A/W-82 P-4, deposited in Department of Animal Morphology (=DAM), Adam Mickiewicz University, Poznań.

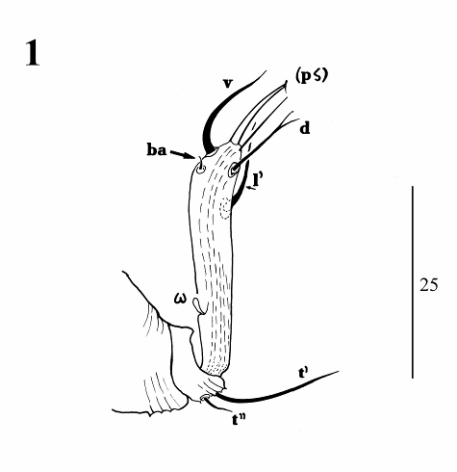
Lorryia subularoides Kaźmierski, 1989 – an allotype male from Poland, Tarnobrzeg province, north-east from Janów Lubelski. Slide T-0266/P-3 kept in DAM.

Lorryia woolleyi (Baker, 1968) – one female from Bulgaria, Varna. Slide BG-006/P-2 kept in DAM.

Lorryia exiguelitterata (Momen and Lundqvist, 1995) – one female from Poland, Kraków province. Slide T-0208/P-1 deposited in DAM.

Lorryia jesionowskii Kaźmierski, 1998 – paratype female from Poland, Kraków province. Slide T-0251 kept in DAM.

Brachytydeus sp. nov. I – one female from Kenya (Mt Kenya slope). Slide EAK 001/P-5 kept in DAM.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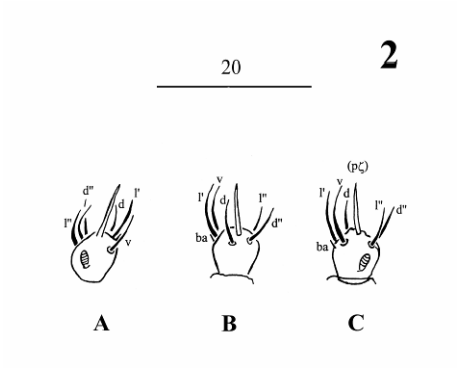
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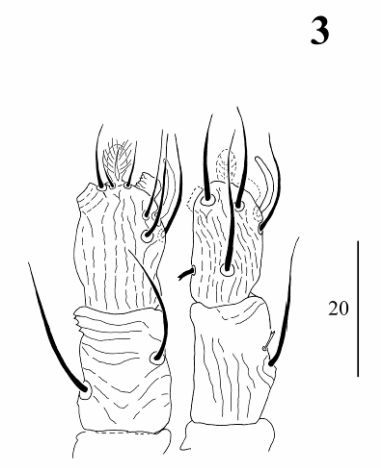
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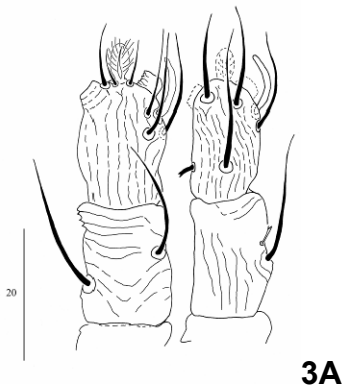
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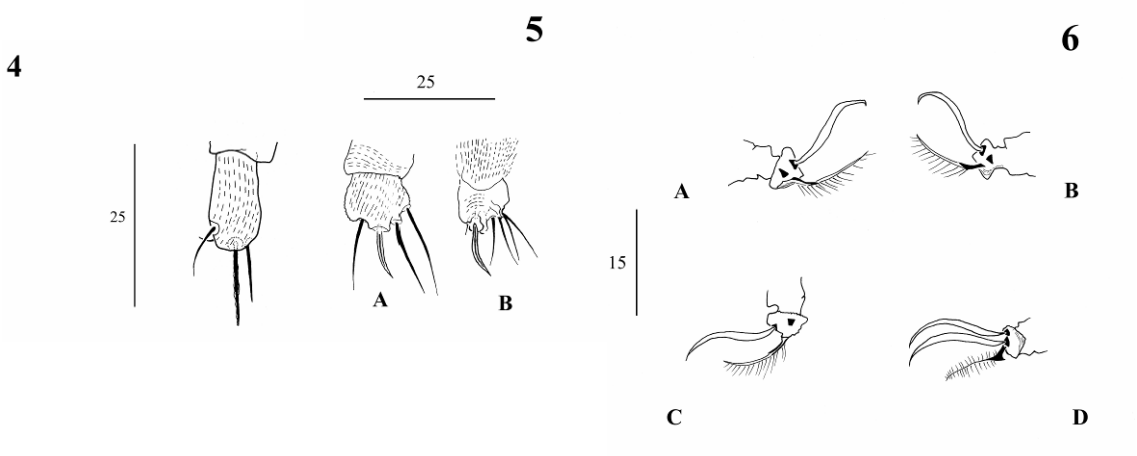
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Figures 1 -6

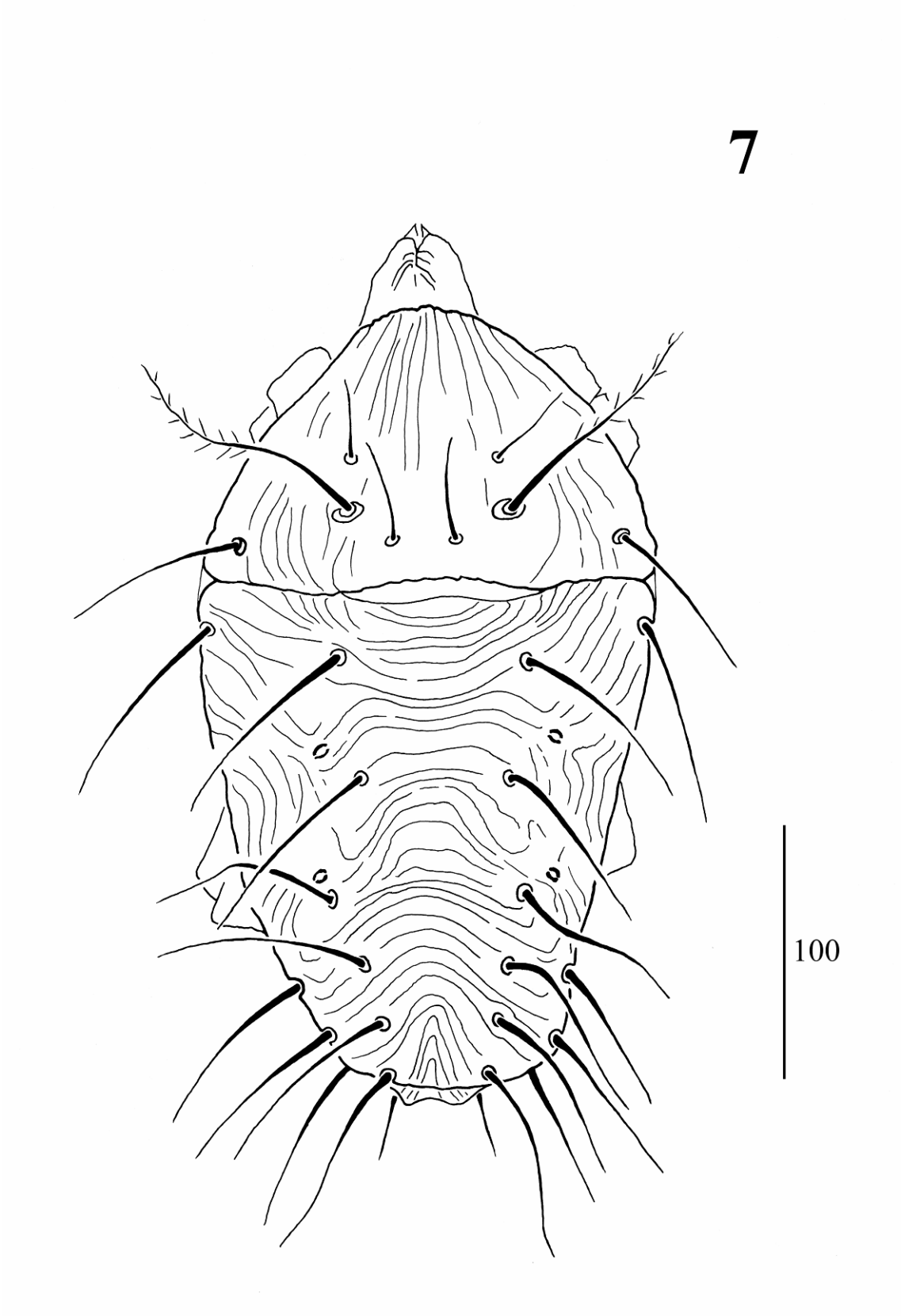


Figure 7

Lorryia tragelaphus Kaźmierski, 1993 – paratype female from Kenya (Mt Kenya slope). Slide EAK 002/P-2 kept in DAM.

Lorryia blaszaki Kaźmierski, 1998 – holotype male from India, Kashmir. Slide originally marked as IND-004/P-3 is deposited in the Zoological Museum of University of Hamburg (=ZMH).

Lorryia reticulata (Oudemans, 1928) – female from Spain, Sierra de Guadarrama, mounted on the slide together with the holotype of *Lorryia draciformis* Kaźmierski, 1998, which is deposited in ZMH (slide A77/99).

Melissotydeus incarum Kaźmierski 1998 – one female, two tritonymphs and two deutonymphs from Peru, Machu Picchu. Paratype female and holotype tritonymph are deposited in ZMH (slide PE-025/P-1); the second tritonymph (PE-025/P-2) as well as both deutonymphs (PE-25/P-4) are kept in DAM.

Tydeus sp. nov. I – a single female from Peru, Machu Picchu. Slide PE-014/P-1 is deposited in DAM.

Tydaeolinae gen. nov. , sp. nov. I – a single female from Peru, Machu Picchu. Slide PE-025/P-3 remains in DAM.

Of course these sixteen specimens were found to be anomalous against a body of thousands.

All the specimens were mounted on slides in modified Berlese liquid and examined under immersion with microscope Zeiss Peraval Interphako. All the photographs were made with Olympus DP71 digital camera under DX51 microscope with Nomarsky contrast phase.

Results

Anomaly on the Palptarsus. Normally developed palptarsus in Tydeinae has six setae on its distal part (Figure 1). These are: (pζ) – double eupathidium set on the very end of segment (fused with a third former seta), seta l' (the most proximal one), l'', d (usually forked), ba (vestigial) and – additionally- small solenidion ω. Only one species is different: *Lorryia globulipalpa* Kaźmierski, 1998 [= *Brachytydeus globulipalpus* (Kaźmierski, 1998) – accordingly with systematic concept proposed by André 2005]. This species was described on the base of a single female, but sufficiently specific because of some combination of characters. Among others, *L. globulipalpa* has eight setae on the left palptarsus and seven on the right one (Figures 2 a, b, c). None of them are cleft or forked distally. There are double setae l'' and d (left side) and double d on the right palp. In opposite to the usual state – setae l'' set more proximally as l'. Moreover, the segment is not longitudinal, but spherical. All the remaining features are typical of the genus. This fact, simultaneously with asymmetry of palpal organotaxy makes us admiting that the unique state mentioned above seems to be as anomal. The question of the essence of this phenomenon remains open.

Anomaly in legs. Similarly to palptarsus, there are some cases of abnormally developed legs in Tydeinae. One of the males of *Lorryia subularoides* Kaźmierski, 1989 [= *Brachytydeus subularoides*] from vicinity of Janów Lubelski in Poland (slide N^{o} T-0266/P-3, author's collection) has underdeveloped (half the length) right legs II and IV. Moreover, its right leg III is ended by abnormal fifth segment (tibia? tarsus?), armed with eight apical setae (one strong and stout, two undersized, five narrowly lanceolated, relatively long, but rather slender). One of the females of *Lorryia woolleyi* Baker, 1968) [=*Brachytydeus woolleyi*] found in Varna, Bulgaria (Slide BG-006/P-2) has left leg I composed with five segments. The last one (tibia? tarsus?) is abnormal, provided for ten different and irregularly distributed setae with addition of long solenidion. Moreover, some kind of quasi-empodium is set on the very end (Figure 3, photo 1). One of the females of *Lorryia exiguelitterata* (Momen et Lundqvist, 1995) [= *Brachytydeus exiguelitteratus*] from vicinity of Kraków, Poland (Slide T-0208/P-1) has abnormal tarsus of left leg IV: without apotel, but with four different setae on the tip (Figure 4). Left leg II of a one specimen of *Lorryia jesionowskii* Kaźmierski, 1998 [= *Brachytydeus jesionowskii*] from Kościelniki near Kraków has no tibia (Slide T- 0251). Undescribed tydein species (a single female) from Kenya, M^{t} Kenya, bamboo forest (Slide EAK 001/P-5) has dwarfish left leg I. Female of *Lorryia tragelaphus* Kaźmierski, 1993 [= *Brachytydeus tragelaphus*] –also from Kenya (Slide EAK 002/P-2) - has left leg I in shape of stump. It is composed with normally developed trochanter and dwarfish distal segment, witch is nude; without any setae (photo 2). Final case relates to the holotype of *Lorryia blaszaki* Kaźmierski, 1998 [= *Brachytydeus blaszaki*] from the vicinity of Srinagar, India (Slide IND-004/P-3). Left leg IV of the holotype male is anomalous: deprived of tarsus and apotel. Distal segment (more or less square-shaped tibia) is ended by nine setae. One of them is distinguished by its thickness and by the shape of sabre. The second one is minute (similar to palptarsal ba). The remaining ones are middle-long and setiform (Figures 5 A, B).

Photo 1A

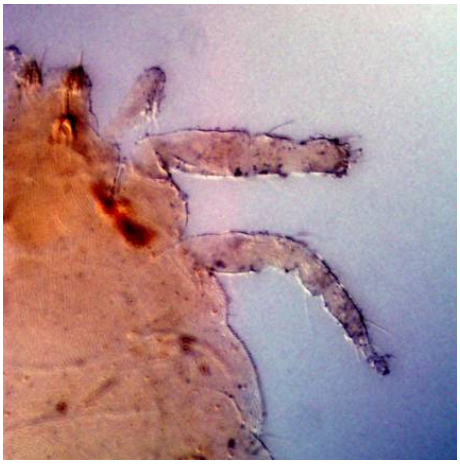
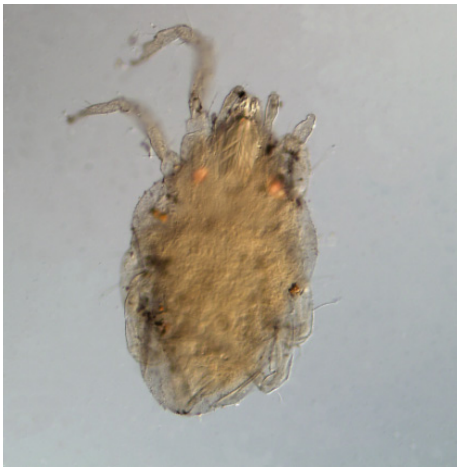


Photo 1B



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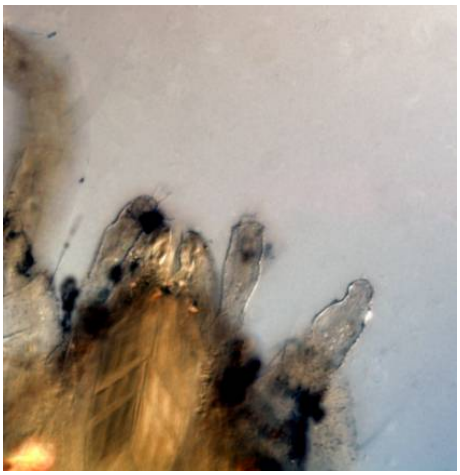

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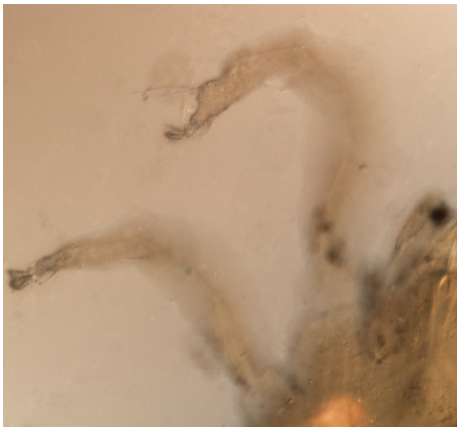
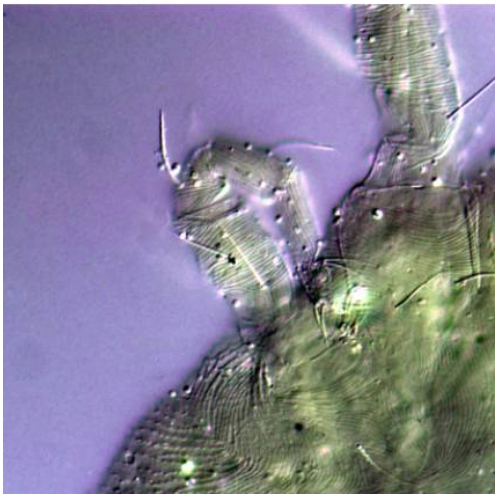


Photo 2C

Photo 3A

Photo 3B



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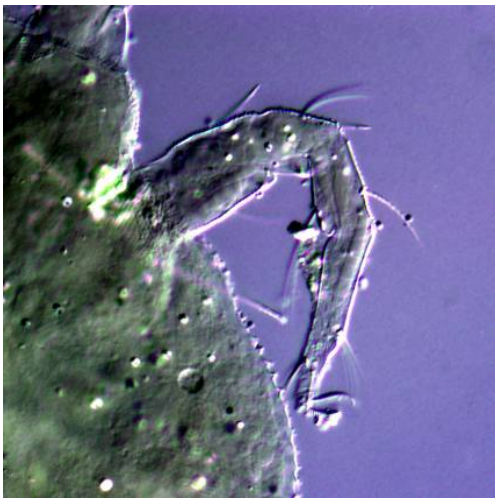

Photo 3D

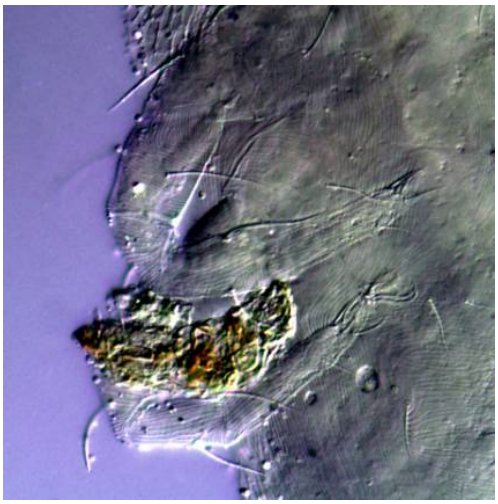

Photo 3E

Maybe the distal parts of legs could have been torn out and anomalous terminal segment shows the possibilities of reconstruction. This is the most probable explanation which concerns these anomalies.

Anomaly of apotel. In the species of the subfamily Tydeinae, each apotel is armed with two claws (ol', ol''), called also "lateral claws" in contrast to single, central empodial hook (om). Exceptionally, a single claw was described and figured by Oudemans (1925) as a characteristic for a newly created species and genus: *Lorryia superba*. This species is mysterious up to present (the holotype of *L. superba* is lost), although, accordingly with Oudemans figure, has the same dorsal reticulation and the same shape of dorsal setae as another and very common species: *Lorryia reticulata*. During the examination of the Spanish material the abnormal specimen (female) of *L. reticulata* has been found (Slide E-006/P-7, deposited in the Zoological Museum in Hamburg as A70/99). It has a single claw on both legs I and II (Figures. 6 A, B, C, D, photo 3). Thus, however tarsi III and IV are normal, the phrase: "(...) if the lack of a second claw in *L. superba* holotype is anomalous, then the possibility of treating *L. reticulata* as a junior synonym of *L. superba* would be worth consideration" (Kaźmierski 1998, p. 301) still remains actual.

Anomaly in body size. Tydeinae are more-less oval in shape. Their idiosoma measured 200-400 µm of length, however, most of them are 280-330 long. During our investigations on the material from Peru (Machu Picchu) two gigantic species of this subfamily have been found. Female of *Melissotydeus incarum* Kaźmierski, 1998 (Slide PE-025/P-1 kept in Hamburg) is 546 µm long and 396 µm broad. Its leg segments are also extremely long. These extreme dimensions can be treated as a specific feature: the remaining specimens (nymphs) of this species are also oversized (holotype tritonymph: 289/222, paratype tritonymph: 386/272, paratype deutonymph I: 269/194, paratype deutonymph II: 313/237). Second (undescribed) species, female (Slide PE-014/P-1), which belongs to the genus *Tydeus* is even slightly bigger (555 µm long and 400 µm broad). An analogous case concerns subfamily Tydaeolinae (Iolinidae, Tydeoidea). The species of this subfamily usually measure 140-150 µm and not longer than 200. One of the species found in Machu Picchu (not yet described – Figure 7) has typical chaetotaxy for the genus *Tydaeolus*, but different dorsal ornamentation (transversal striation), as well as different shape of bothridial setae (whip-like and pilose instead of club-like). What is especially worth mentioning are its measurements: 311 / 178 µm. A question begs to be asked: the coincidence of what factors influences the unnatural dimensions of the mites from Machu Picchu? Can it be that the spirit of Incas ordered them to be the most imposing?

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European Association of Acarolgists, 2008

FUNCTIONAL MORPHOLOGY OF MECHANORECEPTORS IN ASTIGMATIC MITES

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Abstract

Although the mechanoreceptor organs of astigmatic mites are used extensively as taxonomic characters, knowledge of their fine structure is minimal. Mechanoreceptors include setiform sensilla and cupules, and light microscopy, scanning electron microscopy and transmission electron microscopy were used to investigate their morphology. Setiform sensilla are movable and arise from a socket of thin procuticle. The setal base is anchored in the socket by radiating suspension fibers that connect the socket to a thin layer of more heavily sclerotized cuticle surrounding the setal base. Two dendrites terminating in tubular bodies are associated with the sensillum. Four pairs of cupules are found on the lateral margin of the opisthosoma. The cupule consists of a thin layer of cuticle (covering membrane) that overlies a "cup-like" depression within the remaining cuticle. A layer of densely-packed, thick fibers radiate dorsally through the "cup", attaching to its sides and centrally associated with a vacuole-like structure. A dendrite containing a single tubular body enters the cup through an opening in the basal cuticle and connects with the vacuole-like structure rather than with the covering membrane. It is postulated that distortions of the covering membrane are relayed through the fibers and thereby compress the vacuole-like structure which in turn deforms the tubular body and stimulates the dendrite. The cupules are thought to be cuticular receptors that register strains in the cuticle caused by gravity, vibrations, movements of the mite, and/or internal changes in pressure.

Key-words

Cupule, sensillum, seta, slit sense organ, tubular body

Introduction

Although the functional morphology of mechanoreceptors has been extensively investigated in insects and spiders (see McIver 1975, Barth & Blickhan 1984, Keil 1997, 1998, Barth 2002), they have received comparatively little attention in the Acari with the possible exception of ticks (order Ixodida). Since ticks are larger in size than most other mites as well as extremely important in disease transmission and direct damage to both humans and livestock, they are the most intensively studied of the Acari and therefore provide the most detailed knowledge of acarine sensory structures (Coons & Alberti 1999, de Lillo *et al.* 2004). In comparison, a relatively few studies have been conducted on species in the orders Mesostigmata, Prostigmata and Oribatida, and almost none on species in the order Astigmata. The present paper provides a description of the no pore setiform sensilla (np sensilla) of three species of astigmatic mites and the idiosomal cupules of one species. Since the np sensilla of arthropods are relatively well known and those of astigmatic mites show only minor differences, they are only briefly discussed. The idiosomal cupules, however, differ quite radically from the slit sense organs described for other arachnids, including those of other mites,

and are therefore taken up in more detail.

Methods and Materials

Specimens of *Hericia janehenleyi* (Family Algophagidae) were collected from sap flux on oak trees (*Quercus* spp.), and specimens of *Naiadacarus arboricola* (Family Acaridae) and *Algophagous pennsylvanicus* (Algophagidae) from water-filled treeholes, all in eastern Virginia, U.S.A. All three species were used for studying setiform sensilla, whereas only *H. janehenleyi* was used for studying cupules.

For observation using phase contrast and interference DIC microscopy, specimens were cleared in Nesbitt's solution and mounted in Hoyer's medium on microscope slides. Since mite cuticle will autoflouresce, slide mounted specimens were also used for confocal microscopy. Confocal imaging was performed on a Bio-Rad Radiance 2100MP equipped with a Nikon TE2000-E inverted microscope and an HeNe laser with an excitation wavelength of 543 *nm*.

For transmission electron microscopy (TEM), the integument of the idiosoma was first ruptured with a minutin nadle to facilitate fixation and specimens then placed in a fixative of 3.5% gluteraldehyde, 2.5% paraformaldehyde, and 2% acrolein in cacodylate buffer (pH 7.4) for 12 h at 4^{o}C. After several brief cacodylate buffer rinses, they were post-fixed for 1.5 h at 4^{o}C and an additional 1.5 h at room temperature in 1% OsO_4 in cacodylate buffer. Specimens were then briefly rinsed in 50% acetone and soaked overnight in a 2% uranyl acetate 70% acetone solution at 4^{o}C. Dehydration was completed in acetone, and Spurr's medium used for infiltration and embedding. Thin sections were stained in lead citrate, and TEM was performed on a Zeiss EM 109.

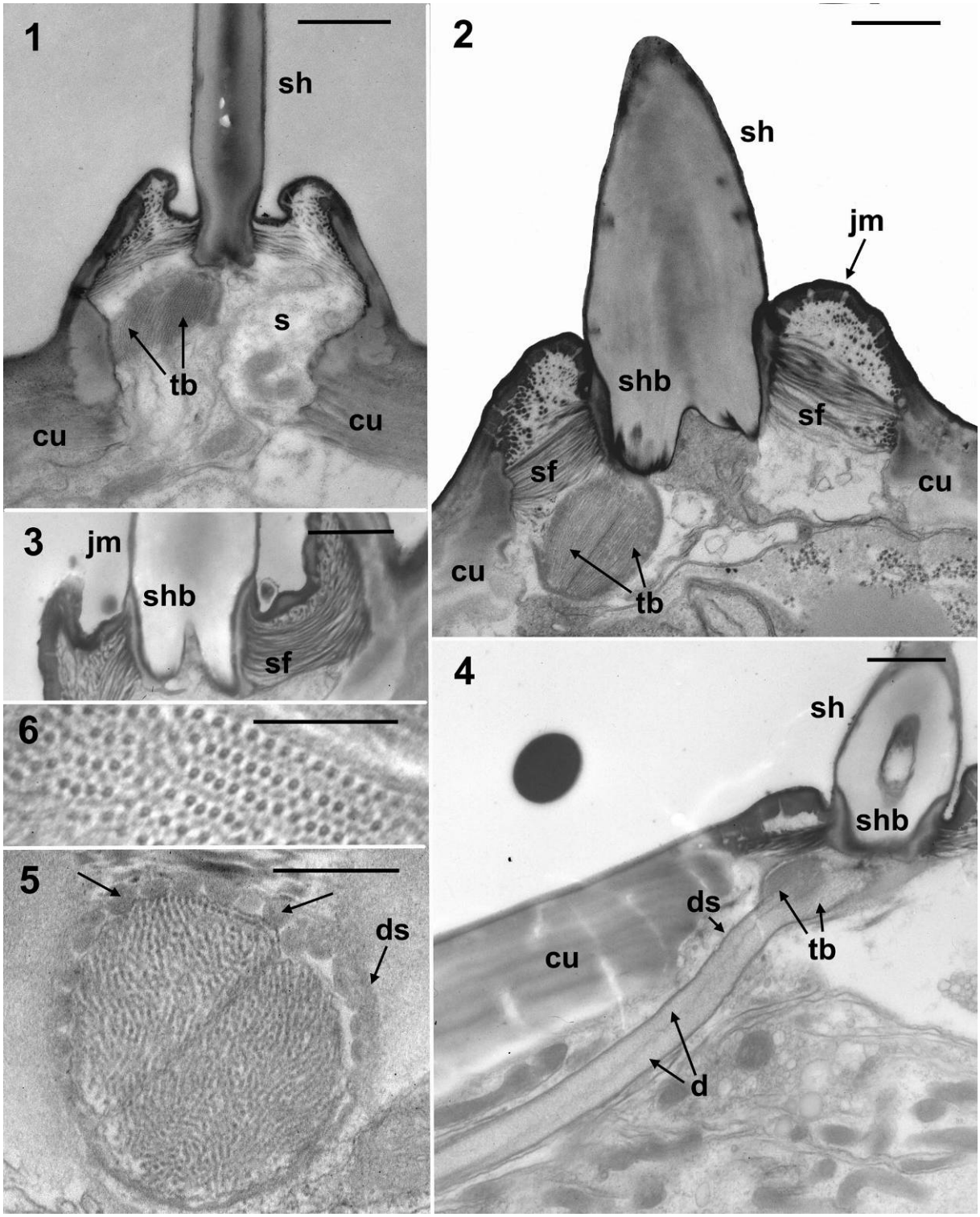
For scanning electron microscope (SEM), living mites were first placed in a bath of distilled water and sonicated at a low frequency. This was repeated several times in an attempt to cleanse them of debris. They were then briefly submerged in distilled water near boiling point in order to force protraction of appendages, dehydrated in ethyl alcohol, dried using the critical point procedure, individually affixed to stubs using double-sided sticky tape, and coated with gold-palladium in a sputter coater. Microscopy was performed on an AMR 1810.

Results and Discussion

No Pore Setiform Sensilla

Detailed descriptions of arthropod setiform sensilla can be found in Altner (1977), McIver (1975) and Keil (1997, 1998), and specifically for mites in Alberti & Coons (1999), Coons & Alberti (1999) and de Lillo *et al*. (2004). From observations in this study, the np sensilla of astigmatic mites appear to be similar in most respects to those found in other arthropods including other mite suborders. They are made up of hair-like or bristle-like setae with a flexible or rigid shaft (fig 1) that is poreless and can vary in size and shape. The setae are distributed on the idiosoma and appendages in a predictable pattern (see Gandjean 1939; Griffiths *et al*. 1990), and are therefore important taxonomic characters. Each seta is located above procuticle that is much thinner than the surrounding cuticle, and is positioned in a flexible socket (= alveolus) (figs 1-4). The seta is anchored in the socket by a surrounding articulation membrane (= joint membrane) as well as by a layer of radiating suspension fibers that attach from the cuticle to a somewhat electron dense layer of procuticle that surrounds the base of the shaft (figs 2-4). Each seta is served by two dendrites and each dendrite terminates in an enlarged tubular body (figs 2, 4) that is tightly packed with microtubules (figs 5, 6). The tubular bodies are surrounded by a dendritic sheath of dense material that inwardly contains conspicuous semicircular bodies (fig 5) a condition observed in other Acari (Alberti & Coons 1999, Haupt & Coineau 1975, Mills 1973). In *Tetranychus urticae* (Tetranychidae) the tubular bodies connect to the articulating membrane (Mills 1973, Alberti & Crooker 1985), and in *Microcaeculus steineri* (Caeculidae) and *Phytoptus avellance* (Eriophyidae) they connect to the base of the seta (Haupt & Coineau 1975, Nuzzaci & Alberti 1996) In some thin sections in our study it appears that one of the tubular bodies connects to the base of the setal shaft and the other to the articulating membrane, however more work is needed to substantiate this. Distinct cell types such as tecagen, trichogen and tormogen could not be discriminated, nor were sheath cells or the cilliary region observed.

Like the np setiform sensilla of other arthropods, those of astigmatic mites are characterized by possessing tubular bodies and are therefore mechanoreceptors. In this regard, they can be stimulated by contact (touch) and movements caused by air and/or water.



Figures 1-6. EM micrographs of np setiform sensilla: 1) *A. pennsylvanicus*, longitudinal section of seta and socket; 2) *N. arboricola,* longitudinal section of setal base and socket; 3) *H. janehenleyi*, longitudinal section through base of socket; 4) *H. janehenleyi*, longitudinal section illustrating dendrites with enlarged tubular bodies; 5) *N. arboricola*, cross section of tubular bodies; 6) *H. janehenleyi* enlarged view of microtubules in tubular body. Abbrev.: cu, cuticle; d, dendrite; ds, dendritic sheath; jm, joint membrane; s, setal socket; sf, suspension fibers; sh, setal shaft; shb, shaft base. Unlabeled arrows in figure 5 point to semicircular bodies of dense material adjacent to dendritic sheath. Scale bar = 1 μm (Figs 1-4), 0.5 μm (Fig 5), 0.25 μm (Fig 6).

With the exception of *Phytoseiulus persimilis* (Phytoseiidae) in which a palpal seta was found to have two pairs of tubular bodies, the Acari have been found to have only two tubular bodies (mechanoreceptor cells) (Alberti & Coons 1999), and the astigmatic mites in our study are no exception. Spiders and scorpions, however, have three and seven tubular bodies respectively (Folex 1985). It is interesting that species in the ricinulid genus *Pseudocellus* were found to have only two tubular bodies (Talarico *et al*. 2006), thus lending support to the theory that the Ricinulei are the sister-group of the Acari (Shear 1999).

Cupules

Astigmatic mites are characterized by four pairs of small, somewhat circular idiosomal organs designated from front to rear as *ia*, *im*, *ip* and *ih* (Griffiths *et al.* 1990). Their cup-like appearance under light microscopy has led to their designation as cupules by many authors, however, in other taxa they are often referred to as slit sense organs (see Alberti & Coons 1999) and sometimes lyrifissures (e.g., Penman & Cone 1974). Although they may sometimes falsely appear to be openings in the cuticle, their lucent appearance under the light microscope is due to a reduction in the thickness of the cuticle; the epicuticle remains intact and covers the cup. Cupules can be classified as "non-setal sensilla" since there is no seta-like shaft extending above the cuticle surface (Alberti & Coons 1999), however they also fit under the "intracuticular receptor" classification of Hess & Vliman (1986). They are considered to be homologous to the lyrifissures (= slit sensilla) of spiders (Alberti 1998), mechanoreceptive organs that have been extensively studied and are therefore well understood both morphologically and functionally (Barth 2002, Barth & Blickhan 1984). Although they are prominent features on the integument, the fine structure of the various intracuticular sense organs has not received much study in the Acari. In this regard, they have been examined in some detail only on the tarsi of the tick *Amblyomma variegatum* (Hess & Vlimant 1984), and to a lesser extent on the chelicerae of a few gamasid mites (Nuzzaci *et al*. 1992, de Lillo *et al*. 1996, 2002), and the opisthosoma of the spider mite *T urticae* (Penman & Cone 1974; Alberti & Crooker 1985) and the oribatid mite *Scutovertex minutus* (Alberti 1998). The following description is based on an examination of the cupules of the algophagid mite *H. janehenleyi*. Cross sections of three individual mites resulted in longitudinal sections of cupules *ih* (3 cupules) and *ip* (4 cupules). Horizontal sections of one individual resulted in cross-sections of all cupules on both sides of the idiosoma (therefore 8 cupules). All cupules were found to have similar morphology.

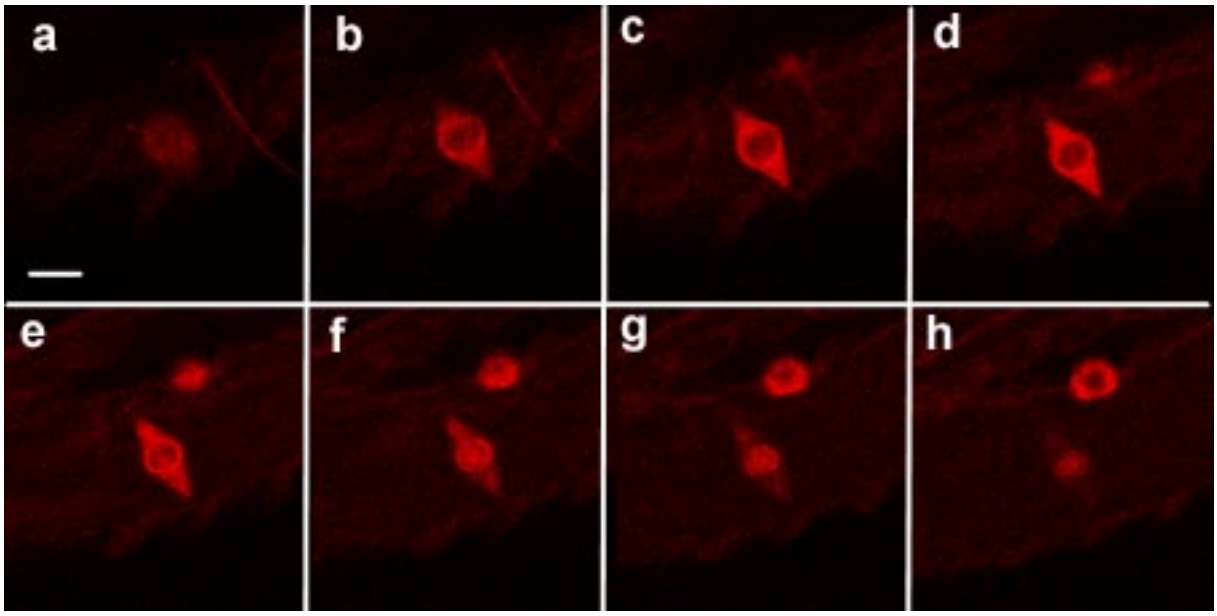
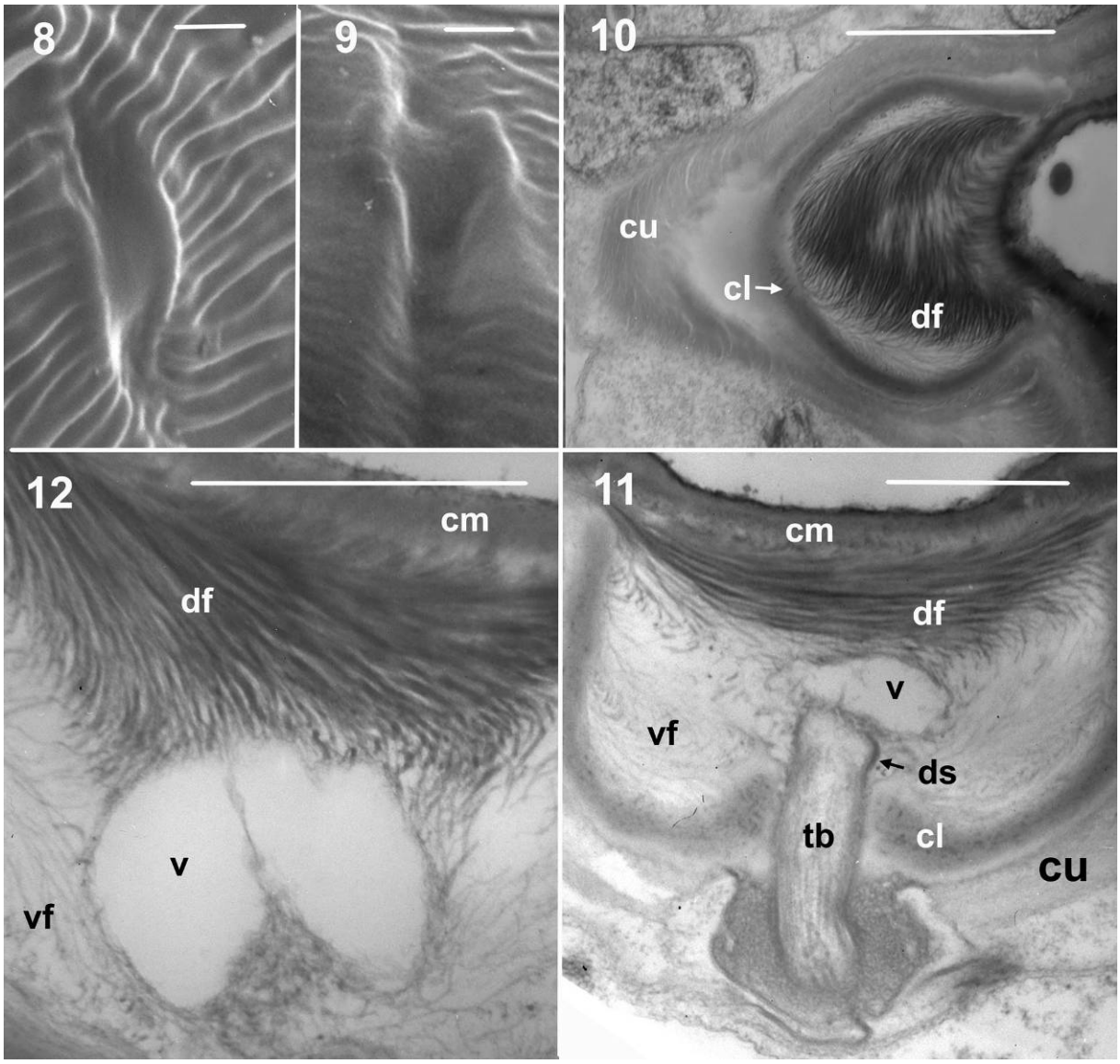


Figure 7. Confocal images of cupula *ip* sectioned from dorsum (a) through to ventrum (h). Note the round "cup" in the center and the extensions of electron dense material responsible for the "eye-shaped" appearance under light microscopy. Scale bar = 3 μm



Figures 8-12. EM micrographs of idiosomal cupules of *H. janehenleyi*: 8-9) SEM images of cupules *ia* and *ih* respectively; 10) TEM cross section of dorsal region of cupule *ia* just below the covering membrane; 11) TEM cross section through center of cupula *im*; 12) cross section through vacuole-like structure in center of cupule *im* presumable formed in part by fibers Abbrev.: cl, electron dense material lining cupula; cm, cover membrane; cu, cuticle; df, thick dorsal fibers; ds, dendritic sheath; tb, tubular body; vc, vacuole-like structure; vf, thin ventral fibers. Scale bar = 3 μm (Figs 8-9), 2 μm (Fig 10), 1 μm (Figs 11, 12).

The orientation of cupules is roughly at right angles with regard to the longitudinal axis of the idiosoma. Under phase contrast, interference DIC and confocal microscopy, a cupule appears as a circular central depression with longitudinal extensions of darkened cuticle in the upper layer that taper to a point, thereby giving it an "eye-like" appearance (fig 7). An examination with the SEM corroborates the eye-like appearance, and also reveals that the cupule is convex in its dorso-ventral axis and slightly concave in its lateral axis (figs 8, 9). In addition, the cupule is flanked by small elevations or ridges of the cuticle on its lateral margins (figs 8, 9). An examination with the TEM verifies the above and provides a detailed analysis of the internal ultrastructure (figs 10-15). The cuticle is separated to form a thin (~0.25 μm) electron-dense, dorsal external covering (= covering membrane) and a thicker, more lucent, internal layer that lines the walls and floor of the cup (figs 11, 13, 14). The circular dorsal portion of the cup measures approximately 3 μm in diameter, and the cup depth in the center is approximately 2.5 μm. Internally, the cup is lined with a somewhat electron dense substance that is presumably more heavily sclerotized cuticle (figs 10, 11, 13, 14). The lining extends past the cup approximately 1 μm as it tapers to a rounded point (fig 14), thereby giving the eye-like appearance seen with light microscopy. Dorsally, just under the

cuticular cover, thick, electron-dense, fiber-like strands transverse the cup at its cross-sectional axis (figs 10-14). In the center of the cupule, the fibers appear to form a vacuole-like, lucent structure or structures (fig 12). It could not be determined whether only one branching vacuole-like structure is formed or multiple vacuoles-like structures. A matrix of thinner and less densely packed fiber-like strands, presumably in a fluid, fill the remainder of the cupule. Only one dendrite serves a cupule and its outer segment containing the tubular body with its associated microtubules enters through the cupule center (figs 11, 13, 15).

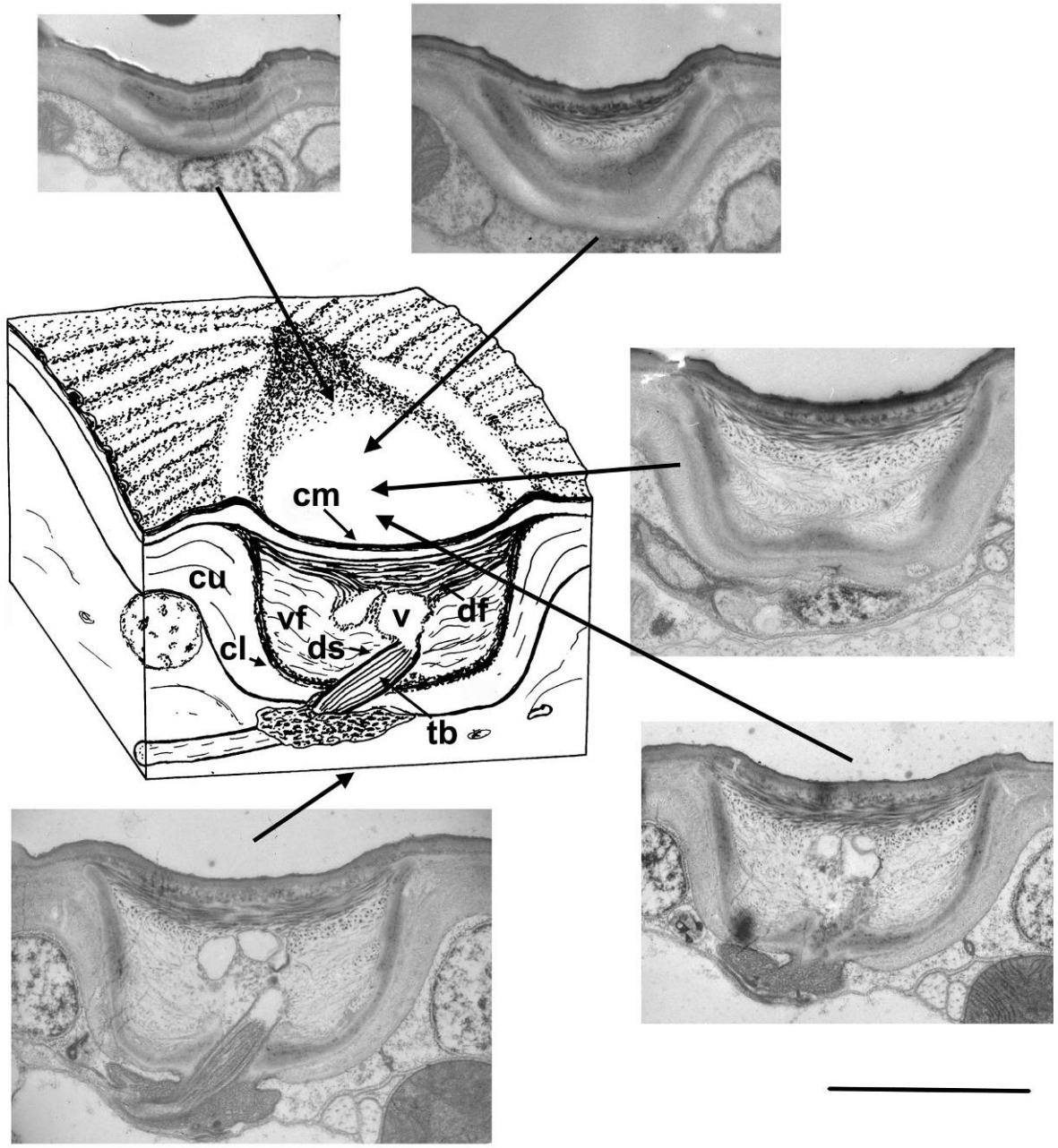


Figure 13. Diagram of *H. janehenleyi* cupula reconstructed from TEM cross sections, and TEM micrographs of cross sections. Arrows point to the approximate locations of TEM cross sections. See caption for Figs 8-12 for abbreviations. Scale bar = *2* μm.

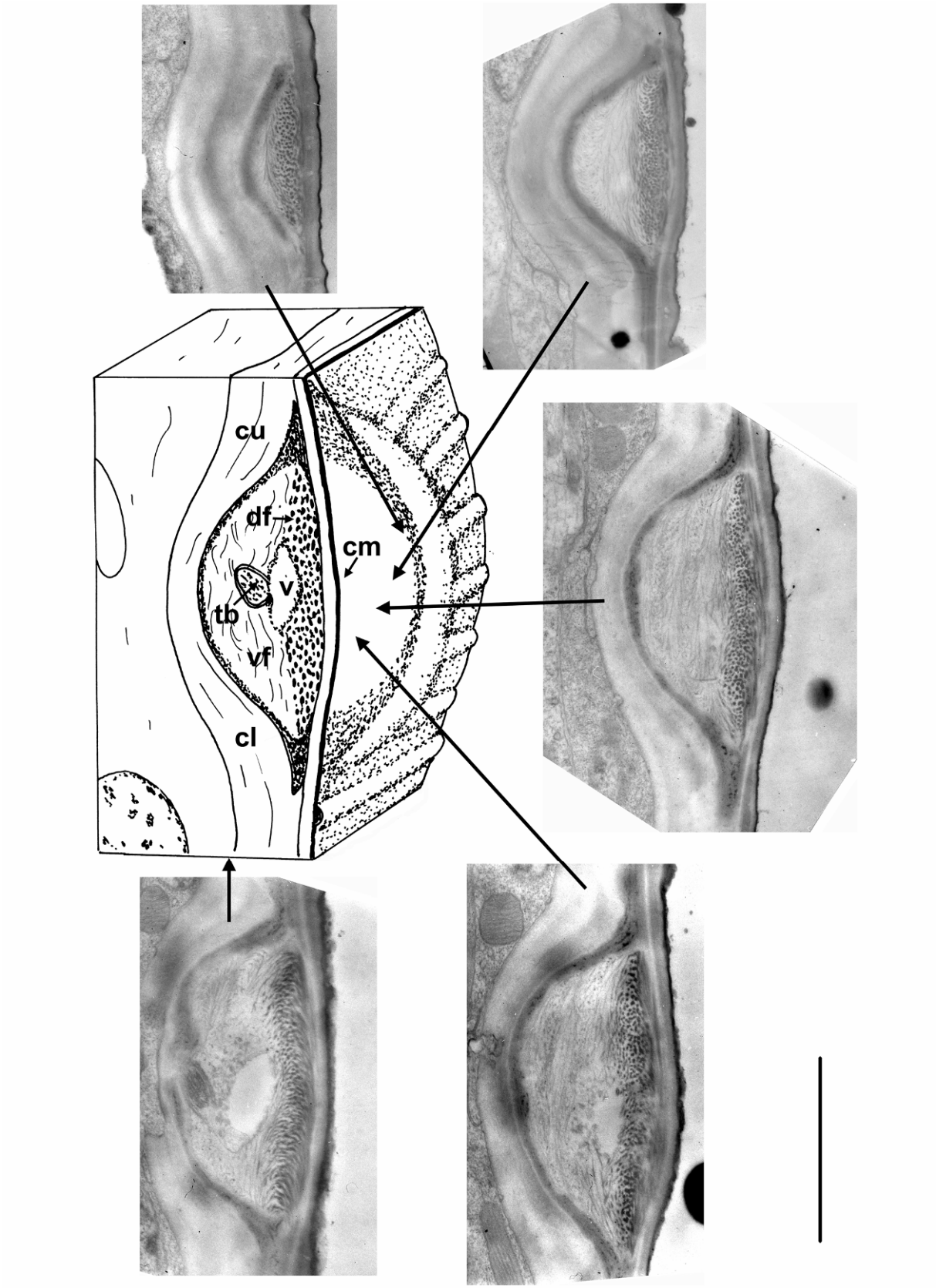


Figure 14. Diagram of *H. janehenleyi* cupula reconstructed from TEM longitudinal sections, and TEM micrographs of longitudinal sections. Arrows point to the approximate locations of TEM longitudinal sections. See caption for Figs 8-12 for abbreviations. Scale bar = *2* μm.

Just below the cupule at the point of entry, the dendrite passes through an electron dense material that possibly functions to seal the entrance hole (figs 11, 13, 15a). The dendrite does not extend to the cuticular cover, but ends approximately mid-way in the cup and in intimate contact with the "vacuole-like" structure (figs 11, 13, 15b-d). In a number of microtome sections the dendrite terminus appears to expand and actually form part of the vacuole wall (fig 15).

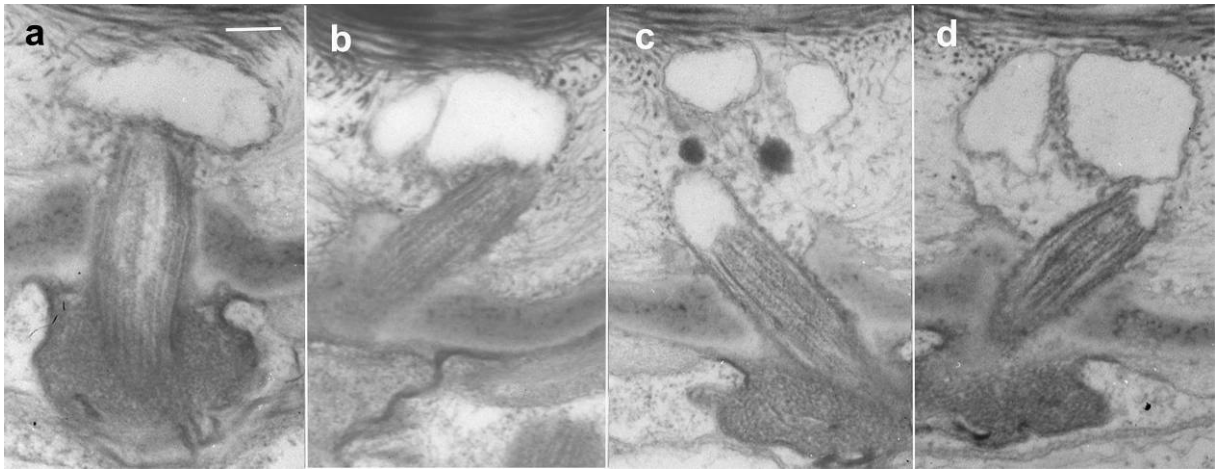


Figure 15. TEM micrographs of cross sections of *H. janehenleyi* cupula illustrating the association of the tubular body with the vacuole-like structure. a = cupule *im*, b = cupule *ip*, c, d = cupule *ih*. Scale bar = 0.5 μm.

Possessing a dendrite with a tubular body, there is no doubt that the cupule functions as a mechanoreceptor that responds to strains in the exoskeleton. It is possible that the longitudinal extensions of more heavily sclerotized cuticle aids in interpreting the directionality of the cuticular deformation. When the cuticle surrounding the cupule is mechanically stressed, the covering membrane is deformed which in turn somehow deforms the tubular body of the dendrite. Since the tubular body does not contact the covering membrane, it is postulated that the deformation of the covering membrane is relayed through the underlying thick fibers, thereby causing the vacuole-like structure to compress which in turn deforms the tubular body and thereby sets off the nervous response. Possible causes of cuticular stress are internal pressures or movements of the mite (proprioception), and/or external forces such as gravity and vibrations caused by substrate or air movement.

The fine structure of idiosomal cupules has been investigated in only two other species, the spider mite *T. urticae* and the oribatid mite *S. minutus*, and studies on both provide only a fragmentary description. The cross sectional images of *T. urticae* cupules published by Penman & Cone (1973) are similar in appearance to those of *H. janehenleyi* in that they have an outer cuticular cover and a cavity filled with loosely packed fibrous material. They did not, however, find a connecting dendrite. Alberti & Crooker (1985) later reported that cupules of *T. urticae* were innervated, but provided no details. In a report on the sensory structures of oribatid mites, Alberti (1998) provided a cursory description of cupules *ia* and *im* of *S. minutus*. He found the cupule to be a narrow portion of modified and flexible procuticle with a small attached cuticular process to which the tubular body of a dendrite purportedly connects. He did not determine the number of receptor cells.

The cupules of *H. janehenleyi* are unlike the cuticular stress detectors described for other arthropods. The tubular body does not connect to the cover membrane as found in the campaniform sensilla of insects and the slit sense organs of other arachnids. Whether this is true for all astigmatic mites and perhaps all actinotrichid mites awaits further investigation.

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European Association of Acarolgists, 2008

THE MORPHOLOGY AND DEVELOPMENT OF A BRAZILIAN CROTONIIDAE (ACARI, ORIBATIDA)

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Abstract

The morphology of immature and adult stages of a Brazilian oribatid mite *Crotonia* is presented. The characters are compared with morphologically similar species *C. capistrata* Luxton, 1987. The Brazilian species possesses only two pairs of setae *c* and well developed h_2 apophyses connected with notogaster by f_1 apophyses. Moreover, fine setae c_1 and d_2, longer prodorsal and lateral notogastral setae and dagger-form setae h_2 identify clearly this species as new for science. *Crotonia* sp. n. was found in wet litter and roots, moss on soil, rocks and branches from the tropical mountain forest and *Araucaria* forest in Brazil Neotropical Serra do Mantiguera, 1300-2100 m a.s.l.

Key words

Crotonia, morphology, juvenile stages, Brazil, Neotropical region.

Introduction

The present paper is part of a study on the ontogeny of crotoniid species. Almost 50 species of the genera *Crotonia* and *Holonothrus* have been described so far. However, immature stages had been described for only 13 of them, often only a single stage for each species. Olszanowski (1997, 2000) started studies on immature stages with precise descriptions and drawings. Lately, all instars of crotoniid mites were described and illustrated (Kuty 2005, Łochyńska 2007, Łochyńska 2008). The analysis of morphology and development of all stages will be useful in phylogenetic analyses, which are planned in the future.

The *Crotonia* genus is represented in the Neotropical region by 5 species: *C. pulcher* (Beck, 1962), *C. flagellata* (Balogh et Csiszár, 1963), *C. chiloensis* Wallwork, 1978, *C. marlenae* Olszanowski, 1997 and *C. blaszaki* Szywilewska, Olszanowski et Norton, 2005. Most other species occur in the Australian and the Ethiopian region. It may suggest that crotoniid mites might have evolved in Gondwanaland (Hammer et al 1979, Domes et al. 2007).

Material and Methods

The description is based on material borrowed from Hungarian Natural History Museum, Budapest, Hungary, collected by Prof. J. Balogh in 1990. All specimens of the new species come from 4 samples from Brazil, and 41 specimens were found: 5 deutonymphs, 17 tritonymphs and 19 adults. This species will be described and named as *C. camillae* in a referenced journal.

The mites were preserved in 70% ethanol and cleared in lactic acid. The layer of debris covering specimens were removed with a small hook. During cleaning process legs of mites were damaged. Body measurements were measured in

dorsal view from the tip of the rostrum to the end of notogastral plate, excluding posterior apophyses.

Five adults, 3 tritonymphs and 1 deutonymph were used in SEM. The specimens were examined with a scanning electron microscope in Electron and Confocal Microscope Laboratory, A. Mickiewicz University, Poznań, Poland. They were mounted on stubs with double-sided stickytabs, coated with gold in a Balzers SPC 050 ion coater, and observed in a Philips 515 scanning electron microscope.

The holotype and 11 paratypes (1 deutonymph, 5 tritonymphs, 5 adults) are stored in the collection of Hungarian Natural History Museum (Budapest, Hungary) and 6 paratypes (3 tritonymphs, 3 adults) – in Natural History Museum of Denmark (Zoological Museum, University of Copenhagen). Reference material is stored in the collection of Department of Animal Taxonomy and Ecology (Faculty Biology, A. Mickiewicz University, Poznań, Poland).

The morphological terminology used in the descriptions follows that developed by F. Grandjean (Travé and Vachon 1975) for references).

Results

Diagnosis

Crotonia sp. n. is characteristic by possessing two pairs of setae *c*, fine and equal in length setae c_1 and d_2, which are set in alveolae, long setae *le* and *in*, dagger-form setae h_2, large and well developed lamellar apophyses, which are as long as the distance from h_1 to the tips of h_2 apophyses. Moreover, posterior apophyses are connected with notogaster by f_1 apophyses.

Locality data

Br.90/B.90 – Brazil Neotropical Serra do Maniguéra; env. of Parati; 1300m; tropical mountain forest; very wet rich litter; L: J. Balogh. (2 juv., 5 ad.)

Br.90/B.91 – detailed data in Hungarian Natural History Museum, (2 juv., 1 ad.)

Br.90/B.126 – Brazil Neotropical Serra do Maniguéra; 2000 m; Berlese samples vegetation type *Araucaria* forest, luxuriant mosses on soil, rocks, branches and trunks; L: J. Balogh. (2 ad.)

Br.90/B.127 – Brazil Neotropical Serra do Mantiguera, near Itateiea; 18.12.1990; 2100 m; *Araucaria* forest in mossy forest Zone; very thick litter and roots; L: J. Balogh. (18 juv., 11 ad.; holotype)

Description

Deutonymph (Figs 1-2, 7-11)

Body length: 670 µm; body width: 330 µm; colour: white-light brown. Whole body covered with a thick layer of debris. Rostrum rounded. Surface of prodorsal plate covered with folds and rare nodes. Rostral setae (*ro*) as long as the distance between their bases. Lamellar setae (*le*) barbed, covered with sheath, bent, one and half times longer than the distance between tips of their apophyses, set on well developed apophyses connected with a ridge of chitin. Interlamellar setae (*in*) smooth, longer than twice the distance between their apophyses, set on small apophyses, which are on longitudinal ridges of chitin. Sensilli well developed, completely contained within bothridia. Setae *ex* lacking. Notogastral plate broadest at the level of setae e_2. Surface of the notogaster covered with small folds and nodes. With 13 pairs of smooth notogastral setae (setae c_2, d_1, e_2 absent). Setae c_1 and d_2 fine, as long as *ro*, set on small tubercles. Setae h_2 ensiform, with extended and lanceolate tips, almost as long as whole length of their large apophyses. Setae p_1 and p_2 two and half times longer than *ro*, set on small apophyses; setae p_3 similar to c_1. Other setae long (the shortest setae c_3 as long as *in*, the longest e_2 slightly longer than *in*). Apophyses of setae c_3 and d_3 located very close to each other; posterior apophyses connected with notogaster by f_1 apophyses; h_1 and h_3 apophyses located on the same level, just under f_1 apophyses. Oval openings of opisthosomal gland (*gla*) situated close to bases of f_2 apophyses. Pairs of epimeres separated; epimeral region punctate, with setation: 2-1-2-2. Genital plates with 4 pairs of setae; 1 pair of aggenital setae. Anal setae lacking; 3 pairs of adanal setae. All legs monodactylous (setation not studied).

Tritonymph (Figs 3-4, 12-14)

Body length: 970 µm; body width: 530 µm; colour: light brown-brown. Whole body covered with a thick layer of debris. Rostrum rounded. Surface of prodorsal plate covered with folds and rare nodes. Setae *ro* almost as long as the distance between their bases. Setae *le* and *in* similar to deutonymphal. Notogastral plate broadest at the level of setae e_2. Surface of the notogaster covered with small folds and nodes. With 13 pairs of smooth notogastral setae. Setae c_1 and d_2 as long as *ro* (c_1 set on small tubercles, d_2 – in alveolae). Other setae and apophyses location similar to deutonymphal. Posterior apophyses connected with notogaster on f_1 apophyses level; h_1 and h_3

apophyses located on the same level, just under f_1 apophyses. Oval openings of opisthosomal gland (*gla*) situated close to bases of f_2 apophyses. Pairs of epimeres separated; epimeral region punctate, with setation: 3-1-2-3. Genital plates with 6 pairs of setae; 2 pairs of aggenital setae. Three pairs of anal setae; 3 pairs of adanal setae (setae ad_1 slightly longer and ad_2 as long as p_3, setae ad_3 fine). All legs monodactylous (setation not studied).

Adult (Figs 5-6, 15-23)

Female – body length: 1180 µm; body width: 690 µm; holotype, male – body length: 880 µm; body width: 540 µm; colour: light brown-brown. Whole body covered with a thick layer of debris. Rostrum rounded. Setae *ro* as long as the distance between their bases. Setae *le* barbed, covered with sheath, bent, one and half times longer than the distance between tips of their apophyses, set on well developed apophyses connected with a ridge of chitin. Setae *in* smooth, twice as long as the distance between their apophyses, set on small apophyses, which are on longitudinal ridges of chitin. Sensilli well developed, completely contained within bothridia. Setae *ex* lacking. Notogastral plate broadest at the level of setae e_2. Surface of the notogaster covered with nodes; one pair of longitudinal ridge of chitin runs from c_3 apophyses in *gla* direction. With 13 pairs of smooth notogastral setae. Setae c_1 and d_2 fine, as long as *ro*, set in alveolae. Dagger-form setae h_2 twice as long as c_1, set on the longest and ensiform apophyses. Setae p_1 one and half as long as *ro*, setae p_2 as long and p_3 slightly shorter than *ro*. Other setae longer (the shortest setae d_3 one and half as long as h_2, the longest c_3 twice as long as d_3). Apophyses of setae c_3 and d_3 located close to each other; posterior apophyses connected with notogaster by f_1 apophyses; h_3 apophyses located in the middle of f_1 and h_1 distance. Oval openings of opisthosomal gland (*gla*) situated close to bases of f_2 apophyses. Pairs of epimeres separated; epimeral region punctate, with setation: 3-1-3-3. Genital plates with 9 pairs of setae; 2 pairs of aggenital setae. Anal plates with 3 pairs of setae; 3 pairs of adanal setae. All legs tridactylous (setation not studied).

Etymology

The new *Crotonia* wil be named after my precious friend – Kamila Tkaczyk.

Remarks

This species is the most similar to the Australian species *C. capistrata* Luxton, 1987. Both species possess narrow, long and well developed h_2 apophyses, which bear small f_1, h_1 and h_3 apophyses, relatively short lateral setae on notogaster, the longest setae c_3 set on apophyses and fine setae c_1 and d_2 in alveolae. What is more, both have similar body dimensions (body lengths of *C. capistrata* females: 1120-1200 µm). However, unlike *C. capistrata*, new species possesses only two pairs of setae *c*, longer prodorsal and lateral notogastral setae. Moreover, posterior apophyses are connected with notogaster by f_1 apophyses, without any stump, and epimeral setation is different (*C.* sp. n.: 3-1-3-3; *C. capistrata*: 3-1-4-2).

In one sample (Br.90/B.90) there was found other adult crotoniid mite – *Crotonia marlenae* Olszanowski, 1997. Nevertheless, new species is really easy distinguished by much shorter antero-lateral notogastral setae, bigger *f* and h_1 apophyses (h_2 after Olszanowski – probably he badly named the posterior apophyses), which bases are connected together and shorter setae *in*. On the holotype drawings Olszanowski matched posterior part of notogaster of tritonymphal exuvium as well. Thanks to this, author could easily distinguish juvenile stages. Immatures of the new species possess much shorter and thicker notogastral apophyses and their setae h_2 are ensiform, with lanceolate tips, almost as long as whole length of their large apophyses.

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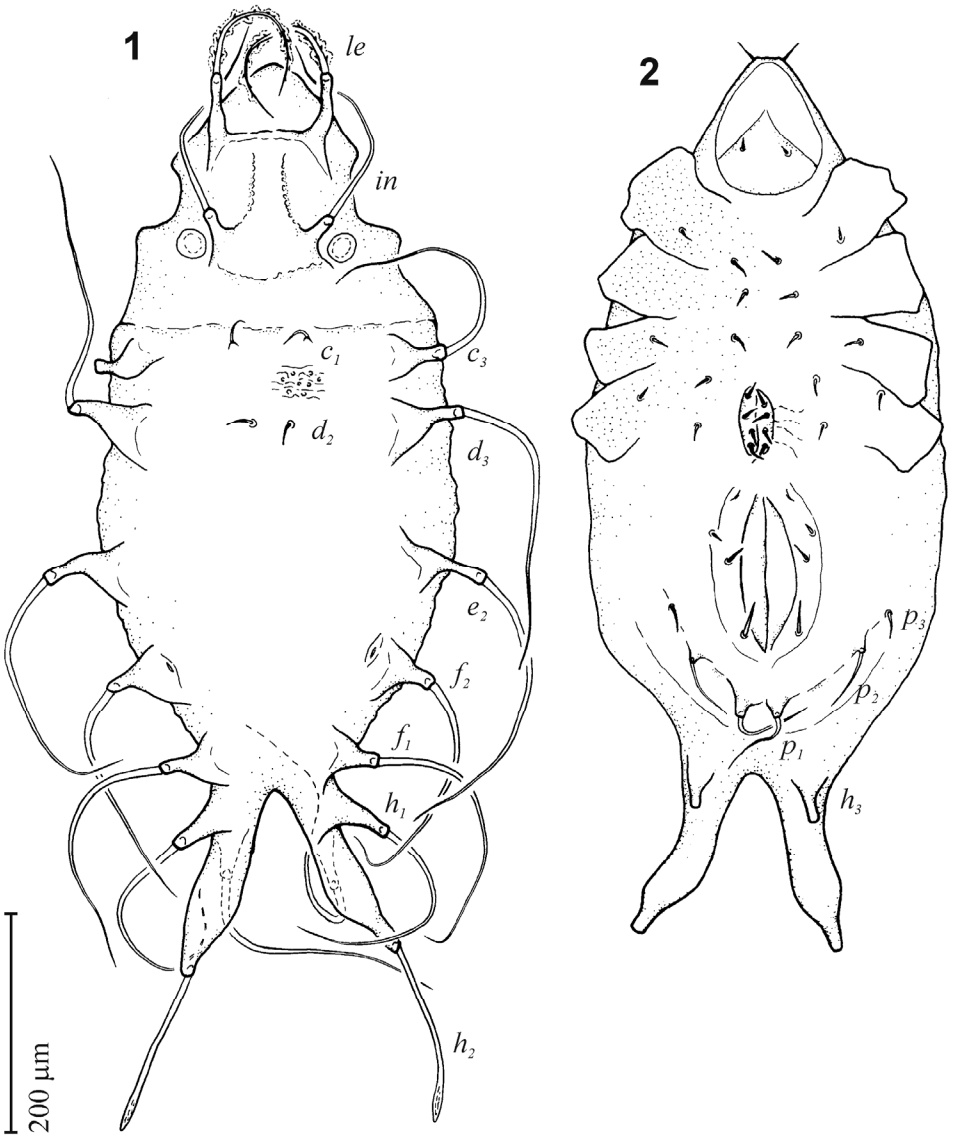
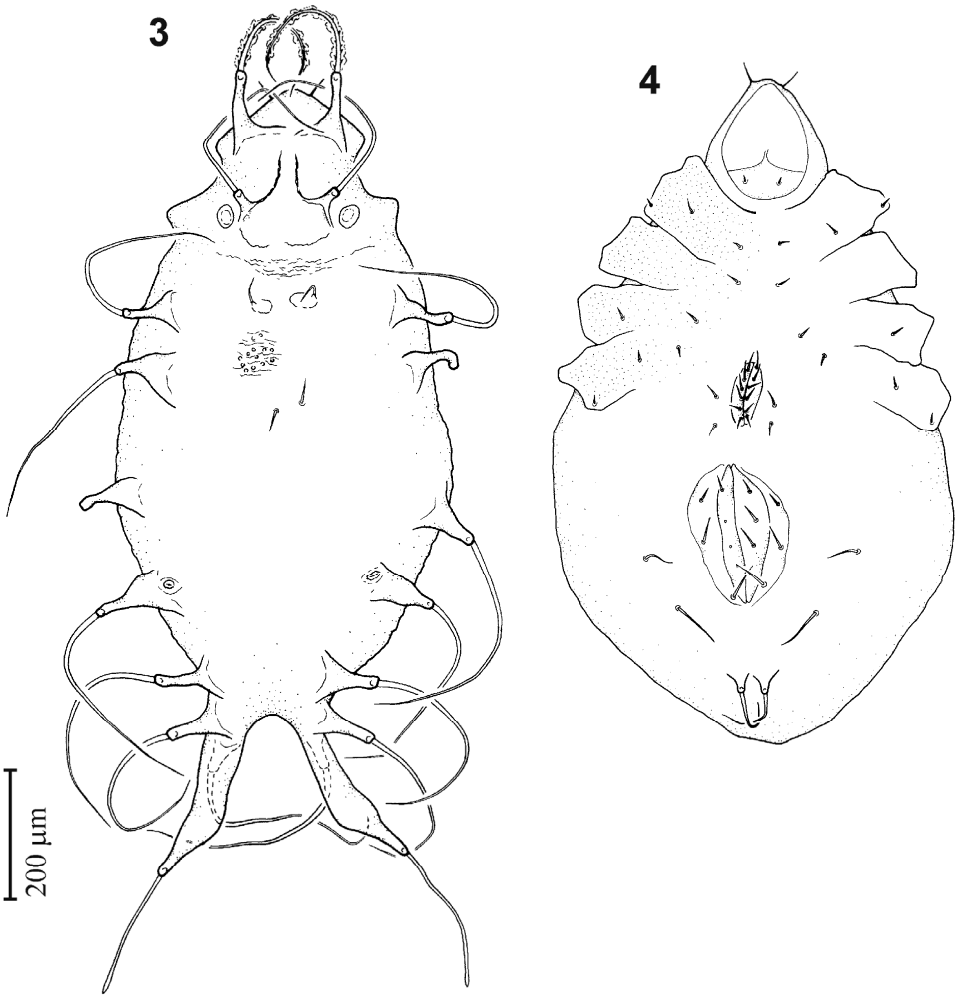


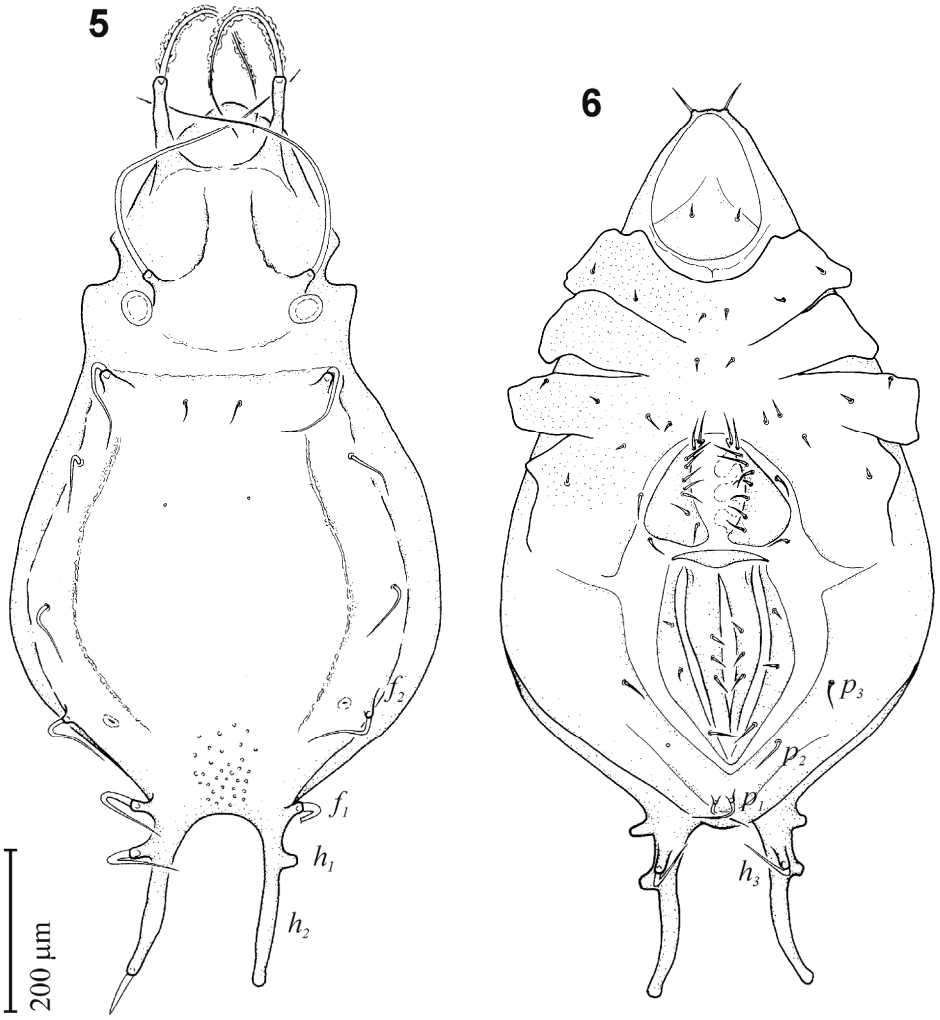
Figures 1-2

Deutonymph. Dorsal view.
Deutonymph. Ventral view.



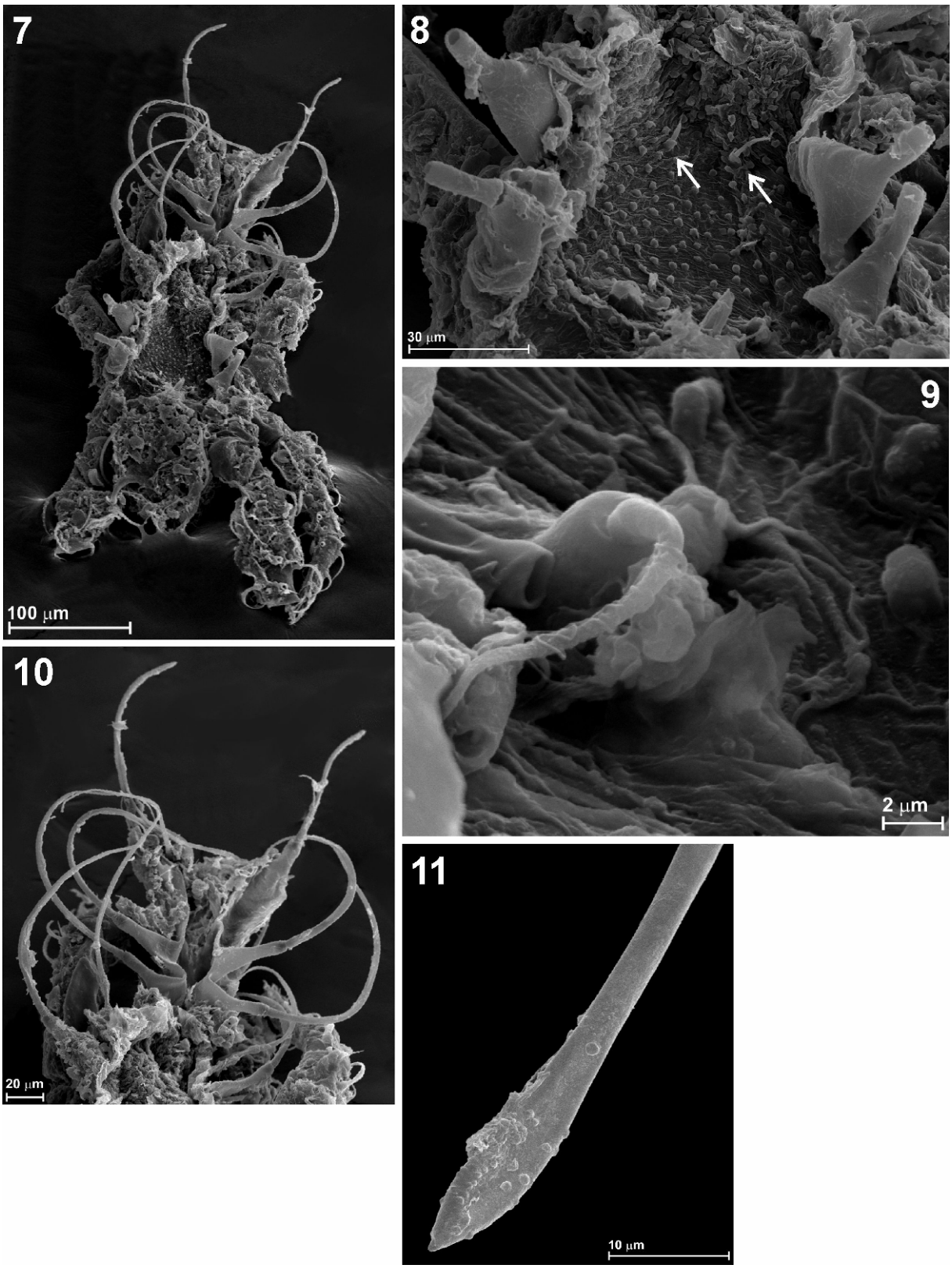
Figures 3-4.

Tritonymph. Dorsal view.
Tritonymph. Ventral view.



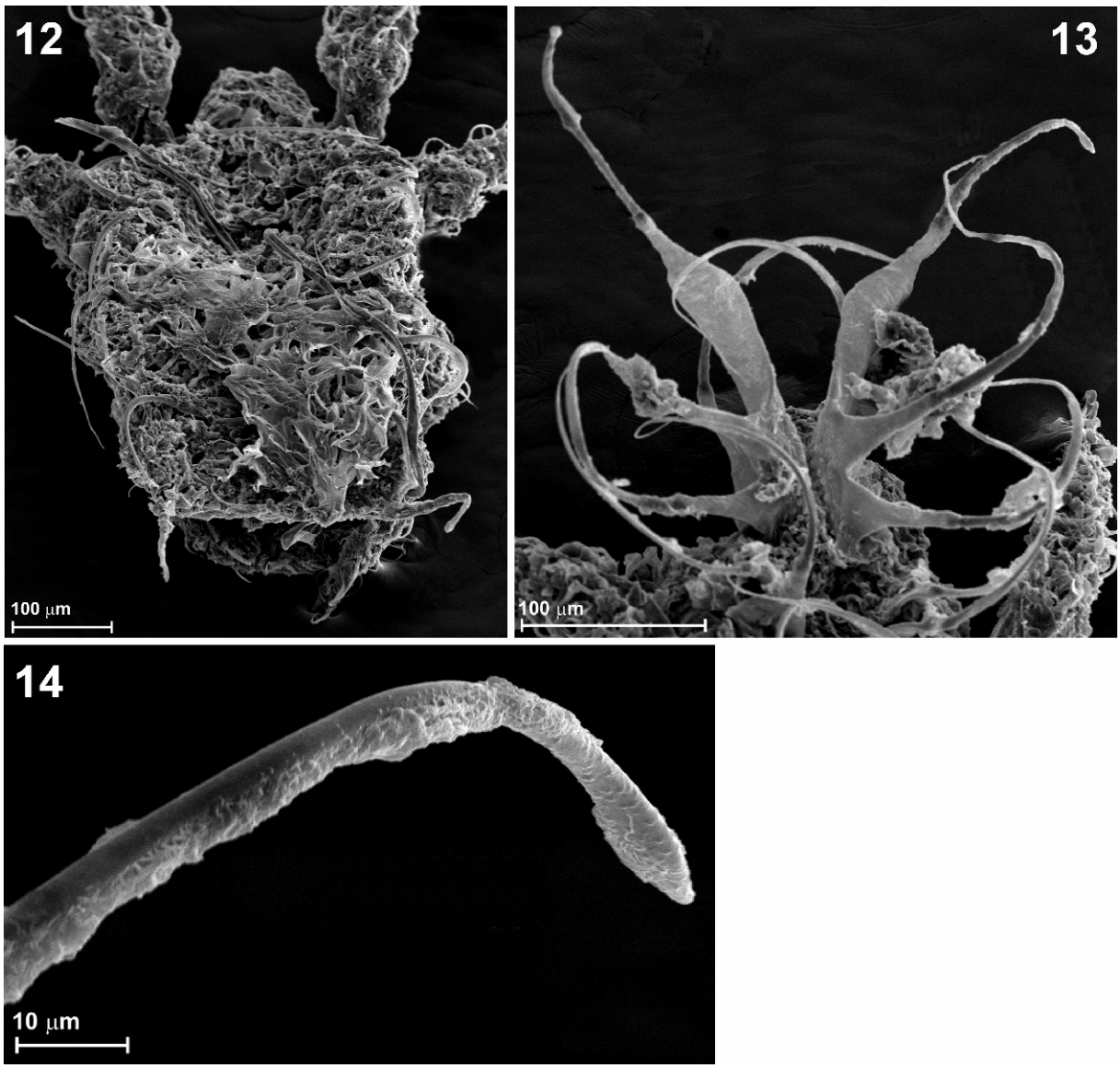
Figures 5-6.

Adult. Dorsal view.
Adult. Ventral view.



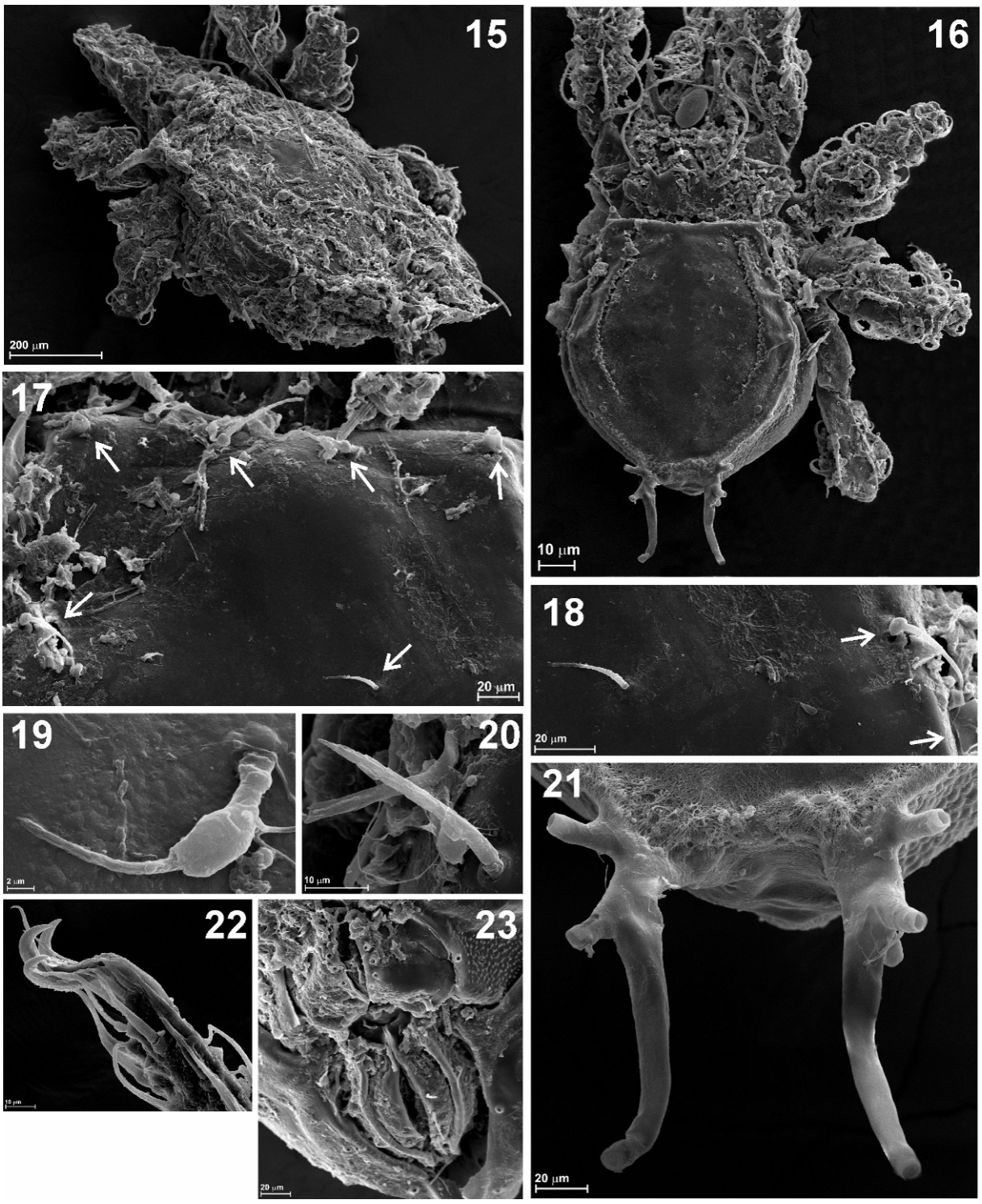
Figures 7-11.

Deutonymph, specimen after cleaning. General view.
Deutonymph. Apophyses of setae c_3 and d_3 (arrows show setae d_2).
Deutonymph. Seta c_1.
Deutonymph. Posterior part of notogaster.
Deutonymph. End of seta h_2.



Figures 12-14.

Tritonymph, specimen before cleaning. General view.
Tritonymph. Posterior part of notogaster.
Tritonymph. End of seta h_2.



Figures 15-22.

Adult, specimen before cleaning. General view.
Adult, specimen after cleaning. General view.
Adult. Setae *c* and *d*.
Adult. Setae *d* (arrows match the base and end of d_3).
Adult. Seta d_2.
Adult. Seta e_2 in tritonymphal exuvium.
Adult. Posterior apophyses.
Adult. Tarsus IV with 3 claws.
Adult. Ano-genital region.

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INFLUENCE OF TEMPERATURE ON LIFE HISTORY PARAMETERS OF THE HOUSE DUST MITE, *DERMATOPHAGOIDES FARINAE* HUGHES (ACARI: PYROGLYPHIDAE)

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Abstract

The life history parameters of the house dust mite, *Dermatophagoides farinae*, were investigated at four constant temperatures under a 14L: 10D photoperiod and 80 ± 5 % RH. Results indicated that the duration period of total life cycle (from egg deposition to adult emergence) averaged 119.2 ± 5.5, 36.9 ± 3.6, 21.1 ± 1.4 and 15.1 ± 1.7 days for female and 129.1 ± 7.1, 35.8 ± 2.9, 19.7 ± 1.8 and 14.6 ± 1.5 days for male at 15, 20, 25 and 30 ºC, respectively. Also, the average incubation periods decreased with increasing temperature for both females and males. Duration of immature stages decreased as the temperature increased from 15 up to 30 ºC for both sexes. Under higher temperatures, daily egg productions were higher than lower temperatures even if the total egg/ female was lower. Preoviposition, oviposition and the total adult survival periods generally decreased with increasing temperature.

Key- words

Mite, Pyroglyphidae, *Dermatophagoides farinae*, biology.

Introduction

House dust is made of organic and inorganic components that may primarily include fungal spores and mycelia, human and/or animal dander, food particles, textile fibers, particles of mites and other arthropods, pollen, algae, bacteria and soil particles (Robinson 1996). House dust mites live in our dwellings and feed on organic constituents and microbes that grow on cast skin flakes, hair, and other detritus. Pyroglyphid house dust mites are the most important and worldwide source of house dust allergens in homes. Exposure to house dust containing mite allergens can cause sneezing, nasal stuffiness, runny nose and asthma (Larson *et al.* 1969; Wharton 1976; Colloff *et al*. 1992; Platts-Mills *et al.* 1992 Solarz 2001a, b). Among several species of the family Pyroglyphidae, *Dermatophagoides pteronyssinus* Trouessart, 1897 and *D. farinae* Hughes, have been found to be the predominant mites of household dust accounting for about 80-90 % of the total mite populations and are important sources of allergens worldwide inside homes in humid geographic areas (Hallas 1991; Arlian *et al*. 1992). The duration of all immature stages and population growth is influenced by both ambient relative humidity and temperature (Matsumoto *et al.* 1986; Arlian *et al.* 1990; Arlian & Dippold 1996; Hart 1998). The life cycle includes five stages, egg, larva, protonymph, tritonymph, and adult (Arlian *et al.* 1998). House dust mite, *D. farinae*, is known as a cosmopolitan species with a world-wide distribution and commonly inhabits house dust and stored products. In Egypt, this mite has been found in homes in various areas where it may cause allergic symptoms especially for asthmatic patients (Rezk 2004). The mite population decreases in winter

and hence, it has been suggested that *D. farinae* favors relatively high temperatures (Rezk *et al.* 1996). However, no quantitative data are available in Egypt on the life history parameters at various temperatures. The aim of this work is to study the effects of temperature on the biological parameters of *D. farinae* under constant photoperiod and relative humidity.

Materials and methods

Rearing*:* house dust mite, *D. farinae* was isolated from mattress dust and grown in clean dried jar on a finely-ground mixture of dust, dried yeast and dried milk (1: 1: 0.5). The stock jars were kept in an incubator at 25 ± 2 ^{0}C and 80 ± 5 % RH. After five months (during a 22- wk period), large numbers of different stages were available for experimentation (Saint Georges 1987; Andersen 1988, 1991; Rezk & Gadelhak 2003).

Developmental time of immature stages: developmental time of immature stages was observed periodically at 15, 20, 25 and 30 ºC under a 14L: 10D photoperiod and 80 ± 5% RH. Females from the stock culture were introduced into vials (n ≥ 20) and allowed to lay eggs during a 24 hours period at each temperature. Deposited eggs were removed and separately cultured to reach adulthood and the developmental time/ day for each immature stage was recorded every 12 hrs at higher temperatures (25 and 30 ºC) or 24 hrs at lower temperatures (15 and 20 ºC).

Oviposition, adult longevity and fecundity: preoviposition, oviposition, postoviposition periods (= Total adult longevity) and daily egg production for individual females (n ≥ 20) were studied at 15, 20, 25 and 30 ºC under a 14L:10D photoperiod and 80 ± 5% RH. One tritonymphal female and two adult males were introduced into a vial for copulation. The number of eggs laid by a female (fecundity) and its longevity were recorded daily. Mites were reared individually and vials checked every day at the same time (± 4 hrs). Eggs laid were removed every day after count.

Hatchability and sex ratio: another experiment was designed to study the hatchability and sex ratio. One tritonymphal female and one adult male were introduced into a vial (n ≥ 20) for copulation, and females were allowed to lay eggs for five days after preoviposition period. Then females and males were removed and eggs laid for 5 days are counted. The eggs obtained from females are separately cultured to reach adulthood. Hatching eggs were counted at 15, 20, 25 and 30 ºC under a 14L: 10D photoperiod and 80 ± 5% RH. All individuals were kept till adulthood then the number of females and males (sex ratio) were recorded per vial.

Statistical Analysis: data were analyzed and means were compared using 1-way analysis of variance (ANOVA) of the software package Super ANOVA (Abacus Concepts 1989).

Results and discussion

Developmental time of immature stages: results showed that the life cycle of house dust mite, *D. farinae*, consists of 5 stages (egg, larva, protonymph, tritonymph, and adult). Quiescent larval, protonymphal and tritonymphal periods were also observed. The duration of development of immature stages of *D. farinae* at various temperatures are shown in table (1) and figure (1). At 15, 20, 25 and 30 ºC, average incubation periods were 29.5 ± 2.5, 8.8 ± 1.4, 4.9 ± 0.9 and 3.6 ± 0.8 days, for female and 30.6 ± 3.4, 9.0 ± 1.6, 4.3 ± 0.7 and 3.4 ± 0.9 days, for male, respectively. Results indicated that larval developmental time was the longest of all other life stages. In the mean time, duration of larval period reached 35.3 ± 4.7, 11.5 ± 1.5, 7.6 ± 1.1 and 5.2 ± 1.1 days for female and 42.5 ± 3.5, 10.6 ± 1.8, 7.5 ± 1.2 and 4.9 ± 1.1 days for male under the previous respective temperature. Duration period of protonymphal stages averaged 26.6 ± 2.1, 8.1 ± 1.1, 4.0 ± 0.7 and 2.9 ± 0.8 days for female and 26.8 ± 2.3, 8.6 ± 1.5, 5.1 ± 1.3 and 3.1 ± 0.9 days for male. Duration of tritonymphal period for female were 27.8 ± 2.9, 8.5 ± 1.4, 4.6 ± 0.8 and 3.4 ± 0.6 days, while it reached 29.2 ± 2.6, 7.6 ± 1.4, 4.3 ± 0.9 and 3.2 ± 0.7 days for male under previous mentioned degrees, respectively. Larson *et al*. (1969) isolated *D. farinae* from house dust and reared the mite on Gaines-burger dog food at room temperature and 75 % RH. They found that the developmental time from egg to egg takes 30 days with egg stage lasting 6-8 days. In 1975, Furumizo reported that the development of *D. farinae* from egg to adult took 33.0, 23.2 and 18.8 days at 22.2, 26.6 and 32.0 ºC, respectively. In addition, the quiescent period represented 28 – 36 % of the total life cycle for all temperatures. In the present study, duration of total life cycle, from egg deposition to adult emergence, for female averaged 119.2 ± 5.5, 36.9 ± 3.6, 21.1 ± 1.4 and 15.1 ± 1.7 days, while it came to 129.1 ± 7.1, 35.8 ± 2.9, 19.7 ± 1.8 and 14.6 ± 1.5 days for male at 15, 20, 25 and 30 ºC, respectively. Statistical analysis proved a significant difference of total life cycle between the four temperatures (table 1). Also, data showed that the percentage of time for the quiescent period of each life stage was

relatively constant for all life stages and was about one-third of the total duration of each life stage (table 1 and figure 2).

Table 1. Effect of temperature on the life cycle of (Mean / day ± SE) of the house dust mite, *Dermatophagoides farinae*, under constant photoperiod (14L: 10D) at 80 ± 5 % RH

Temp. (°C)	Mean/ days (± SE) required to complete development					
	Sex	Egg	Larva	Protonymph	Tritonymph	Life Cycle
15	♀	29.5 ± 2.5	35.3 ± 4.7 (14.6 ± 3.6)	26.6 ± 2.1 (10.7 ± 2.6)	27.8 ± 1.9 (11.1 ± 1.9)	**119.2 ± 5.5 (36.4 ± 2.1)**
	♂	30.6 ± 3.4	42.5 ± 3.5 (18.5 ± 2.4)	26.8 ± 2.3 (11.1 ± 1.8)	29.2 ± 2.6 (13.6 ± 1.7)	**129.1 ± 7.8 (39.2 ± 2.3)**
20	♀	8.8 ± 1.4	11.5 ± 1.5 (5.6 ± 1.2)	8.1 ± 1.1 (3.2 ± 0.9)	8.5 ± 1.4 (3.4 ± 1.1)	**36.9 ± 3.6 (12.2 ± 2.7)**
	♂	9.0 ± 1.6	10.6 ± 1.8 (4.8± 0.9)	8.6 ± 1.5 (3.9 ± 1.1)	7.6 ± 1.4 (3.1 ± 0.8)	**35.8 ± 2.9 (11.8 ± 1.7)**
25	♀	4.9 ± 0.9	7.6 ± 1.1 (3.1 ± 0.9)	4.0 ± 0.7 (1.2± 0.6)	4.6 ± 0.8 (1.6± 0.9)	**21.1 ± 1.4 (5.9 ± 1.9)**
	♂	4.3 ± 0.7	7.5 ± 1.2 (3.3 ± 1.2)	5.1 ± 1.3 (1.7 ± 0.9)	4.3 ± 0.9 (1.7 ± 0.7)	**19.7 ± 1.8 (5.7 ± 1.5)**
30	♀	**3.6 ± 0.8**	**35.3 ± 4.7 (14.6 ± 3.6)**	**2.9 ± 0.7 (0.9 ± 0.6)**	**3.4 ± 0.6 (1.2 ± 0.5)**	15.1 ± 1.7 (4.2 ± 1.3)
	♂	**3.4 ± 0.9**	**42.5 ± 3.5 (18.5 ± 2.4)**	**3.1± 0.9 (1.1 ± 0.8)**	**3.2± 0.7 (1.1 ± 0.8)**	14.6 ± 1.5 (4.1 ± 1.1)

Values in brackets represent quiescent periods.
[#] Means within rows followed by the same letter are not significantly different ($P > 0.05$).

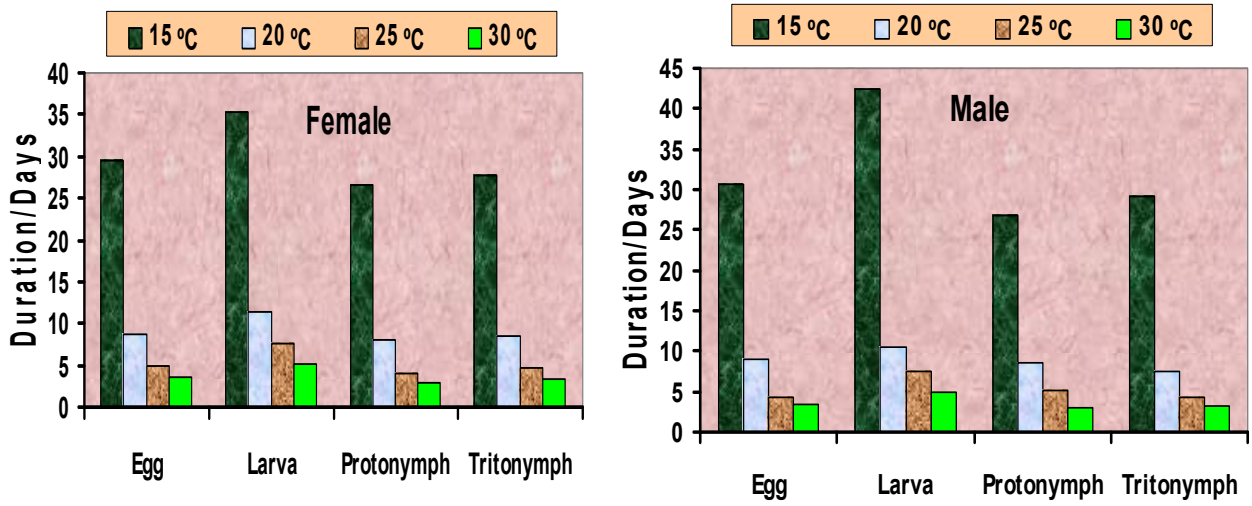

Figure 1. Effect of four constant temperatures on the female and male immature stages of house dust mite, *Dermatophagoides farinae*, under constant photo period (14L: 10D) at 80 ± 5 % RH

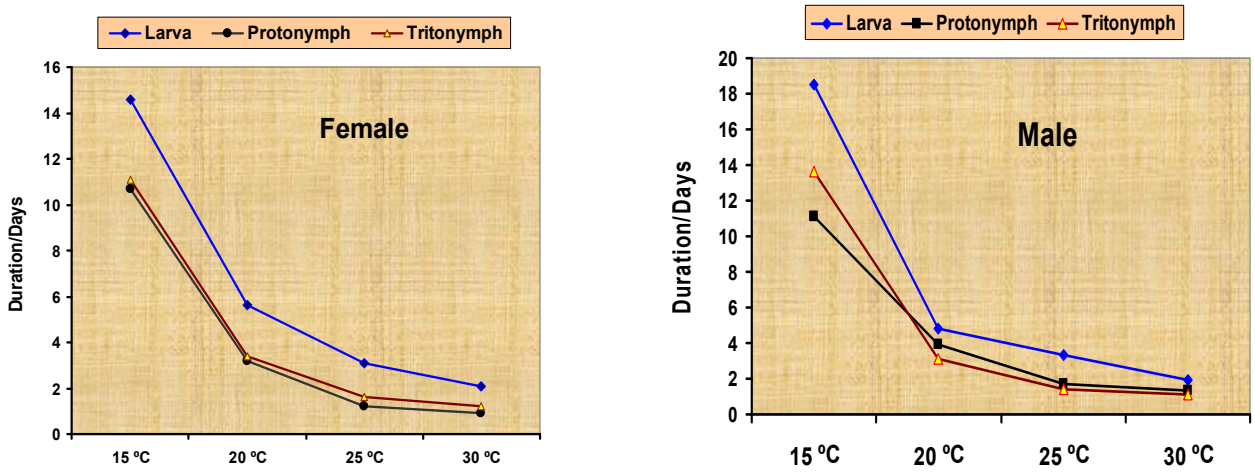

Figure 2. Effect of four constant temperatures on quiescent period of female and male immature stages of the house dust mite, *Dermatophagoides farinae*, under constant photo period (14L: 10D) at 80 ± 5 % RH

Arlian *et al.* (1990) also found that the quiescent period represented 36-47 % of the larval, protonymphal and tritonymphal at all temperatures. For *D. pteronyssinus*, Dobson (1979) reported that at 20, 25, 30 and 35 ºC and 80 % RH, development required 45.2, 16.7, 16.6 and 13.0 days, respectively. Also, Arlian & Dippold (1996) reported the average length of the life cycle to be 122.8 ± 14.5 days at 16 ºC, 34.0 ± 5.9 days at 23 ºC, 19.3 ± 2.5 days at 30 ºC, and 15.0 ± 2.0 days at 35 ºC at 75 % RH. In comparison, for *D. farinae*, few mites complete the life cycle at extreme temperatures of 16 ºC and 35 ºC, but at 23 ºC and 30 ºC, lengths of the life cycle were 35.6 ± 4.4 days and 17.5 ± 1.2 days, respectively (Sakaki & Suto 1991).

Adult longevity and Oviposition: adult female survived for 44.1 ± 5.3, 38.6 ± 2.5, 33.8 ± 2.6 and 22.6 ± 1.7 days, while male survived for 38.6 ± 4.1, 31.5 ± 2.4, 29.7 ± 2.1 and 17.5 ± 1.2 days at 15, 20, 25 and 30 ºC, respectively (table 2). Results have also indicated that the total adult survival period decreased as the temperature rose. The preoviposition, oviposition, postoviposition periods of adult females at various temperatures (shown in table 2) confirmed that the preoviposition and oviposition periods have generally decreased with increasing temperatures. Preoviposition reached 6.3 ± 2.3, 3.2 ± 1.8, 2.6 ± 1.1 and 2.2 ± 0.9 days at 15, 20, 25 and 30 ºC respectively. It was also observed that at 20 and 25 ºC, most of adult females died within 2.6 – 3.1 days after ending oviposition, while at 15 and 30 ºC had a longer postoviposition period, ranging from 3.8 to 4.2 days.

Table 2. Effect of temperature on some biological aspects (Mean /day ± SE) of the house dust mite, ***Dermatophagoides farinae***, under constant photoperiod (14L: 10D) at 80 ± 5 % RH

Biological Aspects	Temperature ºC			
	15	20	25	30
Pre-oviposition	6.3 ± 2.3 a #	3.2 ± 1.8 b	2.6 ± 1.1 c	2.2 ± 0.9 c
Oviposition	33.6 ± 4.2 a	32.8 ± 3.2 a	28.1 ± 2.1 b	16.6 ± 2.4 c
Post-oviposition	4.2 ± 2.1 a	2.6 ± 1.5 b	3.1 ± 1.6 c	3.8 ± 1.1 d
Longevity (♀)	44.1 ± 5.3 a	38.6 ± 2.5 b	33.8 ± 2.6 c	22.6 ± 1.7 d
Life span (♀)	163.3 ± 8.4 a	81.0 ± 5.1 b	54.9 ± 3.2 c	37.7 ± 2.6 d
Longevity (♂)	38.6 ± 4.1 a	31.5 ± 2.4 b	29.7 ± 2.1 b	17.5 ± 1.2 c
Life span (♂)	167.7 ± 9.5 a	67.3 ± 3.2 b	49.4 ± 2.2 c	32.1 ± 2.2 d

Means within rows followed by the same letter are not significantly different ($P > 0.05$).

Fecundity, hatchability and sex ratio: The number of deposited eggs per female averaged 68.3 ± 6.2, 74.3 ± 3.8, 81.5 ± 5.2 and 52.4 ± 1.3 eggs/female with a daily rate of 2.03 ± 1.7, 2.3 ± 1.8, 2.9 ± 0.7 and 3.2 ± 1.1 eggs/female at 15, 20, 25 and 30 ºC, respectively. Egg hatchability was 85.7, 94.8, 96.2 and 97.2 % at same temperatures (table 3). Statistical analysis proved insignificant difference of hatchability between the four temperatures. At 15, 20, 25 and 30 ºC, the sex ratios were 1 ♀: 0.83 ♂, 1 ♀: 0.86 ♂, 1 ♀: 0.91 ♂ and 1 ♀: 0.93 ♂, respectively as indicated in Table 3. Results have also showed that the change in sex ratios were not significant with the change in temperature. Arlian *et al.* (1990) found closer data for *D. pteronyssinus* where female lived 31.2 ± 11.1 days at 23 ºC and produced 2.5 ± 0.8 eggs per day during a reproductive period of 26.2 ± 10.8 days. By comparison, at 35 ºC, female produced 3.3 ± 1.3 eggs per day, but female longevity and the reproductive period were reduced to 15.5 ± 9.6 and 11.6 ± 6.4 days, respectively. During a life time, *D. pteronyssinus* females produced 68.4 ± 30.4 and 48.0 ± 29.6 eggs at 23 and 35 ºC, respectively.

Table 3. Effect of four constant temperatures on fecundity, hatchability and sex ratio of the house dust mites *Dermatophagoides farinae*, under constant photoperiod (14L: 10D) at 80 ± 5 % RH

Biological Aspects	Temperature °C			
	15	*20*	*25*	*30*
Hatchability %	*85.7*	*94.8*	*96.2*	*97.2*
Sex ratio (F ♀♀/M ♂♂)	*1♀♀: 0.83♂♂*	*1♀♀: 0.86♂♂*	*1♀♀: 0.91♂♂*	*1♀♀: 0.93♂♂*
Total No. Eggs/female ♀	*68.3 ± 6.2*[a]	*74.3 ± 3.8*[b]	*81.5 ± 5.2*[c]	*52.4 ± 1.3*[d]
No. Eggs/day/female♀	*2.03 ± 1.7*[a]	*2.3 ± 1.8*[a]	*2.9 ± 0.7*[b]	*3.2 ± 1.1*[c]

[#] Means within rows followed by the same letter are not significantly different ($P > 0.05$).

Conclusion

In conclusion, changing temperatures have critical effects on the biology of *D. farinae*. Larval stage comprised the longest period of all other immature stages. All immature stage durations showed an inverse relationship with increasing temperatures. Quiescent periods were observed in all immature stages with an apparent effect of changing temperatures reaching up to 30% of stage duration. Under higher temperatures, daily egg productions were higher than lower temperatures even if the total egg/female was lower. Fecundity and hatchability showed the same inverse relationship. The data suggests that the best rearing temperature would be 25 ºC.

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COMPARATIVE ULTRASTRUCTURE AND PROBABLE FUNCTIONS OF GENITAL PAPILLAE IN THE PARASITENGONA (ACARIFORMES)

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Abstract

Genital papillae of adult mites *Hirsutiella zachvatkini* (Schluger, 1948) (Trombiculidae), *Platytrombidium fasciatum* (C.L. Koch, 1836) (Microtrombidiidae) and *Teutonia cometes* (C.L. Koch, 1837) (Hydrachnidia: Teutoniidae) were examined by means of transmission electron microscopy. Three pairs of genital papillae are located in these species on the inner sides of genital flaps, are composed of few light tightly adjoined cells and project by their apical portion into the genital atrium being covered by a modified cuticle. Genital papillae are characterized by vacuolar transport that is most conspicuous in a water mite *T. cometes*, moderately expressed in *Pl. fasciatum* and highly reduced in *H. zachvatkini*. Genital papillae are thought to remove superfluous waters and dissolved waste ions from the organism to provide a necessary salt-water balance of these mites.

Key words

salt-water balance, osmoregulation, vacuolar transport

Introduction

Genital papillae and related structures variously named as acetabula, axillary organs, ring organs, etc. are specialized organs, which are characteristically present in the majority of acariform mites and are now thought to function in osmoregulation (Halik 1930; Grandjean 1948; Vercammen-Grandjean 1976; Alberti 1977; 1979; Barr 1982; Fashing 1988; Alberti & Bader 1990; Fashing & Marcuson 1997; Alberti & Coons 1999; Goldschmidt et al. 1999; Witalinski et al. 2002). In primitive condition, genital papillae are situated in a number of one pair in protonymphs, two pairs in deutonymphs and three pairs in tritonymphs and adults on the inner walls of genital flaps and may be progressively modified by multiplication and spreading throughout the ventral body wall and the coxal plates (Grandjean 1948; Alberti & Bader 1990; Alberti & Coons 1999). Generally, genital papillae highly correspond morphologically and functionally to larval Claparède organs (urstigmae) located in a number of one pair between coxae I and II (Grandjean 1946, 1955; Alberti 1979; Baker 1985; Fashing 1988; Shatrov 2004). Both these structures are considered to be homonomous (homotypic) organs (Alberti & Bader 1990) and obey in their distribution through the life cycle stages the Oudemans-Grandjean rule (Johnston & Wacker 1967) with few exceptions (André 1991).

Genital papillae in water mites have been described anatomically and ultrastructurally in a number of previous works (Halik 1930; Grandjean 1948; Alberti 1977, 1979; Barr 1982; Alberti & Bader 1990; Goldschmidt et al. 1999), whereas other representatives of the cohort Parasitengona were not studied in this respect up to now. Nevertheless, it is rather interesting to compare these structures and their possible role in osmoregulation in water inhabiting mites and in

terrestrial forms living in different behavioral conditions. This reason induced me to undertake this study with the main purpose to give a detail ultrastructural description of genital papillae in representatives of three main group of Parasitengona – water mites (phalanx Hydrachnidia), superfamilies Trombidioidea and Trombiculoidea.

Materials and Methods

Adult water mites *Teutonia cometes* (C.L. Koch, 1837) (Teutoniidae) and trombidiid mites *Platytrombidium fasciatum* (C.L. Koch, 1836) (Microtrombidiidae) were captured in natural conditions in the North-Western region of Russia, whereas adult trombiculid mites *Hirsutiella zachvatkini* (Schluger, 1948) (Trombiculidae) were obtained from the first laboratory generation of a laboratory culture originated from fully fed larvae collected from their natural hosts – bank voles (*Clethrionomys glareolus* Schreber 1780) in the same geographic region.

For transmission electron microscopy (TEM), the whole active mites were treated by standard double fixation with 2.5% glutaraldehyde and 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2-7.4) and finally embedded in an araldite mixture. Serial ultra-thin sections after staining with uranyl acetate and lead citrate were examined with LEO-900 and Tesla BS-500 transmission electron microscopes at 60-80 kV. For anatomical observations, serial semi-thin sections were stained with toluidine blue and investigated and photographed under an Amplival and Leica DM LS-2 light optical microscopes.

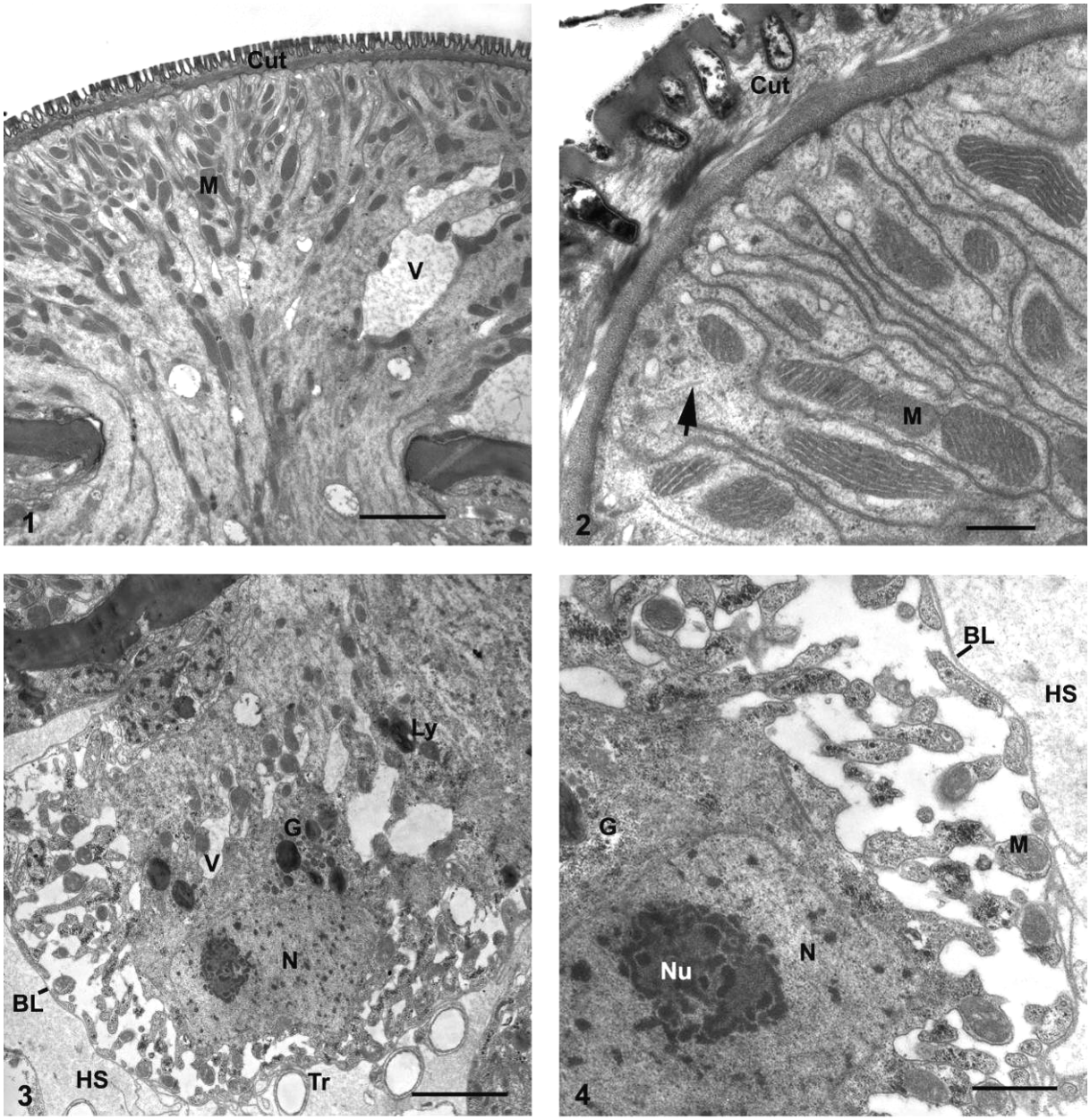
Results

General remarks

In adult mites of the species studied, three pairs of genital papillae are plesiomorphically located on the inner sides of genital flaps looking medially and cannot be observed from the outside. Anatomically, genital papillae are distinctly divided into two different portions – the basal nuclear portion protruding free into the haemocoelic space, and the apical portion projecting semi-spherically into the genital atrium being covered from the outside by a modified cuticle. The middle region of the organ between the basal and the apical portions is narrower and forms a conspicuous waist. Genital papillae are composed of few light tightly adjoined cells running throughout the organ from its basal to the apical zone having corresponding waist in the middle region.

Teutonia cometes *(Hydrachnidia: Teutoniidae)*

There are somewhat differences in the form of papillae in this species – front papillae are relatively narrow and protrude deeper into the genital atrium whereas back papillae are most wide (up to 35 µm) and flattened. Middle papillae have an intermediate shape. The depth of the entire papillae from the cuticle to the basal lamina and, correspondingly, the height of the cells is around 30 µm. The cells of papillae are characterized by deep plications (4-7 µm) of the apical plasma membrane immersed into the cells with characteristically widened terminal parts coming close to the apical cell surface. Among these plications, a large number of oval to elongate electron-dense mitochondria with tightly packed cristae are situated mostly in the axial plane of the cells forming a characteristic mitochondrial pool in the apical portion of the organ. Besides mitochondria, the apical cell zone may contain small clear vesicles emptying into the narrow fine-granular subcuticular space. The apical cell surface itself is slightly wavy. Microtubules are of the most characteristic organelles of the cells and extend throughout the cell volume predominantly in the axial direction. The cells also contain variously expressed small to very large clear vesicles and vacuoles going across the organ from the basal region to the apical cell surface. The middle narrow portion of papillae is poorly provided with organelles containing single mitochondria and microtubules. The basal portion of papillae lies free in the haemocoelic space and may border upon other tissues including tracheae being nearly as wide as the apical potion. The basal cell region in comparison with the apical one has the electron-denser ground cytoplasm and contains large nucleus and nucleolus (5.7 and 2.1 µm in diameter correspondingly), some amount of mitochondria, free ribosomes and glycogen particles, as well as weakly organized Golgi-like bodies and electron-dense lysosome-like inclusions in the perinuclear cell zone. The most characteristic feature of the basal portion of papillae is strongly folded basal plasma membrane leaving large extracellular spaces penetrating nearly the entire basal portion of papillae around the nuclei. Mitochondria and other organelles are placed in the cellular compartment of this region. These basal plasma membrane infoldings just give rise to the clear vacuoles penetrating the cells up to the apical region and fusing with both plications and the



Figures 1-4. Genital papillae of *Teutonia cometes*. 1 – Apical portion of papilla. Scale bar – 3 µm; 2 – Apical cell zone containing mitochondria and modified cuticle. Scale bar – 0.5 µm; 3 – Basal portion of papilla with nucleus. Scale bar – 3 µm; 4 – Nucleus and basal infoldings. Scale bar – 1 µm. BL – basal lamina; Cut – modified cuticle; G – Golgi body; HS – haemocoelic space; Ly – lysosomes; M – mitochondria; N – nucleus; Nu – nucleolus; Tr – trachea; V – clear vacuoles; *arrow* indicates microtubules.

apical plasma membrane itself. The basal lamina surrounding the organ remains flat and well outlines papillae from the side of haemocoelic space.

The modified cuticle covering papillae from the outside is formed of the procuticle with the lamellar basal layer (endocuticle) of moderate electron density and the clear apical layer (exocuticle) with loosely arranged lamellae that is covered by the thin epicuticle with the electron-dense external (cuticulin) layer. The overall width of the modified cuticle is around 1 µm. The exocuticle is characteristically penetrated by deep pits partly filled with an electron-dense flocculent material. The apical portions of the so formed ridges between the pits are filled with the

homogenous material of moderate electron density. No conspicuous secretion via dense vesicles is seen realized through the apical plasma membrane of the cells although small amount of the electron-dense material may be occasionally seen in the very terminal portions of the plasma membrane plications. Special electron-lucent chamber is not obviously observed between the modified cuticle and the cells of papillae. Internal portions of the cuticle forming the waist of papillae (apodeme) are built of the electron-dense cuticle with hardly distinguished lamellae and look like thick valves tightened papillae from both sides in their middle region.

Platytrombidium fasciatum *(Trombidioidea: Microtrombidiidae)*

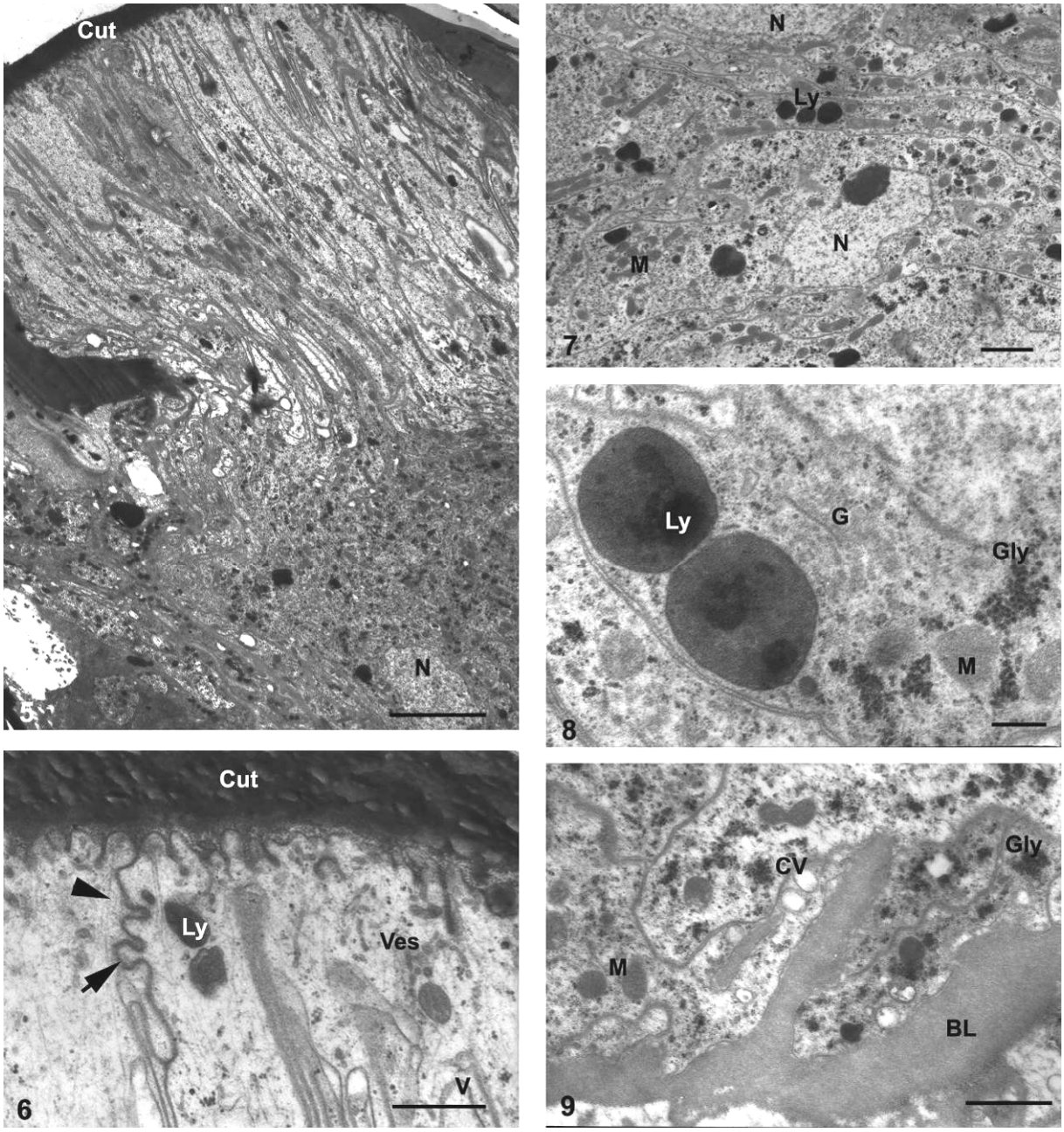
In comparison with the fresh water mite *T. cometes*, genital papillae of the terrestrial mite *P. fasciatum* are wider, up to 45 µm in their external diameter with a significantly more prominent basal portion, so the overall depth of papillae from the basal lamina to the apical cell surface is around 60 µm. The voluminous basal portions typically turn dorsally extending deep into the body cavity. Plications of the apical plasma membrane are not very thick but extremely deep (20-22 µm) occupying the entire volume of the apical portion of papillae. In contrast to *T. cometes*, electron-dense elongate mitochondria in this region are scarce scattered freely throughout the apical portion of papillae. Axially arranged microtubules are numerous. The apical cytoplasm also contains (1) few electron-dense inclusions supposedly of lysosomic nature as well as (2) small vesicles, (3) tubular structures and (4) single not very large vacuoles sometimes with irregular margins all having matrix of moderate electron density, coming close to the apical plasma membrane and apparently fusing with it discharging their contents into the narrow fine-granular subcuticular space. Occasionally, larger clear vacuoles (0.7-0.9 µm) may be seen in the apical cytoplasm and in the vicinity of the apical plasma membrane. The latter shows wavy outline more intensively expressed than that in *T. cometes*. Lateral cell margins are flat without conspicuous extracellular space and become folded only in the apical regions connected with each other via septate junctions with an electron-dense extracellular matrix. These cell contacts are characteristically marked by an electron-dense flocculent material applied to the lateral plasma membrane from the cytoplasmic side.

The modified cuticle covering papillae from the outside, in contrast with *T. cometes*, does not form pits and composed of the lamellar electron-dense procuticle and of the extremely thin electron-light epicuticle. At the same time and in addition to lamellae, the procuticle may show a regular honeycomb structure. The width of the modified cuticle is 1.1-1.2 µm. The lateral and internal portions of the cuticular caps of papillae are formed of the thick electron-dense homogenous apodemes weakly tightening papillae in the waist region.

The basal portion of papillae possesses more electron-dense ground cytoplasm and is provided with the elongated nuclei (5-7x4.5 µm) occupying the middle zone of the basal cell portions as well as with numerous organelles and inclusions. The oval mitochondria are located in small group without obvious gradient. Besides free ribosomes, there are also small Golgi bodies, electron-dense variously shaped lysosomes and small groups of glycogen particles freely intermingled in the basal portion of papillae. Clear vacuoles may be seen only occasionally. The cells are also characterized by the axially orientated double membrane profiles that may be a result of plications of the lateral plasma membranes. It is characteristically that the adjacent cells may have different density of the cytoplasm and so alternate in the basal portion of papillae. The basal cell zones do not differ in any way from the remaining cytoplasm and do not form infoldings of the basal plasma membrane except for the formation of single clear vesicles occasionally blebbing from the plasma membrane into the cells. The basal portion of papillae, in contrast with *T. cometes*, is surrounded by the tightly adjoined thick basal lamina of a moderate electron density, which frequently forms deep intrusions into the cells.

Hirsutiella zachvatkini *(Trombiculoidea: Trombiculidae)*

In contrast with the two above described species, genital papillae of adults of *H. zachvatkini*, living in soil, are significantly reduced both morphologically and supposedly functionally. A semi-spherical cuticular cap of papillae with diameter 26-27 µm covers the reduced organ built of few electron-light cells extremely poorly provided with organelles. The basal portion of papillae is irregularly outlined and has a restricted volume turning dorsally on going out from under the cap. The cells contain a little number of electron-dense mitochondria, single electron-dense granules and clear vesicles uniformly scattered throughout the electron-light ground cytoplasm provided with numerous axially orientated microtubules irrespectively of location within the cells. The latter



Figures 5-9. Genital papillae of *Platytrombidium fasciatum*. 5 – General view of papilla. Scale bar – 4 µm; 6 – Modified cuticle and apical cell zones. Scale bar – 1 µm; 7 – Internal portion of papilla with nuclei. Scale bar – 2 µm; 8 – Golgi body in the basal portion. Scale bar – 0.5 µm. 9 – Basal lamina and clear vesicles detached from the basal plasma membrane. Scale bar – 1 µm. Cut – cuticle; CV – clear vesicles; G – Golgi body; Gly – glycogen; Ly – lysosomes; M – mitochondria; N – nucleus; V – clear vacuole; Ves – small apical vesicles; *arrow* indicates cell contacts; *arrowhead* indicates microtubules.

are also provided with plications of either lateral or basal plasma membrane mostly axially orientated, which apparently give rise to clear vesicles with a double surrounding membrane. These plications do not reach the apical plasma membrane, and the apical cytoplasm mostly looks empty. The cells may also contain single extremely small Golgi bodies, lysosome-like bodies, multivesicular bodies and occasionally larger clear vacuoles throughout the cell volume. Dense vesicles come to the apical cell surface and discharge their contents through the apical plasma membrane into the narrow subcuticular space. The latter is practically absent, and the contents of discharged vesicles so built the modified cuticle from its inner side. The modified cuticle around 0.5 µm width has weakly identified

stratification into the exocuticle with poorly distinguished lamellae of moderate to high electron density and the endocuticle with a slightly wavy basal margin and varying density depending on the activity of the dense vesicles (secretion). The epicuticle is thin dense and practically indistinguishable. The lateral walls of the semi-spherical papillae cap are thickened and look like these apodemes of the other species studied. No subcuticular cavity (chamber) is expressed in papillae of *H. zachavtkini* just as well as in *P. fasciatum*.

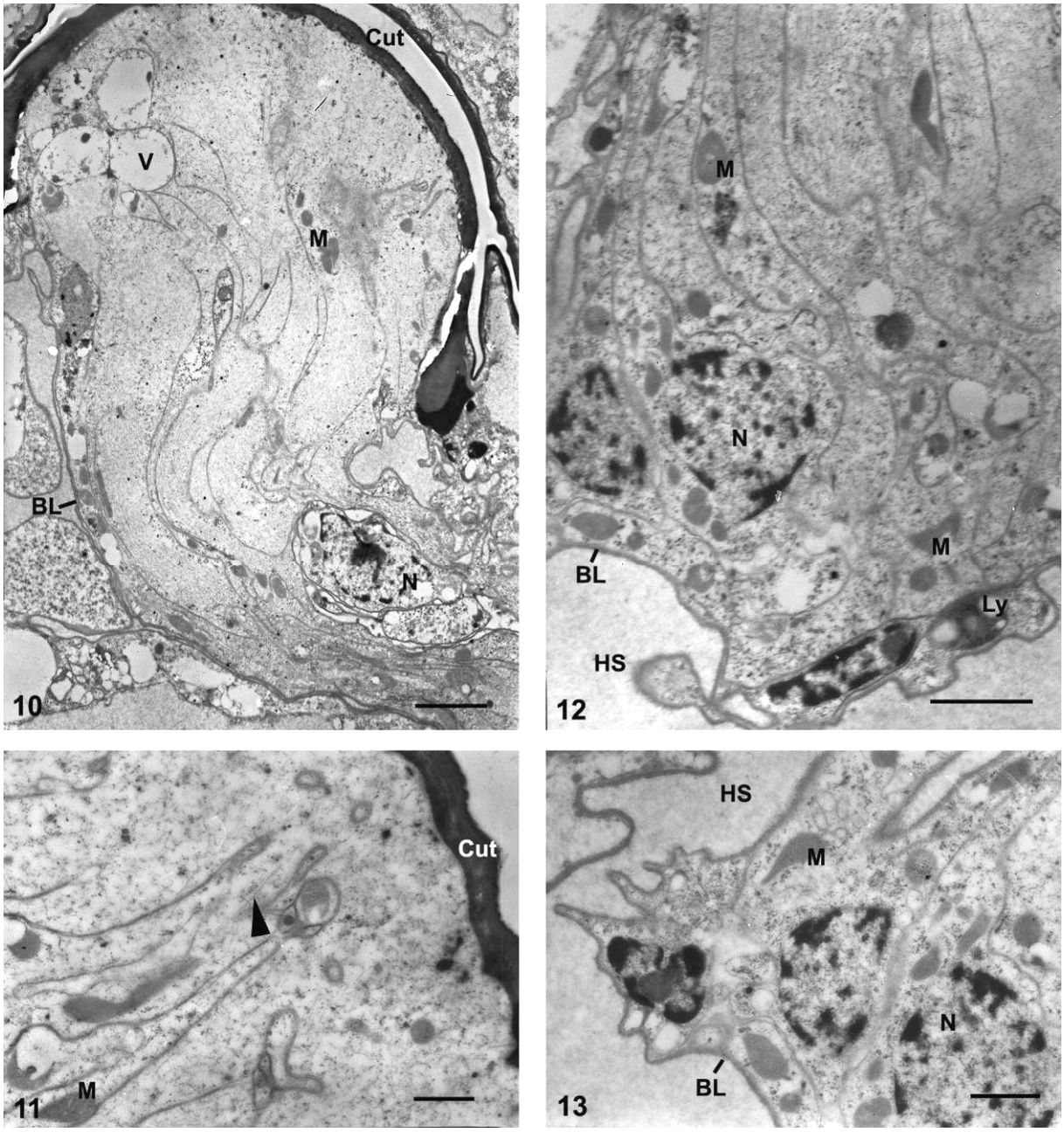
The nuclei 3-5x1.5-3 µm occupy the basal cell portions and may slightly vary in shape and sizes being more flattened in the marginal cell zones oppressed against the basal cell membrane. No any membrane invaginations or blebbings are seen expressed in the basal cell zones. The basal portion of papillae is surrounded by the thin basal lamina, which may form folds repeating configuration of the organ never penetrating into the cells.

Discussion

As seen from this investigation, organization of genital papillae differs significantly in representatives of different groups within the Parasitengona. The most complex structure of papillae that is obviously reflected in their probable functional activity is observed in a fresh-water mite *T. cometes*. The strong basal infoldings, intensive membrane plications and mitochondrial pump in the apical portion as well as clear vacuoles migrating through the cells inevitably indicate the intensive transport of excess water and dissolved ions from the haemocoel to the external milieu through the organ. The same functions were postulated for the ring organs of the water-inhabiting histiostomatid mite, *Histiostoma feroniarum* (Acaridida) (Witalinski et al. 2002). These ring organs correspond to genital papillae, which normally absent from histiostomatid mites, and, supposedly, actively remove the excess water from the haemocoel after taking up water during feeding. However, in contrast to *H. feroniarum*, where the active transport is largely realized only at the basal membrane (Witalinski et al. 2002), in a water mite *T. cometes* the active transport may be expected also in the apical portion of papillae due to the presence of the strongly developed mitochondrial pump among the membrane plications. Nevertheless, this doubtfully changes the outward transport direction of water and solutions. Similar organization of genital papillae has been described earlier in some other water mites studied – *Hydrodroma despiciens* with multiply papillae situated externally on genital valves (Alberti 1977), *Limnesia maculata* with three pairs of papillae (Alberti 1979), *Hydrovolzia placophora* with numerous "genital" papillae located on epimeral plates (Alberti & Bader 1990) and *Neotyrrellia* sp. with acetabula distributed both on the genital flaps and also on the fourth coxae (Goldschmidt et al. 1999). In all these cases, however, the apical region of papillae is not so provided with mitochondria as that in *T. cometes* obviously due to the restricted transportation processes of the large masses of water.

Genital papillae of fresh-water mites were attributed to be closely related to chloride cells of insects (Komnick 1977) and therefore to have a function in the uptake of ions from the external medium (Alberti 1979). For fresh-water inhabiting organisms living in hypotonic medium, both functions – remove of the excess water and the ions uptake are extremely important. Nevertheless, for the mites such as Parasitengona characterized by extra-intestinal digestion and taking up a great amount of the liquid food, the function of remove water seems to be predominant that is indirectly proved by the presence of the large clear vacuoles apparently migrating through the cells of papillae in *T. cometes*.

An electron-lucent chamber between the modified cuticle and the cells of papillae probably filled with highly hydrated solution and found in genital papillae of *Hydrodroma despiciens* (Alberti 1977) and *Acarus siro* (Witalinski et al. 1990) as well as in the axillary organs of *Algophagus pennsylvanicus* (Fashing 1984), in the ring organs of *Histiostoma feroniarum* (Witalinski et al. 2002) and in the Claparède organ of *Naiadacarus arboricola* (Fashing 1988) was not identified both in *T. cometes* and in the terrestrial mites studied by me – *P. fasciatum* and *H. zachvatkini*. Genital papillae of the other water mite species studied to date as well as of *Neomolgus littoralis* (Bdellidae) also lack such a transparent chamber (Alberti 1979; Alberti & Bader 1990; Goldschmidt et al. 1999). Although in all these cases the cells of papillae are firmly attached to the modified cuticle, to which they are connected via the special intermediate zone – the more or less expressed fine granular subcuticular space supposedly important in realizing of some special functions, the potential presence of the chamber may depend on the current physiological state of the organ (Witalinski et al. 2002) and on the particular presence of the transporting water beneath the cuticle at the moment especially on the peripheral regions of papillae.



Figures 10-13. Genital papillae of *Hirsutiella zachvatkini*. 10 – General view of papilla. Scale bar – 3 µm; 11 – Detail of the apical portion of papilla. Scale bar – 1 µm; 12 – Basal portion of papilla. Scale bar – 2 µm; 13 – Basal cell zones of papillae. Scale bar – 1 µm. BL – basal lamina; Cut – cuticle; HS – haemocoelic space; Ly – lysosome; M – mitochondria; N – nucleus; V – clear vacuole; *arrow* indicate microtubules.

Comparison of papillae of *T. cometes* with those of terrestrial mites *P. fasciatum* and *H. zachvatkini* shows significant reduction of transportation activity in the latter. Papillae of these two species are devoid of both basal infoldings and intensive apical plications with mitochondrial "pump" that is extremely obvious in the trombiculid mite *H. zachvatkini*. At the same time in these two cases the vesicular transport across the cells of papillae seems to be also present though in restricted proportions as well as secretion via the electron-dense vesicles into the subcuticular space that is obviously lacking in *T. cometes*. It has been supposed that in terrestrial Actinotrichida, genital papillae, due to the absence of chloride precipitation, do not uptake of ions but are mainly responsible for the uptake of water (Alberti 1979). This is maybe correct for the exceptional dry-air

living mites but is not apparently true for the representatives of Parasitengona living in humid soil condition and taking up the large amount of liquid food during feeding. Moreover, no morphological evidences support such a point of view. Apical surface of the cells in *P. fasciatum* and *H. zachvatkini* is devoid of microvilli, plications as well as mitochondrial activity. Conversely, there has a good reason to believe that the moderate vacuolar transportation of the excess of water across the organ in terrestrial Parasitengona is directed from the haemocoel to the external medium.

As a result, in the line of *T. cometes* (Hydrachnidia) – *P. fasciatum* (Trombidioidea) – *H. zachvatkini* (Trombiculoidea) it is evidently seen the gradual reduction of the transporting processes across genital papillae with the addition of some supplementary functions (lysosomic activity) in *P. fasciatum*. In the latter species, genital papillae are most prominent and reach with organelles. Conversely, in *H. zachvatkini*, genital papillae seem to be mostly reduced morphologically and expectedly functionally. This is well grounded from the general functional analysis. Deutonymphs and adults of trombiculids never come to the soil surface and are devoid of trachea. The main functions of transpiration, respiration and sorption are realized through the entire integument provided with well organized setae equipment retaining permanent volume of moist air (floating chamber) at the integument (plastron respiration) (Shatrov 2000). As a result, functional loading of so small organs such as papillae is seen highly reduced. In trombidiids, invading soil surface in spring-summer period, the problem of retaining of metabolic water and supporting of necessary osmotic conditions is solved by evolvement of the thick complex cuticle (Shatrov unpublished date) as also in the case of water mites. In these instances, the osmotic regulation is sufficiently realized through genital papillae undergoing the additional progressive multiplication in some water mite groups (Alberti & Bader 1990; Alberti & Coons 1999; Goldschmidt et al. 1999). It is interesting that in comparison with “normal” papillae numbered plesiomorphically in three pairs, multiplied papillae demonstrate some morphological regression (smaller number of cells, organelles, etc.) (Alberti & Bader 1990).

Corresponding Claparède organs (urstigmae) in larvae of representatives of trombidiids and trombiculids (Shatrov 2004), apart from their inherent external cuticular armament, possess just the same comparative characteristics as genital papillae in adults. Claparède organs of *P. fasciatum* are comparatively large organs richly provided with the apical mitochondrial pool, dense secretion vesicles and sometimes clear vacuoles; the basal portion is also prominent and rich with organelles, whereas these organs in *H. zachvatkini* are significantly reduced. It should be noted, at the same time, that Claparède organ both of *P. fasciatum* and *H. zachvatkini* (Shatrov 2004) as well as genital papillae of some water mites (Alberti & Bader 1990), have laterally located additional supplementary cells (modified epidermal cells), which are not engaged in transporting processes. In contrast, genital papillae of adult mites of the species studied by me do not possess such cells incorporated into the organ.

To conclude, Parasitengona is the group if Acariformes provided with the most conspicuous osmoregulation organs such as genital papillae and Claparède organs that is obviously resulted of their mode of extra-intestinal feeding. Within the group, however, there are apparently seen an evolutionary tendency to reduction of these organs in trombiculid mites highly specialized to living in soil. It is quite characteristically that this tendency is equally developed as in adult mites so in larvae.

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DETAILS OF MORPHOLOGY OF GAMASID SPECIES *LAELAPS AGILIS* KOCH (ACARI: LAELAPIDAE) USING A SCANNING ELECTRON MICROSCOPY

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Abstract

External morphology of both sexes of gamasid species *Laelaps agilis* Koch, 1836 (Acari: Gamasida: Laelapidae) was examined by scanning electron microscopy. The studies provided information on surface particular anatomical features of this species. The paper includes a SEM photographs of body view and ultrastructural details for the gnathosoma, idiosoma and legs. Morphology of *L. agilis* is illustrated here for the first time in detail.

Key words

Acari, Gamasida, *Laelaps agilis*, SEM, morphology

Introduction

Mites of the genus *Laelaps* (Gamasida: Laelapidae) are important component of the ectoparasitic fauna infecting small mammals, especially rodents. To this time, about 50 species were described from all regions except the Antarctic (Tipton 1960).

The species *Laelaps agilis* Koch, 1836 which is used in the present study is common in Europe and was observed from European part of Russia, Slovakia, Poland, England, Hungary, Germany, Sweden, Ukraine, Yugoslavia, Finland, Bulgaria, Bielorussia and Romania (summarized in Sikora 2006). *Laelaps agilis* is also polixenous ectoparasite with wide spectrum of hosts specificity. To this time this spesies was reported from: *Ondatra zibethica*, *Sorex araneus*, *Clethrionomys glareolus*, *Apodemus flavicolis*, *A. agrarius*, *A. silvaticus*, *A. microps*, *Pitymys subterraneus*, *P. tatricus*, *Microtus agrestris*, *Neomys fodicus*, *Crocidura leucodon*, *Sciurus vulgaris*, *Citellus citellus*, *Mus musculus*, *Glis glis*, *Micromys minutus* (summarized in Sikora 2006).

In the present paper we present the external morphology of both sexes of this species using scanning electron microscopy (SEM). The studies provided information on surface particular anatomical features of this species. The paper includes a SEM photographs of body view and ultrastructural details for the gnathosoma, idiosoma and legs. Morphology of *L. agilis* is illustrated here for the first time in detail.

Material and methods

The mites were collected on rodents (*Apodemus flavicolis*) captured alive from Wielkopolski National Park, July of 2003. The mites were recovered by hand from the host's coat, after the specimens were stored in 100% ethanol. Morphology of 5 specimens (3 females and 2 males) of *L. agilis* were studied using a scaning electron microscopy (SEM). Mites were fixed in glutaraldehyde for 24 hours, then rinsed with cacodylate buffer. Specimens were subjected to

critical point drying with CO2 as the transition fluid, sputtery coated with gold, and then examined using a field emission scanning electron microscope Zeiss Oberkochen DSM 940A3420 in Zoologisches Institut und Museum, Ernst-Moritz-Arndt Universitat in Greifswald (Germany). The terminology of external morphology follows those of Evans (1957, 1992), Evans and Till (1965, 1979) and Krantz (1978).

Results

Species of *L. agilis* are medium sized (600-700um) and well sclerotized mites. Their body is divided on sensory-trophic gnathosoma which are movable connected with flated dorsoventrally idiosoma (Figures 1 and 2).

Gnathosoma includes chelicerae, pedipalps, hypostome and tritosternum (Figure 3). The tectum forms the roof of the gnathosoma and overlies chelicerae (Figure 4). Lying above the buccal cavity, three segmented and retractible chelicerae are preoral trophic appendeages and primary organs of food acquisition. First segment (basal article) is connected with retractor muscles. Second segment (middle article) forming distally the fixed digit which bears hair-like *pilus dentilis*. Third segment is a dentate movable digit. The palps have six free podomers (trochanter, femur, genu, tibia, tarsus and two-pronged apotele) with well defined chaetotaxy (Figures 5 and 6). Palps are sensory appendeages equipped with a variety chemoreceptors and tactile setae. Beak-like hypostome supports the pre-oral trough and is divided into two lobes named as internal mala and external mala. Each of external mala bears antero-laterally horn-like structures termed as corniculus (Figure 7). They appear to act as guides for the chelicerae. The hypostome bears three pairs of subequal in length setae *hyp1-3* and a pair setae *pcx* (Figure 3). Basis capituli is formed by enlarged coxae of the palps which are separated mid-ventrally by shallow capitular groove with longitudinal series of small triangular and sharp-ended deutosternal denticles (Figure 8). Labrum is a long tapering structure situated between corniculi, with median longitudinal grove. Its surface is covered by the sensilla-like structures (Figure 7). Labrum is representing the anterior extension of the dorsal wall of thie pharynx.

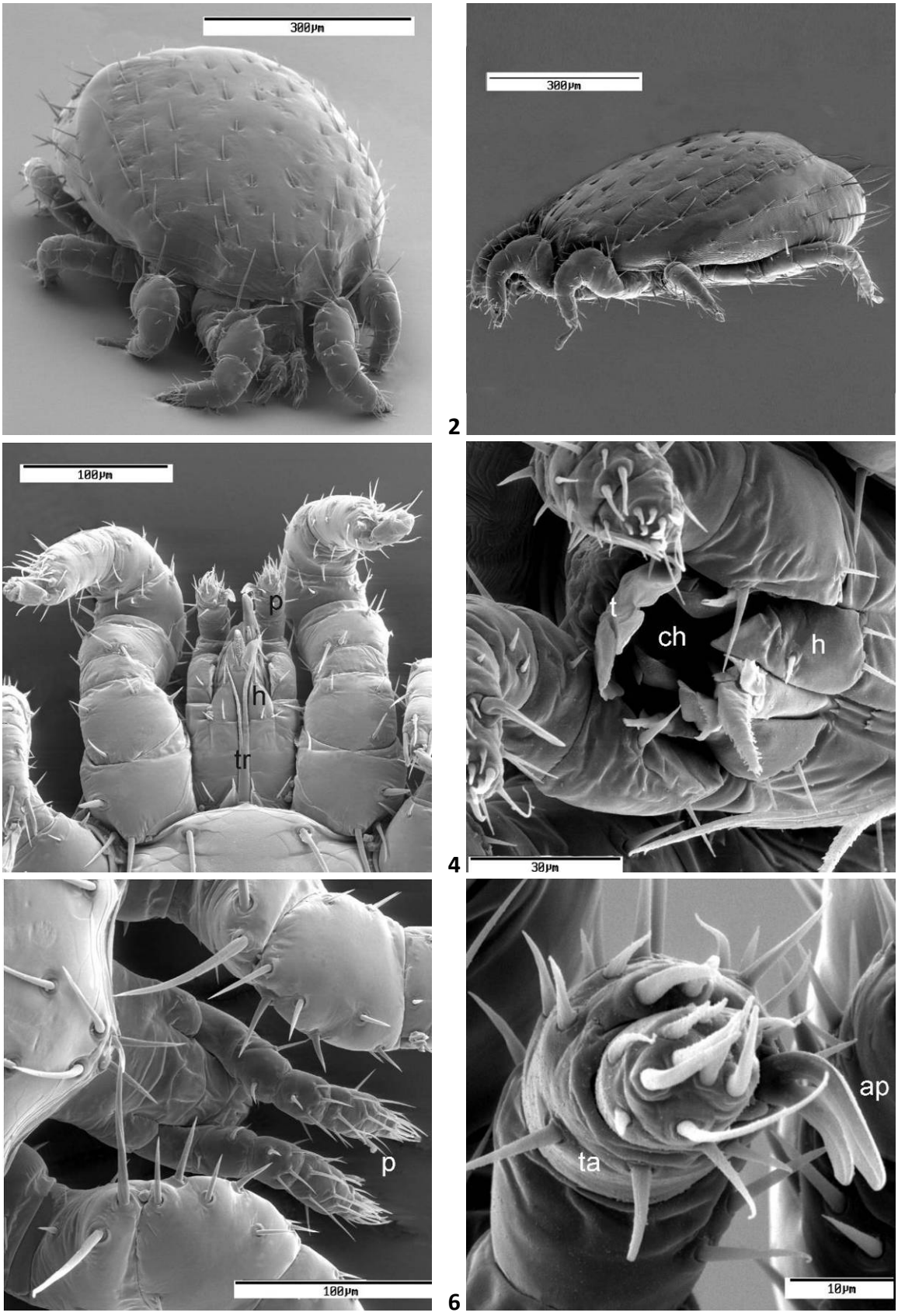
Biramous sensory structure – tritosternum is situated between coxae I and anterior to the sternal shield. It functions together with ventral gnathosomal structures as a fluid transport system during feeding and directing overflow prey fluids to the prebuccal region (Wernz, Krantz 1976). Each pilose laciniae of tritosternum has denticulate border and lie free in the hypognathal groove (Figures 3 and 8).

In both sexes the oval idiosoma is in colour ranges from yellow to dark brown. Its dorsal surface is covered by well sclerotized holodorsal shield (formed by the complete fusion of the podonotal and opisthonotal shield) ornamented by reticulate, unregular patterns of lines (Figures 9 and 10). This shield provided with a distinct pattern of sharp-ended setae arranged in four paired longitudinal series of setae: dorsocentral *J* and *j*, mediolateral *Z* and *z*, lateral *S* and *s* and marginal *R* and *r*. The submarginal series *UR* is restricted to the opisthonotal region. The dorsum bears also pore-like structures (Figure 11).

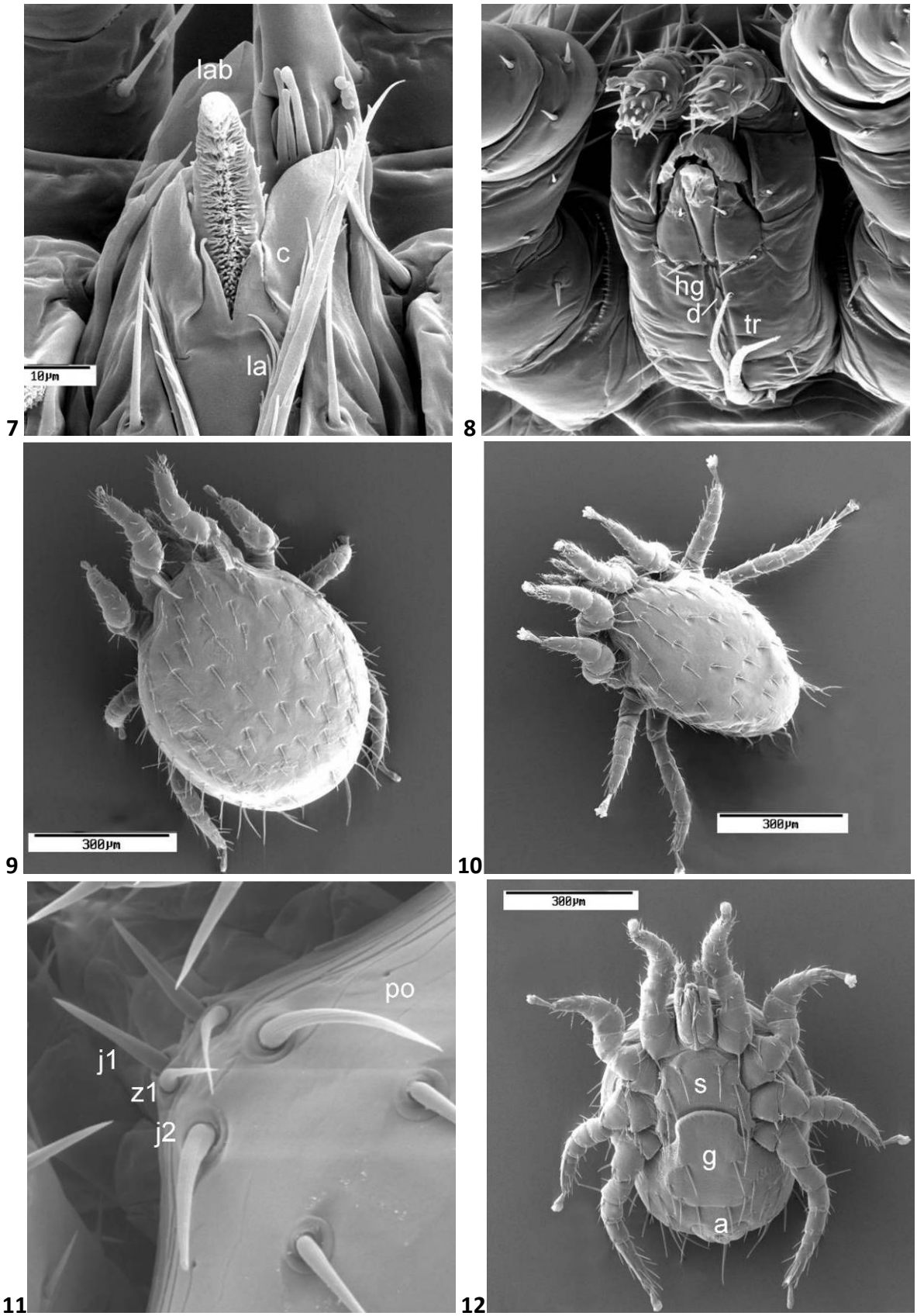
In females the ventral idiosoma is regionally sclerotized to form distinct shields (sternal, metasternal, genital and anal) (Figure 12) which are separated from each other by areas of striate and flexible cuticule. The sub-rectangular sternal shield with convex anterior margin, bears three pairs of setae *st1-3* and two pairs of lyriform fissures (*p1* and *p2*). The more or less pear-shaped genital shield with anteriorly hyaline flaps, bears four pairs of setae (*g, Zv1, Jv1, Jv2*). The female genital orifice is transverse slit situated at the level of the coxae IV (Figure 12). A pair of metasternal shields is situated lateraly to genital shield and between coxae III and IV. Triangular anal shield bearing three setae asociated with the anus (a pair adanal setae and an unpaired postanal seta). Posterior region of this shield is provided with numbers aciculae (Figure 13). In males the ventrum is covered by holoventral shield formed by the fusion of above mentioned shields (Figure 14). The genital orifice is situated presternally at the level of setae *st1*, and is protected and closed by a single valve (Figures 14 and 15).

The rounded posteriorly peritremal shield is fused anteriorly with holodorsal shield (in females) or free on anterior margin (in males). The stigma is situated between legs III and IV on either side of the idiosoma. Each stigma lie at the posterior end of long (in females) or short (in males) peritrematic chanel (Figures 16 and 17).

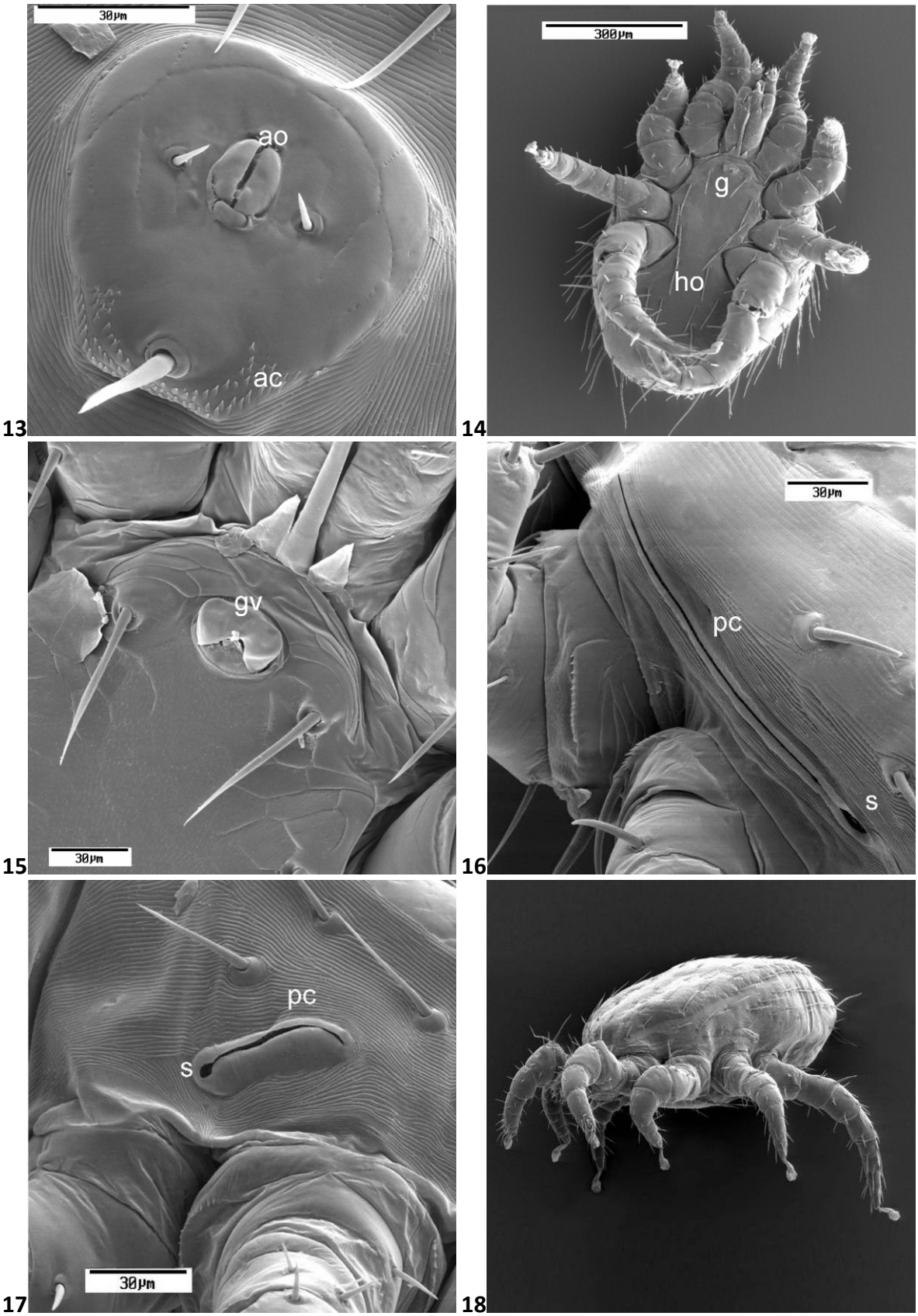
Legs are 7-segmented with terminal segment being represented by the ambulacrum comprising a pre-tarsus, a pair of claws and lobate pulvillus (Figure 18). The striated, sclerotized components of the pretarsus form a rigid sheath (Figures 19 and 20). The ambulacra of legs I are shorter than those on the other legs. Paired of small claws are present on



Figures 1 – 6. *Laelaps agilis*. Female. Fig. 1. Antero-dorsal view. Fig. 2. Lateral view. Fig. 3. Gnathosoma and legs I in ventral view (tr – tritosternum, h – hypostome, p – pedipalps). Fig. 4. Gnathosoma in latero-ventral view (h – hypostome, t – tectum, ch – chelicerae). Fig. 5. Gnathosoma in dorsal view (p – pedipalps). Fig. 6. Pedipalp in anterior view (ta – tarsus, ap – apotel).



Figures. 7–12. *Laelaps agilis*. Fig. 7. Part of gnathosoma of female in ventral view (l – labrum, la – laciniae of tritosternum, c – corniculus). Fig. 8. Gnathosoma of female in antero-ventral view (tr – tritosternum, hg – capitular groove, d – deutosternal denticles). Fig. 9. Female in dorsal view. Fig. 10. Male in dorsal view. Fig. 11. Anterior part of holodorsal shield of female with setae *j1*, *z1* and *j2* (po – pore). Fig. 12. Female in ventral view (g – genital shield, s – sternal shield, a – anal shield, ge – genital orifice).



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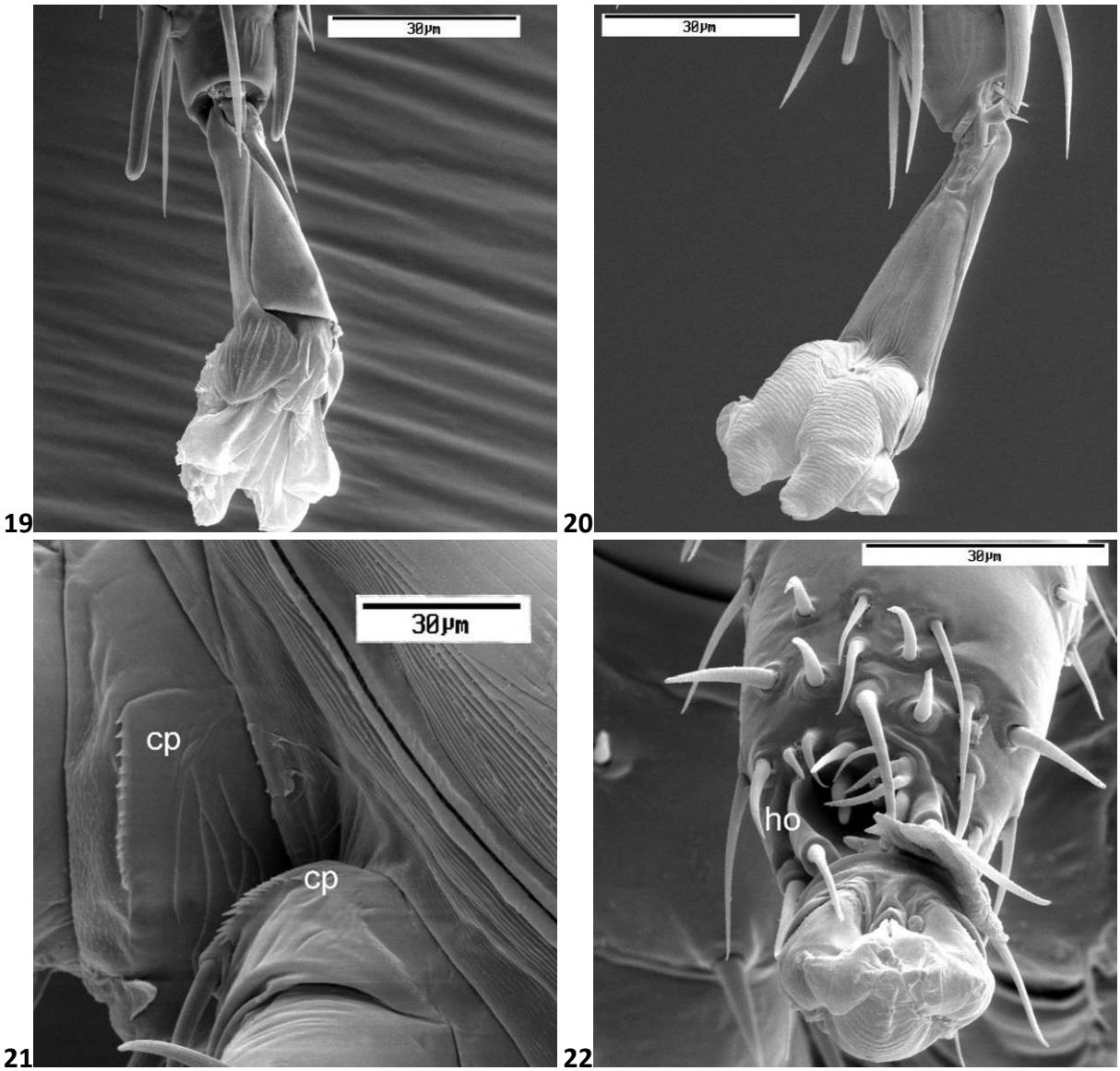
Figures. 13–18. *Laelaps agilis*. Fig. 13. Anal shield of female (ao – anal orifice, ac – aciculae). Fig. 14. Male in ventral view (g – genital orifice, ho – holoventral shield). Fig. 15. Genital orifice of male (gv – genital valve). Fig. 16. Peritreme of female (pc - peritrematic chanel, s – stigma). Fig. 17. Peritreme of male (pc - peritrematic chanel, s – stigma). Fig. 18. Male in lateral view.



Figures. 19 – 22. Laelaps agilis. Fig. 19. Pulvillus in ventral view. Fig. 20. Pulvillus in dorsal view. Fig. 21. Cuticular processes on coxae (cp) of legs I and II of female. Fig. 22. Tarsus of leg I of female (ho – Heller-like organ).

all legs. The legs IV are longer than I-III. The segments from coxa to tarsus have a well defined chaetotaxy. The most of leg setae are simple in form and sharp-ended but some setae especialy on coxa, trochanters and tarsus are enlarge, stout and spur-like structures. Cuticular processes occur at the distal margins of the basal podomers of legs (Figure 21). Legs I is mainly sensory in function and movements at the joints differ in number of setae from those of II-IV, especially on tarsus I where is situated Heller-like organ flanked by numerous setae (Figure 22).

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PHENOTYPIC PLASTICITY IN DEVELOPMENTAL TIME AND BODY SIZE INDUCED BY FOOD LIMITATION IN THREE PHYTOSEIID MITE SPECIES

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Abstract

Juvenile phytoseiid mites born in spider mite patches may essentially adopt two strategies to cope with local food limitation and optimize the trade-offs between survival, age and/or size at maturity: (1) disperse before prey depletion and search for other food resources or (2) remain in the patch until complete prey depletion. We conducted food limitation experiments with three phytoseiid predators differing in their degree of specialization on spider mites *Phytoseiulus persimilis* is highly specialized on spider mites, *Neoseiulus californicus* is a generalist predator with a preference for spider mites, and *Amblyseius andersoni* is a generalist predator without preference for spider mites. Single juveniles of each species were confronted with different prey densities (spider mite eggs) and survival, dispersal (via escaping rates), development and body size of adult females (dorsal shield length) were evaluated. *Amblyseius andersoni* had the highest escaping and mortality rates, followed by *N. californicus* and *P. persimilis*. Food limitation led to faster development in *N. californicus* and *P. persimilis*, whereas development of *A. andersoni* was not influenced by prey densities. Irrespective of species, body size was a function of prey density with smaller body size at decreasing prey densities. Altogether, it seems that *P. persimilis* and *N. californicus* but not *A. andersoni* are able to partially compensate the putative costs of small body size induced by food limitation through faster development. The observed differences in life history of *A. andersoni*, *N. californicus* and *P. persimilis* under food-stressed conditions are discussed in relation to their degree of prey specialization.

Key-words

Life-history evolution, ephemeral food resources, age and size at maturity, survival, dispersal.

Introduction

Life in environments with unpredictable food resources favours adaptive plasticity in key life history components such as age and size at maturity (Stearns & Koella 1986; Roff 1992, Stearns 1992). To maximize juvenile survival under food limitation growth is typically reduced (Abrams *et al*. 1996), which affects age and/or size at maturity (Stearns & Koella 1986). In arthropods three different patterns are reported from literature: (1) most commonly, food limitation prolongs juvenile developmental time and reduces body size at maturity (Knisley & Juliano 1988; Ball & Baker 1995; Dusse & Hurd 1997; Agnew *et al*. 2002; Mikolajewski *et al*. 2005); (2) food limitation does not affect developmental time but reduces size at maturity (Plaistow & Siva-Jothy 1999; Engqvist 2007); (3) food limitation shortens developmental time and reduces size at maturity (Blanckenhorn 1998, 1999).

We assessed the effects of food limitation on dispersal, survival, developmental time and body

size at maturity of the phytoseiid mites *Phytoseiulus persimilis* Athias-Henriot*, Neoseiulus californicus* McGregor and *Amblyseius andersoni* Chant (Acari: Phytoseiidae). These three species constitute a natural guild in Sicilysharing spider mites as prey, but they differ in diet specificity with *P. persimilis* being highly specialized, *N. californicus* being intermediate and *A. andersoni* being the least specialized predator of spider mites (McMurtry & Croft 1997). Spider mites of the genus *Tetranychus* display rapidly succeeding phases of host plant colonization, population growth, dispersal and local extinction (Sabelis 1985a) and are thus an ephemeral food resource for their predators.

Ovipositing predatory mite females deposit their eggs in or close to spider mite patches. Juvenile phytoseiid mites born in spider mite patches have essentially two possibilities to cope with limitations in local spider mite availability: (1) leave the spider mite patch early (before complete depletion) and search for other food resources, or (2) remain in the prey patch until depletion and search for other food resources thereafter. Both strategies have critical consequences on life history traits such as survival, development and body size at maturity. Optimal foraging theories (McArthur & Pianka 1966, Charnov 1976) predict that the first strategy should be more common in generalists, which are able to switch to other food types than spider mites, whereas the second strategy should be more common in specialists. Consequently, everything else being equal at low spider mite densities dispersal rates should be higher in *A. andersoni* than in *N. californicus* than in *P. persimilis*. The theory of life-history evolution in rapidly expanding non-equilibrium populations such as *P. persimilis* predicts that high juvenile survival chances linked with short developmental times should be favored by natural selection (Roff 1992; Stearns 1992). Thus, developmental time of *P. persimilis* should be less affected by food limitation than that of the two generalists, *N. californicus* and *A. andersoni,* with the latter being the least specialized spider mite predator (McMurtry & Croft 1997). Irrespective of species affiliation and diet specificity low prey densities should entail a negative effect on body size. To test the above assumptions, we reared single juveniles of each species at various prey densities and recorded their escaping tendencies (indicative of dispersal), survival, development and body size (dorsal shield length) after reaching adulthood.

Materials and methods

Species origin and rearing

All three predators, *P. persimilis*, *N. californicus* and *A. andersoni,* are indigenous species in Sicily (De Moraes *et al*. 2004) where they were sampled in the State Trapani in 2007. Sampled specimens were used to initiate populations reared in the laboratory. Rearing units consisted of water-saturated foam cubes in plastic boxes half-filled with water. Plastic tiles (*P. persimilis*, *N. californicus*) or detached bean leaves with the lower side up (*A. andersoni*) were placed on the foams. Cotton wool fibers under cover slips served as shelter and oviposition site for *A. andersoni* on the bean leaves. The edges of the tiles or leaves were covered with moist tissue paper to confine the predators to the rearing arenas. To prevent contamination of the predator colonies the rim of the plastic boxes was lubricated with Raupenleim® (Avenarius Agro) and each plastic box was placed in a tray containing water with dishwashing detergent. The predators were fed in two to three day intervals by adding bean leaves infested with *Tetranychus urticae* Koch (Acari: Tetranychidae) (for *P. persimilis, N. californicus*) on to the artificial arenas or by brushing mixed spider mite stages from infested leaves on the detached bean leaves (*A. andersoni*).

Experimental units

Each experimental unit consisted of a single detached bean leaf (mean area ~4 cm^2) placed on a water-saturated foam cube in a plastic box half-filled with water. Leaf area was delimited by strips of moist tissue paper. To obtain a given prey density 1 to 4 spider mite females were allowed to oviposit on each experimental unit for 24h. Afterwards the number of spider mite eggs was adjusted to the predetermined density (6, 8, 10, 12, 16, 18, 20, 24, 28, 32, 36, 40, 44 and 48 for *A. andersoni* with 18-39 replicates per treatment; 5, 6, 8, 10, 12, 14, 20, 28 and 32 for *N. californicus* with 17-24 replicates per treatment; 5, 6, 8, 10, 12, 14, 16, 20, 28 and 32 for *P. persimilis* with 16-24 replicates per treatment). Webbing produced by the spider mite females was carefully removed and single predator eggs (<36h old) of *A. andersoni*, *N. californicus* or *P. persimilis* were placed on the bean leaves. The developmental progress of the mites was observed twice per day in 8 and 16 h intervals until they reached adulthood, died on the leaves or escaped. The number of consumed spider mite eggs was also counted at each observation date. After reaching adulthood the specimens were mounted in a drop of Hoyer's medium and the microscope slides were dried at room

temperature for two days. Then the dorsal shield length of females was measured under the microscope at 200X magnification.

Statistical analysis

All statistical analyses were performed with SPSS for Windows 15.0 (SPPS Inc. 2006). For each species, the influence of prey density on escaping and survival and the influence of the predation rates on the developmental time and dorsal shield length of adult females were analyzed with linear or sigmoid regression . Multivariate analysis (MANOVA) followed by Bonferroni was used to compare female predation rates and developmental times (pooled over prey densities) among species.

Results

Escaping rates, survival probabilities and prey needs for juvenile development

In all predator species, escaping rates decreased and survival probabilities increased with increasing prey densities (Figure 1).

Table 1. Development (from larva to adulthood) and predation (mean ± standard deviation) of juvenile *P. persimilis*, *N. californicus* and *A. andersoni* pooled over prey densities allowing > 75% survival and results of analyses of variance.

Species	Development (days±SD)[1]	Predation (spider mite eggs/day±SD)[1]
A. andersoni	5.50±0.9a	33.28±9.2a
N. californicus	3.48±0.4b	10.06±4.1b
P. persimilis	2.97±0.4c	9.90±4.9b
ANOVA		
d.f. 1,2	2, 166	2, 166
Mean square	90.61.	8834.45
F ratio	342.85	436.75
P	<0.001	<0.001

MANOVA: Pillai trace: d.f. = 2, F= 133.60, P< 0.0001
[1]Different letters accompanying means indicate significant differences among species (post hoc Bonferroni tests).

Mean developmental times pooled over prey densities differed significantly among predator species with the shortest time for *P. persimilis* and the longest for *A. andersoni.* Mean predation rates pooled over prey densities were significantly higher in *A. andersoni* than in *P. persimilis* and *N. californicus* (Table 1). We repeatedly observed that *P. persimilis* and *N. californicus* usually completely sucked out the spider mite eggs, leaving only the empty egg chorion, whereas *A. andersoni* often only partially sucked them out, leaving collapsed eggs.

The effects of food limitation on female developmental time and dorsal shield length

We analyzed the influence of prey consumption on juvenile development and dorsal shield length only at high survival probabilities (>75% survivors) to exclude inadvertent artificial selection on these traits. Prey consumption and developmental time of *N. californicus* and *P. persimilis* were positively correlated below the critical thresholds of ~12 eggs for *P. persimilis* and 20 eggs for *N. californicus*. In contrast, developmental time of *A. andersoni* was not affected by predation rates. In all species, the length of the dorsal shield was a linear to C-shaped function of the consumed prey number with longer shields at higher predation rates (Figure 1).

Discussion

Amblyseius andersoni, *N. californicus* and *P. persimilis* responded differently to food limitation in terms of escaping, survival and prey needs for juvenile development and these differences were related to diet specialization. As predicted, the highly polyphagous predator *A. andersoni* had high escaping rates at low prey densities and the survival probabilities of individuals staying in the patch were rather low. Similar to Sabelis (1981) and Zhang & Croft (1994) we observed that *A. andersoni* juveniles had difficulties in piercing the egg chorion and many eggs were only partially consumed. Thus, the high number of prey eggs needed (including partially consumed eggs) for successful development indicates low profitability of spider mite eggs, due to inefficient handling, for *A. andersoni*. This generalist predator has a preference for spider mite larvae over eggs (Blackwood *et al*. 2001). Their mouthparts seem well adapted to overwhelm mobile prey and to crush pollen but not to pierce spider mite eggs (Flechtmann & McMurtry 1992). Therefore, leaving a patch with an inadequate prey type early seems an appropriate strategy of *A. andersoni* to maximize juvenile survival. The spider mite specialist *P. persimilis* and the generalist but spider mite preferring predator *N. californicus* had much lower escaping rates and higher survival probabilities than *A. andersoni*. The former two species were more efficient in converting food into growth and development, which was reflected in lower prey needs and faster development. However, the response of juvenile *P. persimilis* and *N. californicus* to food limited conditions seem to

reflect the differing degree of prey specialization: (1) at the lowest prey density tested (5 spider mite eggs) 75% of *P. persimilis* juveniles but only 50% of *N. californicus* reached adulthood, (2) the escaping rates of *P. persimilis* were lower and (3) similar consumption rates led to a significant faster development in *P. persimilis*.

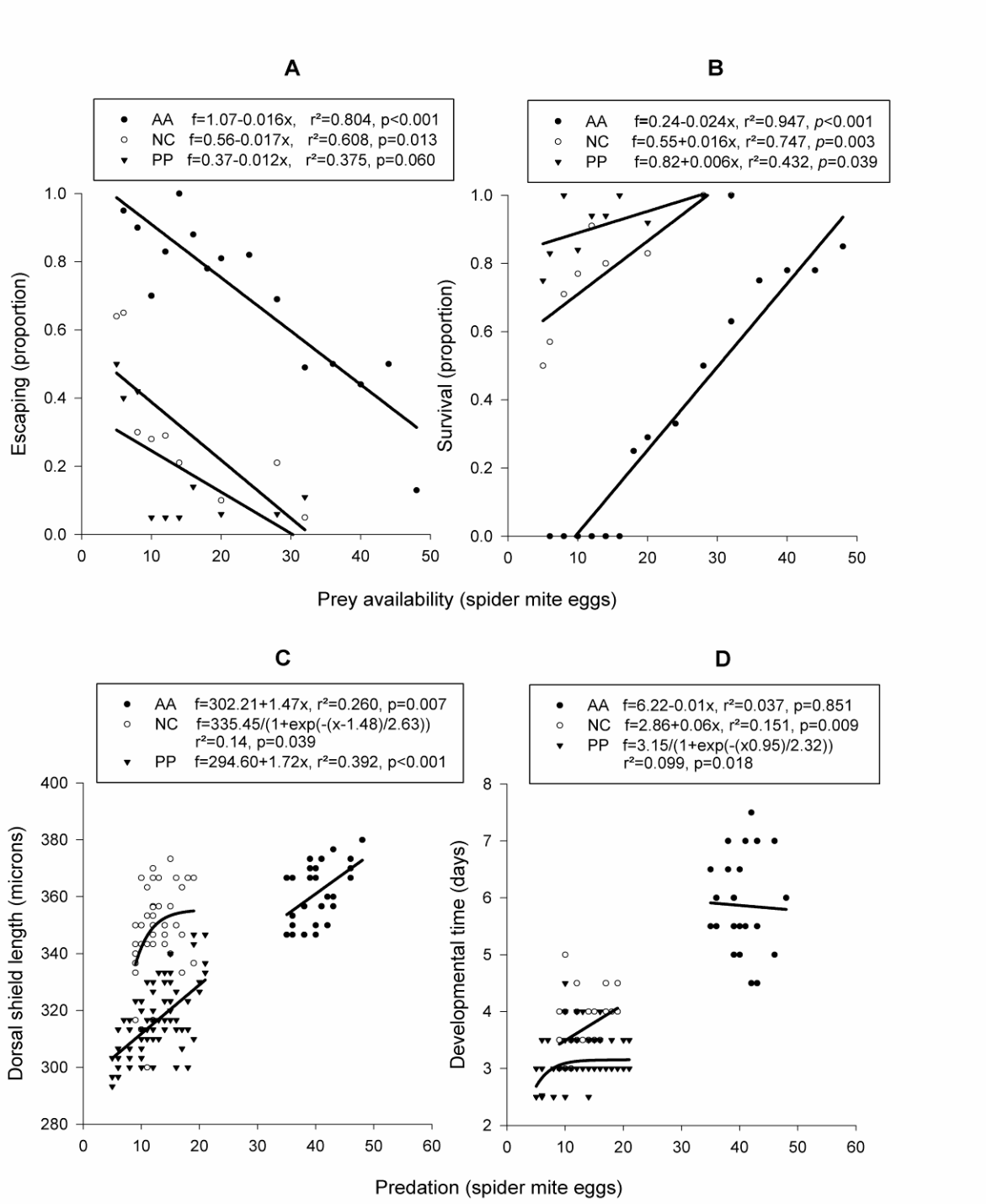

Figure 1. Escaping (A) and survival (B) of *Amblyseius andersoni* (AA), *Neoseiulus californicus* (NC) and *Phytoseiulus persimilis* (PP) juveniles at different prey densities and the effects of prey consumption on body size (C) and development (D) of females of these species.

Age and size at maturity under food limitation

Our results on age and size at maturity partly disagrees with our assumptions and the general prediction of life history models that under food limitation smaller body size should be linked with prolonged developmental times (Berrigan & Charnov 1994; Atkinson & Sibly 1997). As predicted, food limitation reduced female body size in all three species. However, under food limited conditions *P. persimilis* and surprisingly also *N. californicus* flexibly adjusted their development and reached maturity earlier at a smaller size, whereas the developmental time of *A. andersoni* was not influenced by prey densities. These results differ from those obtained by Sabelis (1981) and Zhang & Croft (1994) who found that juvenile development of *P. persimilis*, *Typhlodromus pyri* Scheuten, and *A. andersoni* was prolonged at low prey densities. However, the experimental protocol used by Sabelis (1981) and Zhang & Croft (1994) differed crucially from our experiments. In their experiments the juvenile phytoseiid mites were provided with low but constant prey densities by replenishing the consumed prey in regular time intervals. Under these conditions the juveniles can consume similar quantities of prey items during development at low and high prey densities just over differing time periods. Such homogeneous prey patches may occur when adult spider mite females constantly add eggs to the patch and are not preyed upon by the predators. However, spider mite patches often constitute finite food resources for their predators with prey densities diminishing over time. Our experiments were based on the latter scenario.

The shortened or constant developmental times at food limitation observed in our study raise two important questions: (1) why are the developmental times of the three predators not prolonged as is the case in most animals? (2) what are the possible benefits of faster development at food limitation? First, as in most phytoseiid mites the juvenile developmental phase is only a short period of life in *A. andersoni*, *P. persimilis* and *N. californicus* with a proportion ranging from 7 to 11% of the total lifespan (Amano & Chant 1977; Gotoh *et al*. 2004) indicating that phytoseiid mites are under severe selection pressure for rapid development (Sabelis 1985b, c). Second, food limitation reduced body size at maturity in all three species but shortened development only in *P. persimilis* and *N. californicus*. Faster development at diminishing food availability has also been reported from tadpoles, dung flies and seed beetles (Moller *et al*. 1989; Newman 1992; Fox *et al*. 1994; Blanckenhorn 1998, 1999). A striking similarity of these and our experimental organisms is the ephemeral food resource experienced by the juveniles. The observed plasticity in age at maturity may generally evolve in environments where the distribution, quality and quantity of food patches vary unpredictably in space and time, like those experienced by predators adapted to exploit transient spider mite patches such as *P. persimilis*. Interestingly, we found the same life history pattern in the generalist *N. californicus* probably reflecting its preference for spider mites among the range of accepted food types. In contrast, *A. andersoni* was not able to adjust its development under food limitation indicating low adaptation to spider mite prey (McMurtry & Croft 1997). Moreover, fast development at food limitation may be generally advantageous in several respects: (1) increased probability to reach adulthood because of the short juvenile period, (2) allowing earlier dispersal from low quality prey patches to high quality patches, and (3) earlier mating and reproduction (Stearns 1992). In contrast, it is generally assumed that reduced female body size has negative effects on individual (prolonged preoviposition period, lower fecundity, male preference for larger females, inferiority in contests over resources leading to increased mortality (Andersson 1994)) and/or offspring fitness (negative maternal effects on offspring survival probabilities and body size (Rossiter 1996)).Thus, it seems likely that *P. persimilis* and *N. californicus* are able to partially compensate the putative costs of small body size by faster development. Future studies should clarify whether and how small female size induced by food-stressed conditions during development affects fitness in these species.

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GLOBAL CHANGE AND BIOINVASIONS

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ADAPTATION IN PARASITIC MITES: SPREAD BY THE HOST OR STAY WITH THE HOST?

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Abstract

Pterygosomatidae often show a morphology strongly adapted to life between scales of reptiles, as ectoparasites on some few hosts, eventually specialized on a single host species. The host distribution can be limited (endemic species, i.e; insular species), or wide (cosmopolitan species). Some host species are invasive species, especially in insular habitats. Two examples are chosen: endemic lizards from New Caledonia, and from Mauritius Island house parasitic mites. The invasive lizard (*Hemidactylus frenatus*) brought recently in these ecosystems new loads of parasites belonging to wide spread species: however no parasite transfer was observed. From biogeographic point of view, if compared with other associated hosts and parasites in Pacific Islands or Mediterranean Regions, paleoendemics or neoendemics are more protected against invasive parasite species than invasive hosts, which could capture local parasites in recently invaded countries. However native host species could be more sensible to parasites in presence of invading species.

Keywords

Biogeography, host parasite relastionship, Prostigmata, ectoparasites

Introduction

Pterygosomatidae are specialized on reptiles. The morphology reveals more or less high degree in adaptation (body shape, relative length and shape of legs…). The genus *Geckobia* Mégnin, 1878 is parasitic (exclusive) on Geckoes. The hosts and parasites are well distributed in subtropical and intertropical regions, and hosts belong to three families = Gekkonidae, Pygopodidae and Eublapheridae (Gekkonoidea) (Bochkov & Mironov 2000) (Classification of Gekkota according to Han, Zhou & Bauer 2004). In the genus *Geckobia* several groups of species, notably defined by leg chaetotaxic pattern (Jack, 1964).

The genus *Geckobia* illustrates the problem of strength of the links between host and parasite for at least three reasons:

The parasites are specialized: the genus differentiated on geckoes *sensu lato*, this adaptation being traduced in morphology, by host choice and by location of mites on the host: ventral, dorsal or between scales on the toes… corresponding to morphological types (Hirst 1917);

The distribution of the hosts: some of them are distributed in Pacific and Indian Ocean(Figure 1). Insular faunas are with high rate of endemism, notably the Gondwanian group (Diplodactylini, and notably Australian species), or Ethiopian subgroups (*Afroedura*)…

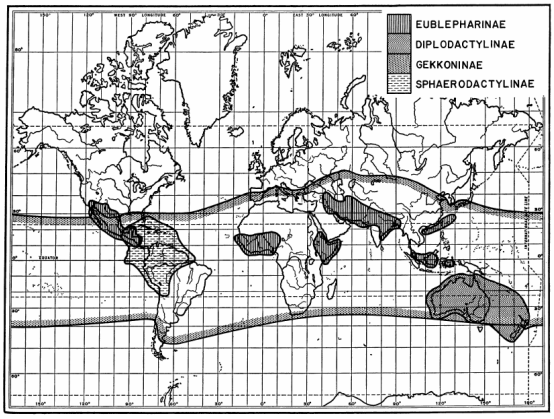

Figure 1. Distribution of Gekkota (Kluge, 1987)

The invading cosmopolitan species of geckoes: tropical islands are invaded by species, helped by anthropic dispersion often more important than natural dispersion. *Hemidactylus frenatus* (Schlegel, 1836), the Asian house gecko, is very adaptable, displacing other reptiles and disseminated all around the world under hot and Mediterranean climatic conditions (Jesus, Brem & Harris 2005) (Figure 2). This invader is known to be infested by some species of *Geckobia* (*G. bataviensis* Vitzthum, 1926 *(=manzanelli)*, *G. dubium* Bertrand, Paperna & Finkelman, 2000, *G. amambavyensis* Haitlinger, 1988, *G. clelandi* Hirst, 1917…), with "primitive" morphology.

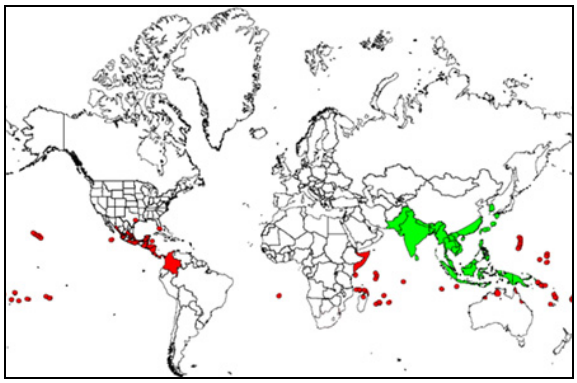

Figure 2. Distribution of *Hemidactylus frenatus:* Native (green) and colonized regions (red).(Cole 2005)

Invasive hosts can be suspected to capture pterygosomatid mites from local reptiles, and to disseminate blood sucking ectoparasites (and zoonoses…). Is there a real "barrier" around the native host and its “specialized” parasites?

Some elements can be given for three questions by analyzing insular faunas:

A) In natura, can the parasite of invasive host switch on endemic hosts?

B) Are the parasites of invading species preying on endemic host? (because the common characters of the invasive species is to be cosmopolitan, and euryecic).

The subsidiary question is:

C) Which consequences on the survival of the endemics (change in fitness) facing the invaders?

New short time data are given by studies on changing fauna. In Pacific and Indian Oceans, herpetological fauna is disturbed by on going invasions, with progressive extinction of endemics. So, how ectoparasites as pterygosomid mites react to this phenomenon?

Material and methods

New Caledonia is peopled by Gondwanian lizards, sharing the same origin than Australian reptiles. Few data are available on the parasite cortege (Womersley, 1941). Mauritius Islands are peopled by endemic reptiles differentiated from continental fauna. Two surveys of parasites were carried on in the general context of the ongoing invasion of the Asian house gecko.

1 The hosts and the parasites

New Caledonia: Amongst the endemic lizards, the genus *Bavaya* Roux, 1913 (Diplodactylini) includes small endemic species being considered as a complex of cryptic or morphologically distinct species from New Caledonia (Bauer *et al* 2000). Until now, none pterygosomatid mites were reported from this genus. *Geckobia naultina* Womersley, 1941 was described on *Naultinus* sp., a species belonging to the same group of Oceanian lizards. *G. gymnodactyli* Womersley, 1941 *G. clelandi* Hirst, 1917 (on *Saltuarius cornutus*) and *G. manzanelli* Domrow, 1983 and *G. haplodactyli* Womersley, 1941 (mismatch) from *Hoplodactylus duveaucelli* (Duméril & Bibron, 1836) from Auckland (New Zealand) were reported from Diplodactylini.

Mauritius Islands: Two genera were studied: 1) the genus *Nactus* Kluge, 1983 (Pacific and Indian Oceans) is present on Mauritius Islands with several endemic species or subspecies (*Nactus serpensinsula durrelli*, Arnold & Jones, 1994, *Nactus serpensinsula serpensinsula*, (Loveridge, 1951), *Nactus coindemirensis* Bullock, Arnold & Bloxam, 1985). Until now, no pterygosomid mite was described hosted by these species. The genus *Phelsuma* Gray 1828 is present in Africa, Madagascar, and Mascarene Islands in Indian Ocean. On Mauritius Islands, Day Geckoes are present with *Phelsuma ornata* Gray, 1825, *Phelsuma guentheri* Boulanger, 1885, *Phelsuma*

cepediana (Merrem, 1820). In previous studies, some *Geckobia* were collected from Madagascan lizards (Haitlinger 1988). In the traditional systematic, *Phelsuma* and *Nactus* were in the same group of genera than *Hemidactylus. Phelsuma* is the genus phylogenetically closer to *Hemidactylus* among all these genera (Han, Zhou & Bauer 2004).

The invader in both cases is *H. frenatus* (Schlegel, 1836), species widely distributed (Figure 2) in tropical and subtropical regions, often found in houses. Parasites were described on *H. frenatus*, (some *Geckobia* can be considered as synonymous by Domrow 1983) the main parasite being *G. bataviensis* Vitzthum, 1926, that cannot be considered as specialized by its morphology. The house gecko is responsible of past and actual disturbance to *P. ornata* and *Nactus* spp. by habitats exclusion, competition for shared habitat and introduction of debiliting parasites (Cole 2005). In past time, these competitions led to actual distribution of species and subspecies in these genera.

2 Collecting hosts and parasites

New Caledonia: (D. Modry collector)

Ectoparasites were collected on the two genera *Rhacodactylus* Fitzinger, 1843 and *Bavaya* Roux 1913, that are closely allied with the New Zealand endemic lizards: the chromosomes of *R. auriculatus, R. sarasinorum* and *B. sauvagei* share a highly derived biarmed 2n=38 karyomorph, which is common to species from both New Zealand and Australia and is believed to be a relic from a previous Gondwanaland distribution. In contrast, *R. leachianus, B. crassicollis* and *B. montana* have karyotypes further modified from this karyomorph by a series of presumed pericentric inversions. (King & Mengden 1990). The Diplodactylini diverged from the remaining taxa prior to the opening of the Tasman Sea and the opening of the Tasman Sea split *Pseudothecadactylus (*Bronsgerma, 1836*)* from the New Caledonian + New Zealand lineage.

Trombiculid mites were frequent but only few scale mites were collected on *Bavaya montana* Roux 1913, *sauvagii* (Boulanger,1883) and *cyclura* (Bavay, 1879*)*, and solely one species of *Geckobia* was collected on *Bavaya* spp.

Mauritius Island: (N. Cole collector)

Lizards were captured in the general frame of a study on competition between local and endemic fauna and invasive lizards. A large number of lizards were captured (more than 400/species), ectoparasites were trombiculids and *Geckobia* specimens.

3 Preparation of material:

Mites were collected from lizard and stored in 70% ethanol. Mites were cleared in hot lactic acid and observed on temporary slides under microscope. The gnathosoma, mouthparts and legs were dissected with pins in lactic acid. Intact mites or dissected structures were mounted in lactic acid on concavity slides (depth 0.6 mm) or on permanent slides in Hoyer's medium. Coloration with Chlorazol Black B was used as a dye. Mites were preserved for descriptions (holotype and paratypes).

Results

Three new for science *Geckobia* were collected : one species on *Bavaya* spp., one on *Nactus* spp., and one on *Phelsuma* spp.; *H. frenatus* on Mauritius Island was found infested by *G. bataviensis* Vitzthum 1926. None *G. bataviensis* was found on *Bavaya* spp., *Nactus* spp. *Phelsuma* spp. None New Caledonian or Mauritian parasite was found on *Hemidactylus frenatus.*

1. The affinities revealed by morphology of the endemic *Geckobia*

(Description of these species will be complete in separate publications)

1.1. The Parasite of *Bavaya* spp. (New Caledonia)

The characters considered as "primitive" are marked in bold letter

Adult female: small species; body enlarged in the posterior part with lobate posterior end. Length without chelicerae (from anterior idiosoma margin to extremity of genital area) 275 µm long (range 250-305 µm); maximal width 230µm (285-170 µm). Triangular scutum, slightly striated with two central pairs of setae, and a posterior pair in median position. Three genital setae. Ventral side with non modified setae. Coxae in two contiguous groups, directed forward with setation [4(=2+2)—5(=2+3)]. Legs chaetotaxy (tibia)-(genu)-(femur)-(trochanter): (5-5-5-5) (1-0-0-1) (2-1-1-1) (1-1-1-1) typical of *Geckobia*'s group 2 (Jack, 1964). Tarsi with usual setae and solenidia corresponding to the Jack's species group A (Jack, 1964).

Characters of this species: One of the smallest species in the genus. It could be confused with *G. boulengeri* Hirst 1917 by body shape, but differs by the smaller size, the density of dorsal setae and no ventral scale-like setae. This species presents a common formula of leg chaetotaxy considered as primitive, a body not greatly modified (body as long

as large), has kept a minute scutum with ocular lens in lateral position. Strong spurs on coxae II, III, IV. The leg chaetotaxy which is the character with the strongest phylogenetic significance, differs from New Zealand species parasitic on *Naultinus* spp. by presence of the seta on genu IV corresponding to the general formula of the Jack's group 1 (Jack 1964). It differs by the position of the dorsal seta of tibia which is backed compared to the usual distal position: *boulangeri*). It differs by coxal setation with three setae on coxae IV (only 2 on *G. naultina* or *G. haplodactyli*); three setae on fourth coxa were found on *G. manzanelli* Domrow 1983 (parasite on *Phyllurus platurus* in New Guinea), and on *G. gehyrae* Hirst 1926, (on *Gehyra oceanica* (Von Tilenau, 1820)), *G. gibbonsi* Bertand & Ineich 1987 (on *Lepidactylus* sp.) and on *G. gymnodactyli* Womersley 1941, all these species being distributed in the Australian region.

1.2. Mauritian species on *Phelsuma* and *Nactus* spp.

The two species found on *Phelsuma* and *Nactus* specimens are easily identified,notably because dorsal setae are different.

Nactus parasite. Female: with long dorsal setae, subcircular posterior edge of scutum, 10 stout scutal setae symmetric with two pairs of posterior setae and 3 or 4 anterior setae. Strong PIV, with spur seta on femora. Epimera with 2-2-2-3 setae, simple on coxae 1. Legs : 5-5-5-5 , 1-0-0-1, 3-2-2-2, 1-1-1-1. *Phelsuma* parasite. Female: *Phelsuma* spp. were found infested by one species with well developed eyes, with dorsal setae differentiated in shape, legs with articles distinct, no spurs on femora IV but with long ciliate setae directed forward. Each epimeral plate with long setae (no stout setae or "spurs"). Epimeral formula is (2-2-2-1 left, 2 right). Legs: 5-5-5-5, 0-0-0-0, 2-1-1-1, 1-1-1-1.

Characters of these species: G. manajaryensis Haitlinger, 1988, G. ifanadianaensis Haitlinger, 1988 and G. andoharonomaitsoensis Haitlinger, 1988 were described from Madagascan Phelsuma sp. The species found in Mauritius on day-gecko differs greatly from the Madagascan material by epimeral chaetotaxy, scutum, dorsal hairs, ventral setae. On Nactus spp., the species found has the same leg chaetotaxy than G. gleodoviana Hirst 1926. A doubt subsists because of confusion in the name of the host being considered now as H. mabouia brooki (= H. tasmani) on which G. tasmanii Lawrence 1936 was described. H. mabouia is African but widespread by human activities. However this species differs by setae on femora and leg chaetotaxy, scutum. 3 setae on the genu I showed conservation of a primitive character shared by Jack's group. Chaetotaxy of parasite found on Phelsuma is shared by the Jack's group 2 that gathers a lot of parasites of Indian and Pacific Islands on endemic hosts.

Discussion

Several groups of species in the genus *Geckobia* were defined by:
a) *The leg chaetotaxy* (Jack 1964), (Annexe Key 2)
b) *The presence of simple or scale-like ventral setae*,

c) *The shape of the scutum* (rectangular triangular, entire or divided),
d) T*he position of ocular formations* (on the scutum, contiguous to the scutum or in lateral position),

e) *The shape of the setae* on the body or on the legs, ciliate or simple setae on palpal tibia,
f) *Contiguous, or clearly separated coxae* [I+II] and [III+ IV],
g) *The number and shape of coxal setae*.

1 Neo Caledonian species

Oceanian fauna is known essentially through Hirst's (1917, 1926), and Womersley's works (1941), and more recently, by publications of Domrow (1983), and Bertrand & Ineich (1986, 1987, 1989).

Domrow (1983) described a species found on the Diplodactylinae *Phylllurus platurus* (Shaw, 1790), the Australian gecko. This parasite shared coxal setation, the discrete scutum, the eye presence with the new species parasitic on *Bavaya* genus. Domrow (op. cit.) noted that the dorsal seta on tibia I was not so basad as in the description of *G. boulangeri* as for *G. bavayae.* Combination of characters made this latter species allied to New Zealand parasites found on *Naultinus* Gray, 1842 or on *Phyllurus* Goldfuss, 1820 and overall closely allied to *G. gymnodactyli* collected from *Saltuarius cornutus* (?) (= *Gymnodactylus*). Considering the phylogenetic relationships of New Zealand geckos studied using DNA techniques (Chambers *et al.* 2001), the two endemic genera of geckos Diplodactylini (*Hoplodactylu*s, the nocturnal brown geckos, and *Naultinus*, the diurnal green geckos) form a monophyletic group with the New Caledonian species and one Australian genus (other Australian taxa are more distantly related). The New Zealand geckos have evolved separately since the development of the Tasman Sea began New Zealand's isolation from the rest of Gondwana around 100 million years ago. The current diversity of gecko species occurred probably after the Oligocene (perhaps 24 million years ago). Chambers

et al. (2001) suggested that the brown geckos, as a group, began to diversify well before the green taxa, which in turn began to speciate less than 5 million years ago. New Zealand was substantially submerged during the Oligocene, which would have produced many isolated populations that could then diverge ecologically and behaviourally from each other, adaptive radiation producing. Distance with New Caledonian species could be explained by this isolation (Han et al, 2004). The parasite species on *Naultina* and *Haplodactylus* are very similar.

If Gekkota is monophyletic (Kluge 1987) the endemics resulted from the drift of isolated population (neoendemism) or from a preserved population (paleoendemism). That the New Zealand parasites of Diplodactylini were used to define the *Geckobia* group 2 (Jack, 1964) is consistent with the history of the host. However, setation on genu IV shows the limit of the Jack's classification founded solely on leg chaetotaxy: the closest species are defined as belonging to the first group only because they have kept plesiotypic pattern of genu, and ancestral coxal chaetotaxy. These plesiomorphies (no loss of setae) did not acknowledge us on the origin. Because of the number of setae, chaetotaxy of first Jack's group could be primitive (5-5-5-5)(1-0-0-1)(3-2-2-2)(1-1-1-1); so other groups of species have lost one or more setae on femora and/or tibia essentially (only the third group is made with "no-typical" *Geckobia* by the significant loss of one tibial seta on the first three pairs of leg: this character is shared by South African species). The species parasite on *Bavaya* spp. is primitive for this character (it lost few setae!) if compared to the species found on *Naultinus* sp. in New Zealand. Considering other characters, the supplementary seta on the fourth coxae, the shape and the median position of the dorsal tibial seta are shared by at least three of the parasitic mites of Diplodactylinae. We can suspect that the parasites of the Diplodactylinae followed two paths: one was followed by *Naultina's* parasites characterized by reduced number of setae, and the second followed by both New Caledonian and Australian ('lato sensu') species. These species have conserved ancestral character.

Macroevolutionnary events which can explain the distribution of the parasites are four (Charleston 1998): 1] COSPECIATION (Host and parasites speciates concurrently: i.e. on *Gehyra oceanica* complex: Bertrand & Ineich 1989), 2] DUPLICATION (Only the parasite lineage speciated and new parasites remain on the host: i.e.: *Geckobia loricata* Berlese, 1892 and *G. latastei* Mégnin, 1878 parasite of *Tarentola mauritanica* (Linnaeus, 1758), 3] LINEAGE SORTING (Even if host lineage speciated, and eventually become extinct, the parasites did not?) or 4] HOST SWITCHING (parasites lineage speciated, and at least one of the new taxa switched the new host?).

It is obvious that on archipelagos the third and fourth categories are of great interest because the local endemic fauna depends on the colonizing process and on how hosts speciated. In Pacific islands, the endemic lizards were found with their adapted parasites, whereas the invading lizards brought their parasites from island to island (Bertrand & Ineich 1986). We observed yet the greatly modified (so, the best adapted?) species on the endemic lizards (Bertrand & Ineich 1987; 1989) whereas some more primitive species are more widely distributed (*G. bataviensis* Vitzthum 1926; *G. keegani* Lawrence, 1953) because of changes in the host's distribution notably due to human activities. New Caledonian fauna showed a double phenomenon:

1) The species belonging to the genus *Bavaya* share the same species of parasite and no "newly switched" parasite was found, (because of recent speciation?)

2) The *Bavaya* parasite is allied to Australian species (New South Wales, Queensland). The hypothesis that Diplodactylinae are parasitized by mites sharing same origin, but that the ectoparasites became highly specialized on *Naultina* whereas Neo-Caledonian and Australian became not, cannot be excluded and must be tested by further studies.

2. *Geckobia* spp. from Mauritius Islands

It is of great interest to consider that both invader and endemic have kept their respective parasites. None host switching and no duplication: the parasites are identical on the different species or subspecies of host. So co-speciation is limited, but we cannot assess (by lack of data) that similarities with continental species (parasites on African and Mauritian *Phelsuma*) are convergences or inherited from a shared origin. This latter hypothesis is consistent with the hypothesis that winds, oceanic currents carried founder animal and plants from Africa, Madagascar and Australasia (Cole, 2005). No cross infection was found (Table1). However, presence of *H. frenatus* increased susceptibility to infestation by *Geckobia* n.sp. (Table 2, Table 3 & Figure 3): infestation by Pterygosomid mites is known to reduce lizard fitness lesions, anaemia and transmission of debiliting blood parasites (Cole 2005; Hanley et al 1995).

Table 1. Prevalence and mean infestation per island (Mauritius Islands) (H.f & P.o.= *H. frenatus* & *P. ornata*) (421 sampled lizards) (Cole, 2005).

	Flat Island		Iles aux Aigrettes		Iles aux Fouquets	Iles de la Passe	Gunners Quoin	Round island
MALES	H. f	P.o.	H. f.	P.o.	H. f.	H. f.	P.o.	P.o.
G. bataviensis	8.1	0.0	32.6	0.0	29.60	56.50	0.0	0.0
G. n.sp.	0.°	25.6	0.0	61.3	0.0	0.0	11.9	14.6
FEMALES	H. f	P.o.	H. f.	P.o.	H. f.	H. f..	P.o.	P.o.
G. bataviensis	3.6	0.0	47.6	0.0	27.3	60.0	0.0	0.0
G. n.sp.	0.0	26.1	0.0	52.6	0.0	0.0	10.0	13.3

Table 2. Number of Geckos screened for parasites (Mauritius Islands) (N. Cole 2005)

Island	Host species	Host sex	nb individuals screened for		
			Ectoparasites	Helminths	Coccidia
Flat Island	*H. frenatus*	Male	37	30	30
Flat Island	*H. frenatus*	Female	28	26	26
Flat Island	*P. ornata*	Male	39	37	36
Flat Island	*P. ornata*	Female	23	19	19
Ile aux Aigrettes	*H. frenatus*	Male	141	122	60
Ile aux Aigrettes	*H. frenatus*	Female	63	43	43
Ile aux Aigrettes	*P. ornata*	Male	150	128	58
Ile aux Aigrettes	*P. ornata*	Female	57	41	31
Ile aux Fouquets	*H. frenatus*	Male	27	21	19
Ile aux Fouquets	*H. frenatus*	Female	33	28	28
Ile de la Passe	*H. frenatus*	Male	92	87	43
Ile de la Passe	*H. frenatus*	Female	30	25	24
Gunners Quoin	*P. ornata*	Male	101	87	45
Gunners Quoin	*P. ornata*	Female	30	20	19
Round Island	*P. ornata*	Male	41	31	22
Round Island	*P. ornata*	Female	30	22	22
Total *H. frenatus* screened			451	382	273
Total *P. ornata* screened			471	385	252

Table 3. Prevalence of *G. bataviensis* in male *H.f.* and of *G.* n.sp. in male and female *P. ornata*. For comparisons between allopatric (Ile aux Fouquets, Ile de la Passe)) and sympatric populations (Flat Island, Iles aux Aigrettes): (significance: a,b,c) (Cole, 2005).

Islands	Male *H. frenatus (G. bataviensis)*	Male *P. ornate (G. n.sp.)*	Female *P. ornata (G. sp. N.)*
Flat Island	**a**	**a**	**ab**
Iles aux Aigrettes	**b**	**b**	**a**
Iles aux Fouquets	**ab**	**a**	**b**
Ile de la Passe	**c**	**a**	**b**

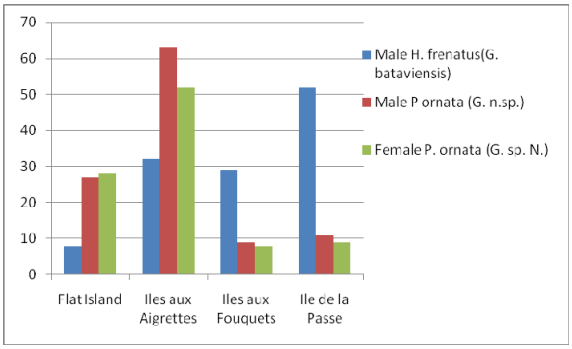

Figure 3. Prevalence of *G. bataviensis* in male *H.f.* and of *G.* n.sp. in male and female *P. ornata*. For comparisons between allopatric (Ile aux Fouquets, Ile de la Passe)) and sympatric populations (Flat Island, Iles aux Aigrettes)(From Cole 2005, modified).

3. Synthesis and perspectives

We can consider the isolation is the rule: Significant co-speciation was observed and instances of host switching were rare. The prevalence of intra-host speciation events was high relative to other such studies and may relate to the large geographical distances over which hosts are spread (i.e. copepods in marine environment: Paterson & Poulin 1999).

We can interrogate on the systematic of scale mites: if the genera were defined on several characters notably the leg chaetotaxy, limited variations of characters forbid to define sub levels or to divide the present genera: "natural groups" can be defined by combined analyses of morphology, legs chetotaxy and hosts. More acknowledgements will be given by compared host and parasite phylogenies. A molecular approach will be of great interest.

According to our present knowledge, 1) "Australian" are the species of the Jack's Group 2 revisited= parasites of Diplodactylini (Annexe, key 3), and "paleoendemic", (because the common characters are essantially primitive). 2) Parasites collected on *Phelsuma* spp. have common characters with African or Madagascan parasites, notwithstanding a necessary re-examination of these species. 3) On host species widely distributed (as *H. frenatus*), different parasites were described. The hypothesis was made that the invading species captured the local and until now undescribed parasites: Data from Mauritius or New Caledonia did not agree with this hypothesis. However, host switching is possible: Bertrand & Pedroño (2000) described a new Madagascan species thought to be captured in natura by a re-introduced tortoise: this species (*G. enigmatica* Bertrand & Pedroño 2000) was described as opportunistic, with unknown host. It is obvious that, paleo or neoendemic geckoes, because they are too different, are partly protected from parasites brought by invasive hosts; if endemic is endangered and becomes extinct, the invasive host could be protected because endemic parasites are too adapted because of the parallel evolution with the host and the necessary constraints of coevolution in insular envionment.

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Annexe

Keys for *Geckobia* groups and Diplodactylini parasites

Key 1: Pterygosomatidae: Key to genera

1. Leg I with at least 3 femoral setae, body usually longer than wide or rounded.....12
1. Three or less femoral setae, body often densely covered by setae, at least dorsally, rounded in shape or as wide as long or wider than long.....2

12. With soft teguments, ...121
12. Skin leathery, large species, 5 genu setae legs I to 4, 3 femoral setae on the legs I to IV, Austral Africa..... *Ixodiderma* Lawrence.
12. Body wider than long, small sized, only 2 to 4 genu setae leg I, Austral African species .... *Scaphothrix* Lawrence.

121. With few long dorsal setae, on Iguanidae.....122
121. 12-13 pairs of very long dorsal setae on dorsal setae base-plates, often on Arthropods, movable digit of chelicerae not hook-like ..... *Pimeliaphilus* Tragardh***
121. With numerous dorsal setae, movable digit hook-like, America .....*Geckobiella* Hirst.

122. Dorsal setae shorter, distinctly placed in rows with or without median plates, inverse pear-shaped scutum with contiguous ocular plates ..... *Cyclurobia** De La Cruz.
122. 13, 14 or 15pairs of dorsal setae, on Reptiles.....*Hirstiella* Berlese**

2. Dorsal scutum absent, eyes lacking, few setae on ventral side, no spurs on coxae, no lateral setae on tibia II, III and IV: 22
22. Body usually wider than long : typically hosted by Agamidae, ... *Pterygosoma* Peters 221
221. 4 setae on genu leg I ..... subgenus *Gerrhosaurobia*
221. less than 4 setae leg I..... sub genus *Pterygosoma*

22. Round in shape, with numerous dorsal setae, on South African Lizards .....*Zonurobia* Lawrence
2. Dorsal scutum present, entire or divided, eyes usually present, usually 5 setae on tibia (leg I to IV) or only 4..3

3. Glabrous genu leg II-III; dense ventral setae (sometimes scale-like), stout setae at least on coxal plates; Typically ex Gekkonids .....*Geckobia* Mégnin
3. Genu leg III with setae, palpal tibial with tuft filamentous seta; ex Xantusiidae (Mexico y Central America) ....*TequisistIana* Hoffmann & Sanchez***

** Poorly know genus described ex* Cyclura, *West Indies, Cuba.*
**** H. dioilii *Baker 1998 collected in Australia and in England and* H. stamii *Jack 1961 both collected on captived iguana could be considered as* Cyclura diolii *(Baker, 1998) and* C. stamii *(Jack 1961) on the basis of generic diagnosis by* De la Cruz *(1984),*
**** Mysterious genus, rarely found, both collected on Arthropods and lizards.*
**** According* Hoffmann & Sanchez *(1980),* Hirstiella otophylla *Hunter & Loomis, 1966 hosted by the genus* Coleonyx, *may be considered as* T. otophylla *(Hunter & Loomis, 1966).*

Key 2: *Geckobia*: Key of the group of species (based on Jack 1964)

1 Five tibial setae on PI, PII, PIII, PIV 2
1 only four tibial setae at PI, PII & PIII.....Jack's group 3: South African species

2 no well visible dorsal genu seta on PI and PV* 3
2 one seta on genu I and IV, or only on PI 5

3 no seta on trochanter IV ..... *keegani*
3 one seta on trochanters I to IV, ...4

4 no seta on genu I and IV.....*bataviensis* (=*gleadovania*)?
4 two setae on femur I..... *oedurae*

5 three setae on femur I 6
5 two setae on femur I.....8

6 one seta on femur III.....8
6 two setae on femur IV.....7

7 no seta genu I.....*diversipilis*
7 no seta genu IV.....*indica (*and Asian species*)* New group 4
7 one seta genu I and IV, two or three (*G. boulangeri*) femoral setae PII-IV, coxae IV often with two-three setae . .....Jack's group1(primitive leg chaetotaxy)**

8 only one seta femur II & III, one seta genu I and IV .....*australis*
8 one or no seta genu I, one or no seta genu IV, two or three setae on coxa IV: — newly defined group2:

Australian group species*** (including diplodactylinae parasites)
8 two setae femur II-IV ..... *simplex*

*some species exhibit a "vestigial" seta reduce in size and hardly visible though they were counted as species with atrichose genu by Jack (1964) i.e. *keegani* or *bataviensis* (observation made by author (M.B.) on Mauritian material and verified by Ricardo Paredes on Mexican material (oral comm.)
** including *G. boulangeri* with 3 setae femora I and II.
*** including new species on *Bavaya*, and *G. gymnodactyli, G. manzanelli.*

Key 3: key to Species (*Geckobia* ssp. found on Diplodactylinae)

1 Coxal setae, 2 2 2 2 4
1 Coxal setae 2 2 2 3, large posterior coxal setae 2

2 Scutum large, eyes bore by the scutum.....3
2 Scutum not well visible, lateral eyes .....The new species on Bavaya

3 Eyes contiguous to the external line, ciliate associated seta.....*G. gymnodactyli*
3 Eyes as above with associated seta elongated .....*G. manzanelli*

4 Scutum with few club shape setae (ca 10) ..... *clelandi*
4 Scutum with more simple setae.....5

5 Scutum not divided posteriorly.....*G. haplodactyli*
5 Scutum short and wide, with convex posterior end .....*G. naultina*

On Diplodactylinae, *G. clelandi, gymnodactyli, haplodactyli*, *manzanelli, naultina* and the new species have been collected. *G. clelandii* and *manzanelli* (the 1rst Jack's group), on *Phyllurus* ssp*., G. gymnodactyli* on *Saltuarius, G. haplodactyli* on *H. duvaucelli, G. naultina* on *Naultinus* sp*.* and the New Caledonian species on *Bavaya* spp.

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PRELIMINARY RESULTS ON PHYLOGEOGRAPHIC PATTERNS OF THE INVASIVE RED PALM MITE, *RAOIELLA INDICA* (PROSTIGMATA: TENUIPALPIDAE)

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Abstract

The red palm mite, *Raoiella indica* (RPM), is a major invasive pest spreading aggressively throughout the Americas. The mite is originally known as a pest of old world palms, but upon arriving to the neotropics has colonized numerous unrelated host plants. Unfortunately, basic biological information regarding RPM, such as origin of the species, dispersal methods, and native predators, is lacking, making it difficult to develop a control strategy. Our research begins to address these inadequacies using population genetics to study the phylogeographic history of the mite worldwide. Partial Cytochrome Oxidase I (*COI*) was sequenced for 15 populations from 12 different countries and results provide interesting patterns about the origin and dispersal pathways of RPM. Analyses also indicate that additional genetic evidence is necessary to truly and confidently elucidate these patterns.

Keywords

invasive, *Raoiella*, molecular, phylogeography, phytophagy

Introduction

The red palm mite *Raoiella indica* Hirst (RPM) was first described from India in 1924 on coconut palms, *Cocos nucifera*, and is recorded as a pest in India, Sri Lanka, Pakistan, the Philippines and Mauritius. RPM is currently spreading across the world on a variety of palm species and has recently been reported throughout the Caribbean region and Central America (for a complete distribution and host plant list see Table 1). Additionally, upon arrival to the Caribbean, RPM has rapidly expanded its host range to include several species in the Musaceae (bananas and plantains), Heliconiaceae (Heliconias), Celastraceae (Bittersweets), Lamiaceae (Mints), Zingiberaceae (Gingers), and Pandanaceae (Screw-pines). RPM was first reported from Martinique in 2004, from St Lucia, Dominica, St Martin and Guadeloupe in 2005, from Puerto Rico, Dominican Republic and Trinidad-Tobago in 2006, from St Thomas, Venezuela, Haiti, and Florida in 2007 (Flechtmann & Etienne 2004; Kane *et al*. 2005; Etienne & Flechtmann 2006; Pons & Bliss 2007; Rodrigues *et al*. 2007), and we report here that RPM has been identified from two more countries in the Greater Antilles region in 2008, Turks & Caicos and Cuba.

The genus *Raoiella* currently contains 12 species distributed from Africa, through Asia to Australia; however, seven of these species, from Pakistan, India, and Sudan, are suspected synonyms of RPM (Mesa *et al*. in press). Australia currently has only one described species recorded, *R. australica*

Womersley, but our collection records so far indicate that this country represents the centre of diversity for this genus as five undescribed species have been collected in association with host plants in the family Myrtaceae. So far RPM has not been collected in Australia.

Table 1. World records of *Raoiella indica* and references

Locality and Year	Year	Initial Host Plant Report	References
Comboitore, India	1924	*Cocos nucifera*	Hirst 1924
Egypt	1942	*Phoenix dactylifera*	Sayed 1942
Mauritius	1958	*Cocos nucifera* L., *Dictyosperma album* (Borg.), *Phoenix dactylifera* L.	Moutia 1958
Sudan	1958	*Phoenix dactylifera*L.	Pritchard & Baker 1958; Smith-Meyer 1979 (as *R. phoenica*)
Pakistan	1974	*Ocimum basilicum* L. (Lamiaceae), *Phoenix dactylifera* L.	Chaudhri *et al*. 1974
Israel	1983	*Phoenix dactylifera* L.	Gerson *et al*. 1983
The Philippines	1997	*Cocos nucifera* L.	Rimando 1996
Sri Lanka	2001	*Cocos nucifera* L.	Mariau 2001
United Arab Emirates	2003	*Phoenix dactylifera* L.	Gassouma 2003
La Reunion Island	2004	*Cocos nucifera* L.	Ueckermann 2004
Martinique	2004	*Cocos nucifera* L., *Veitchia merrilllii* (Becc.)	Flechtmann & Etienne 2004
Saint Lucia, West Indies	2005	*Aiphanes* sp. (Multiple crown palm), *Cocos nucifera* L., *Dypsis lutescens* (H.Wendl.), *Musa* spp., *Syagrus ramanzoffianum* Glassman (Queen palm), *Veitchia merrilllii* (Becc.)	Kane et al. 2005; E. Kane, pers. comm.
Dominica	2005	*Cocos nucifera* L., *Musa* spp.	E. Kane, pers. comm.
Guadeloupe	2005	*Cocos nucifera* L.	Etienne & Flechtmann 2006
St. Martin	2005	*Cocos nucifera* L.	Etienne & Flechtmann 2006
Thailand	2005	*Cocos nucifera* L.	E. Kane, pers. comm.
Saudi Arabia	2005	*Phoenix dactylifera* L.	Alhudaib 2005
Trinidad	2006	*Cocos nucifera* L., *Heliconia psittacorum* L.f., *Musa* spp.	E. Kane, pers. comm.
Dominican Republic	2006	*Cocos nucifera* L., *Musa* spp.	Rodrigues *et al*. 2007
Puerto Rico	2006	*Cocos nucifera* L., *Musa* spp.	Rodrigues *et al*. 2007
Venezuela	2007	*Cocos nucifera* L., *Musa* spp.	M. Quiros, per. comm..
St. Croix	2007	*Cocos nucifera* L.	E. Kane, pers. comm.
St. Thomas	2007	*Cocos nucifera* L.	E. Kane, pers. comm.
Jamaica	2007	*Cocos nucifera* L.	E. Kane, pers. comm.
Haiti	2007	*Cocos nucifera* L.	E. Kane, pers. comm.
United States (Florida)	2007	*Cocos nucifera* L.	C. Welbourn, pers. comm.
Cuba	2008	*Cocos nucifera* L.	Reported here
Turks and Caicos Islands	2008	*Cocos nucifera* L.	Reported here

The apparent rapid spread of this invasive mite prompted our investigation of the phylogeographic history, host plant relationships and population genetics of RPM and its origin. Partial cytochrome oxidase (*CO1*) was sequenced from samples of RPM collected from populations in 12 different countries throughout its distribution area (Table 2), along with samples of two undescribed species of *Raoiella* from Australia. These preliminary analyses represent the first attempt to establish geographic

origins and common dispersal pathways of RPM.

Table 2. Sampled localities of *Raoiella* (25 samples) and *Dolichotetranychus (1 sample)*.

Extraction Code	Species	Country	GenBank Accession
RaIn01	*Raoiella sp.*	Australia	EU682419
RaIn02	*Raoiella sp.*	Australia	EU682420
RaIn03	*Raoiella sp.*	Australia	EU682421
RaIn04	*Raoiella sp.*	Australia	EU682422
RaIn05	*Raoiella sp.*	Australia	EU682423
RaIn06	*Raoiella indica*	India	EU682424
RaIn07	*Raoiella indica*	India	EU682425
RaIn08	*Raoiella indica*	India	EU682426
RaIn09	*Raoiella indica*	India	EU682427
RaIn10	*Raoiella indica*	Reunion	EU682428
RaIn11	*Raoiella indica*	St. Lucia	EU682430
RaIn12	*Raoiella indica*	St. Lucia	EU682431
RaIn13	*Raoiella indica*	Trinidad	EU682432
RaIn14	*Raoiella indica*	Trinidad	EU682433
RaIn15	*Raoiella indica*	United Arab Emirates	EU682434
RaIn16	*Raoiella indica*	Puerto Rico	EU682436
RaIn17	*Raoiella indica*	Dominica	EU682437
RaIn18	*Raoiella indica*	Iran	EU682438
RaIn19	*Raoiella indica*	Philippines	EU682439
RaIn20	*Raoiella indica*	Philippines	EU682440
RaIn21	*Raoiella indica*	Iran	EU682441
RaIn31	*Raoiella indica*	USA	EU682442
Rain34	*Raoiella indica*	Venezuela	EU682443
Rain35	*Raoiella indica*	Venezuela	EU682444
RaIn39	*Raoiella indica*	USA	EU682445
Rain32	*Dolichotetranychus sp.*	Malaysia	EU682418

Methods

Live mites from all stages were freshly collected from host plants and placed directly into 70-100% ethanol. Collections were made from 15 different populations of *Raoiella* in 12 different countries spanning the worldwide distribution of the mite (Table 2). Representatives of each collection were slide mounted to confirm identification. Four mites were pooled from a single population (collection) and extracted using a DNeasy® Tissue Kit and protocols therein with slight modifications to account for the use of whole mite specimens. In order for the dissolved tissue after enzyme incubation to escape the cuticle, a small incision or hole in the cuticle is necessary. This can be produced with a minutun pin and allows recovery of an intact cuticle for vouchering. Additionally, the length of the incubation period has been extended to 12-24 hours, which allows for maximal recovery of DNA. All other steps in the Qiagen protocol were left unaltered.

Amplification of approximately 670bp of the mitochondrial cytochrome oxidase I (*COI*) gene was accomplished using the primer pair COIF 5' GGT CAA CAA ATC ATA AAG ATA TTG G 3' and COIR 5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3' (Folmer et al. 1994). Standard 25µl PCR amplifications were performed using the following reagents and

volumes: 12.5 µl of 10% trehalose; 4 µl ddH_2O; 2.5 µl 10X reaction buffer for Platinum *Taq* (Invitrogen); 1.25 µl 50mM $MgCl_2$ (Invitrogen); 0.25 µl of each primer diluted to 10 µM concentration; 0.125 µl of 10mM dNTP mix (New England Biolabs); 0.12 µl Platinum *Taq* polymerase (Invitrogen); and 4 µl DNA template. All reactions were run on a Bio-Rad DNA Engine under the following conditions: 2 min initial denaturation at 94°C followed by five cycles of 94°C for 30 sec, annealing at 45°C for 40 sec, and extension at 72°C for 60 sec, followed by 35 cycles of 94°C for 30 sec, 51°C for 40 sec, and 72°C for 60 sec, with a final extension of 7 min at 72°C and an indefinite hold at 10°C. Final products were visualized on a 1% agarose gel stained with Ethidium Bromide and successful amplifications were purified. The purified product was sequenced using the BigDye Terminator method (Perkin Elmer, USA) on an ABI 3730xl at the University of Kentucky Advanced Genetic Technologies Center. Sequences were deposited in GenBank (accession numbers in Table 2).

Sequences were aligned using ClustalX (Thompson 1997) and then translated to amino acid sequences to verify alignment. Amino acid sequences were back translated to nucleotides for all analyses. The aligned sequences were analyzed heuristically under the parsimony criterion using PAUP* 4.0b10 (Swofford 2001) to build tree(s) with TBR branch swapping and 10,000 random additions saving all most parsimonious trees. Support values were obtained through parsimony bootstrap of 5,000 pseudoreplicates and subsequent values were mapped onto the appropriate nodes of the strict consensus. Bayesian analysis of the data was done through MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) with the HKY+I+G model of evolution chosen in MrModeltest (Nylander 2004) for 2,000,000 generations. Burnin was determined based on stationarity being reached. MEGA4 (Tamura et al. 2007) was used to determine levels of sequence divergence between samples.

Results

The data matrix consisted of 658 total characters, of which 151 were parsimony informative. Both the parsimony and Bayesian results converge on the same phylogenetic hypothesis (Fig. 1). Parsimony analysis recovers 18 most parsimonious trees (L=231, CI=0.9667, RI=0.9904) with eight distinct haplotypes of *Raoiella* (five haplotypes of RPM). The Bayesian analysis results in the same relationships (Likelihood = -1816.69) and will not be discussed separately from the parsimony results. Support values (parsimony bootstrap and Bayesian posterior probabilities) are high across the phylogenetic hypothesis (Fig. 1). Results indicate that all populations collected and identified as *Raoiella indica* are indeed this species and genetically distinct from other species of *Raoiella* found in Australia (indicated by long branch length separating the groups). Additionally, all populations found in the Americas were genetically identical to populations sampled in Reunion and those found on areca nut in India. These populations were distinct from those found on coconut in India. Populations collected in the Philippines were found to be very similar to the Caribbean populations, differing by a single nucleotide substitution. Populations from the Middle East (UAE and Iran) each contain their own haplotype and cluster together as a more basal clade to the rest of the RPM.

Discussion

Raoiella indica has quickly become a pest of major international significance and our study is the first to provide preliminary molecular evidence in regards to the dispersal and origin of this mite. While it is clear that additional molecular markers are necessary for a complete understanding of the history of RPM, the current results provide a glimpse of the results to come.

India has often been considered the origin of RPM, based primarily on that fact that it was first described from coconut palms in Coimbatore (Hirst, 1924). Additionally, numerous species of Raoiella were subsequently described from Indian palms (Akbar 1990; Akbar and Chaudhri 1987; Chaudhri and Akbar 1985; Hasan and Akbar 2000; Meyer 1979; Mohanasundaram 1985), leading researchers to believe that India may be the epicenter of *Raoiella* diversity. Recent morphological study has indicated that all of these are likely junior synonyms of *R. indica*, decreasing *Raoiella* diversity to a single pest species in India, and casting doubt on an Indian origin of the species. Additionally, all other localities where RPM has been discovered worldwide are in agricultural settings where the mite is determined to be a pest, and it has never been found in what would be considered a more native setting. However, this pattern may simply be due to increased sampling and research in agricultural areas. Recently, numerous undescribed species of *Raoiella* have been found by the authors in Australia, although most of these were not available for molecular analysis in this study.

Analysis of preliminary genetic evidence (Fig. 1) indicates that Australian species are in fact more basal to RPM and RPM appears to have had significant divergence from other *Raoiella* species, based on the extremely long branch linking the two groups. In general, Australian *Raoiella* exhibit about 22-25% sequence divergence from RPM haplotypes included in this study.

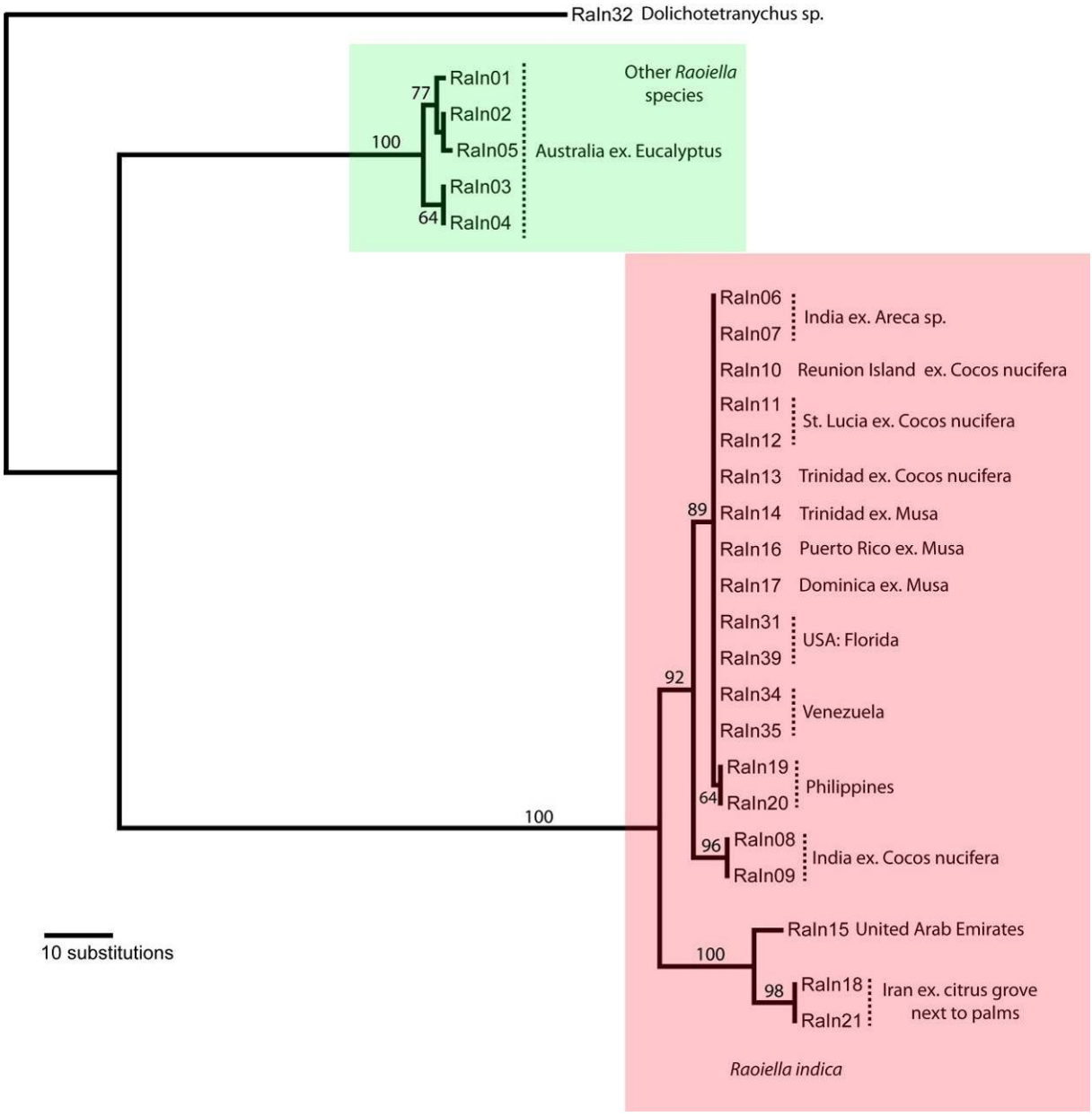

Figure 1. Strict consensus of 18 most parsimonious trees (L=238, CI=0.9667, RI=0.9904) based on 610bp of COI. Numbers associated with nodes are bootstrap values from 5,000 bootstrap pseudoreplicates. Branch lengths correspond to the amount of change that occurs on each branch. Australian *Raoiella* species are highlighted by the green box and populations of *Raoiella indica* are highlighted in the red box.

This amount of sequence divergence (although currently only based on one mitochondrial gene) along with numerous morphological differences, clearly suggests that they are different species from RPM. This amount of divergence is similar to levels of COI divergence found between higher taxonomic levels than species in other taxonomic groups, but the long list of morphological and behavioral synapomorphies clearly indicate the placement of all haplotypes within the genus

Raoiella.

The haplotypes within Australia show little intercontinental divergence, ranging from 0.3-0.9%, values that fall in line with levels commonly found within species or between very closely related species in other arthropod groups (Brown et al. 2000; Favret and Voegtlin 2004; Johnson et al. 2002; Lee et al. 2006; Paquin and Hedin 2004). Due to the diversity of the genus found in Australia (five additional undescribed species not yet sequenced) and that the genetic data, when rooted on Dolichotetranychus, which places the Australian species at the base of the tree, it appears that a hypothesis of Australasian origin for the genus may be more appropriate. The remaining discussion of the results (refer to Fig. 1 and Fig. 2) reflects this assumption.

Figure 2. Hypothesized divergence and dispersal of *Raoiella indica* based upon preliminary results from COI sequence data.1) Origins of the genus in Australasia and early establishment of RPM in the Middle East. 2) Divergence and dispersal within the Middle East and dispersal to India. 3) Divergence of haplotypes associated with coconut and areca nut and potential dispersal to Philippines – followed by divergence of Philippine haplotype from Indian form. 4) Separate dispersal from India to Reunion, but no divergence in COI occurs. 5) Dispersal from Reunion to Martinique, likely in late 2003, again with no divergence in COI. 6) Rapid spread throughout the Caribbean between 2004-2008, including 17 countries with confirmed and established populations.

The most basal of the RPM populations is a clade consisting of two haplotypes, RaIn15 (United Arab Emirates) and RaIn18/21 (Iran), forming a clade divergent from other RPM by 2.7-3.6%. This value is a little high for intraspecies variation, but still falls within the average range found in other arthropods for COI (Brown et al. 2000; Favret and Voegtlin 2004; Johnson et al. 2002; Lee et al. 2006; Paquin and Hedin 2004) and morphologically these populations are RPM. The amount of sequence divergence between the UAE and Iranian haplotypes is 1.5%. This result indicates that RPM was distributed throughout the Middle East before arriving in India, which is contradictory to the often hypothesized Indian origins of RPM. The mite is also known to occur in Israel, Oman, Pakistan, and Saudi Arabia, and we are actively trying to obtain these populations as they may be a critical piece to the puzzle.

From the Middle East, results suggest that the mite dispersed to India where populations further diverged on coconut and areca nut. Two haplotypes exist in India, RaIn08/09 on coconut

and RaIn06/07 on areca nut. The haplotype on coconut is 1.2% divergent from the haplotype on areca nut, which is identical to the haplotype found in the Americas and Reunion. It appears, based on these COI patterns, that the haplotype from areca nut made it to Reunion and then dispersed from there to the Caribbean. It is possible that dispersal was from India to the Caribbean, but political and economic data would suggest the former hypothesis. Reunion is a French Overseas Department and the invasion of the Caribbean occurred in Martinique, another French Overseas Department. Due to these political ties, the likelihood of RPM infested product going from Reunion to Martinique is higher than from areca nut in India to Martinique. From Martinique, RPM has rapidly spread throughout the Caribbean, with recent advances into the United States and Venezuela. All populations sequenced from the Americas were the same haplotype as Reunion and India. With the rapid spread of this mite across the Caribbean in the last four years, COI is not expected to show any divergence among these populations and movement of the mite is simply tracked by its appearance in a new locality.

Interestingly, the specimens from the Philippines, which were initially described in a different genus (Rimando 1996) shows strong similarity to the Caribbean haplotype, differing in a single non-synonymous substitution resulting in an amino acid change from Isoleucine to Methionine. The data corroborates the synonymy of *Rarosiella cocosae* into *R. indica* initially suggested by Kane et al. (2005) and subsequently proposed by Mesa et al. (in press), based on morphological characters. The haplotype found in the Philippines appears to be a result of a separate dispersal from an area containing the Caribbean haplotype, although due to incomplete sampling we cannot be sure of the origination of this dispersal event.

Our preliminary data based on COI sequence data resolves numerous haplotypes and a few major potential dispersal pathways that have led to the spread of RPM. The results also suggest that the origins of the genus appear to be Australasian, which is also hinted at by the total species diversity in Australia (one described and five undescribed species) making up two-thirds of all known *Raoiella* species. Results also indicate that the Middle East may have been one of the earliest occupied regions by RPM, but more thorough sampling needs to take place. An effort in the future will be focused on taxonomic sampling from other Middle Eastern countries as well as exploration of Australia and surrounding areas for more closely related species. The results also indicate that additional molecular markers are necessary for robust tests of RPM dispersal and divergence around the world.

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European Association of Acarolgists, 2008

POTENTIAL DISTRIBUTION OF THE INVASIVE MITE *TETRANYCHUS EVANSI* (TETRANYCHIDAE) IN THE MEDITERRANEAN REGION

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Abstract

Predicting the potential geographical distribution of a species is particularly useful for pests with strong invasive abilities. *Tetranychus evansi*, possibly native of South America, is a spider mite recognized as a pest of solanaceous crops. This mite is considered as an invasive species in Africa and Europe and has been recorded in many parts of the world. To define the potential global distribution of the species, a CLIMEX model distribution was developed using: i) South American records, ii) laboratory life-history parameters and iii) exotic records. The model results fitted the known distribution of *T. evansi* except for some dry locations where host plants develop only with irrigation. High temperatures, dry and wet stresses play a role in limiting the spread of the mite in the tropics, whereas in a large part of North America and Europe, the distribution of *T. evansi* appears to be limited essentially by cold stress. A distribution map is provided for the global potential distribution of *T. evansi*. The Mediterranean region is of particular interest because it is the main area where tomato is grown in open fields in Europe. According to the model, the Mediterranean region will be colonized inexorably by the pest. However, model results indicate a mite distribution being limited to coastal areas.

Keywords

Biological invasions, CLIMEX, climate, predicting species distribution, invasion risk assessment, *Tetranychus evansi*

Introduction

The tomato red spider mite, *Tetranychus evansi* Baker & Pritchard (Photos 1 and 2) is an invasive pest. It belongs to the family Tetranychidae. Gutierrez and Etienne (1986) suggested that the species is native to South America. In the past half-century this mite has been reported from North America, the Indian Ocean Islands, many countries in Africa, the Mediterranean region, as well as from Hawaii and Taiwan in the Pacific area [see Migeon and Dorkeld (2007) for a detailed coverage of its distribution]. The main hosts of *T. evansi* are Solanaceae (Moraes *et al.*, 1987; Bolland *et al.*, 1998; Migeon & Dorkeld, 2007); including numerous cultivated plants (Jeppson et al., 1975). Plants commonly attacked are tobacco, tomato, potato and eggplant, but ornamental plants of the genus *Solanum* are also among the hosts of *T. evansi*. Presently, *T. evansi* is not a pest in Brazil (Furtado, 2006), whereas in the last decade it has become one of the most severe pests of Solanaceae in Africa. The damage caused by outbreaks it causes (Photo 3), can lead to crop losses of up to 90% in South-East Africa (Sibanda *et al.*, 2000; Saunyama & Knapp, 2003) and in West Africa, in Senegal (Duverney & Ngueye-Ndiaye, 2005).

Photo 1. *Tetranychus evansi* adults male (top) and female (bottom).

Rapid expansion of pests represents a threat to agriculture in many countries worldwide. The principal pests of temperate crops are biotic invaders (Mack et al., 2000; Kenis et al., 2007). The expansion of invaders is mostly limited by climatic factors and the assessment of habitat suitability of new areas is regarded as an effective mean to provide basis for pest control and quarantine measures. As one of the ways to address these issues, climate modelling is used to define the bioclimatic range of a given species, as its potential

Photo 2. *Tetranychus evansi* on *Solanum nigrum* leaf - France, September 2005.

distribution based on the bioclimatic envelope (Andrewartha & Birch, 1954; Brown *et al.*, 1996; Beaumont *et al.*, 2005; Guisan & Thuiller, 2005). Predictions based on this envelope integrate the range of a number of climatic variables, and are directly under the control of limiting climatic factors. Climate modelling aims to predict the maximal potential distribution. Introduced species often escape to many biotic factors in the new environment, and can expand their climatic range. In the case of pests, community interactions are limited by the impoverished species diversity encountered in agroecosystems which facilitates their establishment (Baker et al., 2000). Climatic mapping remains a fundamental tool for predicting and analysing the potential distribution of pests (Baker et al., 2000) and is widely used to estimate the risk of invasive species to colonizing new areas (Sutherst et al., 1991; Beaumont et al., 2005; Navia et al., 2005).

Photo 3. Outbreak of *Tetranychus evansi* on protected culture of aubergine (*Solanum melongena*) Canary Is., October 2006.

CLIMEX 2.0 software (Sutherst & Maywald, 1985; Sutherst *et al.*, 2004) was used here to predict the

potential distribution of *T. evansi* across the world on the basis of: i) previously estimated physiological requirements (Moraes & McMurtry, 1987; Bonato, 1999); and ii) the known geographical distribution of the species, together with new records compiled in this study, including exotic records. The relevance of such exotic records in modelling the potential distribution of species has been emphasized (Kriticos & Randall, 2001; Kriticos *et al.*, 2005) for addressing the maximal potential distribution. Analysis focuses on the Mediterranean Basin, where the mite is an important agricultural threat and is of concern to Europe.

Materials and methods

CLIMEX software

The program used in this study was CLIMEX 2.0 for Windows (Sutherst & Maywald, 1985; Sutherst *et al.*, 2004). This software uses a hydro-thermal growth index (GI) to describe conditions that favour the growth of a population, in addition to four stress indices (cold, heat, dry and wet) and their interactions (cold-wet, cold-dry, heat-wet, heat-dry), which describe unfavourable effects of temperature and moisture. All these indices are calculated weekly and then integrated to obtain annual indices. A combination of the growth and stress indices leads to the ecoclimatic index (EI) which describes the potential for population persistence. The EI index provides readily interpretable, simple and concise information in a scale of 0 to 100. Low EI values indicate less suitable habitats that have a low probability of population persistence. The use of stress indices provides useful information for quantifying the climatic stresses that limit species distribution in each location.

CLIMEX 2.0 allows to infer climatic parameters from the known distribution of the species studied and to describe the potential climatic range of this species. The use of parameters calculated from laboratory experiments provides a starting template. We used a template of values based both on known *T. evansi* life-history parameters (Moraes & McMurtry, 1987; Bonato, 1999) and Climex values for tropical species. Detailed methods and workflow are presented in Sutherst et al. (Sutherst *et al.*, 2004). To obtain a fin scale potential distribution, especially useful for contrasted climate and mountainous landscape regions such as Mediterranean Basin, a 10' of arc resolution grid climatic dataset, derived from the Climate Research Unit (CRU) in Norwich (New et al., 2002), was used.

Distribution records of T. evansi

The lack of suitable data on the distribution of organisms has been emphasized by Kriticos and Randall (2001) and Sutherst (2003) as the major obstacle for bioclimatic modelling. This is particularly true for small cryptic species like the red tomato spider mite. While *T. evansi* is considered to have originated in South America, records of the species have been mainly concentrated in managed agricultural systems as

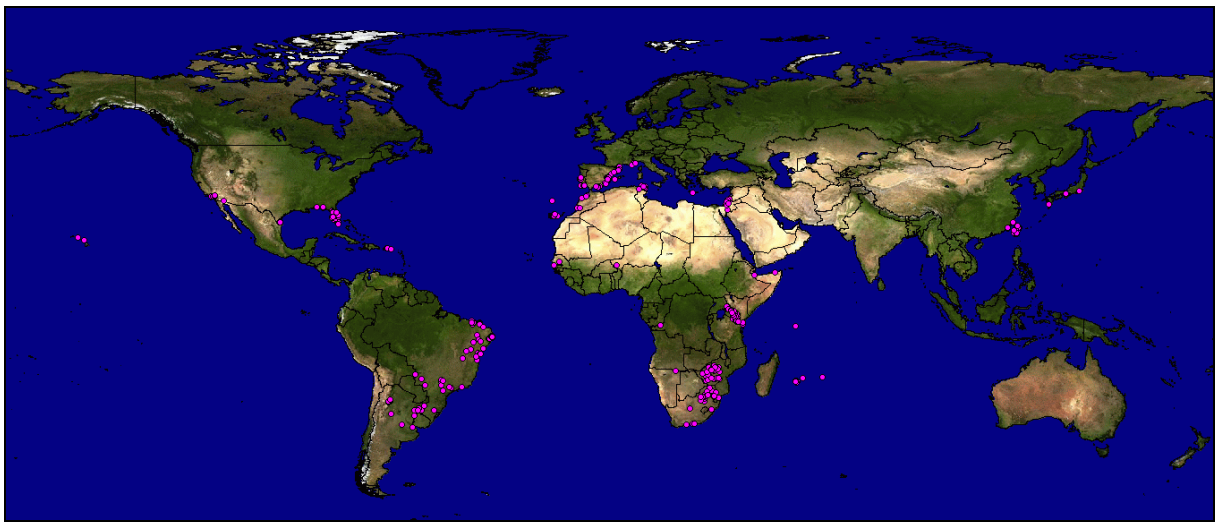

Figure 1. World distribution of *Tetranychus evansi* according to records compiled in this study. ● indicate both, bibliographical and observation records.

the tomato growing areas in Brazil where the first report was in Bahia (Silva, 1954) under the name of *Tetranychus marianae* (see Annex 1 for complete data) and more recently in areas of climatic interest for exploration of natural enemies (Rosa et al., 2005; Fiaboe et al., 2006; Furtado et al., 2006; Fiaboe et al., 2007; Furtado et al., 2007). Knowledge of the species in its area of origin remains still fragmentary for most of South America. The first official report from Argentina, dates back only from 2004 (Furtado et al., 2007). Although the species has long been known there as a 'red spider', *T. marianae*, and sometimes causes considerable outbreaks in tomato crops (Rossi Simons, 1961).

The known world distribution of this spider mite is reported in Annex 1 and shown in Figure 1. The present study includes also *Tetranychus takafujii* data from Japan (Ehara & Ohashi, 2002; Ohashi *et al.*, 2003; Kotsubo *et al.*, 2004), which is now considered as synonym of *T. evansi* (Gotoh et al., submitted).

Fitting parameters of the model

In a first step, thermal, hygrometric and stress parameter values were used and adjusted according to the distribution of *T. evansi* in Brazil and Argentina. In a second step, a fine adjustment was done using both native and invaded areas, according to the methods proposed by Kriticos et al. (2001) and Wharton et al. (2004). Explanations of the parameters significance and values are developed in Sutherst et al. (2004).

Results and discussion

Potential World distribution

We rapidly present here the potential world distribution of *T. evansi* modelled in this work (Figure 2) and the fitted model parameters (Table 1). The model closely agrees on the known distribution of the mite and shows a potential distribution stretching from the tropics to some confined temperate zones. Although *T. evansi* is a tropical species, preferring dry and hot areas, it was also encountered in more wet or cold locations (see Annex 1).

The model projections include the current known distribution in America, Africa, Asia and Europe.

In its native area – South America – the potential distribution of *T. evansi* is wider than the area defined so far by the collection records. The modelled area ranges from north-east (wet coast and dry inlands) of Brazil (State of Maranhão) to central (temperate and dry) Argentina (Province of San Luis), including Uruguay, Paraguay and low lands in Bolivia (Figure 2).

Cold and dry stresses appear to circumscribe the mite in the southern part of Argentina. *Tetranychus evansi* is confined to the East of the Andes Cordillera. The Amazonian Basin does not appear suitable for *T. evansi* due to high values of both wet and hot-wet stresses. Among the potential distribution in exotic areas, almost all sub-Saharan Africa appears suitable to *T. evansi*, with the exception of the Namibian desert, which is too dry, and the Guinean coast, which is too wet. Potentially suitable zones that are not yet infested but have extensive areas with suitable climatic conditions, are i) in Asia: India, a large part of Indochina and south-eastern China; ii) all the

Table 1. CLIMEX parameter values used to model the geographical distribution of *Tetranychus evansi*. See text for the significance of value figures. Temperatures are in °C, moisture is the proportion of soil moisture holding capacity, rates are in week^{-1}, degree-day is the sum of °C*day^{-1} above 10°C.

Variable	Parameter	Value
Temperature	SM0 Limiting low temperature	10
	SM1 Lower optimal temperature	20
	SM2 Upper optimal temperature	34
	SM3 Limiting high temperature	38
Moisture	DV0 Limiting low moisture	0.15
	DV1 Lower optimal moisture	0.22
	DV2 Upper optimal moisture	0.7
	DV3 Limiting high moisture	1.5
Cold stress	TTCSA Temperature threshold (average)	10
	THCSA Temperature rate (average)	-0.002
Heat stress	TTHS Temperature threshold	38
	THHS Temperature rate	0.002
Dry stress	SMDS Dry stress threshold	0.15
	HDS Dry stress rate	-0.01
Wet stress	SMWS Wet stress threshold	1.5
	HWS Wet stress rate	0.02
Cold-wet stress	DTCW Cold-wet degree-day threshold	11
	MTCW Cold-wet moisture threshold	0.9
	PCW Cold-wet stress rate	0.002
Hot-wet stress	TTHW Hot-wet temperature threshold	32
	MTHW Hot-wet moisture threshold	0.9
	PHW Hot-wet stress rate	0.02
Generations	Degree-day per generation	255

Australian coasts, and especially the east coast; iii) many parts of Central America and the Carribean. In the temperate zone, the projections are limited to the relatively restrict areas where mean temperature of the three coldest months is higher than 5°C (Figure 3). Dry stress, wet stress and especially cold-wet stress also limit the distribution of *T. evansi* in the temperate zone.

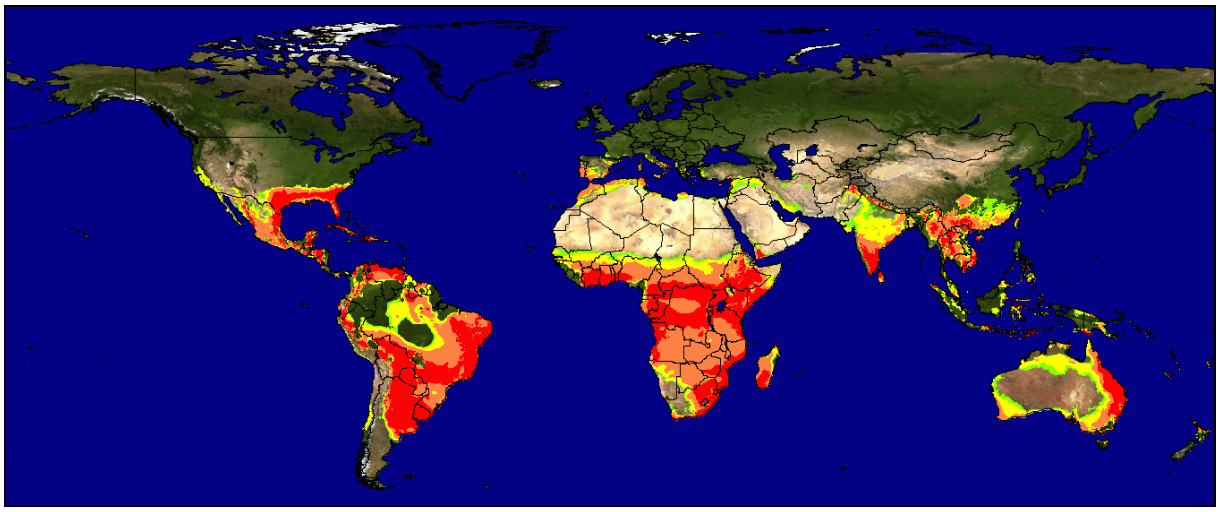

Figure 2. Potential world distribution of Tetranychus evansi as modelled using CLIMEX. EI values: ■ 1-2 - marginal, ■ 3-10 - suitable, ■ 11-25 – very suitable, ■ >25 - excellent.

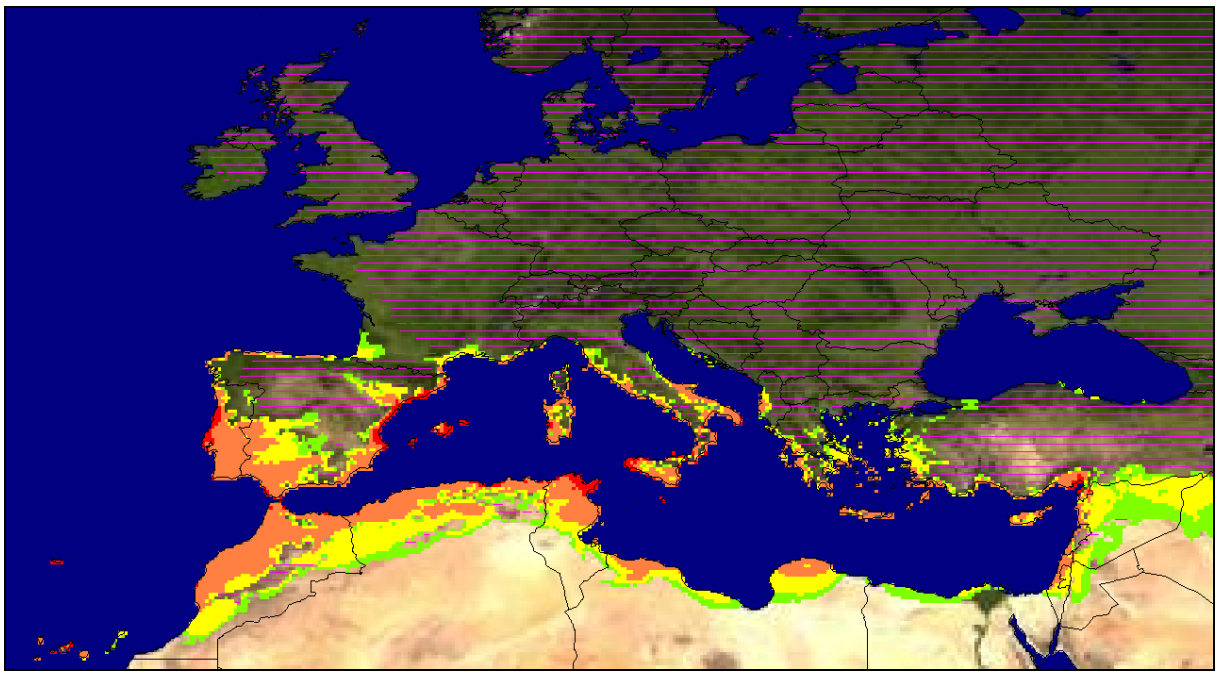

Figure 3. Potential distribution of *Tetranychus evansi* in the Mediterranean Basin as modelled using CLIMEX and mean winter temperature derived from CRU ((New *et al.*, 2002). EI values: ■ 1-2 - marginal, ■ 3-10 - suitable, ■ 11-25 – very suitable, ■ >25 - excellent. Mean winter temperature ≡<5 °C.

Mediterranean Basin

The Mediterranean Basin is an interesting region combining the climate boundaries and the well known distribution of the mite. Study of the distribution maps combined with the stress values allows to analyse these boundaries of the potential climatic zone for *T. evansi*. The European Mediterranean coast (Figure 3) is a well prospected area where *T. evansi* was recorded in 1991. The north coast of the Mediterranean Basin offers mountainous and contrasted landscape due to Alpine barrier. This produces a relatively warm climate, but restricted to the coastal areas, where the sea minimal temperature is 12 °C. The use of a fine scale (10' of arc) allows enough detailed projections in these contrasted areas. In addition, the Iberian potential distribution is restricted to

the Mediterranean climate zone with relatively warm winters. Records are still limited to the coastal areas, regardless that the mite was first recorded more than ten years ago and that the eastern part of Spain has been actively prospected for the presence of *T. evansi*. We can observe, at a fine scale, a strong correspondence between suitable habitats as predicted by the model and observations.

Photo 4. Outbreak of *Tetranychus evansi* on protected culture of tomato (*Lycopersicon esculentum*) France, October 2007. The orange mass under the tunnel are spider mites.

Low temperature is an obvious limiting factor for this tropical mite. Without diapause, the mite survival rate during winter is very low (Ohashi et al., 2003; Migeon, 2007). However *T. evansi* populations have survived for several years and repeated records indicate a stably presence of the mite. Some life history traits of the mite might account for its presence in these areas (Ohashi et al., 2003). *Tetranychus evansi* has a very high intrinsic rate of increase (Moraes & McMurtry, 1987; Bonato, 1999; Kotsubo *et al.*, 2004) and low spring populations can lead to high autumnal densities (Photo 4) as has been observed for example in Europe (Ferragut & Escudero, 1999; Ferragut & Escudero, 2002; Migeon -this study-). At least 6 annual generations of *T. evansi* are expected to occur in these Mediterranean localities, which allow an important population increase. In this way the use of these marginal distribution records allows the model to define the complete bioclimatic range or fundamental eco-climatic niche (Kriticos & Randall, 2001; Wharton & Kriticos, 2004). The cold climatic conditions, and cold stress, encountered in the north Mediterranean basin coast (and also in Japan, not developed here, see Migeon et al. submitted) define the range-limiting factor (Guisan & Thuiller, 2005).

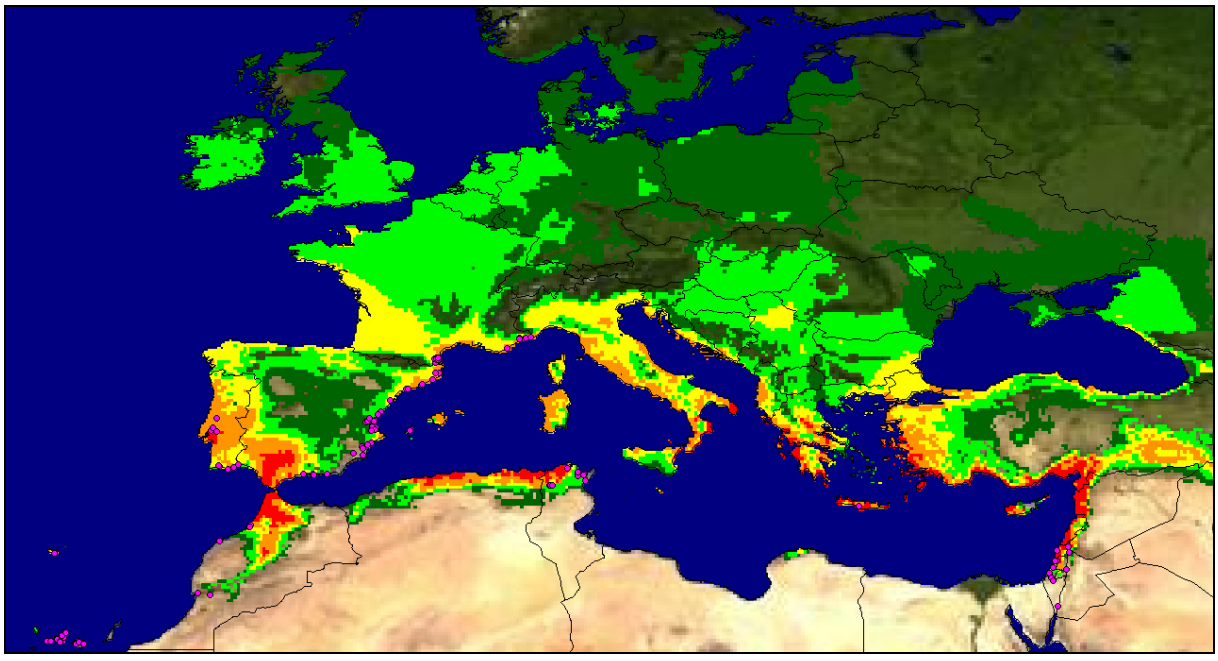

Figure 4. Potential growing of tomato (Lycopersicon esculentum) in the Mediterranean Basin as modelled using EcoCrop modelling, a module implemented in DIVA-GIS (Hijmans et al., 2001; Hijmans et al., 2005). l indicate both, bibliographical and observation records. Values: ■ very marginal, ■ marginal, ■ suitable, ■ very suitable, ■ excellent.

Agricultural issues

The Mediterranean Basin is a zone where tomato and aubergine growing is concentrated in Europe (Costa & Heuvelink, 2005). It represents a major area for field growing of tomatoes (Figure 4).The climate which offers a high-potential for the development of the pest. It is also an area with well established populations, and where new observations are still often recorded (see Annex 1). Mediterranean Basin gathers all the required conditions for a rapid and effective dispersion of the mite, representing a real threat for the agriculture of this region.

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INTRODUCTION OF SOME PORONOTIC ORIBATID MITES OF MAZANDARAN PROVINCE, NORTHERN IRAN

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Abstract

During 2003-2004, in a biodiversity survey of poronotic oribatid mites (Acari: Oribatida: Poronota) in Mazandaran province, North of Iran, 39 species belonging to 29 genera and 18 families were collected. Among them, 5 families, 18 genera and 13 species were new records for Iran. Collection data and distribution of each species are presented together with the map of sampling locations in the Mazandaran province.

Key-words

Acari, Oribatida, new records, Iran

Introduction

Iran is one of the few completely uninvestigated countries in relation to the taxonomic study on its oribatid mite fauna. Faunal lists containing new records have been published by Akrami and Saboori (2001, 2004), Akrami (2007), Akrami et al., (2007), and descriptions of several new species have been published by Bayartogtokh and Akrami (2000a,b), Mahunka and Akrami (2001), Akrami and Subias (2007a,b) and Akrami and Coetzee (2007). In this paper, 5 families, 18 genera and 13 species are added to the Iranian oribatid fauna. During 2003-2004, in a faunistic survey of oribatid mites of Mazandaran province (Figure 1), Northern Iran, soil and litter samples were taken from the surface to a soil depth of 10 cm under trees, crop and weed plants.

Materials and methods

Sampling was done from about -20 to 3000 m height above sea level in 35°30'N-37°N and 50°30'E-54°E.

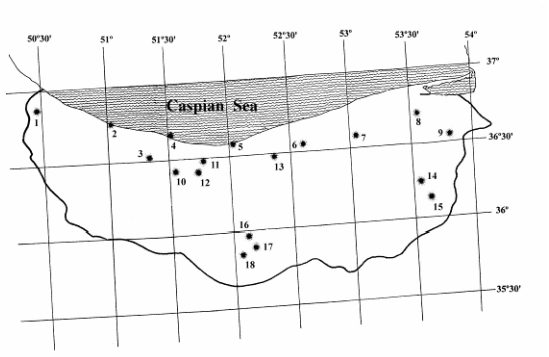

Figure 1. Map of sampling locations in the Mazandaran province of Iran.

Mites were extracted using Berlese funnel and preserved in 75% ethyl alcohol and cleared in lactophenol and mounted in Faur's mixture on glass microscopic slides. Classification followed Balogh and Balogh (1992) and another identification keys. All specimens were deposited in the Acarological collection, of the Department of

Plant Protection, College of Agriculture, Shiraz University, Iran.

Results

During 2003-2004, in a faunistic survey of oribatid mites (Acari: Oribatida) in Mazandaran province, 39 species belonging to 29 genera and 18 families were collected. Among them, 5 families, 18 genera and 13 species (marked by asterisk in the list below) were new records for Iran. All species were new records for the Mazandaran province. All specimens were collected by M. A. Akrami. The list of species follows:

Phenopelopidae*

*Eupelops** *acromios** (Herman, 1804). Material examined: Nashtarood, -20 m. (Fig. 1, No. 2 on map), soil under citrus fruits, 9-IV- 2004; Amol, 600 m. (No. 13 on map), soil of *Arthemisia* sp., 25-V-2004; Behshahr (Aftalet village), 1550 m. (No. 9 on map), soil of pasture, 29-IX- 2004.

*E. torulosus** (Koch, 1839). Material examined: Kojoor road, 1472 m. (No. 12 on map), soil, 18-VII-2003.

Passalozetidae

*Bipassalozetes** *striatus** Mihelcic, 1955. Material examined: Amol (Rine road to Lar), 2300 m. (No. 17 on map), soil of pasture, 5-V-2004; Behshahr (Aftalet village), 1700 m. (No. 9 on map), soil of pasture, 29-IX-2004.

Scutoverticidae

*Scutovertex minutus** (Koch, 1836). Material examined: Amol (Pelomon road to Rine), 1650 m. (No. 17 on map), soil of pasture (*Astragalus* sp.), 5-V-2004. Material examined: Behshahr road to Zaghmarz, 20 m. (No. 8 on map), soil of pasture, 29-IX-2004.

Oribatulidae

Oribatula (*Zygoribatula*) *exarata** Berlese, 1916. Material examined: Amol- Tehran road (35 Km), 600 m. (No. 13 on map), soil of pasture (*Arthemisia* sp.), 5-VI- 2004; Babol, 0 m. (No. 6 on map), soil of Graminae, 29-X-2004.

O. (*Oribatula*) *tibialis** *tibialis* (Nicolet, 1855). Material examined: Many specimens in various parts of Mazandaran province.

O. (*O.*) *pallida** Banks, 1906. Material examined: Kandeloos road, ? m. (No. 3 on map), soil under Cypress trees, 10-IV-2004.

*Pseudoppia** *mediocris** (Mihelcic, 1957). Material examined: Behshahr, Kiasar, Ara village, 2000 m. (No. 14 on map), soil of pasture, 26-IIX-2004.

Haplozetidae

*Brasilobates bipilis** Perez-Inigo & Baggio, 1980. Material examined: Babol, 1 m. (No. 6 on map), soil under moss, 3-VI-2004; Nowshahr, 19 m. (No. 4 on map), soil under moss and weeds, 4-VI-2004; Behshahr road to Zaghmarz, 20 m. (No. 8 on map), soil of pasture, 29-IX-2004.

Hemileiidae*

*Dometorina** *plantivaga** (Berlese, 1895). Material examined: Sari, 52 m. (No. 7 on map), soil, 4-XI-2003.

Scheloribatidae

*Turcibates** *parvus** Ayyildiz & Luxton, 1989. Material examined: Amol, Poloor, 2100 m. (No. 18 on map), soil of pasture, 5-VI-2004.

Mochlozetidae

*Podoribates** sp. Material examined: Ramsar, Javaherdeh road, ? m. (No. 1 on map), soil of pasture, 9-IV-2004.

Oribatellidae*

*Oribatella** sp. Material examined: Behshahr (Aftalet village), 1550 m. (No. 9 on map), soil of pasture, 30-IX-2004.

Ceratozetidae

*Latilamellobates** *naltschicki** Shaldybina, 1971. Material examined: Behshahr (Baladeh village), 2000 m. (No. 15 on map), soil of pasture, 26-IX-2004.

*Trichoribates** sp. Material examined: Amol-Tehran road (35 Km), 600 m. (No. 13 on map), soil of pasture (*Arthemisia* sp.), 7-VI-2004.

*Sphaerozetes** sp. Material examined: Noor, -17 m. (No. 5 on map), soil of forest trees, 19-V-2000.

*Ceratozetes** sp. Material examined: Kandeloos road (No. 3 on map), soil under Cypress trees, 10-IV-2004.

*Ceratozetella** sp. Material examined: Near Damavand mountain, 2800 m. (No. 16 on map), soil of pasture, 5-IIX-2004.

Mycobatidae

*Minunthozetes** sp. Material examined: Ramsar, Javaherdeh road, ? m. (No. 1 on map), soil of forest trees, 9-IV-2004.

Cerasellidae*

*Cyrtozetes** sp. Material examined: Babol, 0 m. (No. 6 on map), soil of Graminae, 29-X-2004.

Euzetidae*

*Euzetes** sp. Material examined: Nowshahr, Diwcheshmeh, 1700 m. (No. 10 on map), soil of forest trees, 11-IV- 2004; Royan road to Firoozkola (after Kodir), 1630 m. (No. 11 on map), soil of forest trees, 11-IV- 2004; Nowshahr, 19 m. (No. 4 on map), soil, 8-VI-2004.

Achipteriidae

*Achipteria** sp. Material examined: Nowshahr, 19 m. (No. 4 on map), soil, 17-VII-2003.

*Parachipteria** sp. Material examined: Nowshahr, Namak-Abrood, 88 m. (No. 4 on map), soil of forest trees, 14-IX-2003.

*Tectoribates** sp. Material examined: Unknown place in Mazandaran province.

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NEW AND RARE SPECIES OF MITES (ACARI: GAMASINA) FROM SOME FOREST ECOSYSTEMS OF THE DANUBE DELTA BIOSPHERE RESERVE

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Abstract

The study contains the redescription of the species *Epicriopsis palustris* Karg, *Arctoseius insularis* (Willmann) and *Neojordensia levis* (Oudemans & Voigts), identified in the context of some ample researches concerning the diversity of the edaphic mites fauna (*Acari: Gamasina, Oribatida*) in the Danube Delta Biosphere Reserve. The first species represents the only one of the *Neojordensia* genus in the Romanian fauna. In our country all these species were identified only in the Danube Delta perimeter. With the above mentioned investigations, it was recorded also a new species of the *Macrocheles* genus. For this one it was given some elements and some illustrations, but this species will be described in a future paper.

Key-words

Gamasid mites, morphology, taxonomy, redescription

Introduction

The priorities of the scientists, including the acarologists, are the revision and revaluation of the systematics, as well as investigation of some less studied zones and habitats, the Danube Delta's ones counted among these. Its territories encompasses besides a great number of islands, marshes, tributaries canals, lakes, dunes, a big mosaic of grasslands and forests. All of these are a great source of biodiversity which must be known and preserved.

Within the framework of some anterior researches it was analyzed the diversity of the edaphic mites fauna (gamasid and oribatid mites) from the forest ecosystems of the Danube Delta Biosphere Reserve. The list of species and the faunistic analysis have been already published in a previous article. In that context it was pointed out for the first time on the Romanian territory *Neojordensia* genera and 7 species of gamasid mites (Ivan *et al.* 2006). Until the present time all these species were identified only in the deltaic perimeter. Among these species are redescribed and illustrated in this paper the following ones: *Epicriopsis palustris* Karg, *Arctoseius insularis* (Willmann) and *Neojordensia levis* (Oudemans & Voigts). Beside these it was identified a new species of the *Macrocheles* genus of which short diagnosis is presented here, a detailed description will be published in a subsequent article.

Material and method

The faunistic material was collected from the soil of the following stations and forest types:
- natural forests:
  - *Quercus robur* and *Q. pedunculiflora* - Letea

forest, Hăşmacul Mare;

- *Quercus robur, Fraxinus pallisae* and *Populus alba* (Letea bank Plăvosu forest);

-anthropized forests : *Salix alba, S. fragilis, Populus alba, Fraxinus pallisae* in theembanked eclosures Uzlina and Sireasa;

- forest plantations:

- Canada poplar – Uzlina Sireasa ; Letea bank, Nebunu forest ;

- *Populus tremula*, *Elaeagnus angustifolia* - Sf. Gheorghe

From each station it was taken 5-7 soil samples with a sourface of 100 cm^2. For the fauna extraction was used Tullgren-Berlese method, modified by Balogh. Lactic acid was used to clear the the specimens. The drawings were made with camera lucida. The specimens stored in 70% alcohol were deposited in the laboratory's collection.The measurements are given in micrometers (µm).

Results

***Macrocheles (Macrocheles)* n. sp. (Figures 1, 2)**

Diagnosis: Dorsal shield oval in shape, with a reticulated ornamentation, with 28 pairs of setae and 18 pairs of pores. The shape of the dorsal setae is characteristic: broadened and barbed distally (j4, Z4); densely feathered along their entire length (J5); thin and barbed (Z4, Z5, S4, S5) (Figure 1A). This species may be distinguished from other known *Macrocheles* species especially by dorsal chaetotaxy (Mašán 2003).

The ornamentation of the sternal shield is a characteristic one, with a discrete foveolar draw that have an oval shape in the posterior part. The ventrianal shield almost round with a foveolar ornamentation distributed in a polygonal net.

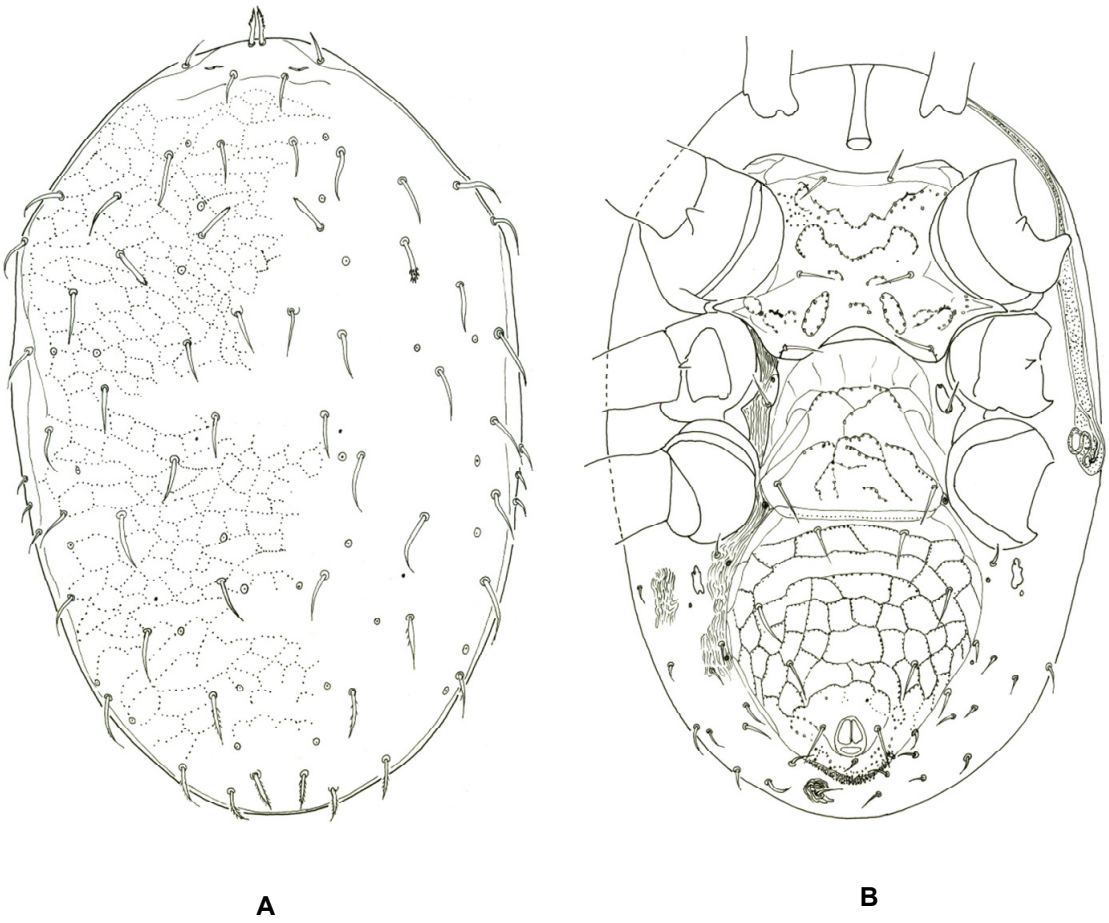

Figure 1. *Macrocheles (Macrocheles)* n. sp. female: A - dorsal aspect; B - ventral aspect

Chelicerae are stout and strongly sclerotized. The fixed digit has three denticles and a thick pillus dentilis, thorn like. A lyrrifisure is extended on the whole length of the fixed digit. The mobile digit has two denticles, the first one being small and the second one stronger and milled. At the base of the fixed digit is a short, broad, milled seta (Figure 2B).

At the male the fixed digit has two denticles; the cheliseta is thin. Mobile digit with two denticles and a spermatodactyl in "S" shape, with a length equal with that of the digit. At the base of fixed digit it is a thorn like seta (Figure 2C).

Dorsal chaetotaxy is that of Lindquist and Evans (1965), recommended by Krantz (1981) and Halliday (1986) for *Macrochelidae* and used also by Mášan (2003).

Examined material. 1 female holotype, 1 male holotype and 2 paratypes (female and male) – 20.08.1993, Nebunu forest, Letea bank .

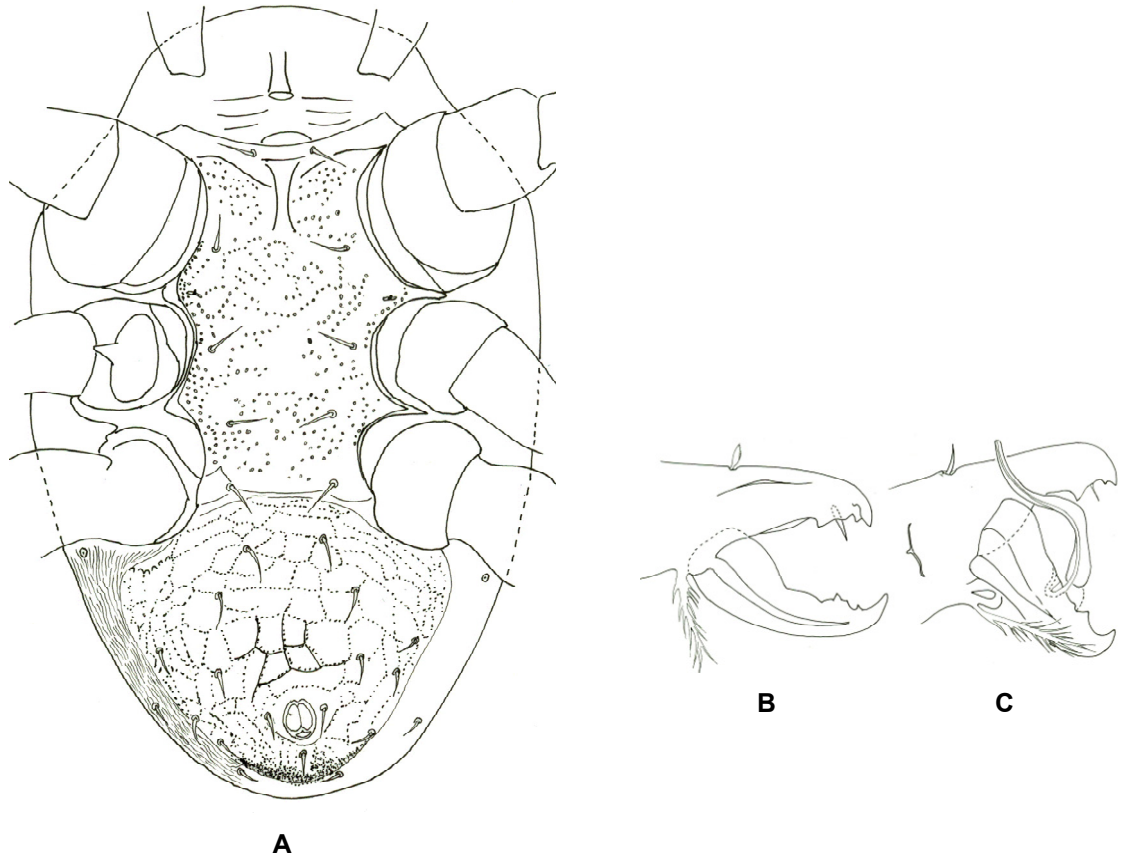

Figure 2. *Macrocheles (Macrocheles)* n. sp. A – male, ventral aspect; B– female chelicerae; C– male chelicerae

***Epicriopsis palustris Karg,* 1971 (Figures 3, 4)**

The species belongs to *Ameroseiidae* Family and *Phytoseioidea* Super-family

Female. Size: 320 - 364 µm long/ 211 - 281 µm wide. The body is ovoid. Dorsal shield. Dorsal shield has 21 pairs of setae with different shapes and sizes. The length of the short setae is between 13-60 µm, and that of long ones 18-197 µm (Figure 3A).The first category of setae are generally smooth and thin, with the exception of i1 which is barbed and very thick; also one pair of posterior and marginal setae are delicate barbed. Long setae are stouter and barbed on their whole length. The ornamentation is made by tubercles in star shape, between them being granulations.

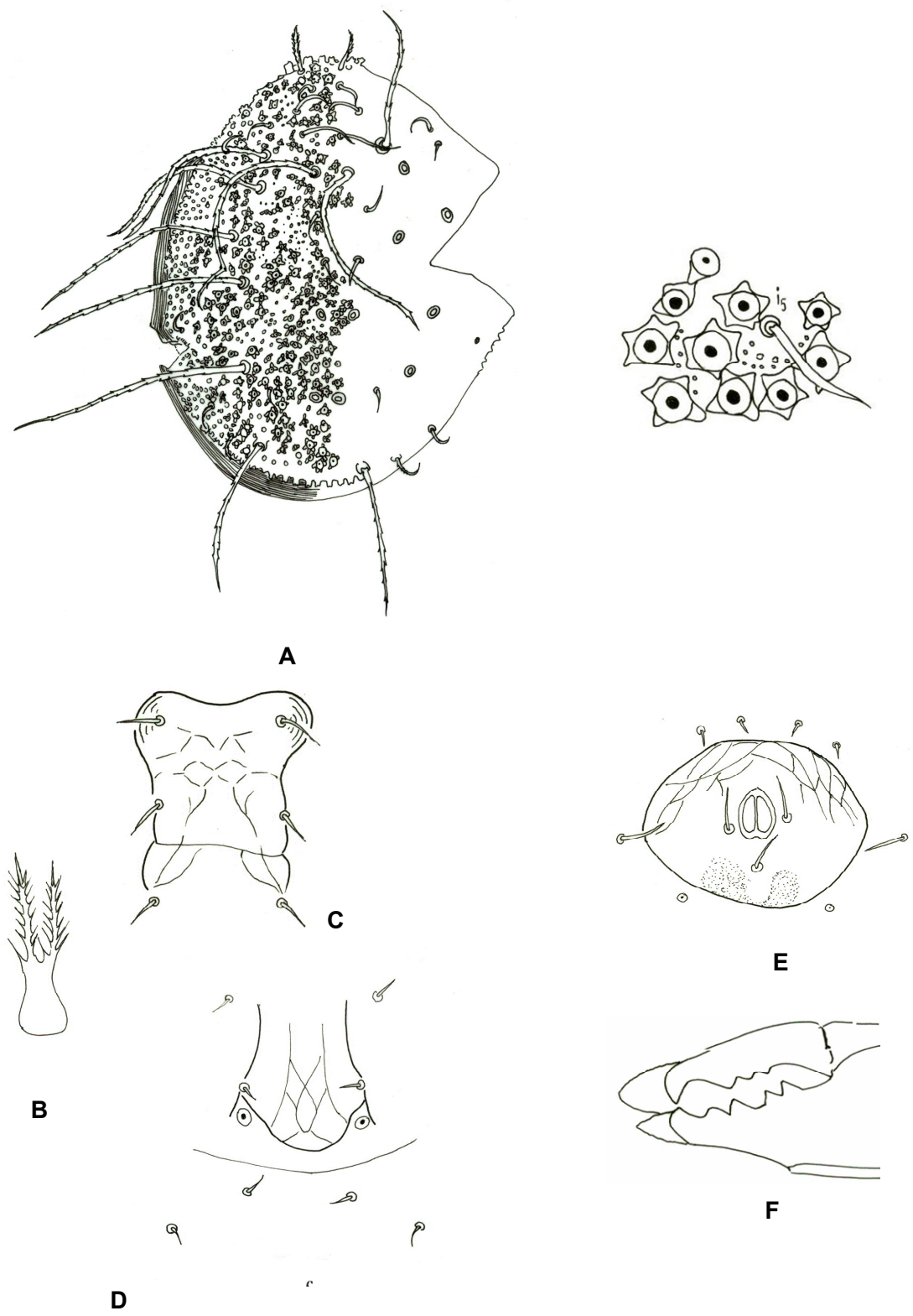

Figure 3. *Epicriopsis palustris* Karg, 1971 female: A - dorsal aspect; B – tectum; C – sternal shield; D – genital shield; E – ventrianal shield; G – chelicerae

Ventral shields. The literature data concerning the ventral morphology are incomplete and even inexact (Karg 1971). Sternal shield has 40 µm in length and 42µm in breadth (at the coxae II level) (the ratio between length and in breadth is 0.95). The first two pairs of setae are on the shield and the next two outside (Figure 3C). All three pairs of setae are simple, short, with the approximate size of 16 µm. The ornamentation of sternal shield is reticulated and discrete (Figure 3C).

Genital shield is a little bit rounds off at the posterior with a very fine net like ornamentation. The pair of genital setae is simple and short, approximately of 13 µm. The shield is flanked in the inferior part by a pair of large and well evidenced pores, and also by two simple and short setae (Figure 3D).

Ventrianal shield wide, with 62 µm in length and 83 µm in breadth (the ratio between length and breadth is 0.74). In the upper part of the shield there are 2 pairs of simple, short setae (16 µm), lateral one pair of setae a little bit longer and simple, too; at the lower part there is a pair of pores well marked. The anal setae are simple and short (16 µm) (Figure 3E).

The chelicerae are small with short digits. The mobile digit have three denticles and the mobile one four. At the both digits extremities was evidenced a fine membranous structure, that does not appear in the original drawing (Karg 1971). At the base of mobile digit is a lyrrifisure.

The tectum has two branches and it is strongly barbed (Figure 3B).

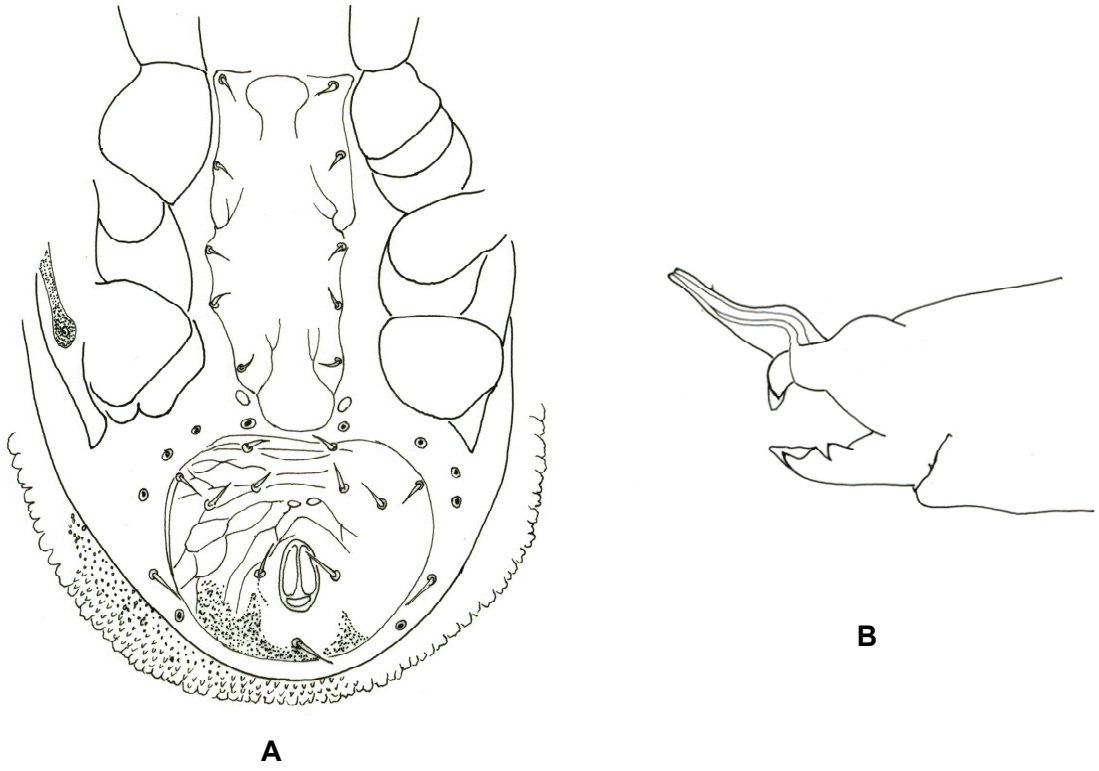

Figure 4. *Epicriopsis palustris* Karg, 1971 male: A - ventral aspect; B - chelicerae

Male. Size: 256 µm long/ 179 µm wide. Dorsal shield's chaetotaxy is the same as at the female. Sternogenital shield has 5 pairs of simple and short setae (10 µm); the ornamentation is represented by a very fine striation. Ventrianal shield has 4 pairs of simple, short setae (10 µm); the ornamentation is net like. Around the shield it can be observed some large pores well evidenced. On the ventral side of dorsal shield are many spicules and granulations (Figure 4A).

The chelicerae are small with short digits. The mobile digit has two sharp denticles and the mobile one has a spermatodactyl with a bigger

length than the digit. At the base of fix digit is a lyrrifisure. As well as at the female the digits extremities have a fine membranous structure that does not appear in the original drawing (Karg 1971). (Figure 4B).

Remarks. *Epicriopsis palustris* was not found before in Romania (Stănescu & Juvara-Balş 2005) and was recorded for the first time in the deltaic habitats (Ivan *et al.* 2006).. In Europe this species has been identified in swamp zones and in the rivers beds (Karg , 1993). So, as it is suggested by its name this species is hygrophilous. Thus, its observation in the forests of the deltaic biome is not so surprising.

Examined material: 2 females, 1 male – 10.06.1995 - Letea forest, Hăşmacul Mare; 1 female, Sf. Gheorghe – 24.05.1994.

***Arctoseius insularis* (Willmann, 1952) (Figure 5)**

The species belongs to *Ascidae* Family and *Ascoidea* Super-family

Female. Dorsal shield. Size:320-333 µm long/180-186 µm wide. Dorsal shield is unique, with a reticulated ornamentation. Under s7 setae, little lateral incisions exist. The dorsal setae are simple, elongated (16-18 µm) (Figure 5A).

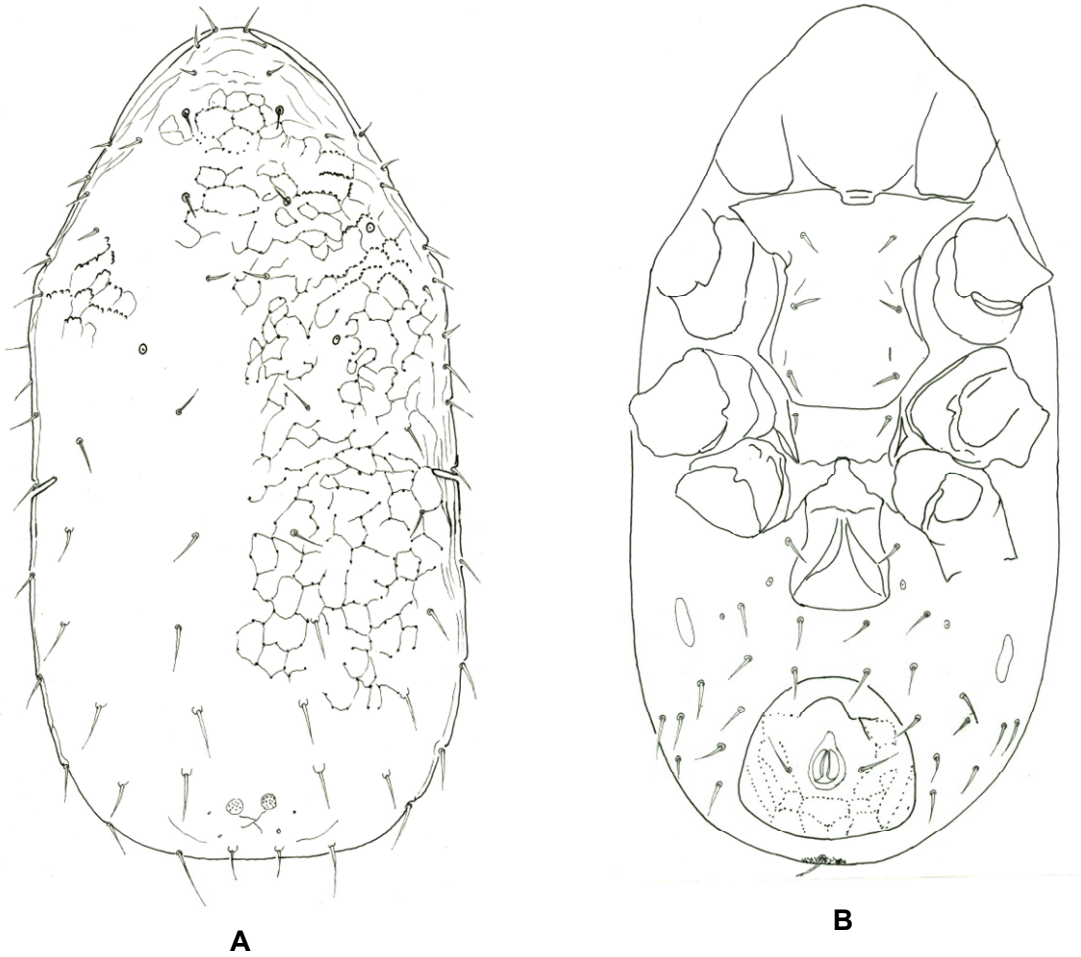

Figure 5. Arctoseius insularis (Willmann, 1952) female: A - dorsal aspect; B - ventral aspect

Ventral shields. Sternal shield has 78µm in length and 52 µm in breadth (at the coxae II level). The three pairs of setae are simple (11-15 µm).

Genital shield has a straight posterior edge and a very discreet ornamentation formed by fine lines; a pair of simple and short setae (13 µm) is visible, too. Some muscle insertions are evidenced. Paragenital pores are in the posterolateral sides of the shield (Figure 5B).

The ventrianal shield has a reticulated microsculpture and 2 pairs of simple short setae.

Remarks. The identification of *A. insularis* in some

deltaic forests (Ivan *et al.* 2006) and the literature data concerning the species' autecological peculiarities (Karg 1993) indicate the preferences of this species for humid habitats.

Examined material: 2 females – 10.06.1995 - Letea bank, 3 females – 9.06.1995 -embanked enclosure Sireasa; 2 females – 24.06.1994 - Uzlina forest plantation.

***Neojordensia levis* Evans, 1957 (Figure 6)**

The species belongs to *Podocinidae* Family and *Phytoseioidea* Super-family.

Female. Dorsal shield. Size: 390 µm long/ 256 µm wide. The body is ovoid. Dorsal shield has 42 setae, short, smooth (22 pairs anterior and 20 posterior). In the anterior part of the body, under the first pair of setae are two big lyrifisures, well evidenced. The ornamentation is net like. In the anterior part are visible muscle insertions (Figure 6A). The slength of the setae are between 5-13 µm.

Ventral shield. Sternal shield has 102 µm in length and 72 µm in breadth (at the coxae II level). The three pairs of setae are simple, short (approximately 12 µm). The ornamentation of sternal shield is polygonal, net like. The first pair of setae is on the presternal shields (jugulare). In the anterior part of sternal shield is a pair of lyrrifissures. The main characteristic of this species is the absence of the metasternal shields, the fourth pair of setae being inserted in the cuticle. Between st2 and st3 and behind st3, there are little pores (Figure 6B)

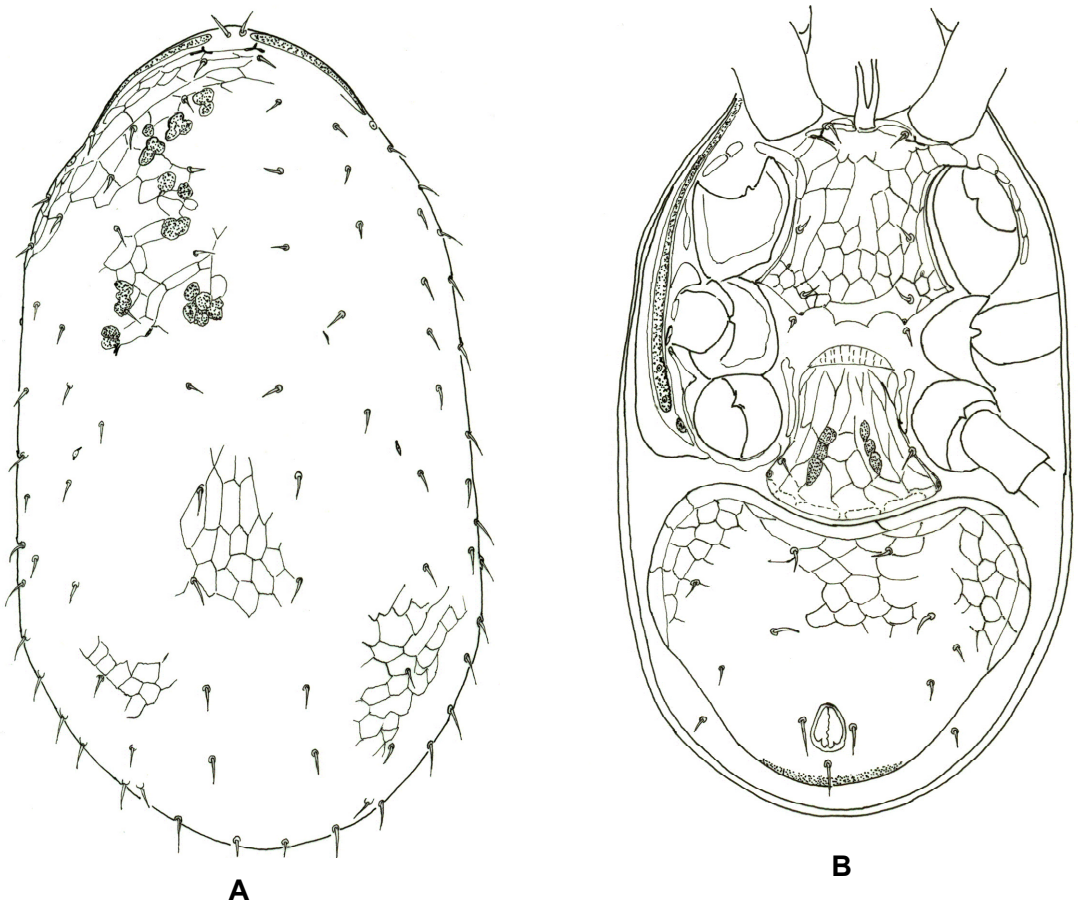

Figure 6. Neojordensia levis Evans, 1957 female: A - dorsal aspect; B - ventral aspect

The genital shield is a little bit round off at the posterior, with a net like ornamentation and has a pair of simple and short setae (13 µm). There are visible muscle insertions. Paragenital pores are in the posterolateral sides of the shield (Figure 6B).

The ventrianal shield (147µm long/205 µm wide; ratio length/breadth 1.19), has a reticulated microsculpture and 4 pairs of simple short setae (8-

10 µm). In the posterior part of the shield outside on the body is a pair of simple, short setae (8-10 µm) (Figure 6B).

The peritremal shield is one-legged at its posterior edge. Especially, at the coxa I level are visible little exopodal shields. Endopodal shields of irregular shape are well evidenced at the coxae IV level.(Figure 6B).

Remarks. The characters showed are in accordance with the literature data (Bregetova et al 1977). *Neojordensia* genus, implicitly *N. levis* were pointed out for the first time in the Romanian fauna in D. D. B. R. territory (Ivan *et al.* 2006; Stănescu, Juvara-Balş 2005).

Examined material: 3 females – 16.07.1995 - Letea bank.

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THE FAMILY SCHELORIBATIDAE GRANDJEAN, 1933 IN ROMANIAN FAUNA

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Abstract

A review of the species belonging to Scheloribatidae family Grandjean, 1933 has been performed. Nine species of Scheloribates s. str. Berlese, 1908, recorded on Romanian territory, were analyzed. A diagnosis, the main biometrical data and original illustrations are given for each of them. An identification key, including both morphological and biometrical characters, is proposed. In this context were also described two new species: Scheloribates (S.) longisensillus n. sp. and Scheloribates (Topobates) vasiliui n. sp.

Key words

Scheloribatid mites, morphology, biometry, diagnosis.

Introduction

The family *Scheloribatidae* Grandjean, 1933 (sensu Balogh&Balogh, 1984; Perez-Iñigo, 1993; Subias, 2004), widely distributed throughout the world, is represented in European fauna by a relatively limited number of species. Their characters are often very similar and it is therefore difficult to differentiate one species from another.

The comparative morphological analysis carried out in this paper takes this into consideration, as well as some biometrical characters. On this basis, an identification key for the species of *Scheloribates* s. str. Berlese, 1908, has been elaborated.

Scheloribates (Scheloribates) barbatulus Mihelčič, 1956 (Figs. 1, 2)

Literature used for identification: Ghiliarov & Krivolutsky (1975), Mihelčič (1956), Perez-Iñigo (1974, 1993).

Diagnosis. Medium sized species, but comparatively, one of the smallest representatives of the genus (Table 1). Yellowish colour, tegument without ornamentation. Prodorsum with lamella, prolamella, and sublamella, constituting a characteristic complex for this genus. Prodorsal setae robust and barbed. Sensillus fusiform elongated, with the distal part narrow and a pointed apex. Notogaster oval, elongated, the length/breadth ratio having a comparatively high value. Pteromorphs are narrow and less conspicuous. 10 pairs of notogastral setae – very small and hardly observable – are present (Table 2). 4 pairs of sacculi, typically positioned. Epimeral region with the characteristic configuration, and the setae placed according to the formula 3:1:3:3. Genito-anal region with the usual setal formula 4:1:2:3.

Distribution and autecology. Southern Palaearctic species (Subias, 2004).

The species has been recorded in the North-

Eastern part of Romania in some silvo-steppe hayfields. Probably, it is a thermo-xerophilous, lawn species.

Scheloribates (S.) fimbriatus Thor, 1930 (Figs. 3, 4)

Literature: Ghiliarov & Krivolutsky (1975)

Diagnosis. Species of medium size, yellowish in colour; it is one of the smaller scheloribatid species recorded in Romania (Table 1). Tegument smooth, without ornamentation. Prodorsum with narrow, convergent lamellae; sublamella and prolamella present. Prodorsal setae robust and barbed. Sensillus long, reclinated, fusiform elongated, with a long apical thorn. Notogaster oval elongated, the length/breadth ratio having a value similar to that of the preceding species. 4 pairs of sacculi in habitual position. 10 pairs of notogastral setae, simple, thin, but easily observable (Table 2). The epimeral and genito-anal regions with the characteristic aspect in this genus and the typical chaetotaxy.

Distribution. Cosmopolitan species (Subias, 2004).

Scheloribates fimbriatus was cited in several counties from Southern and Eastern regions of Romania, in lucerne and cereal crops, and in some sowed and natural lawns as well. Some of the earlier records need revision (Vasiliu et al., 1993).

Scheloribates (S.) labyrinthicus Jeleva, 1962 (Figs. 5, 6)

Literature: Csiszár & Jeleva (1962), Ghiliarov & Krivolutsky (1975), Miko (1987), Perez-Iñigo (1993).

Diagnosis. Species of medium size, dark chestnut in colour; it is one of the larger species of this genus (Table 1). Cuticle shows a characteristic, reticulate ornamentation with a labyrinth-like aspect. The lamellar complex (lamella, prolamella, sublamella) is present on the prodorsum. Prodorsal setae robust and barbed (Table 2). Sensillus reclinated, fusiform elongated, with asymmetrical distal part and lanceolate end. Notogaster oval, with well developed pteromorphs. 10 pairs of notogastral setae – simple, thin, but easily observable. 4 pairs of sacculi typically positioned. Epimeral region strongly sclerotized, with the setal formula 3:1:3:3. Both the ventral plate and the anal valves show reticulate ornamentation, similar to that present on the notogaster. Genito-anal region with the typical chaetotaxy.

Distribution and autecology. European (Ghiliarov & Krivolutsky, 1975) or Mediterranean species (Subias, 2004).

In Romania, *S. labyrinthicus* has been recorded in the Southern and Eastern zones; no citations inside the Carpathians' arc. The species is frequent in varied types of lawn ecosystems: meso-xerophilous plain lawns, mesophilous mountainous meadows, up to alpine ones. It was also recorded in eutrophic bogs, and only accidentally in deciduous forests (Vasiliu et al., 1993). We consider that it is a eurytopic, lawn species.

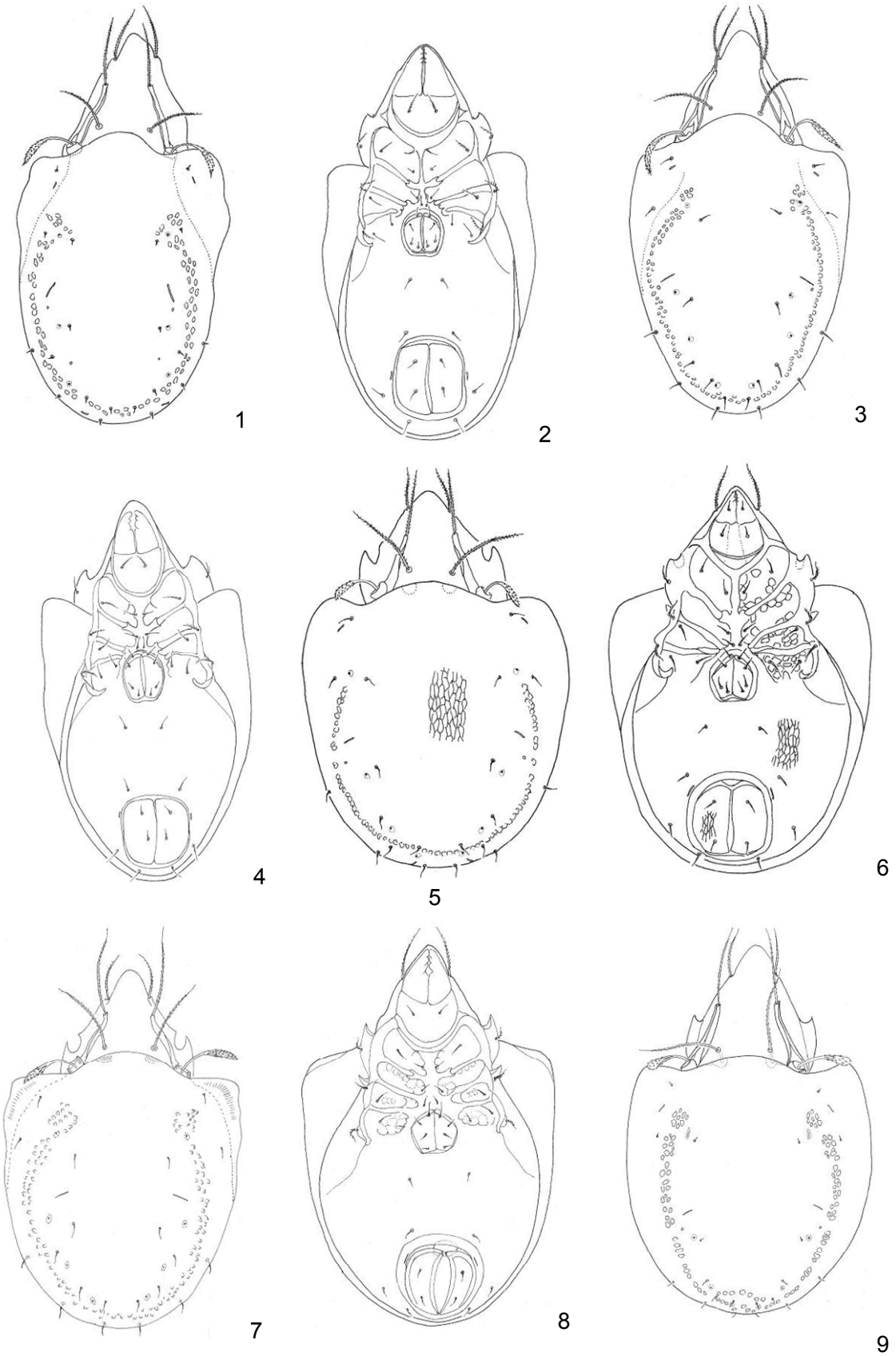
Scheloribates (S.) laevigatus (C. L. Koch, 1836) (Figs. 7, 8)

Literature: Ghiliarov & Krivolutsky (1975), Perez-Iñigo (1974, 1993), Weigmann (1969, 2006), Wunderle & al. (1990).

Diagnosis. Medium sized species with a large variability of this feature (Table 1), chestnut in colour. Cuticle smooth, without obvious ornamentation. Prodorsum with convergent lamellae; prolamella and sublamella present. Prodorsal setae robust and barbed. Sensillus reclinated, with the distal part elongated and fusiform in shape and with a lanceolate apex. Notogaster with well developed pteromorphs. 10 pairs of notogastral setae – simple, thin, but easily visible (Table 2). 4 pairs of sacculi in the typical position. The ventral side with the characteristic aspect and chaetotaxy.

Distribution and autecology. Semi-cosmopolitan species (Subias, 2004).

S. laevigatus has a wide distribution in Romania, occurring frequently in various lawn types, from the plains region up to the alpine level, in deciduous and coniferous forests, in alpine, saxicolous habitats, in peat bogs, in some wet habitats in the Danube Delta, and in cultivated soils (Vasiliu et al., 1993). Therefore, it may be considered as a species with a large ecological valence. Nevertheless, some of the earlier citations need revision.



Figures 1-9. *Scheloribates barbatulus* Mihelčič, 1956: 1-dorsal view, 2-ventral view; *Scheloribates fimbriatus* Thor, 1930: 3-dorsal view, 4-ventral view; *Scheloribates labyrinthicus* Jeleva, 1962: 5-dorsal view, 6-ventral view; : *Scheloribates laevigatus* (Koch, 1836): 7-dorsal view, 8-ventral view; *Scheloribates latipes* (Koch, 1844): 9-dorsal view

Scheloribates (S.) latipes (C. L. Koch, 1844) (Figs. 9, 10)

Literature: Ghiliarov & Krivolutsky (1975), Perez-Iñigo (1993), Weigmann (1969, 2006).

Diagnosis. Medium sized species (Table 1), light chestnut in colour, and cuticle smooth, without ornamentation. Prodorsum with the characteristic, convergent lamellae; prolamella and sublamella present. All prodorsal setae robust and barbed. Sensillus reclinated, fusiform with rounded end. Notogaster oval, rounded with well developed pteromorphs. 4 pairs of sacculi typically positioned. 10 pairs of short notogastral setae, extremely thin and hardly observable. Epimeral region strongly sclerotized, with the setae arranged according to the formula 3:1:3:3. Genito-anal region with the characteristic aspect, and the setal formula 4:1:2:3.

Scheloribates latipes is very similar in its characters to *S. pallidulus* (Koch, 1841); therefore, it was considered as synonym of this species (Subias, 2004). We have found the both species in the same samples; thus, it is possible to tell one from the other and to show the differentiating features.

Distribution and autecology. Holarctic species (Mahunka, 2004; Weigmann, 2006).

In Romania, this species is widely distributed and has been recorded in hilly and mountainous lawns, in deciduous and coniferous forests, in subalpine shrubs, in alpine, saxicolous habitats, in oligotrophic and eutrophic bogs, but rarely in cultivated fields (Vasiliu et al., 1993). Although some of the earlier records need revision, *S. latipes* may be considered a euryplastic form.

Scheloribates (S.) pallidulus (C. L. Koch, 1841) (Figs. 11, 12)

Literature: Ghiliarov & Krivolutsky (1975), Perez-Iñigo (1974, 1993), Weigmann (2006), Wunderle & al. (1990).

Diagnosis. Species of medium size, light yellowish in colour; it is one of the smaller representatives of the genus (Table 1). Cuticle smooth, without ornamentation. Prodorsum with narrow, convergent lamellae; prolamella and sublamella present. Prodorsal setae robust, finely and rarely barbed. Sensillus reclined, fusiform, with rounded end; its distal part is longer than the stalk. Notogaster oval, rounded, with a low length/breadth ratio. Pteromorphs are small, and the 4 pairs of sacculi in the usual position. 10 pairs of simple and thin notogastral setae, which are hardly observable, but relatively longer than in preceding species (Table 2). Ventral side has the aspect and chaetotaxy characteristic for the genus.

Distribution and autecology. Holarctic species (Weigmann, 2006).

Scheloribates pallidulus has a wide distribution in Romania; It has been cited in various types of lawns, from plains up to mountainous zones, in deciduous, coniferous and mixed forests, in alpine, saxicolous habitats, in peat bogs, and accidentally in cultivated soils (Vasiliu et al., 1993). Some of the records are probably erroneous, thus it is difficult to state the ecological peculiarities of this species.

Scheloribates (S.) quintus Wunderle, Beck et Woas, 1990 (Figs. 13, 14)

Literature: Weigmann (2006), Wunderle & al. (1990).

Diagnosis. Medium sized species (Table 1), yellowish or light chestnut in colour. Cuticle smooth, without ornamentation. Prodorsum with narrow lamellae, the prolamella and sublamella are also present. Prodorsal setae robust and barbed. Sensillus reclinated, fusiform, with a lanceolate apex. Notogaster oval, rounded, with small pteromorphs. 10 pairs of notogastral setae – simple, thin, and relatively long (the higher relative length – Table 2). 4 pairs of sacculi in the usual position. Epimeral and genito-anal regions with the characteristic aspect. Chaetotaxy of these regions are also characteristic for the genus.

Distribution. SW Germany.

In Romania the species was recorded for the first time in a sub-mountainous zone (the Argeş county) in some beech forests and, also, in a saxicolous habitat; recently, it was found again in a *Quercus petraea* forest (Eastern Sub-Carpathians, the county of Bacău).

Scheloribates (S.) rigidisetosus Willmann, 1951 (Figs. 15, 16)

Literature: Ghiliarov & Krivolutsky (1975), Willmann (1951).

Diagnosis. Medium sized species (Table 1), chestnut coloured. Cuticle smooth, but a thin cerotegument layer with polygonal ornamentation can be observed on the notogaster. Prodorsum with convergent and slightly sinuous lamellae; prolamela and sublamella present. Prodorsal setae are robust and rarely barbed, except the lamellar ones, which are simple. Sensillus short, proclinated, club-shaped (the shortest prodorsal

Table 1. Biometric data of the *Scheloribates* species (µm).

Species	Idiosoma		Prodorsum	Notogaster		Genital foramen		Anal foramen	
	L	b	Lp	Ln	Ln/b	D	d	D	d
Scheloribates barbatulus	367 - 386	193 - 205	90	295	1.44	40	38	68	65
Scheloribates fimbriatus	379 - 434	204 - 241	88	291	1.42	58	48	83	80
Scheloribates labyrinthicus	528 - 592	336 - 432	138	409	1.22	66	60	114	108
Scheloribates laevigatus	433 - 524	295 - 331	84	283	1.23	53	50	88	83
Scheloribates latipes	445 - 456	277 - 307	127	325	1.17	60	55	95	88
Scheloribates pallidulus	355 - 403	223 - 259	84	271	1.15	53	50	85	80
Scheloribates quintus	385 - 403	259 - 294	84	319	1.18	55	50	83	80
Scheloribates rigidisetosus	409 - 434	247 - 271	108	313	1.21	55	48	88	80
Scheloribates xylobatoides	470 - 494	235 - 241	114	379	1.61	48	40	88	83

Legend: L – length of idiosoma;b - breadth; Lp – prodorsum's length; Ln - notogaster's length; D – longitudinal diameter; d – transversal diameter.

Table 2. Average dimensions of the setae (µm).

Species	Prodorsum				Notogaster		Epimeral region			Genito-anal region				
	in	le	ro	ss	ls	ls/Ln (%)	a	b	c	g_1	g_2-g_4	ag	an	ad
Scheloribates barbatulus	55	58	50	58	8	2.7	10	15	20	8	8	10	8	10
Scheloribates fimbriatus	68	75	58	80	20	6.8	15	23	25	18	10	15	15	15
Scheloribates labyrinthicus	120	120	75	80	18	4.4	20	25	25	20	13	23	13	23
Scheloribates laevigatus	102	120	66	72	18	5.2	15	25	25	20	13	15	15	15
Scheloribates latipes	96	108	66	72	10	3.1	20	30	30	15	10	15	18	23
Scheloribates pallidulus	70	73	50	58	13	4.8	15	20	20	15	10	15	10	15
Scheloribates quintus	72	90	60	72	40	13.3	18	25	25	18	13	18	15	18
Scheloribates rigidisetosus	93	100	65	40	10	3.2	20	25	25	15	10	15	15	15
Scheloribates xylobatoides	48	88	63	78	10	2.6	15	25	25	23	8	10	13	13

Legend: in – interlamellar setae; le – lamellar setae; ro – rostral setae; ss - sensillus; ls – length of the notogastral setae; Ln - notogaster's length; a, b, c, - epimeral setae; g_1 - g_4 – genital setae; ag – aggenital setae; an – anal setae; ad - adanal setae.

seta) (Table 2). Notogaster is oval, rounded, and the length/breadth ratio has a low value. 10 pairs of short, simple, and thin notogastral setae. 4 pairs of sacculi, typically positioned. Ventral aspect and chaetotaxy characteristic for the genus.

Distribution and autecology. Southern Palaearctic species (Subias, 2004), recorded in Austria, Hungary (Mahunka, 2004) and in the former Soviet Union.

In Romania this species has only been recorded in very wet habitats in the Danube Delta Biosphere Reserve. It is probably a typical hygrophilous species.

Scheloribates (S.) xylobatoides Mahunka, 1977 (Figs. 17, 18)

Literature: Mahunka (1977)

Diagnosis. Medium sized species with elongated idiosoma, yellowish or light chestnut in colour. Cuticle smooth, without ornamentation. Prodorsum with narrow lamellae; prolamella and sublamella present. Prodorsal setae robust and barbed. Sensillus reclinated, fusiform elongated; its distal part is narrow, with a pointed apex and ends with a long thorn. Notogaster is oval, elongated, and the length/breadth ratio is comparatively higher than the other *Scheloribates* species (Table 1). 4 pairs of sacculi in usual position. 10 pairs of notogastral setae – very short and thin (Table 2). Epimeral region, strongly sclerotized, has the characteristic aspect. All the epimeral setae are barbed and arranged according to the formula 3:1:3:3. Genito-anal region has the typical aspect and setation.

Distribution. Greece (Mahunka, 1977, Subias, 2004).

There is only one record of this species in Romania, found in cultivated soil in the Southern part of the country (Slatina, Olt county). The material was inventoried as *S. fimbriatus*; in the context of this study it was possibly to distinguish the two species. The main differences between the two species are: the size, the length/breadth ratio, the relative length of the notogastral setae, and also the aspect of the epimeral setae.

Identification key for *Scheloribates* s. str. species

1.a. Sensillus reclinated, equal or longer than the rostral setae **2**
b. Sensillus proclinated, short (the shortest prodorsal seta) ..... ***S. rigidisetosus*** **Willmann, 1951**

2.a. Cuticle smooth, without ornamentation **3**
b. Cuticle with reticulate ornamentation visible on the notogaster, on the ventral shield, and on the anal valves ..... ***S. labyrinthicus*** **Jeleva, 1962**

3.a. Relatively small species, often under 450µm in size **4**
b. Medium sized species, often over 450µm in size **7**

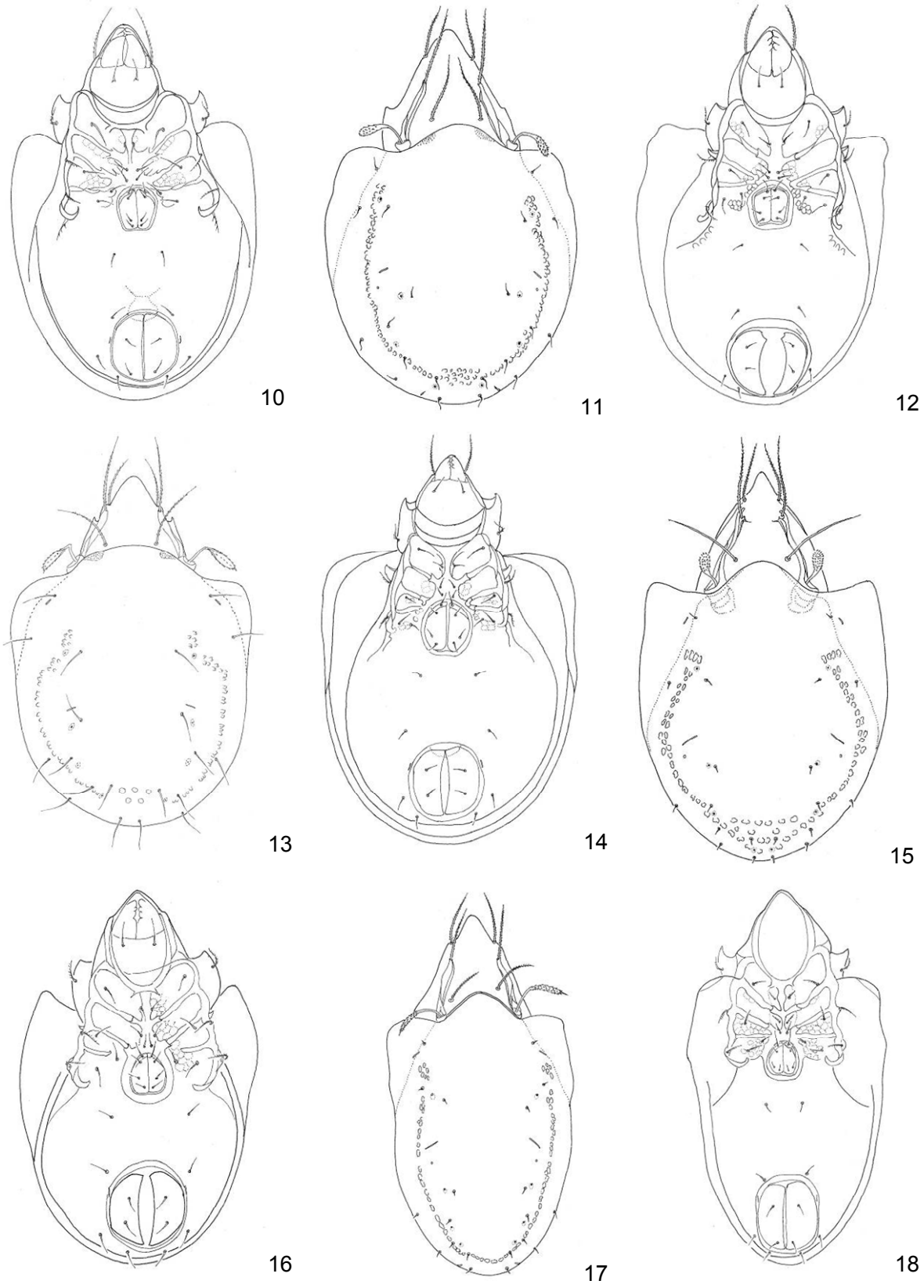
4.a. Notogastral setae relatively short **5**
b. Notogastral setae long (about 40 µm, representing approximately 13.3% of the notogaster's length) ..... ***S. quintus*** **Wunderle, Beck et Woas, 1990**

5.a. Sensillus with the stalk shorter than the distal, clavate part; the distal end is usually rounded ..... ***S. pallidulus*** **(C. L. Koch, 1840)**
b. Sensillus with the distal part fusiform elongated, and a pointed apex **6**

6.a. Notogastral setae very short (about 8µm, representing 2.7% of the notogaster's length) ..... ***S. barbatulus*** **Mihelčič, 1956**
b. Notogastral setae longer (about 20µm, representing 6.8 % of the notogaster's length); sensillus with an evident terminal thorn ..... ***S. fimbriatus*** **Thor, 1930**

7.a. Notogaster very elongated, with the length/breadth ratio over 1.6 ..... ***S. xylobatoides*** **Mahunka, 1977**
b. Notogaster relatively rounded, with the length/breadth ratio under 1.3 ..... **8**

8.a. Notogastral setae minute; sensillus with a rounded apex ..... ***S. latipes*** **(C. L. Koch, 1844)**
b. Notogastral setae longer and easily observable. Sensillus with the distal part fusiform elongated, with lanceolate end; notogastral setae represent about 5% of the notogaster's length ..... ***S. laevigatus*** **(C. L. Koch, 1836)**



Figures 10-18. *Scheloribates latipes* (Koch, 1844): 10-ventral view; *Scheloribates pallidulus* (Koch, 1840): 11-dorsal view, 12-ventral view; *Scheloribates quintus* Wunderle, Beck et Woas, 1990: 13-dorsal view, 14-ventral view; *Scheloribates rigidisetosus* Willmann, 1951: 15-dorsal view, 16-ventral view; *Scheloribates xylobatoides* Mahunka, 1977: 17-dorsal view, 18-ventral view.

Conclusions

Among the species of *Scheloribatidae* cited in Romanian fauna, there are some widely distributed and with large ecological plasticity (*S. laevigatus, S. latipes, S. pallidulus*); certain other species have a limited distribution and, are probably more exigent or even stenotopic forms (*S. rigidisetosus, S. xylobatoides*).

The present study has shown that in Romania, the scheloribatid mites are well represented compared to other Central European countries (Mahunka & Mahunka-Papp, 2004; Schatz, 1983; Weigmann, 2006), with twice the number of species. Considering the presence of certain species with typically more Southern distributions, and not recorded in other Central Europe locations, we can suggest that Romania likely represents an interference territory from a zoogeographical point of view.

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CONTRIBUTION TO THE BIODIVERSITY OF MITES (ACARI: MESOSTIGMATA) OF TARCHANKUT PENINSULA (CRIMEA)

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Abstract

Mesostigmata (Acari) mite communities from various microenvironments of Tarchankut Peninsula (Crimea) were studied. Over 500 soil samples, 50cm^3 each, were taken from 2nd to 9th June 2004. Over 3000 specimens of Mesostigmata were identified to the species level. Altogether 47 species were found. The dominant species were: *Asca nova* Willmann and *Leitneria pugio* (Karg) (litter from shelterbelts with *Crataegus pojarkoviae* (Kossych)), *A. nova* (Willmann) and *Amblyseius levis* (Wainstein) (*Sedum* sp. patches, grass and moss patches), *Trichouropoda elegans* (Kramer) (litter from herb patches) as well as *T. elegans* (Kramer) and *Zercon athiasi* Vincze (decaying wood). Zoogeographical and ecological aspects of studied fauna are discussed.

Key-words

Mesostigmata, Crimean Peninsula, microenvironments, zoogeography

Introduction

Microhabitat conditions shaping animal communities result from the geographical position, soil and hydrological conditions, as well as the lie of the land, which all model the ecological factor complex that is significant for the organisms.

Areas that are especially ecologically and zoogeographically precious are those characterised by a rich variety of microhabitats and influenced by various climatic conditions. Therefore, the Crimean Peninsula is an interesting area of study in regard to ecological requirements and the range of species occurrence. Its location on the influence border of temperate and Mediterranean climates, different from the rest of the Ukraine oceanic climate type as well as the insular character of Crimea, all influenced of the occurrence of a rich variety of plant and animal communities with high values of endemic species.

The aim of the research was to determine the richness of Mesostigmata soil communities in the Tarchankut Peninsula, as well as the comparison of community diversity of the studied arachnida within selected microenvironment types.

Study area

The Tarchankut Peninsula is located in the north-western part of Crimea (Fig. 1). The northern, north-eastern and southern coasts of the peninsula are covered by semi-desert steppes and saline lands. The central part, as well as the western and north-western coasts, are covered by true steppe (Elaboration of Priorities, 1999). There are 50 priority protection areas within the Crimean Peninsula which are included in three priority classes. The Highest Priority Area embraces 15 localities, including the north-western part of the peninsula (Dzhangul and Bolshoi Kastel). The area covers the grounds of the planned Tarchankut

National Park and Nature Reserve, characterised by a high biodiversity level of plants, birds, mammals, and invertebrates as well as a high endemism level.

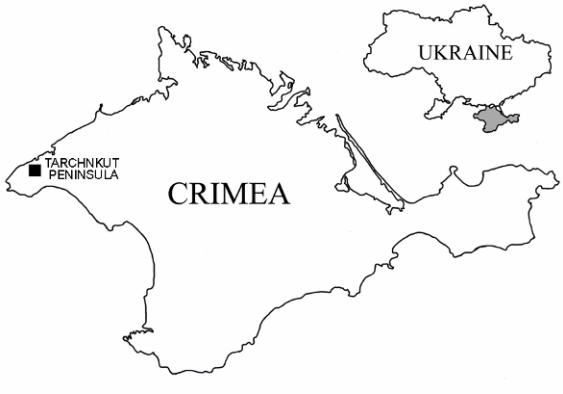

Figure 1. Location of the studied area (marked with ■).

Material and methods

Acarological samples were collected between 2nd and 9th June 2004 in the north-western part of Tarchankut Peninsula (Fig. 1). Overall, 513 soil and litter samples were collected, 50cm^3 each, from 37 plots representing 6 types of microhabitats: decaying wood (2 plots, 30 samples), grass patches (8 plots, 93 samples), litter from under herbs (3 plots, 32 samples), moss patches (12 plots, 163 samples), *Sedum* sp. patches (4 plots, 73 samples), and litter from shelterbelts with *C. pojarkoviae* (Kossych) (8 plots, 122 samples). Altogether, 3005 Mesostigmata specimens belonging to 47 species were found. Mites were marked using the work of Micherdziński (1969), Błaszak (1974), Bregetova (1977), Kuznetsov and Petrov (1984) and Karg (1993). Mesostigmata communities were characterised using: abundance (A in ind./50cm^3), dominance (D in %), number of species (S), Shannon-Weaver's species diversity (H'), and Pielou's community evenness (J'). The dominance structure analysis was based on the classification proposed by Błoszyk (1999). The quality-quantity similarity of the studied communities was analysed (percent similarity based on Bray-Curtis index), as well as the zoogeographical and ecological character of Mesostigmata communities.

Results

A total of 47 Mesostigmata species were recorded within the studied area (Tab. 1). The number of species ranged from 14 in herb patches, to 35 in the litter of shelterbelts with *C. pojarkoviae*. The lowest Mesostigmata abundance characterised moss patches, whereas their highest density was found in patches with dominant *Sedum* sp. The lowest indices of species diversity and community evenness were recorded with the studied mite community penetrating the patches with *Sedum* sp., whereas their highest indices characterised the communities inhabiting the litter of shelterbelts with *C. pojarkoviae*.

The microenvironment of decaying wood was inhabited by a Mesostigmata community dominated by *T. elegans* (D=25.27%) and *Z. athiasi* (D=21.25%), both appearing in the dominant class, and a relatively high dominance within this community was reached by *Ameroseius plumea* (D=14.65%).

The eudominant class of grass patches soil included *A. nova* (D=48.20%), *A. levis* (D=23.94%) was recorded in the dominant class, and *Amblyseius* sp. (D=9.89%) reached a relatively high dominance. The eudominant class of the soil collected from herb patches listed only *T. elegans*, which constituted 46.78% of the community. In that patch, there were also relatively high dominance values of *Amblyseius* sp.

and *Gamasellodes bicolor*, which constituted respectively 10.43% and 8.48% of the community.

The community of the mites occurring in moss patches included the eudominant *A. nova* (D=41.29%) and the dominant *A. levis* (D=16.90%). The species with a relatively high dominance in the moss patches was *L. pugio* (D=9.01%). *Sedum* sp. patches were dominated by populations of *A. levis* and *A. nova*, which occurred in the eudominant class (respectively 34.51% and 33.28% of dominance). A relatively high dominance in that community, ca. 10%, was reached by *Hypoaspis praesternalis*.

Mesostigmata communities inhabiting the litter from under *C. pojarkoviae* were dominated by *A. nova*, *L. pugio* and *Hypoaspis zachvatkini* (respectively 15.53%, 13.46% and 11.06%). Other species within that microenvironment failed to surpass 8% of the dominance.

The highest quality-quantity similarity (54.26%) was recorded between Mesostigmata communities inhabiting the grass and moss patches (Fig. 2). The communities of the studied mites recorded in the decaying wood and herb patches were, from the quality-quantity perspective, different from the others (barely 22.66% of similarity).

Discussion

In terms of ecology and zoogeography the Crimean Peninsula is an extremely interesting area. In view of its location between the Black and Azov Seas as

well as the only connection with land being the Isthmus of Perekop the area has a very insular character. Simultaneously, the Crimean Peninsula, as opposed to a considerable part of Ukraine, is influenced by marine climate. Major part of the peninsula is covered by steppe-like vegetation communities. Only the southern part of Crimea (in view of the lie of the land) is covered by forest stands extending along the Crimean Mountains, mountain forests, mountain meadows, steppes, and a vegetation strip of around-Mediterranean character (along the southern coast) (Elaboration of Priorities, 1999).

The occurrence of true and semi-desert steppe elements within the studied area as well as saline vegetation patches along the coast influence the extremly rich microenvironmental mosaic that is being shaped there.

The biggest number of species, as well as the highest values of the indices of community diversity and evenness, were recorded in the litter of shelterbelt with dominant *C. pojarkoviae*. That community was shaped in natural land hollows characterised by a humid microclimate that is different from that occurring in the neighbouring patches of the dry and grassy steppe vegetation. The outcome is connected to the selectivity of most mites, which prefer microhabitats with proper food base, thermal conditions and humidity, generally avoiding extremely dry places (Wallwork, 1967).

The species that reached high values of dominance in most of the studied microenvironments (between 15.53% and 48.20%) was *A. nova*, whose dominance failed to exceed 5% only in decaying wood and herb patches. *Asca nova* is an eurytopic species so far recorded in Europe and North America. It is mainly encountered in Mesostigmata soil communities of cultivable fields and meadow ecosystems, although it also appeared in smaller numbers in the litter of different types of forest stands and decaying wood (Bregetova 1977, Karg 1993, Kalúz & Fenďa 2005).

Table. 1. Dominance (in %) of selected Mesostigmata species, total abundance (A in ind./50cm^3), number of species (S), Shannon-Weaver species diversity index (H') and Pielou evenness index (J') of Mesostigmata communities within studied microenvironments: I – decaying wood, II – grass, III - litter under herbs, IV – moss, V - patches with *Sedum* sp., VI – litter from shelterbelts with *C. pojarkoviae* (Kossych). Other species and their occurrence within the studied microenvironments are given in the footnote of the table.

	microenvironment					
species	I	II	III	IV	V	VI
Amblyseius levis Wainstein, 1960	1.10	23.94	3.13	16.90	34.51	5.66
Amblyseius sp	9.89	3.13	10.43	2.99		5.71
Ameroseius plumea Oudemans, 1930	14.65	0.95				2.98
Asca nova Willmann,1939	1.83	48.20	4.40	41.29	33.28	15.53
Gamasellodes bicolor (Berlese, 1918)	0.73	1.16	8.48	1.11	1.26	0.83
Hypoaspis zachvatkini Buyakova et Goncharova, 1972	4.03	3.13	1.77	0.25	1.37	11.06
Hypoaspis praesternalis Willmann, 1949	2.93	3.11	1.04	3.48	10.04	2.65
Leitneria pugio (Karg, 1961)	4.03	3.11	6.00	9.01	9.48	13.46
Nenteria stylifera (Berlese, 1904)						7.66
Trichouropoda elegans (Kramer, 1882)	25.27		46.78	0.39		6.83
Zercon athiasi Vincze, 1965	21.25	0.19	6.18			4.82
A	9.10	5.06	2.91	2.37	12.07	6.55
S	20	21	I 14	22	20	35
H'	2.268	1.785	1.944	2.048	1.722	2.819
J	0.757	0.586	0.737	0.663	0.575	0.787

Others: Amblyseius aurescens – I, II, III, IV, V, VI; Ameroseius corbicula – VI; Antennoseius sp. – I, VI; Antennoseius sp.2 – VI; Arctoseius minusculus – I, VI; Discourella cordieri – VI; D. modesta – I, VI; Holoparasitus excipuliger – VI; Hypoaspis aculeifer – I, II, III, IV, V, VI; H. austriaca – I, II, IV, V, VI; H. karawaiewi – II, IV, V; H. miles – V, VI; H. vacua – II, III, IV, V, VI; Laelaspis imitatus – II, IV, V; Macrocheles sp. – I, VI; Ololaelaps placentula – IV; Olopachys scutatus – IV; Oplitis punctata – III; Pachylaelaps denticulatus – VI; P. furcifer – VI; P. imitans – VI; P. ineptus – V; P. karawaiewi – II, VI; P. pectinifer – VI; Pergamasus holzmanae – IV; Prozercon carsticus – II; Pseudolaelaps doderoi – II, IV, V, VI; Pseudoparasitus dentatus – II, IV, V; Rhodacarus olgae – II, IV, V, VI; R. coronatus – I, II, IV, VI; Trachytes pauperior – IV, VI; Trichouropoda ovalis – VI; Urodiaspis tecta – V; Veigaia decurtata – I, III, V, VI; Zercon foveolatus – I, Z. peltatus peltatus – V.

Decaying wood and herb patches were dominated by T. elegans – a north- and central-European species recorded so far in under-bark microenvironments, tree hollows, soil, fallen leaves, anthills, and bird nests (Bregetova 1977, Wiśniewski & Hirschmann 1993, Błoszyk 1999, Mašán 2001). The central-European Z. athiasi species was the second in terms of community abundance among the studied mites inhabiting decaying wood. Taking into consideration the microenvironments in which it was recorded to date (steppes, forest-steppes, thermophile oak-woods), it can be listed among the polithermophile species tolerating low levels of humidity (Błaszak 1974, Bregetova 1977, Mašán & Fenďa 2004).

Amblyseius levis reached high dominance values in the community of grass patches, moss patches and Sedum sp. patches – until now, it was recorded on plants from Crimea, Georgia, Armenia, Estonia, Lithuania, and Latvia (Kuznetsov & Petrov 1984).

Ameroseius plumea appeared in grass patches, litter from under C. pojarkoviae and decaying wood, where it reached the highest dominance in the community. Until now, that west-European species was recorded in forest litter, anthills, decaying tree hollows, as well as rodent nests and on rodents themselves from the areas of Ukraine and Moldavia (Bregetova 1977).

Gamasellodes bicolor reached a relatively high dominance in the soil of herb patches and was present in all the studied microenvironments. That European species, occurring in a wide range of humidity, was recorded so far in the soils of cultivable fields, meadows, woods and forests as well as animal droppings (Karg 1993).

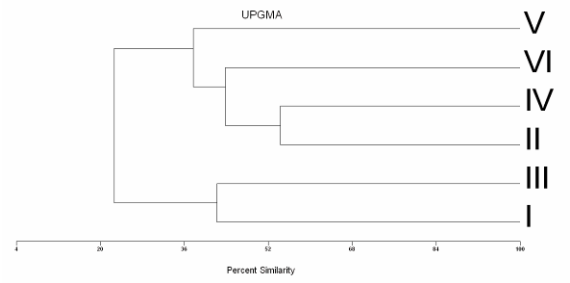

Figure 2. Percent similarity of Mesostigmata communities within the studied microenvironments: I – decaying wood, II – grass, III - litter under herbs, IV – moss, V - patches with Sedum sp., VI – shelterbelts with C. pojarkoviae (Kossych).

Species with a relatively high dominance value in the Mesostigmata community of the litter under C. pojarkoviae, except for the aforementioned A. nova, were H. zachvatkini, L. pugio and Nenteria stylifera. The two former were listed in all the studied microenvironments, whereas N. stylifera penetrated exclusively the litter under C. pojarkoviae. Hypoaspis zachvatkini is an east-Siberian species listed so far in the nests of small mammals, farm buildings and food warehouses (Bregetova 1977). As for L. pugio, it is a central-European species with a big tolerance towards humidity, until now recorded in the soils of meadows and cultivable fields (Karg 1993). The occurrence of N. stylifera exclusively in the litter under hawthorn scrubs indicates its preference towards a higher level of humidity. That European species prefers open environments (waterlogged meadows, xerothermic turfs, steppes, and forest-steppes), although it was occasionally encountered in forest ecosystems and merocenoses (Bregetova 1977, Wiśniewski & Hirschmann 1993, Błoszyk 1999, Mašán 2001).

Among the less abundant species, Discourella cordieri is noticeable. The limitation of that species' occurrence to only hawthorn scrubs confirms its formerly described forest character (Błoszyk 1999, Mašán 2001). As for Laelaspis imitatus it is an example of a south-European species, listed so far in Crimea, Azerbaijan and Georgia (Bregetova 1977). A species with a central- and south-European range was Pseudoparasitus dentatus, and Olopachys scutatus had a south-European range (Bregetova 1977). Prozercon carsticus, on the other hand, is a south- and east-

European fauna element, possibly spreading into central-Europe (Błaszak 1974, Mašán & Fenďa 2004). The myrmecophillic Oplitis punctata should be considered an interesting species – it was recorded in the soil from under herbs and to date registered exclusively within Slovakia (Mašán 2001).

Conclusions

Out of the 47 Mesostigmata species recorded within the studied microenvironments of Tarchankut Peninsula, most were constituted by species of the west-European range. Asca nova, which was dominant in most of the studied microhabitats, is a typical element of agro-ecosystem and meadow fauna. Species such as A. levis, G. bicolor and L. pugio can be included in the same ecological group. Nenteria stylifera, which is typical for open environments, appeared exclusively in hawthorn scrubs, which is undoubtedly connected to its preference of microhabitats characterised by increased humidity. As for D. cordieri, it clearly avoided microhabitats of open ecosystems. Amblyseius levis, L. imitatus, P. dentatus, O. scutatus, P. carsticus, and O. punctata should be listed among the typically south-European elements of Mesostigmata fauna of Tarchankut Peninsula.

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MESOSTIGMATA (ACARI) OF ECOTONE ZONES WITHIN BAGNO STAWEK RESERVE (TUCHOLA FOREST, POLAND)

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Abstract

Acarological investigations were performed during a three-year study in Bagno Stawek Reserve within the area of Zaborski Landscape Park (Tuchola Forest, Poland). Soil samples were taken from three pine forests – a Scots pine forest (*Leucobryo-Pinetum*), a moist pine forest (*Molinio-Pinetum*), and a wet pine forest (*Vaccinio uliginosi-Pinetum*) – as well as a transitional fen (*Caricetum lasiocarpae*) and the ecotone zones between them. Additionally, soil samples from a wide ecotone zone (*Ledo-Sphagnetum magellanicum*) between the wet pine forest and the transitional fen were taken. Total abundance of Mesostigmata (Acari), number of species, community evenness, species diversity, selected species abundance and dominance were analysed in the studied habitats as well as the ecotone zones between them. Apart from habitat generalists, some ecotone-specific species were found.

Key-words

Biodiversity, soil ecology, pine forest, fen, protected area

Introduction

Wide spectrum of habitat selectivity of Mesostigmata results from the diversity of their ecological preferences. As a consequence many species belonging to this group, clearly react in unique conditions prevailing in transition zones between ecosystems. Dissimilarity of ecotone zone conditions makes possible, in some cases, for the so called ecotone-specific species or communities to occur, which highlights the role of transition zones in the enrichment of natural species diversity. Until now, studies have described borderline effect included organism groups or singular species, and presenting various levels of intensity. The recorded reactions considered differences in spatial distribution, abundance, species composition of the community, as well as the level of species diversity and some animal behaviour. Among the studied species, some preferred transition zones whereas some avoided them.

Unique conditions, dynamic character and ecological importance of natural and artificial ecotone zones make them interesting case studies (i.e. van der Maarel 1990, Di Castri & Hansen 1992, Hanel 1993, Murcia 1995, Kent *et al.* 1997, Seniczak *et al.* 1998, Kotze & Samways 1999, Gascon *et al.* 2000, Schilthuizen 2000, Laurance *et al.* 2001, Niemielä 2001, Falińska 2004, Ries *et al.* 2004, Ginsberg 2006, Rosenblum 2006, Baker *et al.* 2007, Dąbrowska-Prot & Wasiłowska 2008).

Study area

The peat-bog Bagno Stawek Reserve is located within the area of Zaborski Landscape Park, north of Bory Tucholskie National Park. It covers a

relatively shallow peat-covered melt-out hollow, surrounded by pine forests, located slightly aside of the meridionally arranged set of trough-like melt-out hollows filled with lakes. Average annual precipitation within the area ranges 500-600mm, average annual temperature fluctuates between 7.0°C and 8.0°C, average January temperature ranges from –3.0°C to 3.5°C, average July temperature ranges from 17.0°C to 17.5°C, and the vegetative period averages between 190 and 200 days (Lorenc 2005). The soils of the studied reserve are represented by peat soils of transitional fens, often with the decaying process already started, podsols, rusty soils, peat soils of low mires, peat and gley soils, as well as mud and decay soils (Kowalewski et al. 1997).

The Scots pine forest (*Leucobryo-Pinetum*) is made of relatively young pine trees (*Pinus sylvestris*) in a rather high density (80%). The shrubs layer is poorly developed and created by common juniper (*Juniperus communis*). Relatively poor ground cover spreads to 30-60%, and the moss layer to 40-50%. The closer to the peat bog, the forest communities become more humid – first clearly relating to *Molinio-Pinetum*, then turning into a wet pine forest (*Vaccinio uliginosi-Pinetum*) created as a result of successive pine self-seeding on peat moss. Between the wet pine forest and the transitional fen (*Caricetum lasiocarpae*) there is a relatively wide strip of raised mire (*Ledo-Sphagnetum magellanicum*) sparsely covered by short pine trees, creating a wide transition zone between the two habitats.

Material and methods

Soil samples, 250 cm3 each, were collected from 27 plots located within the studied habitats and transition zones between them (Fig. 1) in autumn seasons (in years 2002, 2003 and 2004) and spring seasons (in years 2003, 2004 and 2005). The repetition rate was 10 each time. After a six-day extraction in Tullgren funnels, the mites were preserved in 70% ethyl alcohol and mounted in Hoyer's liquid. Overall, 16244 Mesostigmata specimens belonging to 74 species were collected. The reaction analysis within transition zones was performed at the order level as well as selected species of the studied mites.

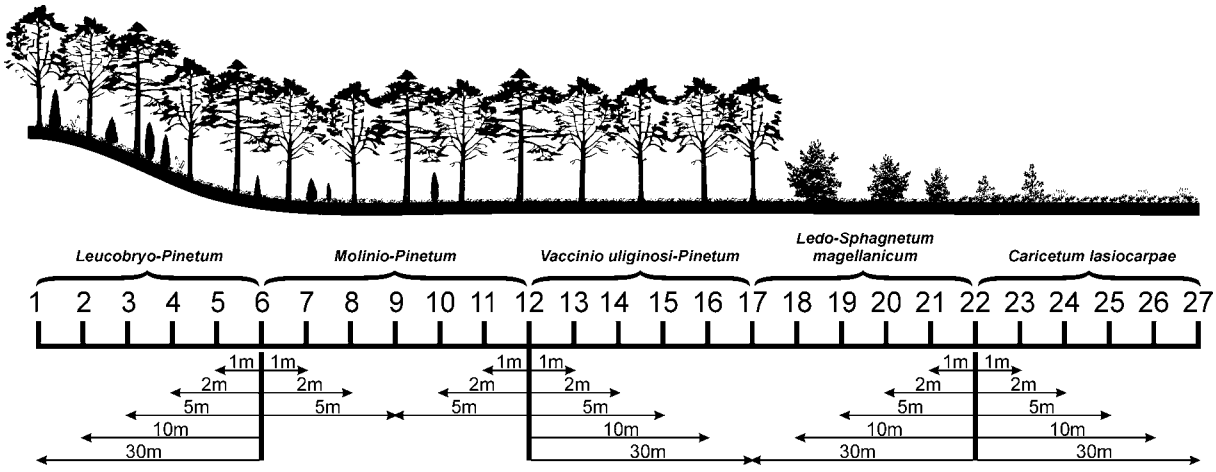

Figure 1. Location of the studied plots within Bagno Stawek Reserve. 1-27 – plot numbers. The figures next to arrows mark the distances from border areas (6, 12, 22) in metres.

Results

There were 74 species belonging to the Mesostigmata recorded within the studied area, and their number ranged from 11 on plot 27 (*Caricetum lasiocarpae*) to 36 on plot 1 (*Leucobryo-Pinetum*) (Fig. 2). Outside the Scots pine forest, large numbers of Mesostigmata species were recorded on plots 10 and 11 in the moist pine forest (in both cases 34) as well as, relatively more compared to the neighbouring plots, on plots 18 and 19 in the raised mire. General Mesostigmata abundance ranged between 2950 ind/m^2 (plot 21 – *Ledo-Sphagnetum magellanicum*) and 25510 ind/m^2 (plot 2 – *Leucobryo-Pinetum*). Except for the relatively high densities in the central part of the Scots pine forest, there was an increase on plots 10, 13 and 14 in the transition zone between the moist pine forest and the wet pine forest. The values of the species diversity index (H') fluctuated between 0.870 (plot 24 – *Caricetum lasiocarpae*) and 2.840 (plot 19 – *Ledo-Sphagnetum*

magellanicum), reaching also relatively high values on plot 18 in the raised mire (2.710) as well as on plots 9 and 11 in the moist pine forest (respectively 2.510 and 2.570). The level of community evenness took a similar shape and it ranged from 0.79 (plot 21) to 0.88 (plot 19) in the raised mire, whereas

Mesostigmata communities on plot 24 (*Caricetum lasiocarpae*) realised merely 35% of maximum diversity.

In case of *Paragamasus runciger*, there were increases recorded in both the abundance and dominance on plots from 12 to 14 in the transition zone between the moist pine forest and the wet pine forest (Fig. 3). The abundance of *P. runciger* was over twice higher there in comparison to the neighbouring plots. A similar reaction was observed in case of *Prozercon kochi* – this species numerously occurred in the Scots pine forest community, whereas in the moist pine forest it appeared exclusively on plot 10. An increase in abundance and dominance of that species was recorded on plots from 12 to 14 (respectively A=560 ind/m^2 , D=5.61%, and A=1710 ind/m^2, D=10.94%) in the transition zone between the moist pine forest and the wet pine forest. Apart from that, *P. kochi* appeared abundantly in the transition zone between the wet pine forest and the raised mire as well as in the raised mire. There were merely several specimens of that species found in the transitional fen.

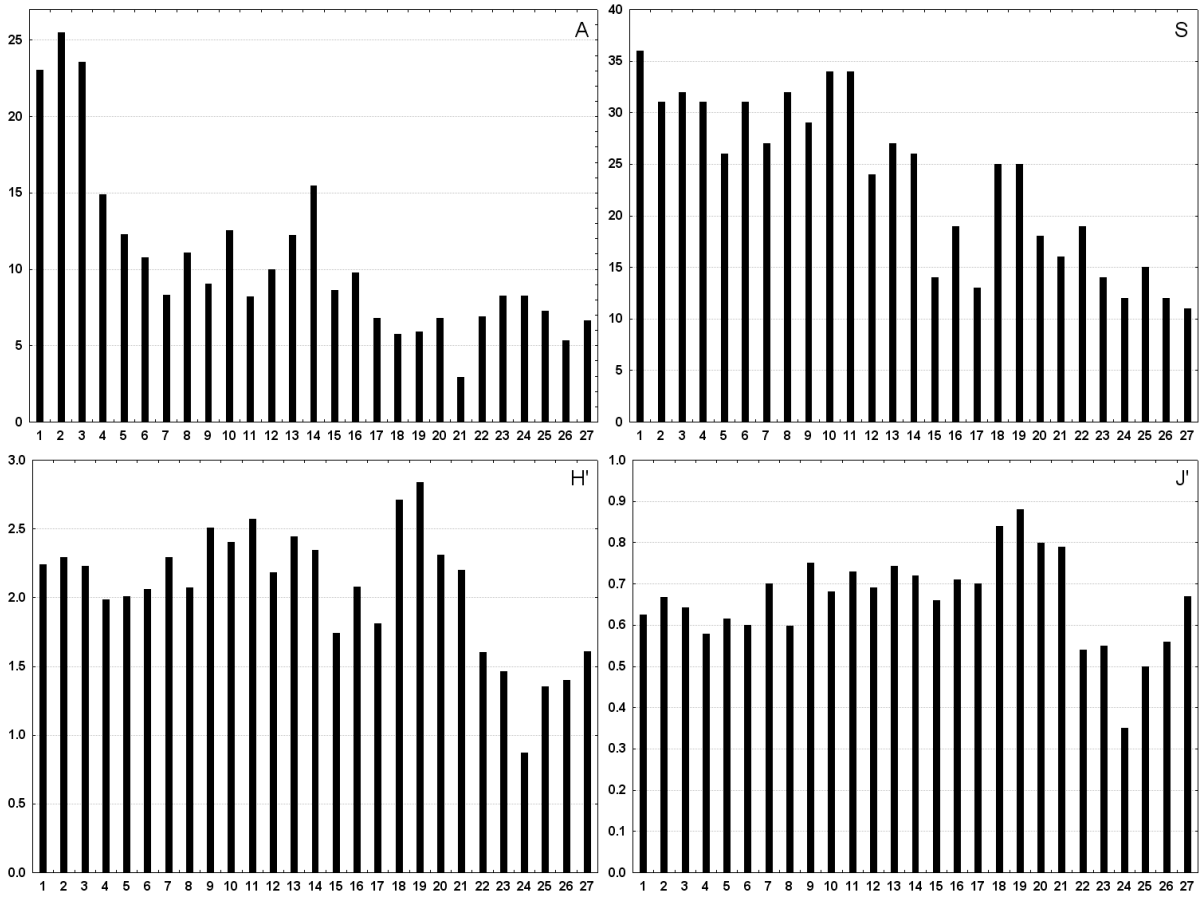

Figure 2. Abundance (A in thousands ind/m^2), number of species (S), Shannon Weaver species diversity index (H') and community evenness index (J') of the Mesostigmata within the studied plots in Bagno Stawek Reserve.

High density of *Uropoda minima* population was recorded on plot 11 in the transition zone between the moist pine forest and the wet pine forest (A=560 ind/m^2, D=6.77%) as well as on plot 20 in the raised mire (A=440 ind/m^2, D=6.21%). On other plots, dominance of that species failed to surpass 3%, and the abundance 370 ind/m^2.

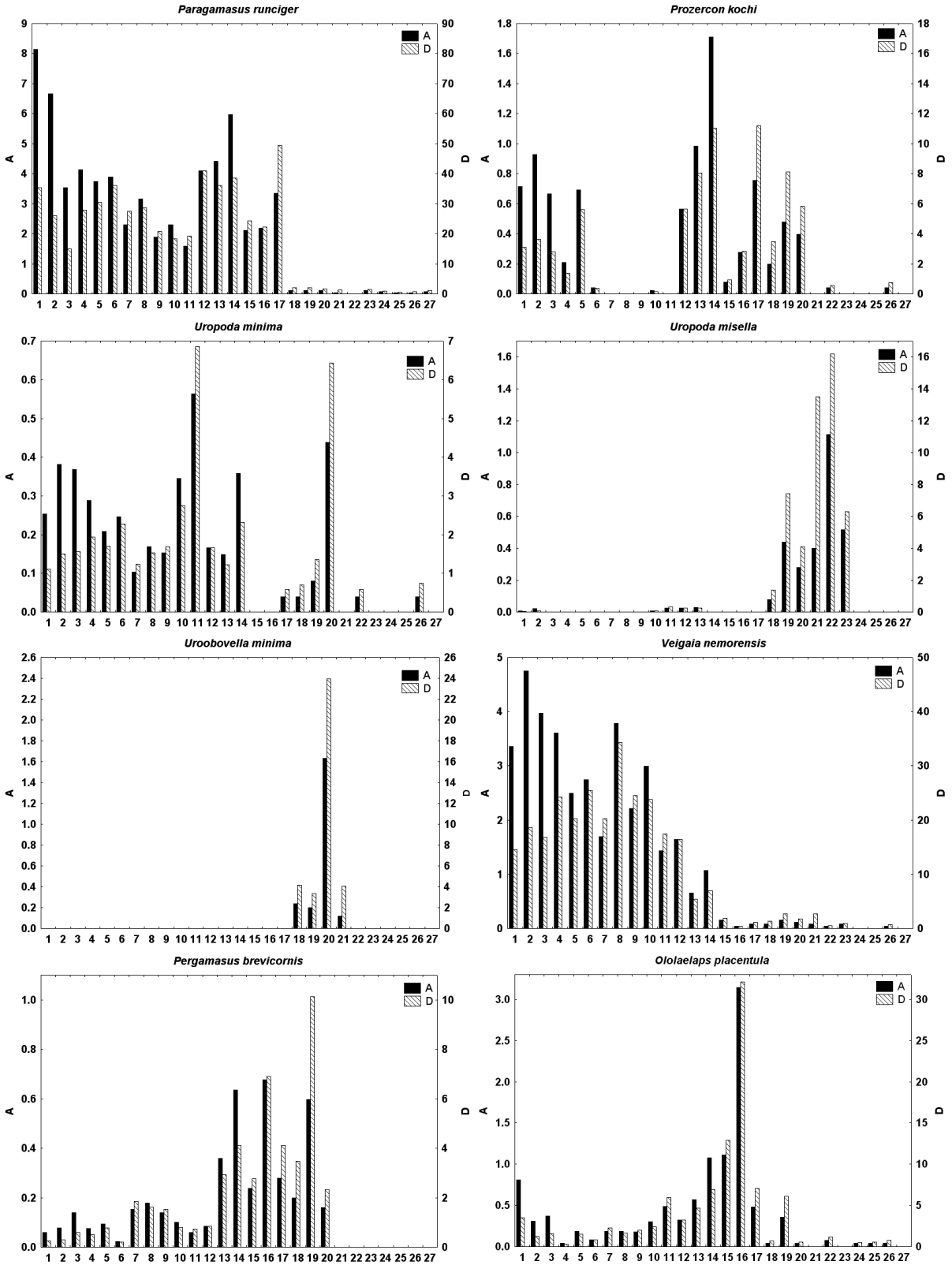

Figure 3. Abundance (A in thousands ind/m^2) and dominance (D w %) of selected Mesostigmata species within the studied plots in Bagno Stawek Reserve.

The species whose occurrence was practically limited to the raised mire were *Uropoda misella* and *Uroobovella minima*. The former reached its highest density and dominance in the community on plot 22 in the raised mire (A=1110 ind/m^2, D=15.30%). Apart from that, *U. misella* appeared

with relatively high abundance on plots 18-21 and 23 also in the

raised mire, and on the remaining plots (2 in the Scots pine forest and 10-13 in the transition zone between the moist pine forest and the wet pine forest) its population density was meagre. *Uroobovella minima*, on the other hand, was recorded exclusively on plots 18-21 in the raised mire, its highest density and dominance occurring in the community on plot 20 (A=1630 ind/m^2, D=23.16%).

Within the studied reserve, *Veigaia nemorensis* was the most abundant on plot 2 in the Scots pine forest (A=4750 ind/m^2, D=18.47%) and its density was relatively high on plots 1, 3 and 4. Moreover, there was and increased density of that species observed on plots 8 and 10 in the moist pine forest (respectively A=3790 ind/m^2, D=34.12% and A=2990 ind/m^2, D=23.38%), with a simultaneous drop in abundance in both directions (plots 5-7 and 11-14). On plots 15-27, the populations of *V. nemorensis* reached meagre densities (A < 200 ind/m^2, D < 3%).

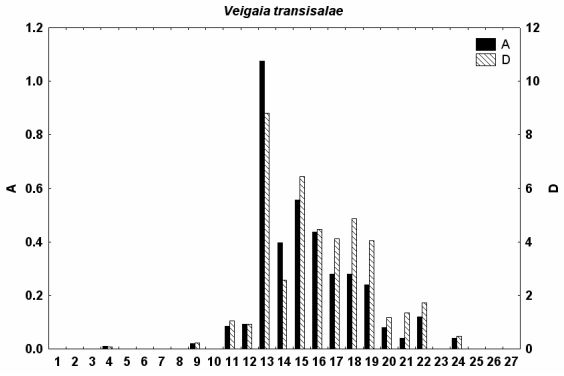

Figure 4. Abundance (A in thousands ind/m^2) and dominance (D w %) of *V. transisalae* within the studied plots in Bagno Stawek Reserve.

Pergamasus brevicornis and *Ololaelaps placentula* were recorded with rather low abundance in the Scots pine forest and the moist pine forest, whereas they were altogether absent in the transitional fen (*P. brevicornis*) or their abundance was relatively low (*O. placentula*). In case of *P. brevicornis*, clearly higher densities were recorded on plots 14 and 16 in the wet pine forest (respectively A=640 ind/m^2, D=4.07% and A=680 ind/m^2, D=6.80%) as well as 19 in the raised mire (A=600 ind/m^2, D=10.07%). As for *O. placentula*, it was the most abundant on plot 16 (A=3140 ind/m^2, D=31.60%), yet there was an increase in its abundance and dominance compared to the borderline plot between the moist pine forest and the wet pine forest (plot 12).

Veigaia transisalae was listed mainly in the wet pine forest and the raised mire (Fig. 4). Moreover, singular specimens of that species were found in the soil of the Scots pine forest (plot 4), the moist pine forest (plots 9 and 11) and the transitional fen (plot 24). The species reached its highest abundance on plot 13 in the transition zone between the moist pine forest and the wet pine forest (A=1070 ind/m^2, D=8.74%).

Discussion

The areas where communities change, have always been of great interest to biologists. In this context, borderline zones, hybrid areas, ecotones or ecoclines have been and still are intensely studied. Within those special ecological systems a mosaic of microenvironments and ecological niches is created, in which unique plant and animal communities appear. Transition zones are also places of gene exchange between the neighbouring ecosystems; it is also plausible that the intensity of speciation increases there (Schilthuizen 2000, Rosenblum 2006).

Literature offers two definitions of the main transition zone categories: ecotones (Livingstone 1903, Clements 1905) and ecoclines (Whittaker 1960, van der Maarel & Westhoff 1964). Ecotone is a relatively narrow ecological zone, a sharp transfer between two different and relatively homogeneous systems. Because of the ecological tonus occurring in such an area, such zone is characterised by a considerable dynamics of ecological processes as well as ecological instability (Clements 1905, van der Maarel 1990, Hanel 1993, Kent *et al.* 1997, Gascon *et al.* 2000). Ecological tonus in a transition zone is connected to the increased abundance and population diversity in relation to its density and diversity recorded in the ecosystems adhering the borderline zone (Trojan 1981). According to Odum (1982), a transition zone is rich in plant and animal species, which migrate from the bordering ecosystems. As a result of the species richness shaping within those areas, they are significant in the maintenance of natural biodiversity (Di Castri & Hansen 1992, Kotze & Samways 1999). Such places, therefore, are backgrounds for the creation of distinct ecological systems, which, despite their transitory character, should be treated as separate spatial units (Di Castri & Hansen 1992). In terms of botany, an ecotone zone is considered to be the area between two communities, where neither of them occurred, and where simultaneously appear elements of both neighbouring communities (Falińska 2004). Ecoclines are much wider zones than ecotones,

characterised by gradient structure changes, therefore more ecologically stable than ecotones (van der Maarel 1990). Transition zones are characterised by the presence of multiplicity of diversified ecological niches, which enable the species from the neighbouring ecosystems to settle down (Robinson 1981) as well as supply them with proper feeding and reproductive conditions etc. (Murcia 1995). Creating such areas and differentiating the structure of ecological systems (structural diversity) is one of the methods that increase biological diversity of forests (Ginsberg 2006). Different communities shaping in the areas of borderline zones indicate the occurrence of so called edge or ecotone effect. Transition zones can be permeable for species with a wide range of ecological tolerance, whereas for others they are the borderlines preventing their migration. Some ecotone zones additionally modify the quality and quantity structure of individual species and whole communities supplying unique habitat conditions which are not a simple average of the conditions occurring in the bordering ecosystems (Laurance *et al.* 2001). Ecological effects taking place in transition zones are not a constant function of the distance from the border between the neighbouring ecosystems (Gascon *et al.* 2000). It means that the changes in population structure can appear in a specific transition zone in different places depending on the time (Hanel 1993). Additionally, the influence range of the edge effect described by means of DEI index (depth of edge influence) (Ries *et al.* 2004), is largely different depending on the considered biotic or abiotic factors, and its intensity varies even between very closely related species (Laurance *et al.* 1997, Peltonen *et al.* 1997, Ries *et al.* 2004). Negative results of the edge effect are known as well, e.g. a decrease in the ecological homogeneity of neighbouring ecosystems (especially if they are not particularly wide) or a negative influence on the species avoiding transition zones (Yahner 1988, Laurance & Yensen 1991, Soulé & Gilpin 1991, Murcia 1995, Niemielä 2001, Ries *et al.* 2004). A decrease in the abundance of some species within transition zones can result from unfavourable changes in their food base, a larger number of natural enemies or individual habitat requirements. Van der Maarel (1990) suggests that species in ecotone zones can be scant, which is associated with a low number of species capable of adjusting to particular conditions prevailing on the borderline of two different ecosystems. Lack of species that are characteristic for transition zones can also be the result of the unnatural character of ecosystem discontinuity (Baker *et al.* 2007).

The research on ecotone zones to date mainly considered the influence of forest surfaces fragmentation on the animal populations inhabiting them, including the effect of the sharp transition zone between the closed and open biotopes (e.g. Murcia 1995, Didham 1997, Kotze & Samways 1999) as well as the importance of intra-meadow forest and shrub stands as specific refuges enabling re-colonisation of regularly interrupted agro-ecosystems, and giving shelter to natural enemies of crop pests (e.g. Banaszak & Cierzniak 2000, Dąbrowska-Prot 2000, Kaczmarek 2000, Wiśniewski 2000).

Most of the aforementioned publications state the occurrence of the edge effect, however, they refer to organism groups or separate species, indicating different levels of intensity. The recorded reactions included differences in spatial distribution, abundance, species composition of the community, as well as the level of species diversity and some animal behaviour. Among the studied species, some preferred transition zones and some avoided them. Only in few cases the occurrence of ecotone species population was recorded.

The studies on the Mesostigmata within ecotone zones involved transition zones between an intra-meadow tree stand and a cultivable field (Seniczak *et al.* 1996, 1996a, 1997, 1998, Kaczmarek & Ratyńska 1998), an intra-meadow stand and a meadow (Seniczak *et al.* 1996b), a pine forests and a meadow (Seniczak *et al.* 2000), as well as a pine forest and a birch forest (Seniczak *et al.* 2005). In the ecotone zones between tree stands and cultivable fields an ecotone effect was revealed that consisted in the increase of density and species richness of the mites, mainly in the areas of herb borders. Similar results regarding spiders were obtained by Łuczak (1997) in borderline zones between tree stands and cultivable fields in Pojezierze Mazurskie lake district. The ecotone effect was also recorded at the level of individual species. For *Alliphis siculus* (Karg 1971, Trojanowski & Błaszak 1981, Seniczak *et al.* 1998), numerously occurring in cultivable soils, the edge zone constituted an area filtrating (rarefying) its abundance. In the transition zone, that species reached a noticeably lower density than in the cultivable fields, whereas it was almost absent in the tree stands. Similar reactions, though reversed, were observed for *Zercon peltatus*, *Holoparasitus calcaratus* and *V. nemorensis*. It is worth pointing out that in the case of narrow belting tree and shrub stands the aforementioned reaction of *A. siculus* was not observed (Seniczak *et al.* 1996b, Kaczmarek & Ratyńska 1998), which indicates distinct influence of narrow ecological islands on

the communities of that species. In the research within the borderline zone between a pine forest and a meadow the ecotone effect was listed with respect to *V. nemorensis*, *Trachytes aegrota* and *Zercon triangularis*, and in the transition zone between a pine forest and a birch forest (Seniczak *et al.* 2005) the ecotone effect was recorded for *Z. triangularis* and *Rhodacarus coronatus*. Simultaneously, there was observed a preference of some species towards the conditions prevailing in transition zones. Such reaction was recorded with regard to *Ameroseius corbicula* (Seniczak *et al.* 1997), *Antennoseius bacatosimilis* and *Trichouropoda ovalis* (Kaczmarek & Ratyńska 1998), all of which were especially numerous in the ecotone zones, whereas they occurred with noticeably lower indices of abundance and dominance in the neighbouring ecosystems.

Mesostigmata communities within the studied reserve reacted at the level of abundance and species diversity in the moist pine forest (*Molinio-Pinetum*) as well as the number of species, the level of species diversity and community evenness in the raised mire (*Ledo-Sphagnetum magellanicum*). Reactions at the level of Mesostigmata abundance can be observed in the transition zone between the moist pine forest and the wet pine forest (plot 14). Therefore, the influence of ecological tonus prevailing in the transition zones on community parameters of the studied arachnida is already visible at the level of the studied mite order. Those observations are confirmed by the recorded parameters of selected Mesostigmata species. It seems that a narrow, barely 10-metre wide strip of the moist pine forest constituted in whole an ecotone zone for *V. nemorensis* populations. In the transition zone between the moist pine forest and the wet pine forest, there were recorded reactions by *P. runciger*, *P. kochi* and *Uropoda minima*. The last of the species also reacted in the raised mire, which additionally was the habitat of the two species characteristic exclusively of that area of the studied reserve – *Uroobovella minima* and *U. misella*. The former species occurred only in that habitat, whereas *U. misella* also appeared, with small density and dominance, in the transition zone between the Scots pine forest and the moist pine forest as well as in the central part of the Scots pine forest. *Veigaia transisalae* reacted in the transition zone between the moist pine forest and the wet pine forest, whereas *P. brevicornis* reacted in the wet pine forest and the raised mire. *Ololaelaps placentula* populations reacted in the transition zone between the wet pine forest and the raised mire with an increase in the abundance and dominance of the Mesostigmata community.

Conclusions

Ecotone zones, as areas of ecological tonus, are very interesting, yet difficult, case studies. Because of the multiplicity of simultaneously operating, connected and often very changeable ecological factors, it is not an easy task to investigate their influence on the population parameters. As a consequence it is impossible, in natural conditions, to study their influence in a continuous way, especially in the situation of ecological tonus that is always present within transition zones. However, in a properly selected system of gradually changing abiotic parameters influencing the alternations in vegetation followed by changes in microhabitat conditions, it is possible to indicate certain tendencies of parameter changes at selected taxonomic levels. The most interesting habitats in the Bagno Stawek Reserve, from the perspective of this paper were the moist pine forest (*Molinio-Pinetum*) and the raised mire (*Ledo-Sphagnetum magellanicum*), which functioned as transition zones altogether. The moist pine forest, in the form of a narrow strip between the habitats of the Scots pine forest and the wet pine forest, influenced the zoocenological parameters at the level of both order as well as some species (*V. nemorensis*). Additionally, there was a reaction recorded at the order level in the raised mire. Moreover, the conditions prevailing in this habitat – a naturally shaped transition zone between the wet pine forest and the transitional fen – favour the populations of *Uropoda minima*, *U. misella* and *Uroobovella minima*. The two latter can be considered ecotone-specific, which probably explains their rather rare appearance in acarological studies. The recorded changes of the population parameters of other analysed species show how diverse the character and intensity of microhabitat conditions influence on arachnida of the order Mesostigmata can be in a transition zone.

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PHYTOSEIID MITES (ACARI: MESOSTIGMATA) FROM TUNISIA: CATALOGUE, BIOGEOGRAPHY AND KEY FOR IDENTIFICATION

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Abstract

The authors give a report on results of several surveys carried out to collect Phytoseiid mites, between 1994 and 2007, in main crops and surrounding wild vegetation in several regions of Tunisia. A catalogue of all species found is provided with some information on their biology, when available, and biogeography. Almost all species are new to Tunisian and African fauna, one genus and one species being new to Science. Finally, a key for the identification of the species found is provided.

Key words

Survey, collection, taxonomy, systematics, key

Introduction

For a long time, only one species of phytoseiid mite, *Phytoseiulus persimilis* Athias-Henriot, was reported from Tunisia (Gafsa region: Rambier 1972). Recently, a previous paper (Kreiter *et al.* 2002) reported results of surveys carried out during seven years in five regions of Northern Tunisia (North, West Center, Cap-Bon, Bizerte and Sahel regions), in four main crops: vegetable production in greenhouses, apple and citrus orchards, and grapevines. Among the thirty-seven species of mite found, belonging to 8 families, 30 species were new to Tunisian fauna and thirteen species of phytoseiid mites were identified. Another paper also reported results of other surveys carried out in 2000 and 2001 in the Southern part of Tunisia, mainly in date palm production areas. Twelve species of phytoseiid mites were found, including a new genus and a new species to Science (Kreiter & Tixier 2006) and 11 already known species, 6 being new for the Tunisian fauna (Kreiter *et al.* 2006). We report here a synthesis of the knowledge on the Tunisian fauna with some additional data resulting from surveys carried out from 2005 to 2007.

Material and Methods

Plant inhabiting mites were collected from various cultivated or uncultivated plants from 1994 to

2007, during different seasons. Mites were directly collected on leaves with a brush using a stereoscopic microscope, by using the leaf dipping-shaking-washing method (Boller 1984), mites being collected on a filter at the end of the process, or by beating shrubs and trees. Mites were then transferred with a fine paintbrush into small plastic vials containing 70° alcohol. They were mounted on slides using Hoyer's medium and identified using a phase and interferential contrast microscope. The sub-family and generic classifications of Chant & McMurtry (1994, 2007) are used for taxonomic considerations and the catalogue of Moraes *et al.* (2004) for faunistical and biogeographical aspects. The chaetotaxy terminologies used in this paper were proposed respectively by Rowell *et al.* (1978) for dorsal and by Chant & Yoshida-Shaul (1991) for ventral idiosomal setae. Adenotaxy and poroidotaxy terminologies are those proposed by Athias-Henriot (1975). Specimens of each species are deposited in the mite collections of Montpellier SupAgro / INRA Acarology laboratory. The following abbreviations are used in this paper: INRA (Institut National de la Recherche Agronomique; Centre de recherche de Montpellier, France), MSA (Montpellier SupAgro, France), INAT (Institut National Agronomique de Tunisie).

Results and discussion

Thirteen species of phytoseiid mites were found in a first survey (Kreiter *et al.* 2002). In a second one, twelve species were found (Kreiter *et al.* 2006), including a new genus and a new species for Science (Kreiter & Tixier 2006) and 11 already known species, 6 being new for Tunisia. Few species were then added from 2005 to 2007. A total of 25 species, belonging to 13 genera, are reported from Tunisia and included in the following catalogue. These species belong to the three sub-families: Amblyseiinae, Typhlodrominae and Phytoseiinae. The most diverse genera were *Neoseiulus* (sub-family Amblyseiinae, with 5 species) and *Typhlodromus* (*Anthoseius*) (sub-family Typhlodrominae, with 6 species).

Catalogue of the species, distribution and specimens collected

Amblyseiinae

***1. Amblyseius graminis* Chant**

Amblyseius graminis Chant 1956, 34.

Previous Records: Algeria, Armenia, Australia, Azerbaijan, Denmark, France, Germany, Greece, Mmoldavia, Morocco, Norway, Poland, Russia, Spain, Turkey, Ukraine, USA (Moraes *et al.* 2004).

Specimens examined: Menzel Bou Zelfa, Grombalia, Mraïssa (Cap Bon Region), on *Citrus* sp. (lemon, navel and orange), July 2006.

***2. Euseius scutalis* (Athias-Henriot)**

Typhlodromus scutalis Athias-Henriot 1958a, 183.

This species seems very common all around the Mediterranean Sea. It was described from Algeria (Athias-Henriot 1958a). This species seems to be common in the driest regions of the northern Tunisia.

Previous Records: Algeria, Canary Islands, Cape Verde, Ghana, Israel, Egypt, India, Iran, Israel, Italy, Jordan, Lebanon, Morocco, Pakistan, Spain, Turkey (Moraes *et al.* 2004).

Specimens examined: Mateur (North region), Sousse (Sahel region), Tekilsa and Slimane (Cap-Bon region), on *Malus domestica*, July 2000; Sousse (Sahel region), on *Citrus* sp., April 2000; Sidi Saheb, near Kairouan (Sahel region), on *Hibiscus* sp. near citrus orchard, May 2001; Tozeur City, on *Hibiscus* sp., *Musa paradisiaca*, *Ricinus communis*, *Malus pumila* and *Vitis vinifera*, April 2000; Tozeur, Jardin du Paradis, on *Hibiscus arboreus*, April 2000; Douz, Hôtel Sahara, on *Hibiscus* sp. and an unknown Verbenaceae, April 2000, and on *Lantana camara*, April 2001; Oasis de Douz, n *R. communis*, April 2000; Tataouine, Hôtel Dak Yanus, on *Althea rosea*, April 2000; Palmeraie Ibn Chabbat, on *Cynodon dactylon*, July 2000; Palmeraie de Tozeur, on *Prunus persica*, *V. vinifera*, *Punica granatum*, *Morus* sp., *Ficus carica*, *C. dactylon* and an unknown Asteraceae, July 2000; Nefta, on *Phoenix dactylifera,* April 2000; Segdoud, on gombé, November 2006; Degache, on *Urtica dioica*, October 2005, August 2006, on *Vitis vinifera*, June 2004, September 2006, on *Phoenix dactylifera* cv. Deglet Noor, May 2004, May 2005, on *Ficus carica*, November 2005, on *Punica granatum*, May 2005; Mraïssa, Soliman, Tekilsa (Cap Bon Region), on *Citrus* sp. (oranges, Thomson Navel and lemon), May 2006.

***3. Euseius stipulatus* (Athias-Henriot)**

Amblyseius stipulatus Athias-Henriot 1960a, 294.

This species was described in Algeria (Athias-Henriot 1960a, b). *Euseius stipulatus* seems to feed on red spider mites and eriophyid mites. This species also consumes pollen (Ferragut *et al.* 1987).

Previous Records: Algeria, Canary Islands, France, Greece, Italy, Montenegro, Morocco, Portugal, Spain, Turkey (Moraes *et al.* 2004).

Specimens examined: Bou Argoub, Hammamet, Maamoura, and Tazarka (Cap-Bon region), on *Citrus* sp., July 1994; Beni Khalled, Bou Argoub, Grombalia, Hammamet, Menzel Bouzelfa, Nabeul, Soliman (Cap-Bon region), on *Citrus* sp., November 1994; Intilaka, Korba, and Takilsa (Cap-Bon region), on *Citrus* sp., October 1995; Mateur (North region), Sousse (Sahel region)**,** Tekilsa and Slimane (Cap-Bon region), on *Malus domestica*, July 2000; Degache, on *Olea europea*, July 2005; Soliman, Grombalia, Mraïssa (Cap Bon Region), on *Citrus* sp. (clementine, Malti and lemon), May 2006.

***4. Iphiseius degenerans* (Berlese)**

Seiulus degenerans Berlese 1889, 9.

Described in Italy during the 19^{e} century (Berlese 1889), this species has a wide distribution in Africa and around the Mediterranean Sea.

Previous Records: Algeria, Benin, Brazil, Burundi, Canary Islands, Cape Verde, Egypt, Georgia, Greece, Israel, Italy, Kenya, Lebanon, Madeira Islands, Madagascar, Malawi, Morocco, Nigeria, Portugal, Rwanda, South Africa, Tanzania, Turkey, Yemen, Zaire, Zimbabwe (Moraes *et al.* 2004).

Specimens examined: Sousse (Sahel region), on *Citrus* sp., April 2000; Sousse (Sahel Region), on *Hibiscus syriacus* near citrus orchard, April 2001; Chott-Mariem, on *Citrus* sp., April 2000; Hammamet, Soliman, Beni Khalled (Cap Bon Region), on *Citrus* sp. (Thomson, Navel and oranges), May 2006.

***5. Neoseiulus barkeri* Hughes**

Neoseiulus barkeri Hughes 1948, 142 and 1976, 343; *Typhlodromus barkeri* Nesbitt 1951, 35; *Typhlodromus barkeri* Chant 1959, 61; *Amblyseius barkeri* Athias-Henriot 1961, 440; *A. mckenziei* Schuster & Pritchard 1963, 268.

Neoseiulus barkeri is widespread throughout the world (Moraes *et al.* 2004). Its biological characteristics have been documented because of its use in controlling thrips on Cucurbitaceae in greenhouses (Castagnoli 1989). It also feeds on red spider mites and eriophyid mites. This species was found in Israel on *Citrus* sp. (Porath & Swirski 1965).

Previous Records: Algeria, Australia, Brazil, canary Islands, Cape Verde, China, Finland, France, Georgia, Germany, Ghana, Greece, Guinea, Hawaï, Israel, Italy, Japan, Jordan, Netherlands, Nigeria, Norway, Reunion Island, Russia, South Africa, South Korea, Spain, Sweden, Turkey, Ukraine, United Kingdom, West Bank, Yemen (Moraes *et al.* 2004).

Specimens examined: Beni Khiar (Cap Bon region), on *Oxalis* sp. in citrus orchard, October 1995; Palmeraie Ibn Chabbat, on *Cynodon dactylon*, July 2000; Segdoud, *Phoenix dactylifera* cv. Alig, January 2006.

***6. Neoseiulus californicus* (McGregor)**

Typhlodromus californicus McGregor 1954, 89.

This very widespread species (Moraes *et al.* 2004), which McMurtry & Croft (1997) consider to be specialised, migrates from the grassy layer to fruit trees or grapevines and vice-versa (Auger *et al.* 1999). It is a specialist predator of *T. urticae* on annual plants and woody species, and of *P. ulmi* (and perhaps eriophyid mites) on trees and less frequently on grapevines. These biological features have only recently been studied (Castagnoli *et al.* 1995; Auger *et al.* 1999).

Previous Records: Algeria, Argentina, Brazil, California, Chile, Colombia, Cuba, France, Guatemala, Italy, Japan, Mexico, Peru, Spain, Taïwan, Uruguay, USA, Venezuela (Moraes *et al.* 2004).

Specimens examined: Sousse (Sahel Region), on *Lycopersicon esculentum* in greenhouses, April 2000; Mateur (North Region), on *Malus domestica*, July 2000; Chekmo oasis, on *Malva* sp., June 2005; Hammamet, Mraïssa, Grombalia, Menzel Bou Zelfa (Cap Bon Region), on *Citrus* sp. (lemon, Clementine and Malti), July 2006.

***7. Neoseiulus cucumeris* (Oudemans)**

Typhlodromus cucumeris Oudemans 1930, 69; *Amblyseius cucumeris* Athias-Henriot 1957: 336; *Typhlodromus* (*Amblyseius*) *cucumeris* Chant 1959: 78.

The biological characteristics of this Palaearctic species have been well documented because of its use as a commercial agent used in controlling thrips on various cultivated plants in greenhouses.

Previous Records: Numerous records, including Europe, Middle East, North Africa, Asia, North America, Australia (Moraes *et al.* 2004), in Morocco (Kreiter *et al.* unpub. data).

Specimens examined: Palmeraie Ibn Chabbat, on *Cynodon dactylon*, July 2000; Palmeraie de Chekmo, on *C. dactylon*, July 2000; Palmeraie de M'Rah Lahouara, on *Digitaria communata* and *C. dactylon*, July 2000; Palmeraie de Tozeur, on *C. dactylon*, July 2000; Segdoud, *Pheonix dactilifera* cv. Alig, November 2005, September 2007; Segdoud, on *Sorghum vulgare*, June and October 2006; Segdoud, Sur *Setaria* sp., June 2006.

***8. Neoseiulus mumai* (Denmark)**

Cydnodromus mumai Denmark 1965, 91; *Neoseiulus mumai* Muma & Denmark 1971, 10; *Amblyseius mumai* Schicha 1981, 209.

The biology of this species remains unknown. It seems to be common on various herbaceous plants in the American continent.

Previous Records: Brazil, Hawaii, USA (Arizona, Florida) (Moraes *et al.* 2004).

Specimens examined: Palmeraie de Tozeur, on *Cynodon dactylon*, July 2000.

***9. Neoseiulus paspalivorus* (De Leon)**

Typhlodromus paspalivorus DeLeon 1957, 143; *Neoseiulus paspalivorus* Muma & Denmark 1971, 110; *Amblyseius paspalivorus* Schicha 1981, 210.

The biology of this species remains unknown. It seems to be common on various herbaceous plants (Moraes *et al.* 1986) and could be a Gondwanian species because of its currently known area of repartition: Caribbean, India, Oriental region and Africa.

Previous Records: Gualeloupe (Moraes *et al.* 1999), India, Jamaica, Philippines, USA (Florida) (Moraes *et al.* 2004).

Specimens examined: Palmeraie de M'Rah Lahouara, on *Cynodon dactylon*, July 2000.

***10. Paragigagnathus tamaricis* Amitai & Grinberg**

Paragigagnathus tamaricis Amitai & Grinberg 1971, 327.

The biology of this species remains unknown. It seems to be common on *Tamarix* sp. in Northern Africa and Middle East (Moraes *et al.* 2004).

Previous Records: Israel, Jordan (Moraes *et al.* 2004).

Specimens examined: Chekmo oasis, on Tamarix sp., June 2005.

***11. Phytoseiulus persimilis* Athias-Henriot**

Phytoseiulus persimilis Athias-Henriot 1957, 347; *Phytoseiulus riegeli* Dosse 1958, 48.

This species was the only species of phytoseiid mite known from Tunisia before these surveys (Gafsa: Rambier 1972). *Phytoseiulus persimilis* was first collected in Algeria in 1955 and is known mainly from mediterranean climates around the world (Takahashi & Chant 1993). Many studies deal with this specialist predator (McMurtry & Croft 1997) because of its economic importance, especially in greenhouses to control *T. urticae* populations (Van Lenteren & Woets 1988).

Previous Records: Algeria, Australia, Canary Islands, Chile, China, Costa Rica, Finland, France, Greece, Guatemala, Hungary, Israel, Italy, Jordan, Lebanon, Lybia, Morocco, New Caledonia, Peru, Reunion Island, South Africa, South Korea, Spain, Tunisia, Turkey, Venezuela, USA (California) (Moraes *et al.* 2004).

Specimens examined: Sousse (Sahel region), on *Lycopersicon esculentum* Miller and *Cucumis sativus* L. in greenhouses (but not introduced), April 2000; Hammamet, Menzel Bouzelfa and Mraïssa (Cap-Bon region), on *Citrus* sp., November 1994, October 1995, and July 2001; Metline (Bizerte region), on *Malus domestica*, June 2000; Mraïssa, Soliman (Cap Bon region), on *Citrus* sp. (Thomson, Navel and lemon), July 2006.

***12. Typhloseiella isotricha* (Athias-Henriot)**

Amblyseius isotricha Athias-Henriot 1958b, 37-38.

This species was described from Algeria on Inula viscosa L. in 1958 (Athias-Henriot 1958b). Up to now, it has been reported only under Mediterranean climatic conditions. Furthermore, in the present survey, this species is recorded on I. viscosa, where this species has almost exclusively been found up to now (Moraes et al. 1986; Tixier et al. 2003). Specific relationships could occur between this mite and I. viscosa; this plant is especially sticky, odorant, with hairy leaves. However, the mechanisms involved in this relationship have not been investigated.

Previous Records: Algeria, Canary Islands, France, Greece, Israel, Jordan, Lebanon, Portugal (Moraes *et al.* 2004), Morocco (Tixier *et al.* 2003).

Specimens examined: Matmata, on *Inula viscosa*, May 2001.

Phytoseiinae

***13. Phytoseius finitimus* Ribaga**

Phytoseius finitimus Ribaga 1904, 178.

The specimens found in Tunisia belong to the species *P. finitimus*. This species was confused in many studies with *Phytoseius plumifer* Canestrini & Fanzago. The confusion between these 2 species has existed for a long time. The solution was provided recently by the contribution of Duso & Fontana (2002). *Phytoseius finitimus* seems to feed on *Panonychus ulmi* (Koch) (Duso & Moretto 1994) and various eriophyid mites (Rasmy & El-Banhawy 1974), and consumes pollen (Zaher *et al.* 1969; Rasmy & El-Banhawy 1975). Local conditions in Corsica, i.e. high relative humidity and very hairy-leaved grapevine varieties, seem to be very suitable for this species (Rasmy & El-Banhawy

1974; Duso & Moretto 1994; Kreiter *et al.* 2000).

Previous Records: Algeria, Egypt, France, Greece, Iran, Israel, Italy, Montenegro, Spain, Turkey, USA (California) **(Moraes *et al.* 2004).**

Specimens examined: several vineyards in Cap-Bon region, *Vitis vinifera* L., July 1995; Palmeraie de Tozeur, on *Ficus carica*, July 2000; Degache, on *F. carica*, July 2005; Degache, on *V. vinifera*, September 2006; Degache, on *Urtica dioica*, October 2005; Soliman, Hammamet, Beni Khalled and Grombalia (Cap Bon region), on *Citrus* sp. (lemon, Clementine), May 2006.

Typhlodrominae

***14. Africoseiulella flechtmanni* Kreiter**

Africoseiulella flechtmanni Kreiter 2006, *in* Kreiter & Tixier 2006, 5-12.

This new genus and new species are described in another paper with a phylogenetic discussion concerning its position within the family Phytoseiidae (Kreiter & Tixier 2006).

The biology of this species remains unknown. It seems abundant on small weed plants inside oases, but these plants seem to be present only for short periods during the year. This mite is therefore probably submitted to extreme environmental conditions.

Specimens examined: Chekmo oasis, on an unknown small weed, July 2000.

15. Neoseiulella perforata *(Athias-Henriot)*

Typhlodromus perforatus *Athias-Henriot 1960b, 72.*

Biological characteristics of this species remain unknown.

Previous Records: Algeria (Moraes *et al.* 2004).

Specimens examined: Sousse (Sahel Region), on *Malus domestica*, July 2000.

***16. Neoseiulella tiliarum* (Oudemans)**

Typhlodromus tiliarum Oudemans 1930, 51.

This species is found worldwide and is commonly encountered in Mediterranean countries.

Previous Records: Algeria, Austria, Azerbaijan, Canada, Denmark, England, France, Georgia, Germany, Greece, Hungary, Iran, Italy, Moldavia, Montenegro, Netherlands, Norway, Poland, Russia, Spain, Switzerland, Turkey, Ukraine, USA (Massachusetts) (Moraes *et al.* 2004).

Specimens examined: Sousse (Sahel region), on *Malus domestica*, July 2000.

***17. Paraseiulus soleiger* (Ribaga)**

Seiulus soleiger Ribaga 1904, 176; *Paraseiulus soleiger* Chant & Yoshia-Shaul 1982, 3027; *Paraseiulus incognitus* Wainstein & Arutunjan 1967, 1768; *Typhlodromus trimediosetus* Xin, Liang & Ke 1980, 469.

This species is very widespread in the palaearctic and nearctic regions and could be considered as a Laurasian species. Despite this very wide area of distribution, its biological characteristics have still to be documented. Kropczynska *et al.* (1988) investigated the development parameters of this species; it seems to be an arboreal generalist predator, feeding preferentially on red spider mites. Kropczynska *et al.* (1988) concluded that it was the only species that could control spider mite populations on lime trees under their study conditions in Poland.

Previous Records: Europe, North America, Middle East, China, Japan (Moraes *et al.* 2004).

Specimens examined: Chott-Mariem (Sahel Region), on *Convolvulus arvensis*, April 2000.

***18. Typhlodromus* (*Anthoseius*) *athenas* Swirski & Ragusa**

Typhlodromus (*Anthoseius*) *athenas* Swirski & Ragusa 1976, 111.

Previous Records: Greece, Israel, Italy, Morocco (Moraes *et al.* 2004).

Specimens examined: Gafsa, on *Olea europea*, March 2004 and March 2007; Segdoud, on *Phoenix dactylifera* cv. Alig, November 2005, October 2006 and October 2007; Segdoud, on *P. dactylifera* cv. Deglet Noor, November2005, October 2006 ; Segdoud, on the trunk of *P. dactylifera* cv. Deglet Noor, November 2005 ; on Segdoud, on *Solanum melongena*, April 2006.

***19. Typhlodromus* (*Anthoseius*) *foenilis* Oudemans**

Typhlodromus foenilis Oudemans 1930, 70; senior synonym of *Typhlodromus cryptus* Athias-Henriot 1960b, 89.

Previous Records: Azerbaijan, Belgium, Canada, England, France, Greece, Ireland, Israel, Italy, Norway (Moraes *et al.* 2004).

Specimens examined: Degache, on *Punica granatum*, May 2005; Cap Bon, on Citrus sp., June 2005.

***20. Typhlodromus* (*Anthoseius*) *kazachstanicus* Wainstein**

Typhlodromus kazachstanicus Wainstein 1958, 203.

The biology of this species remains unknown. It seems common on various trees (Moraes *et al.* 1986), especially vines and Rosaceae in the eastern Europe.

Previous Records: Armenia, Georgia, Iran, Kazakhstan, Kyrgystan, Russia, Tajikistan, Uzbekistan (Moraes *et al.* 2004).

Specimens examined: Palmeraie Ibn Chabbat, on *Ficus carica*, July 2000; Palmeraie de Tozeur, on *Vitis vinifera*, July 2000.

***21. Typhlodromus (Anthoseius) rhenanoides* Athias-Henriot**

Typhlodromus rhenanoides Athias-Henriot 1960b, 85; McMurtry & Bounfour 1989, 16; *Neoseiulus rhenanoides* Schuster & Pritchard 1963, 205.

This species has been described in Algeria (Athias-Henriot 1960a,b). Its biological characteristics have been poorly investigated. It seems to be polliniphagous (Ragusa & Tsolakis 1998) but specific diets have not yet been documented.

Previous Records: Algeria, Canary Islands, France, Hawaii, Italy, Les Saintes, Morocco, Spain, USA (California) (Moraes *et al.* 2004).

Specimens examined: Hammamet (Cap-Bon Region), on *Citrus* sp., November 1994; Bou Argoub (Cap-Bon Region), on *Citrus* sp., October 1995; Chekmo oasis, on *Tamarix* sp. June 2005.

***22. Typhlodromus (Anthoseius) rhenanus* Oudemans**

Typhlodromus rhenanus Oudemans 1905, 128.

This species was found in Israel on *Citrus* sp. (Porath & Swirski 1965) and on grapevines in France (Kreiter *et al.* 2000). The biolocal characteristics of this species remain unknown.

Previous Records: Algeria, Azerbaijan, Belgium, Byelorussia, Canada, Cyprus, Denmark, England, Finland, France, Germany, Hungary, India, Iran, Israel, Italy, Kazakhstan, Madeira Island, Moldavia, Montenegro, Netherlands, Northern Ireland, Norway, Poland, Portugal, Russia, Switzerland, Turkey, Ukraine, USA (California) (Moraes *et al.* 2004).

Specimens examined: Mateur (North Region) and Sousse (Sahel Region), on *Malus domestica*, July 2000; Tunis (INAT, North Region), on *Urtica dioica*, June 2000; Mraïssa, Grombalia, Soliman (Cap Bon Region), on *Citrus* sp. (Clementine, maltaise, lemon), July 2006.

***23. Typhlodromus (Anthoseius) recki* Wainstein**

Typhlodromus recki Wainstein 1958, 203.

The biology of this species has not been investigated.

Previous Records: Algeria, Armenia, Azerbaijan, Caucasus Region, France, Georgia, Greece, Hungary, Israel, Italy, Kazakhstan, Lebanon, Moldavia, Russia, Turkey, Ukraine (Moraes *et al.* 2004).

Specimens examined: several vineyards in Cap-Bon Region, *Vitis vinifera*, July 1995.

***24. Typhlodromus (Typhlodromus) exhilaratus* Ragusa**

Typhlodromus exhilaratus Ragusa 1977, 380.

Many studies deal with this species in Italy because of its dominance in several Italian vineyards (Castagnoli & Liguori 1986; Castagnoli *et al.* 1989; Liguori & Guildi 1990).

Previous Records: France, Greece, Israel, Italy, Morocco, USA (Moraes *et al.* 2004).

Specimens examined: Sousse (Sahel Region), on *Malus domestica*, July 2000; Cap Bon Region: Hammamet, Tekilsa, Beni Khalled, on *Citrus* sp. (Maltaise, Thomson and Lemon), May 2006; Gafsa, on *Olea europea*, March 2007.

***25. Typhlodromus (Typhlodromus) phialatus* Athias-Henriot**

Typhlodromus phialatus Athias-Henriot 1960b, 100.

This species is known to feed on red spider mites and to consume pollen (Ferragut *et al.* 1987).

Previous Records: Algeria, France, Germany, Hungary, Israel, Italy, Jordan, Moldavia, Morocco, Norway, Russia, Spain, Ukraine (Moraes *et al.* 2004).

Specimens examined: El Gobba and Hammamet (Cap-Bon Region), on *Citrus* sp., June 1994; Monastir (Sahel Region), on *Citrus* sp., November 1994; several vineyards in Cap-Bon region, on *Vitis vinifera*, July 1995; Slimane (Cap-Bon Region) and Sousse (Sahel Region), on *Malus domestica*, July 2000; Grombalia, Tekilsa, Menzel Bou Zelfa (Cap Bon Region), on *Citrus* sp. (Maltaise, Thomson), September 2006.

Key to the species of Phytoseiid mites of Tunisia

1. Podonotal region of the dorsal shield (anterior to setae R1) with 4 pairs of “lateral” setae : j3, z2, z4 and s4

present, z3 and s6 absent ..... **Amblyseiinae: 3**
1'. Podonotal region with 5 or 6 pairs of "lateral" setae : j3, z2, z4 and s4 always present and z3 and/or s6 present **2**

2 (1'). Posterior "lateral" setae Z1, S2, S4 and S5 absent. Setae r3 usually inserted on the dorsal shield .... **Phytoseiinae: *Phytoseius finitimus***
2' (1'). At least one of setae Z1, S2, S4 and S5 present. Setae r3 usually in soft cuticule next to dorsal shield (rarely on shield) ..... **Typhlodominae: 14**

3 (1). Macrosetae usually present only on leg IV (rarely missing on this leg) but sometimes also on leg III. Lateral dorsal setae other than Z5 usually subequal. J2, Z1, S2, S4 and S5 always present. Sternal shield usually with concave or straight posterior margin. Ventrianal shield usually longer than wide, often pentagonal. Line between JV1 and JV2 subparallel to main idiosomal axis. Internal margin of fixed cheliceral digit never markedly concave **4**
3' (1). Macrosetae usually present on legs II, III and IV, and sometimes also on leg I. Lateral dorsal setae often of quite different lengths. J2, Z1, S2, S4 or S5 may be missing. Sternal shield sometimes with a lobe on posterior margin. Ventrianal shield may be wider than long, often not pentagonal. Line between JV1 and JV2 may not be subparallel to main idiosomal axis. Internal margin of fixed cheliceral digit may be markedly concave **9**

4 (3). Female ventrianal shield reduced and/or markedly wider at anus level, with a marked waist. Movable and fixed cheliceral digits with 1 and 1-3 distal teeth, respectively ..... ***Paragigagnathus tamaricis***
4' (3). Female ventrianal shield not reduced and/or markedly wider at anus level, without a marked waist. Movable and fixed cheliceral digits with larger number of teeth, which are not confined to apical region .... ***Neoseiulus*: 5**

5 (4'). Spermatheca with atrium forked for at least half its length at juncture with major duct, or atrium appering thick-walled, vacuolated ..... ***Neoseiulus barkeri***
5' (4'). Spermatheca with atrium not deeply forked at juncture with major duct, nor appearing thick-walled, vacuolated **6**

6 (5'). Female ventrianal shield large, square or rectangular, rounded posteriorly (L/W ratio : 1.0-1.3:1.O). Dorsal shield with marked "shoulder"at level of seta r3
***paspalivorus* species group: 7**
6' (5'). Female ventrianal shield pentagonal or with lateral margins slightly rounded. Dorsal shield without marked "shoulder"at level of seta r3 ..... ***cucumeris* species group: 8**

7 (6). Seta ZV1 absent ..... ***Neoseiulus mumai***
7' (6). Seta ZV1 present ..... ***Neoseiulus paspalivorus***

8 (6'). Ventrianal shield with large prominent crescentic preanal pores close to the central part. Setae Z4 longer than S4. Cervix of the spermatheca open and short ..... ***Neoseiulus californicus***
8' (6'). Ventrianal shield with very small punctiform preanal pores close to JV2. Setae S4 and Z4 of almost the same size. Cervix of the spermatheca closed and long ..... ***Neoseiulus cucumeris***

9 (3'). Setae j6 2-3 times longer than the distance between their bases. Ventrianal shield with 0-1 pairs of preanal setae ..... ***Phytoseiulus persimilis***
9' (3'). Setae j6 less than twice as long as the distance between their bases. Ventrianal shield with one (rare), 2 or 3 pairs of preanall setae **10**

10 (9'). Heavily sclerotised, brown, with separate anal shield and subrectangular ventral shield .... ***Iphiseius degenerans***
10' (9'). Lightly sclerotised and ventrianal shield entire **11**

11 (10'). Sternal shield sometimes indistinct, always with a median lobe on posterior margin. Preanal setae of the ventrianal shield of the female on a same line ..... ***Euseius*: 12 & 12'**
11' (10'). Sternal shield distinct. Posterior margin always without a median lobe, usually straight or concave medially. Preanal setae never on a same line **13**

12 (11). Peritreme long, extending at level of j3 or between j3 and z2. Macrosetae relatively short (54 µm).

Spermatheca tubular with a long cervix of 20 to 25 µm long ..... ***Euseius stipulatus***
12' (11). Peritreme short, extending at level of z4 or between z2 and z4. Dorsal setae long. Macrosetae of leg IV long (77 µm). Cervix of the spermatheca tubular, thin, long and sinuous (43 µm) and atrium globular ..... ***Euseius scutalis***

13 (11'). Setae Z2 present, VAS reduced..... ***Typhloseiella isotricha***
13' (11'). Setae Z2 absent, VAS non reduced..... ***Amblyseius graminis***

14 (2'). Setae z6 present (relatively rare taxa). Ventrianal shield sole-shaped, with 2 pairs of preanal setae. Setae Z1 and JV2 absent and setae R1 present ..... ***Paraseiulus soleiger***
14' (2'). Setae z6 absent..... **15**

15 (14'). Most species with both S4 and JV4 present ..... **Tribe Typhlodromini: 18**
15' (14'). Both dorsal shield setae S4 and JV4 absent ..... ***Africoseiulella flechtmanni***

16 (15). Setae Z1 present. ..... ***Neoseiulella*: 17**
16' (15). Setae Z1 absent ..... ***Typhlodromus*: 18**

17 (16). Four large and one small solenostomes on the dorsal shield. Ventrianal shield not reduced, with 4 pairs of preanal setae and without solenostomes. Setae on dorsal shield mostly slender. Peritreme short, extending anteriorly between z3 and z4 and punctuate. Cervix of the spermatheca saccular. Leg IV without macroseta..... ***Neoseiulella tiliarum***
17' (16'). Six small round solenostomes on the dorsal shield. Ventrianal shield reduced, with 4 pairs of preanal setae and without solenostomes. Peritreme long, extending anteriorly to z2. Cervix of the spermatheca saccular. Leg IV with a macroseta..... ***Neoseiulella perforata***

18 (15). Seta S5 present..... ***Typhlodromus* (*Anthoseius*): 19**
18' (15). Seta S5 absent ..... ***Typhlodromus* (*Typhlodromus*): 24**

19 (18). Presence of 3 pairs of solenostomes on a strongly reticulated dorsal shield..... ***Typhlodromus* (*Anthoseius*) *recki***
19' (18). Presence of 5 pairs of solenostomes on a less reticulate dorsal shields **20**

20 (19'). Leg IV with a long knobbed macroseta (54-57 µm). Two teeth on the digitus mobilis. Dorsal shield not strongly reticulated..... **21**
20' (19'). Leg IV with a shorter macroseta not knobbed. One tooth on the digitus mobilis **22**

21 (20). Spermatheca with a long narrow cervix (25 µm) ..... ***Typhlodromus* (*Anthoseius*) *rhenanoides***
21' (20). Spermatheca with a shorter cervix (< 20 µm) ..... ***Typhlodromus* (*Anthoseius*) *foenilis***

22 (20'). Macroseta of the Leg IV short, < 30 µm ..... ***Typhlodromus* (*Anthoseius*) *rhenanus***
22' (20'). Macroseta between 40-45 µm and knobbed **23**

23 (22'). Spermatheca with a globulous atrium at the basis of the cervix, with sometimes a thick neck. Ventrianal shield elongate and not pentagonal..... ***Typhlodromus* (*Anthoseius*) *kazachstanicus***

23' (22'). Spermatheca with the atrium on the cervix, no neck and a long major duct. Ventrianal shield subpentagonal and large..... ***Typhlodromus* (*Anthoseius*) *athenas***

24 (17'). Calix of the spermatheca squared basally, with a short neck ..... ***Typhlodromus* (*Typhlodromus*) *exhilaratus***
24'(17'). Calix of the spermatheca rounded basally, without neck ..... ***Typhlodromus* (*Typhlodromus*) *phialatus***

Conclusion

Twenty-five species in total were found across the different surveys carried out, with a new genus and a new species to Science. Among them, twenty one species are new to the fauna of Tunisia and 2 are new to the fauna of Africa.

The number of known species of phytoseiid mites for the Tunisian fauna is now 25, which is very low for a whole country (but only one was known before 2002). The number of species is probably

strongly underestimated if compared to the fauna of Algeria (53 species) and Morocco (47 species). The flora and fauna of Tunisia seem less diverse than that of the two larger countries. Most of the species found are common Mediterranean species or palaearctic species. *Neoseiulus mumai* and *N. paspalivorus* are reported for the first time from North Africa, these two species being mainly reported from Nearctic and Neotropical regions (but recently from Africa on coconuts).

Surveys were done mainly in crops and in the surrounding areas which probably explains the very low diversity found. It is thus now very important to also investigate uncultivated areas. However, one new genus and one new species were found in the palm tree growing area of Tozeur, in the South of Tunisia. Oases are probably habitats in this part of the World with a high level of endemism. New surveys are consequently needed. A stronger effort should be made in the southern and western parts of Tunisia, in this last case along the Algerian border and the East and North Mediterranean Coasts. In general, all regions of this country very poorly investigated for now.

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SPIDER MITES WEB: A DATABASE DEDICATED TO THE KNOWLEDGE OF AN ACARINE PEST FAMILY, THE TETRANYCHIDAE

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Abstract

Building databases to collect information regarding pest families is particularly useful for an overall knowledge of their biology. Spider Mites Web is dedicated to the Tetranychidae, a family including about 1,250 species, of which 100 are considered as pests. Spider Mites Web is an online database, free, regularly updated, and continuously improved. Built to provide information on all described spider mites worldwide, the database was originally developed from work initiated by J. Gutierrez (Bolland *et al.*, 1998). It was first devoted to taxonomic knowledge, so that it includes all the taxonomic data relating to the history of the nomenclature. It also includes all the host plants and geographical records. We have recently introduced some improvements by updating the user interface. Particularly, we have added a cartographic display of the distribution. This new tool is useful for understanding at a glance the world distribution of each species. Spider Mites Web presently includes (December 20, 2007): 1,250 valid species recorded, 1,256 references, 11,435 host records on 3,877 different plants and 5,219 records of geographic distribution. The Spider Mites Web provides an interactive user interface built for an easy and instinctive use. Three types of queries are available to retrieve information: i) by species, ii) by author and iii) by a cross search allowing multiple combinations of nomenclatural, host plant and geographical data. The Spider Mites Web also has a download section consisting of valid species, host plants and references. The Spider Mites Web is the result of a community work and will grow with the continued participation of this community.

Keywords

Database, free, Tetranychidae, pest, host plant, map, geographical distribution

Introduction

Online databases are one of the most important innovative tools used in current biology and systematics. They constitute a repository of the secular knowledge built by previous taxonomists and biologists. They also constitute a basis for further taxonomic improvements. Present taxonomic databases are developed in two ways. On the one hand, there is a push to collect and assemble large amounts of data with wide taxonomical and/or geographical ranges. This is exemplified by Fauna Europaea (Fauna Europaea Web Service, 2004), dealing with all animals encountered in Europe. On the other hand, there is a need for databases regarding small groups of interest. These latter databases need the contribution of specialists and experts. ScaleNet (BenDov *et al.*, 2006) and the Biosystematic Database of World Diptera (Thompson, 2005) are both examples of such specialised databases. They constitute taxonomical references. To ensure their continued existence and to guarantee widespread access and expertise, they have to be set-up within institutional networks. Our team is currently working on the future development of the CIRES

(Centre International de Recherches en Systématique). This French structure aims to build a network of specialists for both pests and auxiliaries, combining classical taxonomical knowledge, biogeography, phenology and new advances in bar-coding, molecular identification, but also phylogenetic and phylogeographic molecular aspects.

Among pest families, Tetranychidae are an important group due to both their agronomical impact and the fundamental knowledge already acquired on the group, such as the recent whole genome sequencing of the emblematic *Tetranychus urticae*. This Acari family totals about 1,250 phytophagous species and includes several ubiquitous pests. Despite the presence of major pests and a large number of studies on these mites, no database was available until now. The current knowledge of the Tetranychidae is sufficiently advanced to ensure adatabase of great value (Shimano 2004). Indeed, a substantial amount of literature on spider mites has contributed the general description of the family. The first of these studies was by McGregor (1950), followed by the work by Pritchard and Baker (1955) which is still used. More recently the catalogue by Bolland *et al.* (1998) gives a list of species of the family. These studies have been complemented by other regional studies such as Baker and Pritchard (1960), and Meyer (1974; 1987) for Africa, Tuttle *et al.* (1976) for Mexico, Baker and Tutlle (1994) for USA, Mitrofanov *et al.* (1987) for former USSR, and Ehara (1999) for Japan. The study by Bolland *et al.* (1998) is a catalogue of the systematics of the family, which was reviewed and updated by Migeon and Flechtmann (2004). This catalogue remains an extremely useful reference; however it contains limitations inherent to traditional publications as static knowledge and difficultly in retrieving available information.

The presented Spider Mites Web (http://www.montpellier.inra.fr/CBGP/spmweb/) has been designed to provide comprehensive information for every species of Tetranychidae, including taxonomy, distribution and host plant with reference to the literature since 1758. In addition to the advantages of interactivity, Spider Mites Web, like other databases, provides cross-analyses, giving a synthetic view of the biodiversity of the Tetranychidae ranging from host plant to continent. It also provides a continuous framework, always including new features such as an improved user interface and map distribution display.

Materials and methods: database workflow

The database was developed and is updated using Microsoft Access. For web applications, the database was transferred to Unix/Linux System. Site development was performed using Php language, version 5.04, in complement to Html. PostgreSQL was used for the database engine and Php-PostgreSQL for database queries. Php-PostgreSQL is a universal, free, well-documented and standard pack for developing such applications. The database relational and functional structure is shown in figure 1.

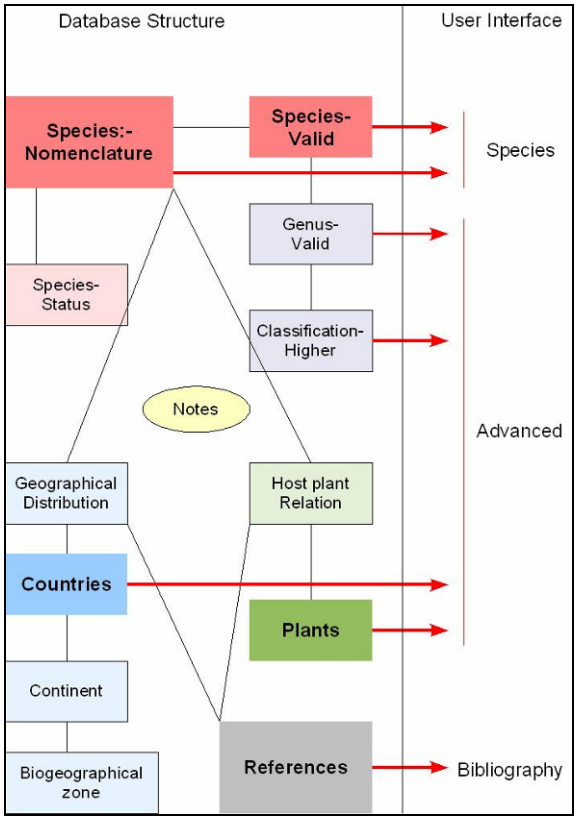

Figure 1. Schema of the database showing the data structure in relation to the user interface.

References. Bibliographic references constitute the unique data source. All publications regarding Tetranychidae and especially taxonomy, host plants and distribution are analysed. The heterogeneity of specialised, generalist and agriculturally oriented publications used requires the validation of each record by a specialist. 1,256 publications are referenced in Spider Mites Web and are available as a list, via a pdf document, in the site.

Nomenclatural information. One of the major difficulties encountered when building such databases is to model the nomenclatural and taxonomic information. The tables Species-Nomenclature and Status allow the description of the nomenclatural history of each taxon. Statuses (table 1) are derived from the International Code of Zoological Nomenclature (1999). This information keeps with standard definitions and describes the nomenclatural data available in each publication. Genus and higher classification ranks are also used for retrieving information, but we did not represent all the complexity of the taxonomic relationships. 1,250 valid species and 3,865 records are included in the database. A systematic list of all species is presented (pdf document) to help in finding a particular species.

Table 1. The status used for recording names in Species-Nomenclature.

Valid species status :
Valid name: the name use for taxonomical information
Valid nomenclatural act: the publication of a valid name
Valid name in invalid publication: the publication of a valid name
Change of status: a change to subspecies to species...
New combination: change of generic assignment
Emendation: a justified change in the original spelling
Replacement name: a new name for a valid taxon with an invalid name
Synonyms status :
Synonym description: the publication of a junior synonym
Reinstatement: a name considered as junior synonym and now valid species
Synonymy by: reference for a synonymisous work
Miscellaneous status :
Homonym: an already used valid name
Unjustified emendation: an unjustified change in the original spelling
Misspelling: a valid citation with an erroneous spelling
Misidentification: an erroneous citation
Corrected identification: a correction of above

Host plants. The table Plants contains all information for host plants including their classification ranging from phylum to species. We give a list (pdf document) of all the 3,877 plants recorded in the database. Sometimes information is lacking and limited to a family or to a genus. We have used up-to-date plant nomenclature according to the IPNI (International Plant Name Index 2004). 11,435 references to taxon-host plant relationships are included in the database.

Geographical information. Representation of geographic knowledge ranges from biogeographical area to country. This representation follows the recommendations and schemes proposed by the Taxonomic Database Working Group (TDWG). Data entries are performed using maximal precision and correspond to 609 basic units, grouped into 264 countries, 9 continents and 7 biogeographical areas. For countries belonging to more than one continent or biogeographical area, such as China or Russia, searches can be performed by "sub-country". Former Czechoslovakia and Yugoslavia are still used because records corresponding to the current countries are not yet completed. The database includes 5,219 references to taxon-geographical unit relationships.

Results and discussion: user interface and displaying results

General information. The home page (figure 2) displays general information regarding the database (short presentation of the family Tetranychidae, how to use the database, last update, etc). A background page explains the general structure of the database and the repositories used for nomenclature, host plants and geographical information. These pages are complemented by a download section, offering pdfs for species, host plants and references lists, a photo gallery, showing a small part of the diversity of the family and a list of web links regarding French agricultural research, acarology, biodiversity and tools used in the database. We are glad to have a special page listing all the colleagues who helped us by sending references and reprints. All pages show a left menu allowing rapid navigation and queries.

By species search and display. A simple and rapid search can be performed by entering a species name, valid or not. The results give valid names in blue with hypertext links. Other names are displayed in black with the valid name indicated in brackets (figure 3). Clicking the hypertext link takes the user to the species page.

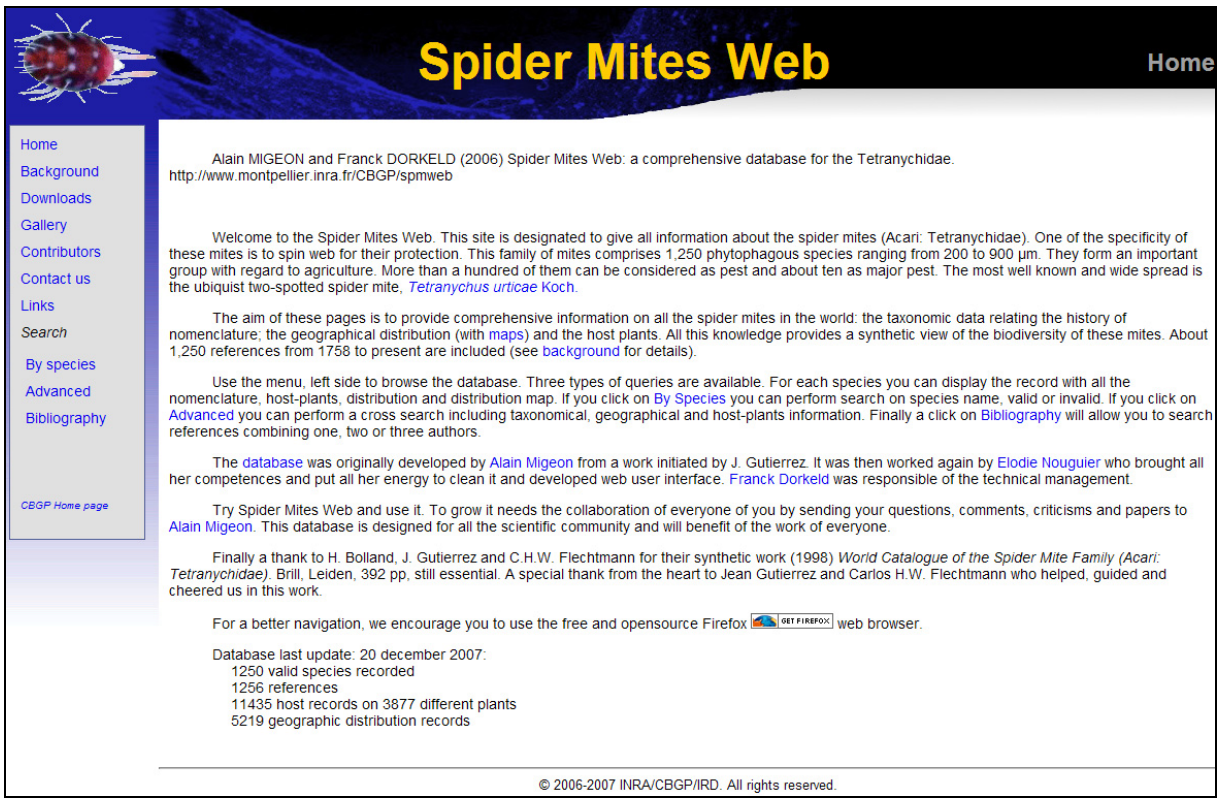

Figure 2. Screen copy of the Home page, showing the left menu and providing concise information.

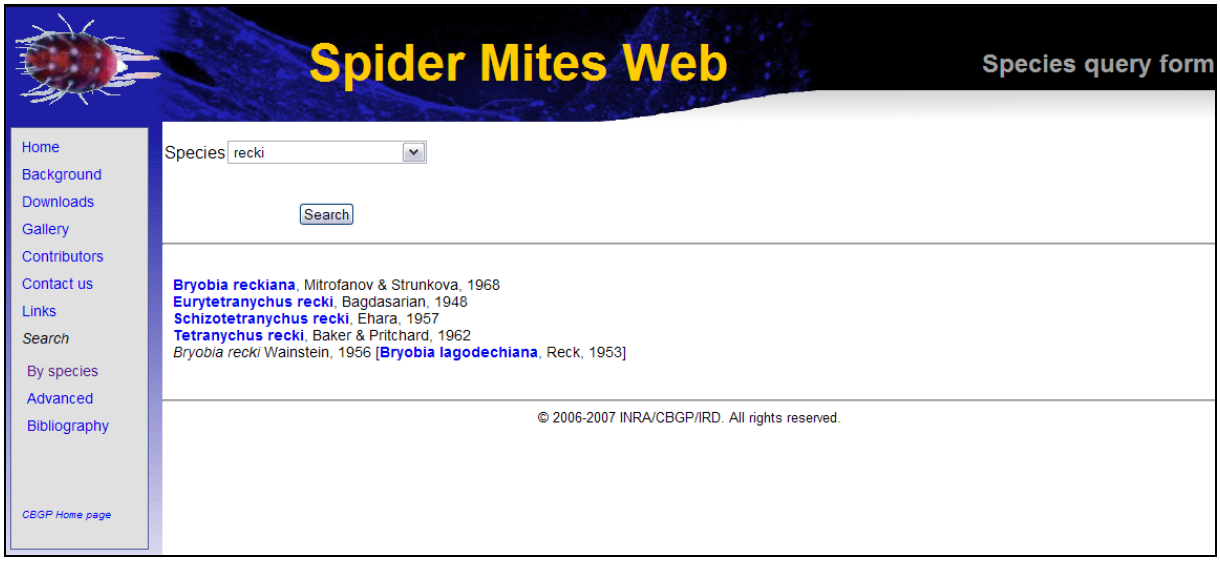

Figure 3. Screen copy of a By Species search, showing the results for *recki.*

The species page is divided into five parts. All of these parts offer a rapid hypertext link to cited references. The first part (figure 4) is dedicated to summarising the original data: original description with type host plant and type locality (country), completed by the current valid name. The second part (figure 4) is a summary, allowing the rapid navigation in sub-pages for species having numerous data. This section also includes a link opening a new window and displaying the world distribution map. The third part (figure 5) is dedicated to the nomenclatural history of the studied species and lists all synonyms, synonymy references, replacement names, emendations, and combination changes. The fourth part (figure 6) displays a list of host plants arranged by family, genus and plant species. On the same page, the fifth (figure 6) part is then devoted to the distribution of the species and is arranged by biogeographic area and country.

Figure 4. Screen copy of the first species page, with the original description reference, type country and host plant for *Tetranychus evansi*.

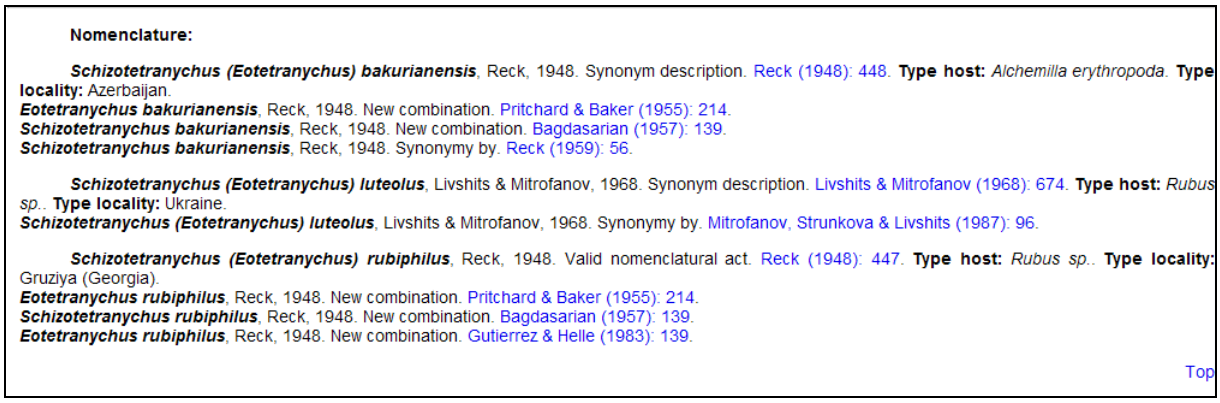

Figure 5. Screen copy of nomenclatural data of *Eotetranychus rubiphilus*.

Figure 6. Screen copy of host plant and geographic distribution for *Eurytetranychus admes*.

Advanced search. A cross search including taxonomical, geographical and host plant information can be performed. At least one of the proposed fields must be completed. For example, as presented figure 7, 4 species of the genus *Tetranychus* are present in France and have been reported from Solanaceae as host plants. Only valid names are displayed and hypertext links take the user to the species page as in "By Species" search.

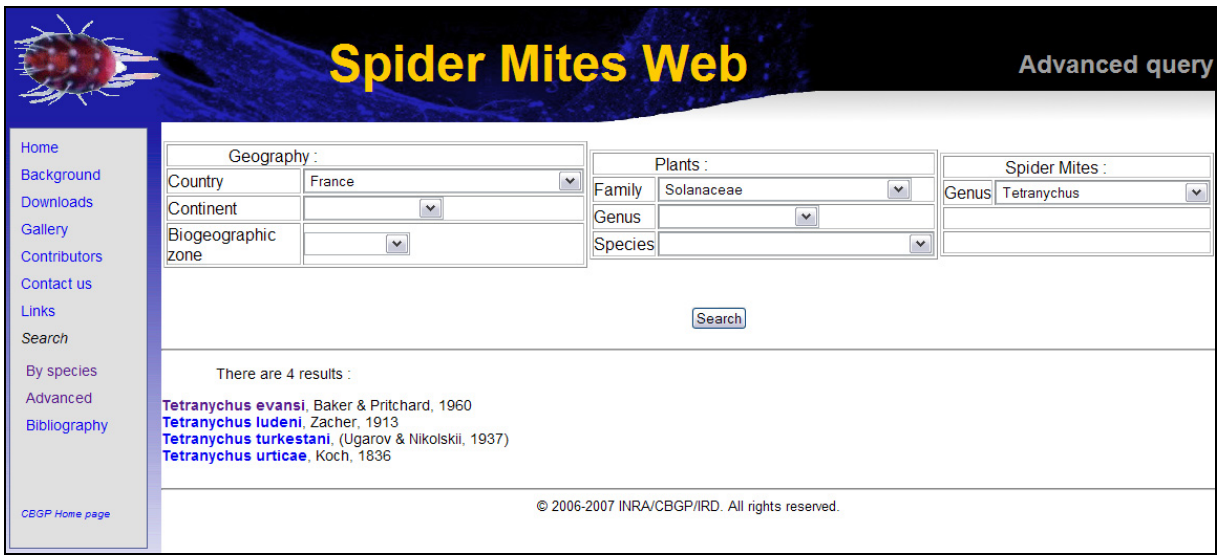

Figure 7. Screen copy of an Advanced search, showing results for all the species belonging to the genus *Tetranychus* found in France and previously recorded on plants of the family Solanaceae.

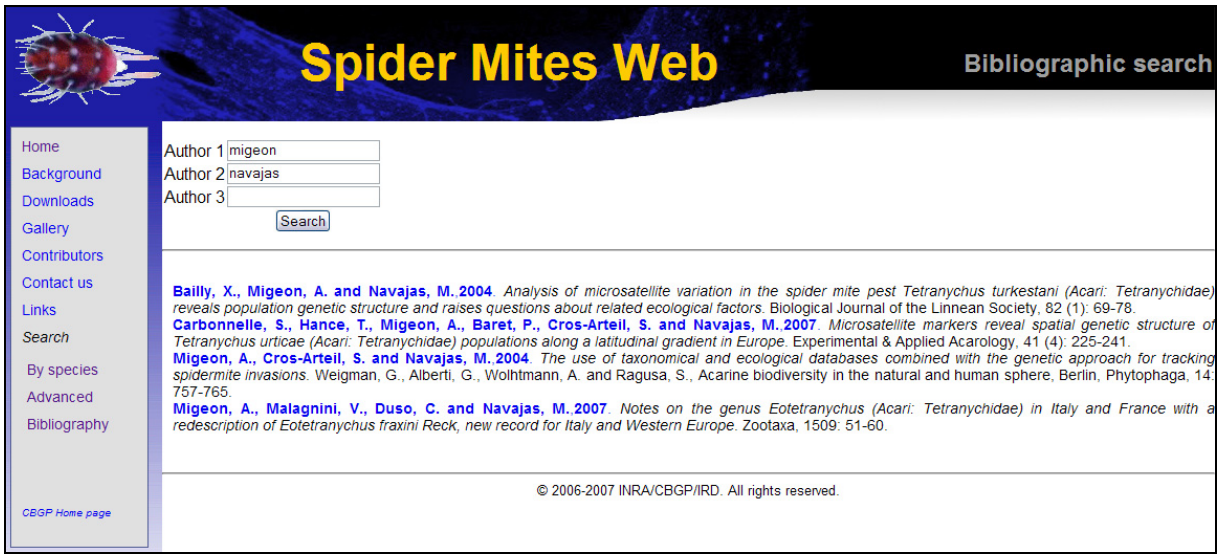

Figure 8. Screen copy of a Bibliographic search showing results for a combination of two authors Migeon and Navajas.

Bibliographic search. This query allows the user to search references combining one, two or three authors (figure 8). The result is a list of references, each with hypertext. If the user follows the hyperlink, the complete reference is given at top of the page, followed by nomenclatural data, host plants and distribution. A hypertext link can then take the user to the cited species page.

Map display. Displaying the worldwide distribution is a brand new tool. The default view provides a satellite background and a worldwide scale (figure 9). Pre-selected zoom levels to continental scales are implemented, but free scale magnifying is also possible. The background can be changed to biogeographical areas, or countries. A neutral grey background is also possible, which is particularly useful for showing records from small countries.

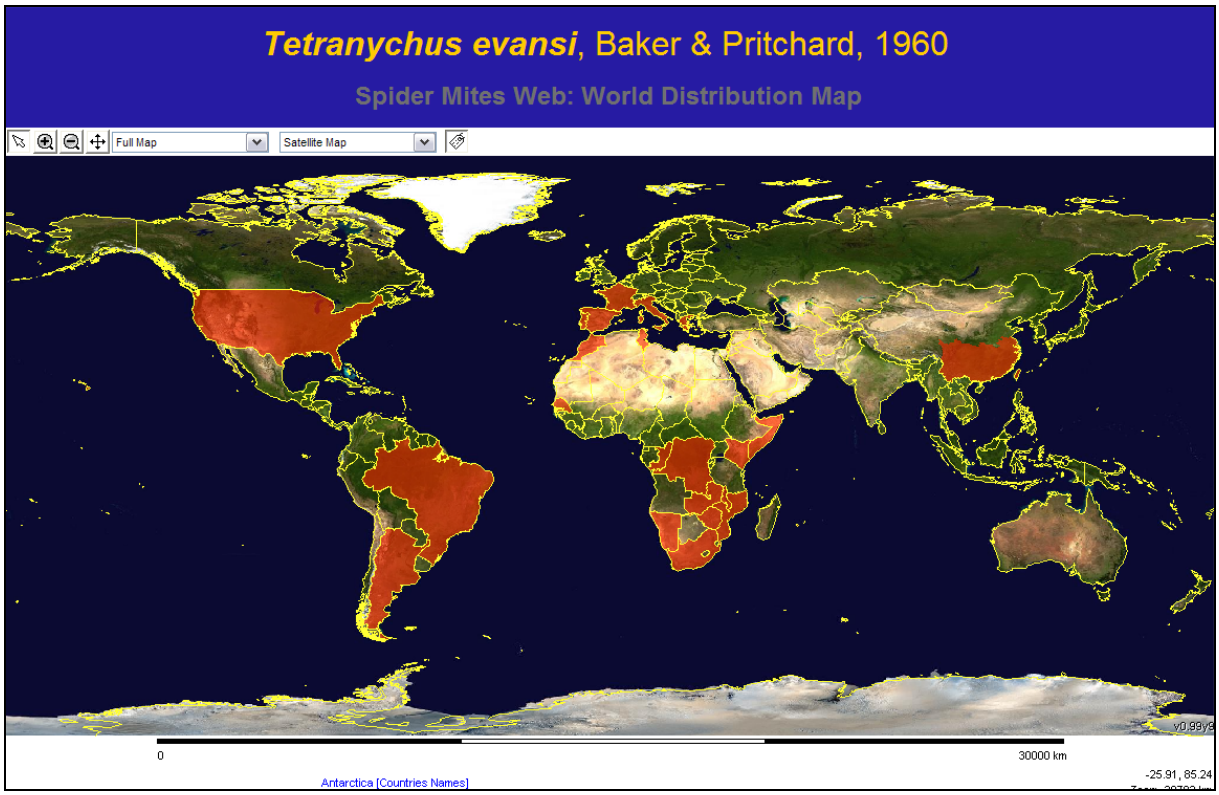

Figure 9. World distribution map of *Tetranychus evans*i. Red colour indicates country records.

Conclusion

The Spider Mites Web site is aimed at providing comprehensive information on the spider mites of the world, including data referenced in the literature on taxonomy, distribution and host plants. In addition to the advantage provided by the interactivity and cross analyses of an electronic database, this project gives a synthetic view of the biodiversity of the Tetranychidae covering scales from the continent to the host plant. New tools are continuously being added. The latest improvement is the integration of a world distribution map which provides the user with an immediate understanding of the biogeographical range of a species. In the present world context of global trade, such a synthesis is an essential facility that instantly provides lists of potentially harmful organisms. This information should contribute to evaluating the risk of bio-invasions and guiding managing decisions.

Finally, try Spider Mites Web, use it and send any comments, criticisms and further information to the authors. This database is designed for use by all members of the scientific community and will benefit from your input.

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THE EFFECT OF FIRE DISTURBANCE ON ORIBATID MITE COMMUNITIES

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Abstract

The effect of fire disturbance on the composition of oribatid mite communities was studied in riv. Vere canyon (Tbilisi, Georgia). Oribatid mites in a severely burnt shrub area and a less severely burnt artificial pine forest were investigated. Two unburnt sites were used as controls. 105 species were registered. *Graptoppia paraanalis* was new for Caucasian fauna. Analyses showed an increase of species dominance corresponding to an increase in mite density and the recovery of plant cover. The recovery of oribatid mite fauna was slower in the severely burnt shrub-strand than in the less severely burnt pine forest. Our research suggest that season, fire intensity, post- fire age and soil characteristics (pH, humidity, humus) all likely influence the composition of oribatid mite communities, supporting their use as indicators of environmental quality.

Key-words

Oribatid mites, fire, Simpson's index of diversity, Tbilisi.

Introduction

Fire has affected terrestrial ecosystems since ancient times. Thus, it is considered as a significant ecological factor and ecosystems have become adapted to frequent fires. Fire is considered as an important limiting factor as well, but unlike other limiting factors, humans can control its intensity. Two major types of fire disturbance are known: the first type of fire completely destroys the plant cover, whereas the second is more selective and supports the development of fire stable vegetation (Odum, 1971).

In natural ecosystems, fire influence may be considered as a positive factor and it is frequently followed by pyrogenic succession. In urban conditions, where the creation and maintenance of forest patches is associated with significant expenses, the destruction of these forests by accidentally or carelessly induced fires is great loss for society.

In the end of August 2006, in the riv. Vere canyon, an accidental, human-caused fire destroyed part of an artificial pine forest and a shrub- area. The fire resulted in the destruction of the soil litter and organic layer. According to the literature data, fire induces changes in the abundance and composition of soil microarthropod communities (Radea C., Arianoutsou M., 2000, Henig-Sever N. et al., 2001, Migliori M. et al., 2004, Dress W. J., Boerner R. E. J., 2004). The main goal of our investigation was to study the response of oribatid mites to fire disturbance at different time of burning and at different seasons.

Material and Methods

Research was carried out in the riv. Vere canyon (Tblisi, Georgia). Oribatid mites in a severely burnt shrub area and a less severely burnt artificial pine forest were investigated. Two unburnt sites were used as control. The first sampling was performed 10 days after the fire in 08.09.2006. Variations in oribatid mite communities were then monitored in soil samples collected from under the burnt vegetation every month during one year. At each site, three soil samples of 10 cm^3 were taken with a distance of 10-15m between samples. The mites were extracted by use of modified Berlese funnels and preserved in 70% ethanol for further studies. After clearing, the specimens were studied in lactic acid in an open hollow-ground microscope slide. For identification the keys of Weigmann (2006) and Ghilarov, Krivolutsky (1975) were primarily used, as well as typed specimens preserved in collections of Institute of Zoology. The densities of oribatid mites and the dominance index for each species were determined. Simpson's index of diversity (1-D) was calculated. The value of this index ranges between 0 and almost 1; the greater the value, the greater the sample diversity. The index represents the probability that two individuals randomly selected from a sample will belong to different species (Simpson, 1949). The average annual density, index of diversity and number of species were calculated for each sampling site.
In burnt and control sites, soil pH and humidity were measured and the percent of humus in soil was determined at the Laboratory of Analytical Chemistry of the A. Tvalchrelidze Institute of Mineral Raw Materials.

Ecological characteristics of the sites were as follows:

P1 – Control: unburnt pine forest. *Pinus eldarica*, understorey represented by *Cerasus incana, Paliurus spina-christi, Lonicera sp., Cotoneaster sp., Quercus iberica, Rhamnus pallasii, Prunus spinosa, Carpinus orientalis, Jasminum.* N 4174742; E 4468042; Elevation – 695m.s.l.

P2 – Burnt pine forest. Fire completely destroyed the understorey, but the pine trees remained in tact.. N 4171742; E 4468042; Elevation – 695m.s.l.

Sh1 – Control: unburnt shrub area. *Paliurus spina-christi, Cerasus incana, Cotoneaster sp., Festuca pratensis, Andropogon ischaemum, Asparagus, Papaver sp.* N 4171626; E 4468707; Elevation – 640m.s.l.

Sh2 – Burnt shrub area. Fire destroyed all vegetation. N 4171429; E 4468321; Elevation – 638m.s.l.

Results

During one year of study 105 species of oribatid mites were identified. Three species – *Licnodamaeus costula, Epimerella smirnovi* and *Simkinia tianschanica* were new to Georgian fauna and *Graptoppia paraanalis* was new to Caucasian fauna.

Widely distributed oribatid mites characterized all studied sites. Eurytopic species, such as *Oppiella fallax, Ramusella clavipectinata, Ceratoppia quadridentata, Tectocepheus sarekensis, T. velatus* and *Punctoribates punctum* predominated throughout the entire year. These species increased in dominance with the recovery of plant cover in burnt sites. *Spherochthonius splendidus* showed strict seasonal dependence and appeared only in fall and spring months (Sept-Nov; Mar-June), whereas in summer and winter it was totally absent (Jan-Mar; July) or presented in very low quantities (Dec, Aug) (tab. 1). Xerophilous species *Passalozetes africanus, Epilohmannia cylindrica, Thrypochthnius tectorum, Licnodamaeus costula and Scutovertex sculptus* were constantly found during the whole year, but at a lower abundance. The whole faunal composition seems very similar to Mediterranean maquis oribatid mite fauna, which is adapted to frequent fire disturbance (Migliori et al., 2004).

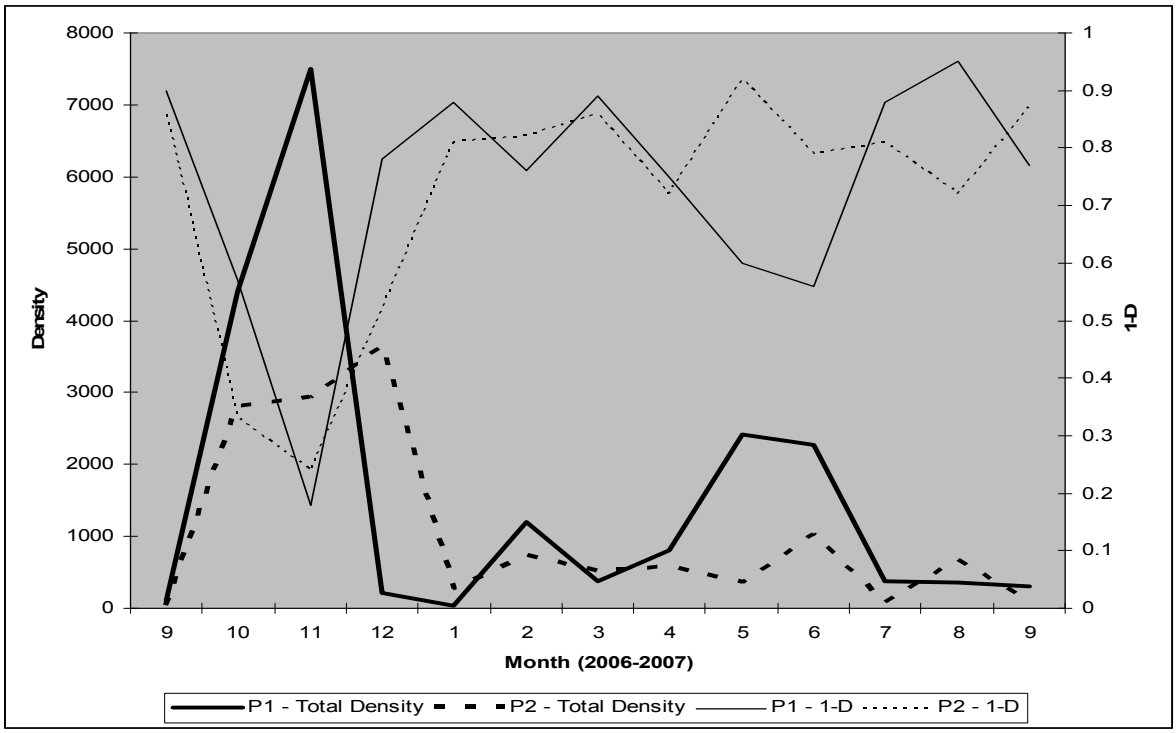
Changes in the densities of oribatid mite communities over the year showed a reduction in the number of mites immediately after a fire. Plant cover in the shrub-area was completely destroyed; the first sampling date showed only 12 species with a total density of 92n/m^2 (fig. 1a,b). In the pine forest (P2), where fire only destroyed the understorey, 7 species were registered with a density of 29n/m^2. Recovery of oribatid mite fauna in the pine forest was evident four months after fire. The number of species and densities increased and exceeded the same indices in the control site (P1). In the severely burnt shrub area, faunal recovery was seen five to six months after the fire (fig.1a, b).

Changes in the Simpson's index of diversity correlated negatively with changes of density. In almost all studied sites Simpson's index of diversity (1-D) was low, when total faunal density was high. In most cases, high density was caused by the increase of wide-spread, dominant species, whereas faunal diversity remained low. For example, during the second sampling date (10.06) in the control pine forest (P1), the density of oribatid mites equaled 4407 n/m^2, due to high density of *Oppiella fallax* – 2367n/m^2 and *Tectocepheus velatus* – 1633n/m^2. At the same

sampling time, the total density of oribatid mites in the burnt pine forest site (P2) equaled 2816n/m^2 and the density of *Oppiella fallax* – 2300 n/m^2 (Fig.1a, b).

Table 1. Changes in the percent dominance of common species in the sampled sites over 1 year. P1 = control pine forest; P2 = burnt pine forest; Sh1 = control shrub area; Sh2 = burnt shrub area.

species	site	09.06	10.06	11.06	12.06	01.07	02.07	03.07	04.07	05.07	06.07	07.07	08.07	09.07
Oppiella fallax	P1		54	91	43		11	97	17	62	65	14	20	1
	P2	10	82	2	59	32	35	32	51	20	41	3	10	3
	Sh1	9	12	17	23	93	39	34	45	21	48	15	8	10
	Sh2		5	13		19	25	55	74	40		7	5	
Ramusella clavipectinata	P1		2	2	9		38	14		4	6	9		
	P2		1	<1	36	26	4	6	2	6	11	3	3	
	Sh1	1	1	5			25	5	4	5	<1	2	1	
	Sh2	28	3	6	5	5			<1	4	8		2	
Tectocepheus sarekensis	P1	25	1	<1	15			2	12	7	<1	17	28	
	P2		1	87	1	10	2	1	8	3		29	5	
	Sh2	29	35			1	6		6	5	5	7	14	
	Sh2	11	1	3			2		6	5			8	20
T. velatus	P1		37	1			4	2			9	5	5	10
	P2		6	1			16	2		5	10		50	7
	Sh1		1	52	33	1		2			27		8	26
	Sh2	7	32	9	17	23	21	2		1	22			7
Ceratoppia quadricarinata	P1	9	1	<1	5	17	<1	2	2	2	4	3	3	43
	P2			<1	<1		14	5	8	6	6		1	3
	Sh1	1	<1	<1		<1	3	4	5	<1	1	5	1	14
	Sh2			2		4	4	3	<1	5	1	16		7
Sphaerochthonius splendidus	P1	9	<1						2					
	P2		<1	<1	<1				1				1	
	Sh1	5	32	<1						<1	<1		2	
	Sh2	11	8	9						2	1			
Punctoribates punctum	P1		<1	<1	3				1	<1	<1		4	
	P2		<1	2		1		1	5	1	1			
	Sh1			3		<1			<1	1	1		31	1
	Sh2					2	<1	2	<1	3	7		3	20



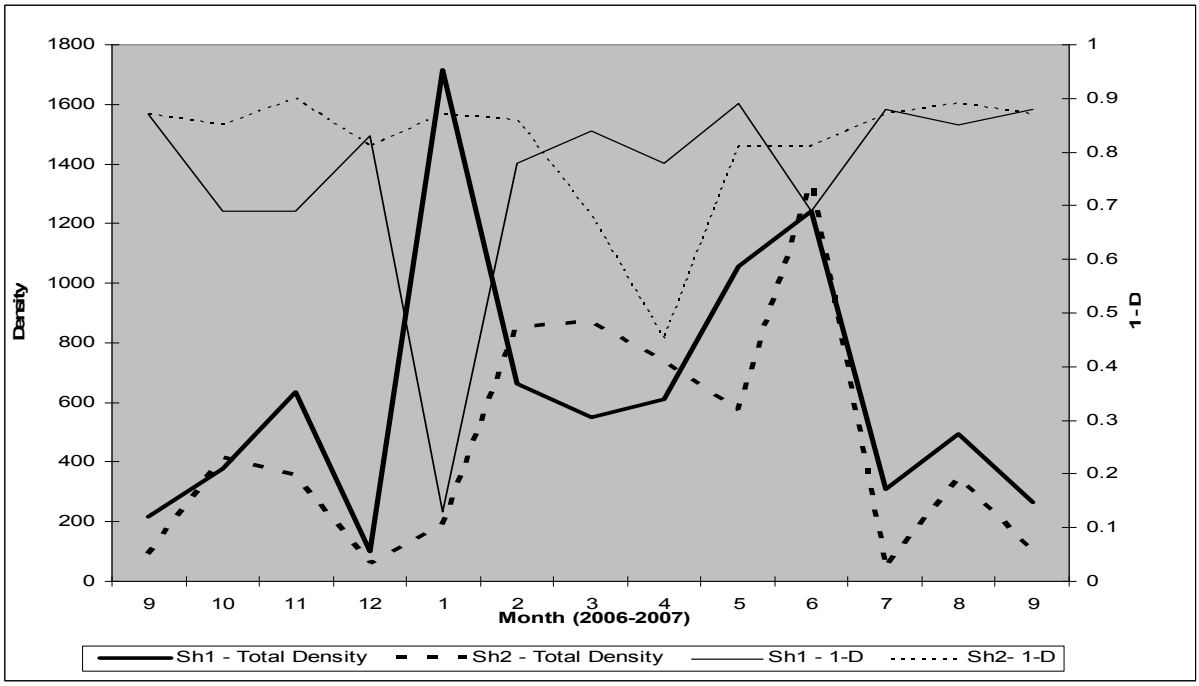


Figure 1. Changes in the densities of oribatid fauna and Simpson's index of diversity (1-D) in a)-top- control (P1) and burnt (P2) pine forest sites and b)-bottom- control (Sh1) and burnt (Sh2) shrub areas.

The number of species collected per site per month ranged from 7 species (P2 at 09. 06) to 33 (P1, P2 at 11. 06). The high number of species corresponded to high density during spring and fall; this pattern is likely determined by the two periods of vegetation growth that characterize ecosystems around the Tbilisi region and are associated with increased humidity (Darejanashvili Sh. D., Gomelauri L. A., 1975; Murvanidze M., 1999).

The average annual density calculated for each site, showed that the annual densities of control sites (P1, Sh1) were higher than the burnt sites (P2, Sh2). Average annual Simpson's index of diversity (1-D) and number of species per site showed no such difference (tab. 2).

Table 2. Average annual densities, number of species and Simpson's index of diversity (1-D) for the studied sites (P1 = control pine forest; P2 = burnt pine forest; Sh1 = control shrub area; Sh2 = burnt shrub area).

sampling sites	density n/m^2	number of species	1-D
P1	1569	21	0,73
P2	1061	22	0,71
Sh1	633	23	0,75
Sh2	459	19	0,81

Chemical analyses performed on soil samples showed that the pH was close to neutral in pine forest, whereas in shrub area it was slightly alkaline. Humus percentage in soil was higher in burned sites than in control sites due to an increasing amount of humus due to the addition of organic matter after the fire (tab. 3).

Table 3. Measurements of soil pH and humus percentage in burnt (P2, Sh2) and control (P1, Sh1) sites.

sites	soil humidity (%)	pH	humus (%)	humus per dry mass (%)
P1	3,49	7,46	6,73	6,97
P2	3.95	7,40	10,62	11,05
Sh1	4,77	8,20	6,5	6,8
Sh2	4,48	7,80	8,9	9,3

Discussion

The study of fire influence on the oribatid mite communities of the semi-arid ecosystems of Tbilisi showed that the fire resulted in an immediate drop in the population density and in moderate changes in the mite species composition. The effect of fire reflected changes in species inhabiting litter and soil surface, while animals living in deeper layers survived. Community recovery begun by increasing the number of ubiquitous and wide spread species that can tolerate extreme conditions: members of family *Oppiidae, Tectocepheus sarekensis, T. velatus, Punctoribates punctum* and *Fosseremus laciniatus.* Indeed, recent studies have shown that *T. velatus* is the most heat-tolerant species within Oribatida. It can stand temperatures of 40^{0}C during 4 hours (Malmström, 2008).

Litter dwellers such as *Nothrus biciliatus, Liacarus brevilamellatus, Eupelops torulosus, Peloptulus phaenotus* and *Pilogalumna crassiclava* gradually increased their numbers, most likely due to hidden microhabitats where they survived the fire (under stones, deep in moist moss, on the tree bark etc.). This also occurred for typical pine species – *Eniochthonius minutissimus, Jacotella ornata* and *Oribatula tibialis* that composed an important part of the pine forest fauna.

The recovery of plant cover begun after the rains in October- November 2006 and resulted in new grass cover that was gradually followed by new shrub vegetation; increasing mite densities coincided with this period. Recovery of oribatid mite fauna was slower in the severely burnt shrub-strand than in the less severely burnt pine forest (four months after the fire in the pine forest and six months after the fire in the shrub-strand). Soil analyses showed an increase in humus mass in burnt plots due to the addition of organic matter after burning. This resulted in an increase in faunal composition and density after the fire.

Our research showed that season, fire intensity, post- fire age and soil characteristics (pH, humidity, humus) all influenced the composition of oribatid mite communities, supporting the use of these species as indicators of environmental quality.

Acknowledgements

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DISTRIBUTION OF PTYCTIMOUS MITES (ACARI, ORIBATIDA) IN THE MOUNTAIN RAIN FOREST LA SELVA, COSTA RICA

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Abstract

The analysis of the distribution of ptyctimous mites in the mountain rain forest La Selva (Costa Rica) was based on 5045 individuals belonging to 47 species. Several methods were applied: Chao estimators, accumulation curves, one-way analysis of variance and correspondence analysis. Thirty-five species have been found in qualitative samples, whereas twenty-five species in quantitative samples. No species has been found exclusively in quantitative samples. The hypothesis tested has been that ptyctimous mites are microhabitat specific (leaf litter, dead wood, epiphytes, mushroom spawn) in the mountain rain forest. Only nine species have been described as stenotopic (occurring in one microhabitat) and three species as eurytopic (occurring in all studied microhabitats). The abundance and species richness of ptyctimous mites did not differ significantly between microhabitats. Analysis of the vertical distribution over the altitude range from 50 to 2160 m a.s.l. has permitted classification of ten species as low-altitude ones, thirteen species as high-altitude ones and the identification of three species whose occurrence was not related to altitude. A significant fluctuation of the number of species and number of individuals, independent of the altitude, has been observed.

Key-words

Acari, Ptyctima, rain forest, microhabitat, altitude

Introduction

Costa Rica is a small country in the south of Central America. According to the zoogeographic division, it belongs to the Neotropical Region. The study reported was conducted at the biological station La Selva (10˚26”N and 84˚01”W) in the Heredia Province, at altitudes ranging from 50 to 150 m. The study was performed within the international project ALAS (Arthropods of La Selva) (started in 1991) under the auspices of the National Institute of Biodiversity. The studied area is one of the two fragments of the primeval rain forest preserved in the country of Costa Rica and one of the four regions in which the rain forest fauna is studied. Topographically, the area of La Selva covers low and steep part of the foot of the Central Cordilleras stretching to the vast coastal plainland from Rio San Juan to the north and Tortuguero.

Ptyctimous mites have specific, ptychoidal construction of body. They are able to fold the aspidosoma against the opisthosoma to protect their appendages and in this way they avoid predation. Ptyctimous mites occur wherever there is decaying organic matter. They are secondary decomposers and as macrophytophagous they play an important role in the mechanical fragmentation of organic matter. In the Neotropical Region this group is characterised by relatively high abundance and species diversity. The number of Ptyctimous mite species described from this region is

estimated as 305 (Niedbała 2004). In Costa Rica the number of Ptyctimous mite species found is 76 (Niedbała 2003). The analysis presented in this paper is based on the results reported in the papers (Niedbała 2003, 2004) supplemented with additional data from additional samples.

Materials and methods

The material analysed included 5,045 individuals representing 47 species, collected between 1991 and 2005. The material was collected at selected sites along the carefully selected tracks of 250 m each during subsequent research expeditions. The sites of sample collection have been selected to represent a possibly greatest number of microhabitats at every 5 m along the track. In subsequent years (1991-2005) two-week expeditions were organised in February, March and April, and each year one trip was carried out to the constant monitoring site at each track. The qualitative samples of 1.5 l in capacity each (organic matter) were collected by hand from the unmeasured area and unmeasured depth in a given microhabitat, whereas the quantitative samples were collected with the use of a metal cylinder of 14.5 cm in diameter, submerged to the depth of 10cm from the squares of the area from 60x60cm to 100x100cm. The soil fauna was extracted with Tullgren apparatuses, the procedure lasting 7 days, and the animals collected were conserved in 75% ethanol. The types of microhabitats represented in the samples included: leaf litter, dead wood, epiphytes, mushroom spawn and mixed samples (leaf litter and upper layer of soil).

The analyses of the qualitative and quantitative samples and the occurrence of mites in the microhabitats were performed on 148 samples. The analyses concerning the effect of altitude were performed on 318 samples. Besides 148 above mentioned Berlese samples, 170 so called „Winkler samples" were included into the analyses. Winkler samples are an efficient method of sampling leaf litter ants. The method involves sifting bulk samples of leaf litter and rotten wood by agitating them vigorously in a bag above a coarse mesh screen. Litter arthropods are concentrated in the finer "siftate" that passes through the screen. Arthropods are then extracted from the siftate by a passive extraction method, in which the siftate is placed in thin mesh sacks and then suspended and enclosed within an outer cloth "Winkler bag." The samples were collected at the following altitudes a.s.l.: 50–150 m, 250–350 m, 450–550 m, 1050–1150 m, 1450–1550 m, 1760–2160 m.

Four univariate measures were used to assess the fauna characteristics of ptyctimous mites: abundance (number of specimens per sample), species richness per sample, total number of species, Shannon index of diversity (H') and equitability (J). Theoretical total species richness for quantitative and qualitative samples was calculated using first- and second-order Jackknife and Chao estimators (Chao quantitative data richness, Chao presence/absence richness). Furthermore, the theoretical and observed species accumulation curves were computed for both quantitative and qualitative samples. One-way ANOVA was performed to identify statistically significant differences in abundance and species richness, Shannon index of diversity (H') and equitability (J) between studied sites. Data were transformed to log (x+1) to minimize violations of parametric statistics. When a statistically significant difference ($p < 0.05$) was noted differing pairs were identified with the Tukey post- hoc test. Levene's test was used to verify homogeneity of variance. The level of significance for all statistical tests was accepted at $\alpha=0.05$. Differences between mean species richness of Ptyctima in quantitative and qualitative samples were tested for significance using the "t" test.

Multivariate analysis was used to determine relationships between species occurrence and sites. Correspondence analysis (CA) was chosen as the ordination method to explore the compositional variation between microhabitats and the altitude. We restricted the interpretation to the ordination space determined by the first two axes. Rare species (less than 2 individuals) were excluded from the analysis because they do not improve the CA analysis and this was confirmed in an initial analysis with all species. The numbers of individuals were log (x+1) transformed and equal weight was applied to all the species. All statistical calculations for this research were done in STATISTICA 7.1 and Species Diversity and Richness IV software.

Results and Discussion

Ninety-one qualitative and fifty-seven quantitative samples have been collected in the mountain rain forest La Selva. The numbers of individuals collected from qualitative and quantitative samples were 1,798 and 443, respectively. Representatives of the following 10 species were found only in the qualitative samples: *Acrotritia brasiliana*., *Arphthicarus pararidiculus*, *Arphthicarus saucius*, *Austrophthiracarus admirabilis*, *Euphthiracarus comteae*, *Euphthiracarus dlouhyorum*,

Euphthiracarus serengos, *Mesoplophora (Mesplophora) hauseri*, *Microtritia simplex* and *Protophthiracarus varius*. No species was found only in the quantitative samples. The number of qualitative samples collected was almost twice as high as that of quantitative samples. Nevertheless, the fact of finding 10 species only in the qualitative samples suggests that the ecological studies based on the quantitative samples may not ensure representation of all species occurring in a given area.

Estimates of species richness in qualitative samples (mean species number per sample – 2.2) exceeded those of quantitative samples (1.3) (t=4.427, p=0.0000). The observed total numbers of species in qualitative and quantitative samples were 35 and 25, respectively. Also, theoretical species richness using first- and second-order Jackknife and Chao estimates were markedly higher in qualitative samples compared with quantitative samples (Table 1). Species accumulation curves for qualitative and quantitative samples also demonstrated that mite species richness was greater for qualitative samples than quantitative samples (Fig. 1). Both analyses proved that the unrevealed number of species in qualitative samples was only slightly higher in comparison with number of species in quantitative samples.

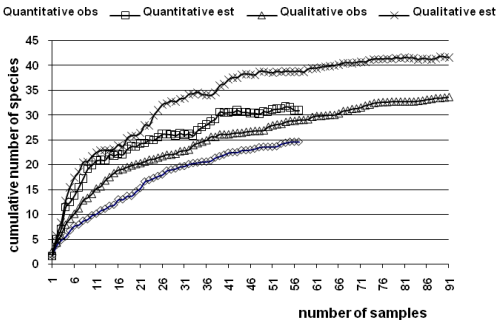

Figure 1. Observed and estimated ptyctimous mite species richness on species accumulation curves for qualitative samples and quantitative samples in the mountain rain forest La Selva, Costa Rica.

The observed and estimated species accumulation curves for both qualitative and quantitative samples approached an asymptote. Buddle *et al.* (2005) warn that accumulation curves can give biased results in sample-based data, if there is inequality in sampling effort (i.e. number of samples collected or individuals caught). In the present study, the number of qualitative samples (91) and individuals collected from these samples (1,798) is higher than from quantitative samples (57, 443). However, both curves reached an asymptote and theoretical species richness in both types of samples was only slightly higher than the observed ones. So it seems that the biodiveristy was well assessed with the samples carried out.

In total 148 samples were collected in five microhabitats (leaf litter, dead wood, epiphytes, mushroom spawn and mixed samples) in the mountain rain forest La Selva. 2,240 individuals belonging to 35 species were collected from this material. Total abundance of ptyctimous mites recorded in studied microhabitats varied from 7.4 individuals per sample (epiphytes) to 22.4 indiv./sample (leaf litter). However, the one-way ANOVA did not reveal any significant differences in oribatid abundance among the microhabitats (Table 2). The mean species numbers and the values of indices of diversity (H' and J) were similar and no statistically significant differences were noted. It is noteworthy that, mushroom spawn displayed the lowest general number of species (9) and the highest species richness was observed in mixed samples (30) (Table 2).

A correspondence analysis (CA) was performed in order to evaluate relationships between species abundances and microhabitats. The scores for the first 2 axes are shown in Fig. 2. The eigenvalues (the dispersion of the sites/species distribution along the ordination axis) of the ordination axes 1 and 2 were $\lambda_1 = 0.191$ and $\lambda_2 = 0.168$, respectively. Over 72% of the variance was explained by the first two axes. Overall, sites are well separated on the ordination plot.

Axis 2 appears to divide the fauna in mushroom spawn from the fauna found in other microhabitats. Species associated with mushroom spawn, e.g. *Protophthiracarus varius*, *Mesoplophora (M.) gaveae* are situated close to the negative part of the axes. Axis 1 differentiates species between four other microhabitats. Three clusters of species can be distinguished in the diagram. The first cluster of species are those associated with dead wood and epiphytes, e.g. *Atropacarus (Hoplophorella) hamatus, Austrophthiracarus admirabilis, Arphthicarus pararidiculus, Arphthicarus saucius, Austrophthiracarus retrorsus, Austrophthiracarus ridiculus, Phthiracarus bryobius*, *Phthiracarus pygmaeus*. Several species assiociated with leaf litter, e.g. *Arphthicarus paraallocotos, Atropacarus (Atropacarus) antrosus, Acrotritia clavata,*

Table 1. Observed and theoretical total ptyctimous mite species richness for qualitative samples and quantitative samples in the mountain rain forest La Selva, Costa Rica.

	Qualitative samples	Quantitative samples
Observed total number of species	35	25
Observed mean species number	2.2 ± 1.9	1.3 ± 1.5
Jackknife 1st order	41.1 ± 2.6	31.6 ± 3.2
Jackknife 2nd order	46.7	29.4
Chao quantitative data richness estimator	45.9 ± 11.2	25.7 ± 1.1
Chao presence/absence richness estimator	44.4 ± 4.9	28.1 ± 1.2

Values are mean estimates (± S.D.)

Table 2. General characteristics of Ptyctima oribatid mites in microhabitats. Mean abundance of oribatids (indiv./sample ± S.E.) is tested by one-way analysis of variance. The results are compared by the Tukey *post-hoc* test for differences between microsites.

	Microhabitats					ANOVA	
	Leaf litter	Dead wood	Epiphytes	Spawn	Mixed samples	F	p
Abundance	24.2 ± 9.2	11.7 ± 3.4	7.4 ± 224	12.5 ± 9.0	13.7 ± 3.7	0.912	0.4587
Mean species numbers	2.3 ± 0.3	2.4 ± 0.4	2.1 ± 0.3	3.0 ± 1.2	2.1 ± 0.2	0.800	0.6263
Shannon index (H')	0.520 ± 0.092	0.464 ± 0.099	0.447 ± 0.120	0.701 ± 0.409	0.405 ± 0.062	0.509	0.7290
Evenness index (J)	0.448 ± 0.073	0.416 ± 0.082	0.428 ± 0.106	0.442 ± 0.255	0.379 ± 0.053	0.166	0.9550
Total species richness	22	24	16	9	30		

Table 3. General characteristics of Ptyctima oribatid mites with regard to the vertical distribution over the altitude range from 250 to 2160 m a.s.l. Mean abundance of oribatids (indiv./sample ± S.E.) is tested by one-way analysis of variance. The results are compared by the Tukey *post-hoc* test for differences between altitudes.

	Altitude						ANOVA	
	50-150	250-350	450-550	1050-1150	1450-1550	1750-2160	F	p
Abundance	43.5 ± 22.3 [b]	62.3 ± 25.5 [b]	38.1 ± 11.7 [b]	3.2 ± 0.3 [a]	51.8 ± 16.4 [b]	10.3 ± 1.8 [a]	**9.488**	**0.0000**
Mean species numbers	1.8 ± 0.2 [a]	5.9 ± 0.6 [c]	3.7 ± 0.5 [b]	1.4 ± 0.1 [a]	3.5 ± 0.6 [b]	2.0 ± 0.1 [a]	**46.724**	**0.0000**
Shannon index (H')	0.238 ± 0.070 [ab]	1.269 ± 0.101 [d]	1.001 ± 0.147 [d]	0.190 ± 0.026 [a]	0.666 ± 0.139 [c]	0.422 ± 0.052 [b]	**27.633**	**0.0000**
Evenness index (J)	0.207 ± 0.057[a]	0.763 ± 0.050[c]	0.711 ± 0.089[c]	0.241 ± 0.032[a]	0.425 ± 0.086[b]	0.419 ± 0.047[b]	**9.306**	**0.0000**
Total species richness	10	12	12	14	18	15		

Bold typed values denote significant differences between abundances at the 0.05 and lower probability level. The results of the Tukey test are given by letters. Means sharing a common letter (a, b, c or d) do not differ significantly from other means at the 5% level.

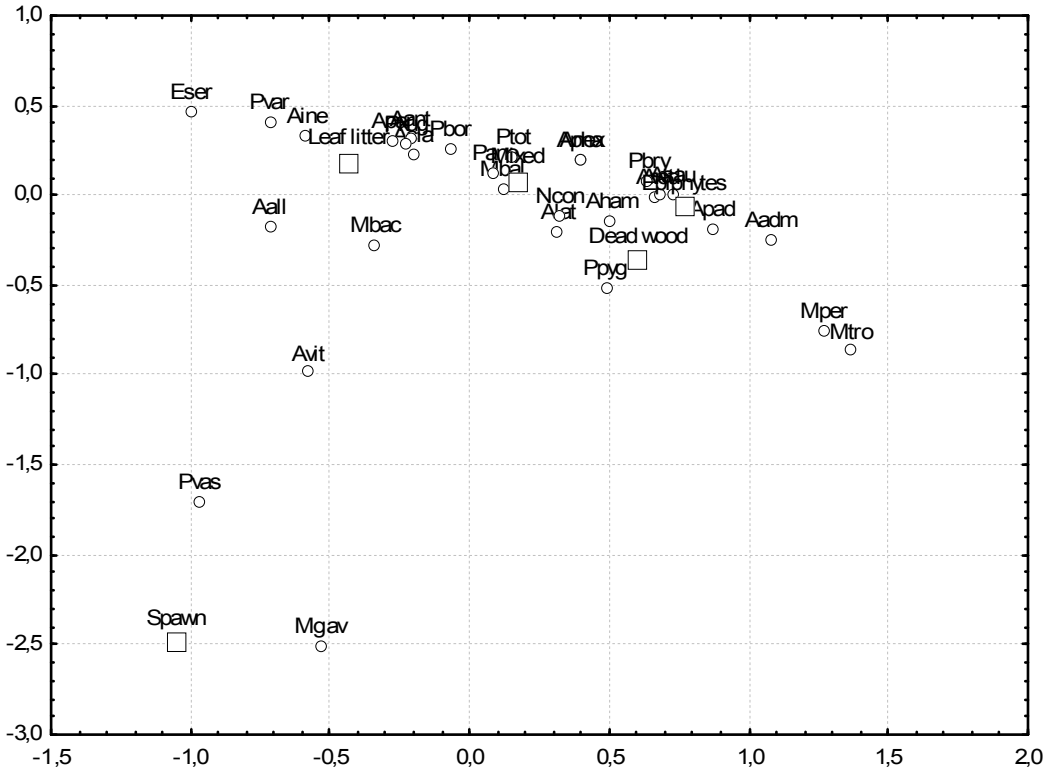

Figure 2. Plot of the first two axes of correspondence analysis (CA) of the of 30 ptyctimous species and 5 sampling sites in the mountain rain forest La Selva, Costa Rica**.** Codes: see Appendix I

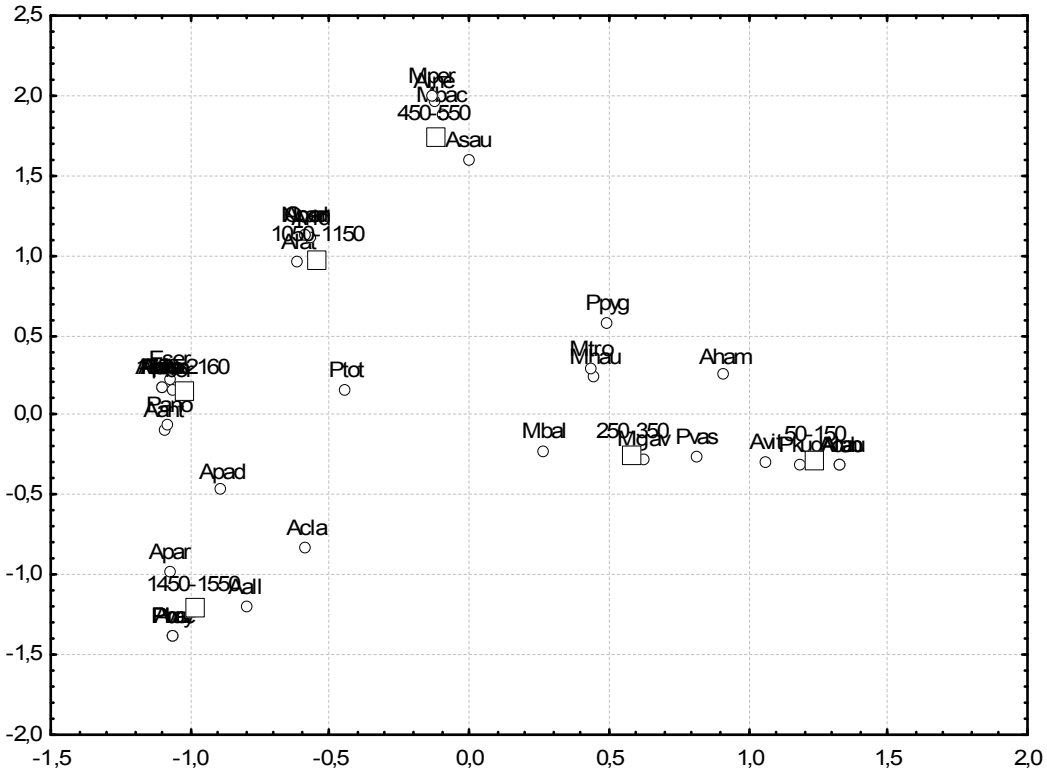

Figure 3. Plot of the first two axes of correspondence analysis (CA) of the of 39 ptyctimous species and 6 sampling sites at the different altitude in the mountain rain forest La Selva, Costa Rica**.** Codes: see Appendix I

Plonaphacarus kugohi. The last cluster of species, which are crowded around the microhabitat – mixed samples, is ordinated between two previous groups of ptyctimous mites. *Austrophthiracarus phaleratus, Austrophthiracarus nexilis, Mesoplophora (Parplophora) bacula, Phthiracarus anonymus* were among the most numerous species in this microhabitat (Fig. 2).

We had expected that significantly different microhabitats in the mountain rain forest La Selva would be reflected in different population densities of ptyctimous mites, species richness and diversity, however, this was not the case for the five microhabitats studied. The species composition of ptyctimous fauna collected in different microhabitats differs markedly between microhabitats (indicated by the correspondence analysis). Only few ptyctimous oribatids were found not strictly confined to some microhabitats in the study.

Samples were collected from six different altitudes from 50 to 2160 m a.s.l. In total 318 samples were collected and 5,045 individuals belonging to 47 species of Ptyctima were found. The lowest abundances were found in the samples collected at the altitudes 1050-1150 and 1750-2160 m a.s.l., respectively 3.2 and 10.3 individuals per sample. The abundance of oribatids at the other altitudes was significantly higher (one-way ANOVA, Tukey HSD test) (Table 3). With regard to other characteristics of Ptyctima fauna, the one-way ANOVA revealed significant differences in the mean species number at different altitudes. The lowest mean species number per sample was found at the altitude of 1050-1150 m (1.4 species/sample) and the highest at the altitude of 250-350 m (5.9). The value of diversity indices varied strongly between sites and the highest value of both indices was noted at the altitude of 250-350 m (Table 3). In total 47 ptyctimous species were recorded at all altitudes and the total number of species per site varied only slightly between sites; from 10 (50-150 m) to 18 (1450-1550) species.

Ordination by Correspondence Analysis (CA) was used to assess community similarities and relations between species and communities at different altitudes. The scores for the first 2 axes are shown in Fig. 3. The eigenvalues of the ordination axes 1 and 2 were λ_1 = 0.866 and λ_2 = 0.762 (both significant), respectively. Over 54% of the variance was explained by the first two axes.

Overall, axis 1 appears to reflect a gradient from low altitude (right) to high altitude (left). The species and sites could be grouped into 6 clusters, as the number of studied sites. Species associated with lower altitude are ordinated along the positive part of axis 1. They were *Atropacarus (Hoplophorella) vitrinus*, *Atropacarus (Hoplophorella) lanceosetus* and *Plonaphacarus kugohi* (50-150 m) and *Mesoplophora (Mesoplophora) bacilla*, *Mesoplophora (Mesoplophora) gaveae* and *Protophthiracarus varius* (250-350 m). Species associated with higher altitudes are located along the negative part of axis 1 and 2. Following species were most numerous observed at the highest altitude (1750-2160): *Atropacarus (Atropacarus) antrosus*, *Austrophthiracarus admirabilis*, *Austrophthiracarus nexilis*, *Euphthiracarus serengos*, *Phthiracarus anonymus* and *Phthiracarus boresetosus*. Four ptyctimous species appeared to be largely restricted to the altitude from 1450-1550: *Arphthicarus allocotos*, *Arphthicarus paraallocotos*, *Austrophthiracarus retrorsus* and *Protophthiracarus varablancus*. Species more abundantly observed at the altitude of 450-550 and 1050-1150 m a.s.l. were located along axis 1 between two above mentioned groups of species. *Arphthicarus saucius*, *Arphthicarus inelegans*, *Mesoplophora (Mesoplophora) bacilla* and *Mesoplophora (Mesoplophora) permodica* may be included to low-altitude species, characteristics at the altitude of 450-550 m. *Arphthicarus latebrosus*, *Austrophthiracarus ridiculus* and *Notophthiracarus conspersus* were recorded with highest abundance at the altitude of 1050-1150 m. Species which do not show any significant preference for any of the altitudes were few, e.g. *Acrotritia clavata*, *Phthiracarus totus* or *Phthiracarus pygmaeus* (Fig. 3).

The fauna of ptyctimous mites, e.g. population densities, mean species numbers and diversity indices, differs significantly with regard to the altitude. The highest values of these parameters were recorded for the fauna at the altitude of 250-350 m a.s.l. However, a significant fluctuation of population densities and species richness of Ptyctima, independent of the altitude, has been observed. As regards the species composition of ptyctimous fauna collected at different altitudes, correspondence analysis revealed strong preferences for most species. Only few ptyctimous oribatids were found not strictly confined to some altitudes in the study. It is noteworthy that the species composition changes smoothly from the lowest to the highest altitudes (see Fig. 3). The height above sea level appeared to be the most important factor for the distribution of Tardigrada

during the studies in Costa Rica (Kaczmarek 2008). Microhabitat type had significantly feeble influence on the Tardigrada fauna. Taking into consideration the results of studies on Tardigrada and ptyctimous mites in Costa Rica, it may be concluded that climatic conditions, characteristic of different altitudes, have the direct influence on the invertebrate fauna. And their influence is much stronger than conditions caused by a microhabitat type. Ecological studies on the mountain distribution of ptyctimous mites at different altitudes and microhabitats have not been conducted so far. Above results may be a good example for future comparisons.

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Appendix I.

Check-list of the ptyctimous species in the mountain rain forest La Selva, Costa Rica

Species	Codes	Species	Codes
Arphthicarus iubatus Niedbała	Aiub	*Euphthiracarus dlouhyorum* Mahunka	Edlo
Acrotritia brasiliana Mahunka	Abra	*Euphthiracarus serengos* Niedbała	Eser
Acrotritia clavata Markel	Acla	*Euphthiracarus tumidus* Niedbała	Etum
Acrotritia monodactyla Niedbała	Amon	*Mesoplophora (Mesoplophora) bacilla* Niedbała	Mbac
Arphthicarus allocotos Niedbała	Aall	*Mesoplophora (Mesoplophora) gaveae* Schuster	Mgav
Arphthicarus inelegans (Niedbała)	Aine	*Mesoplophora (Mesoplophora) hauseri* Mahunka	Mhau
Arphthicarus latebrosus (Niedbała)	Alat	*Mesoplophora (Mesoplophora) permodica* Niedbała	Mper
Arphthicarus paraallocotos Niedbała	Apar	*Mesoplophora (Parplophora) bacula* Niedbała	Mbal
Arphthicarus pararidiculus Niedbała	Apad	*Microtritia simplex* (Jacot)	Msim
Arphthicarus pervalidus Niedbała	Aper	*Microtritia tropica* Markel	Mtro
Arphthicarus saucius (Niedbała)	Asau	*Notophthiracarus conspersus* Niedbała	Ncon
Atropacarus (Atropacarus) antrosus Niedbała	Aant	*Oribotritia alajuela* Niedbała	Oala
Atropacarus (Hoplophorella) hamatus (Ewing)	Aham	*Oribotritia attenuata* Niedbała et Schatz	Oatt
Atropacarus (Hoplophorella) lanceosetus (Balogh et Mahunka)	Alan	*Oribotritia partita* Niedbała	Opar
Atropacarus (Hoplophorella) vitrinus (Berlese)	Avit	*Phthiracarus anonymus* Grandjean	Pano
Austrophthiracarus admirabilis (Niedbała)	Aadm	*Phthiracarus boresetosus* Jacot	Pbor
Austrophthiracarus caudatus (Balogh et Mahunka)	Acau	*Phthiracarus brevisetae* Jacot	Pbre
Austrophthiracarus diazae Ojeda	Adia	*Protophthiracarus clandestinus* Niedbała	Pcla
Austrophthiracarus nexilis Niedbała	Anex	*Phthiracarus pygmaeus* Balogh	Ppyg
Austrophthiracarus phaleratus (Niedbała)	Apha	*Phthiracarus totus* Niedbała	Ptot
Austrophthiracarus radiatus *(Balogh et Mahunka)*	Arad	*Plonaphacarus kugohi* (Aoki)	Pkug
Austrophthiracarus retrorsus Niedbała	Aret	*Protophthiracarus varablancus* Niedbała	Pvar
Austrophthiracarus ridiculus (Mahunka)	Arid	*Protophthiracarus varius* Niedbała et Schatz	Pvas
Euphthiracarus comteae Mahunka	Ecom		

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PTERYGOSOMATID MITES (ACARI: PROSTIGMATA) OF MEXICO

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Abstract

Family Pterygosomatidae is worldwide in distribution, ectoparasite with high host specificity. The family is divided in 11 genera: nine associated to lizards (*Cyclurobia*, *Geckobia*, *Geckobiella*, *Hirstiella*, *Ixodiderma*, *Pterygosoma*, *Scaphothrix*, *Tequisistlana*, and *Zonurobia*), one collected on arthropods (*Pimeliaphilus*) and one was found on a bird (*Bharatoliaphilus*). Mexico is considered a megadiverse country but until now, only 14 species, and five genera of pterygosomatid mites have been recorded. In recent studies, some undescribed species pertaining to the genera *Geckobia*, *Geckobiella* and *Hirstiella*,. have been recognized. These species are associated to native lizards of the genera *Aristelliger*, *Coleonyx*, *Phyllodactylus* and *Thecadactylus* (Gekkonidae), and *Sceloporus* (Phrynosomatidae)

Key-words

Mexico, Megadiverse Country, Pterygosomatidae, Diversity, Ectoparasitic, Scale Mite.

Introduction

Pterygosomatidae Oudemans 1910 includes nine genera of scale mites parasitic on lizards (*Cyclurobia* Cruz 1984, *Geckobia* Mégnin 1878, *Geckobiella* Hirst 1917, *Hirstiella* Berlese 1920, *Ixodiderma* Lawrence 1935, *Pterygosoma* Peters 1849, *Scaphothrix* Lawrence 1935, *Tequisistlana* Hoffmann & Sánchez 1980 and *Zonurobia* Lawrence 1935), one genus parasitizing arthropods (*Pimeliaphilus* Tragardh 1905), and one monotypic genus found on a bird (*Bharatoliaphilus* Prasad 1975) (Jack 1964; Prasad 1975; Hoffmann & Sánchez 1980; Cruz 1984). This family is distributed worldwide and 150 species are described, most of them in the genera *Geckobia* and *Pterygosoma* (Table 1).

According to Hoffmann & López-Campos (2000) in Mexico 2,343 species of Acari have been recorded, 14 of them correspond to five genera of Pterygosomatidae; however, this number does not reflect the expected biodiversity, bearing in mind that Mexico is considered a megadiverse country.

In this review, we show the historical progress and update the knowledge about the diversity of pterygosomatid mites in Mexico.

The pterygosomatid mite fauna of Mexico

The study of Pterygosomatidae in Mexico began in the early XXth century when Berlese (1920) described the genus *Hirstiella*, with *H. trombidiformis* as type species, based on Mexican specimens of an unknown host (probably a lizard) from the state of Guanajuato (Hirst 1926; Cunliffe 1952). Posteriorly, Cunliffe (1949; 1952) described two new species of *Hirstiella* (*H. pelaezi* and *H. bakeri*). Jack (1959) recorded to *Geckobiella texana*

from some Mexican states, representing all of them new records for the country. Beer (1960) described a new species of *Pimeliaphilus* (*P. rapax*) parasitic on scorpions. Newell & Ryckman described one species of *Hirstiella* (*H. pyriformis*) and three species of *Pimeliaphilus* (*P. gloriosus*, *P. peninsularis* and *P. plumifer*) (Newell & Ryckman 1964; 1966). Hunter & Loomis (1966) described another species of *Hirstiella* (*H. otophila*) from geckonid lizards.

Table 1. Genera included in Pterygosomatidae, number of species, distribution and host groups..

Genus	Number of species	Distribution	Hosts group
Bharatoliaphilus	1	India	Aves
Cyclurobia	1	Cuba	Iguanidae *s.str.*
Geckobia	70	Africa, America, Asia, Australia and Europe	Gekkonidae *s.l.* Testudines
Geckobiella	2	America	Iguanidae *s.l.*
Hirstiella	11	Africa, America, Asia, Australia and Europe	Gekkonidae *s.l.* Iguanidae *s.l.* Teiidae
Ixodiderma	4	South Africa	Cordylidae Lacertidae
Pimeliaphilus	13	Africa, Asia, North America (Mexico and USA) and South America (Colombia)	Arthropoda
Pterygosoma	37	Africa, Asia and South America (Argentina)	Agamidae Gerrhosauridae Iguanidae *s.l.*
Scaphothrix	1	South Africa	Cordylidae
Tequisistlana	1	Mexico	Xantussidae
Zonurobia	9	South Africa	Cordylidae
	150		

The first Mexican researcher that made contributions to this group of mites was A. Hoffmann, whom in 1969 added some new locality records for *G. texana*. Later, Hoffmann and collaborators described a new genus and species *Tequisistlana oaxacensis*, and a new species of *Geckobia* (*G. leonilae*) (Hoffmann & Sánchez 1980; Hoffmann & Morales-Malacara 1986). After, new records of two species of *Pimeliaphilus* (*P. podapolipophagus* and *P. triatomae*) were added by Hoffmann & López-Campos (2000). Finally, the most recent contribution includes the works of parasitic mites of geckonid lizards from the Neotropical Region of Mexico that includes six species of *Geckobia* (*G. bataviensis*, *G. keegani* and four undescribed species) and four of *Hirstiella* (undescribed) (Paredes-León 2006; Paredes-León & Morales-Malacara 2007).

Results

The knowledge of the diversity of Pterygosomatidae in Mexico has had a slow progress in the last 88 years (from 1920 to 2008), since only 25 species has been reported. The first great increase occurred in 1966 when four species were described (Hunter & Loomis 1966; Newell & Ryckman 1966), and the second great contribution was 30 years later when ten species were added (Paredes-León 2006) (Figure 1).

Up to date, 25 species of Pterygosomatidae occur in Mexico, nine of them undescribed; this represents around 17% of the described species of the family. The knowledge of the pterygosomatid mite fauna from Mexico is very heterogenous for the different states of Mexico. Although the family is represented in 29 of the 32 mexican states, most of the studies include southern states (Neotropical). The state of Guerrero present the highest number of species (15); on the other hand, in the States of Chihuahua, Coahuila, Durango, Nuevo León, Querétaro, Tamaulipas and Tlaxcala only one species has been recorded (Table 2).

Pterygosomatid mites occur is high, considering the wide distribution of some lizards that have

been reported as hosts of these mites in another states (*e.g. Hemidactylus turcicus* and several species of *Sceloporus* and *Phrynosoma*). The differences found in the pterygosomatid fauna among Mexican States, could be caused by a high degree of endemism, but more likely is an effect of the poor knowledge of this mites.

Aguascalientes, San Luis Potosí and Zacatecas are the only three States without species records for this family. These states have been poorly explored, but the probability that pterygosomatid mites occur is high, considering the wide distribution of some lizards that have been reported as hosts of these mites in another states (*e.g. Hemidactylus turcicus* and several species of *Sceloporus* and *Phrynosoma*). The differences found in the pterygosomatid fauna among Mexican States, could be caused by a high degree of endemism, but more likely is an effect of the poor knowledge of this mites.

Pterygosomatidae in Mexico has been recorded parasitizing 39 host species: 31 lizards (Gekkonidae, Iguanidae, Phrynosomatidae, Crotaphytidae and Xantusidae), 4 kissing bugs (Reduviidae), 3 scorpions (Vaejovidae) and 1 cockroach (Dyctioptera).

Ptyerygosomid diversity ranks Mexico country at the second rank, the first place is for South Africa, where during the first half of the XXth century there was a hard effort on the knowledge of these mites, and at least 36 parasitic species on lizards were reported (Lawrence 1935; 1936; 1951; 1959). In India and Philippines 9 and 5 species have been recorded respectively (Abdussalam 1941; Hiregaudar *et al*. 1959; Cuy 1979). Unfortunately, the poor diversity in most of the countries is due to lack of collecting.

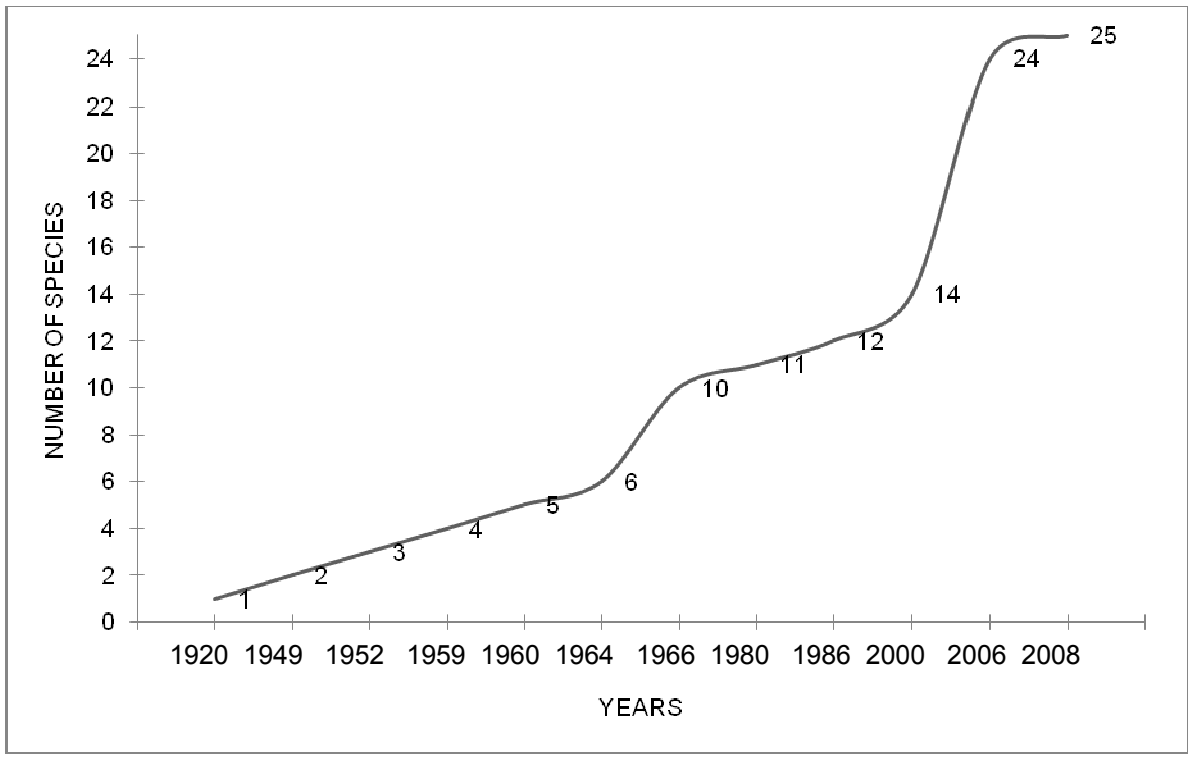

Figure 1. Cumulative snumber of species reccorded through time. Horizontal axis represents the year of publication as follow: Berlese (1920), Cunliffe (1949; 1952), Jack (1959), Beer (1960), Newell & Ryckman (1964), Hunter & Loomis (1966), Newell & Ryckman (1966), Hoffmann & Sánchez (1980), Hoffmann & Morales-Malacara (1986), Hoffmann & López-Campos (2000), Paredes-León (2006) and Paredes-León & Pérez (this study).

Table 2. Diversity of pterygosomatid mites of Mexico

State and species	Hosts	Reference
BAJA CALIFORNIA		
Hirstiella otophila	*Coleonyx variegatus*	Hunter & Loomis 1966
Hirstiella pyriformis	*Sauromalus ater*, *S. hispidus* and *S. varius*	Newell & Ryckman 1964
Pimeliaphilus plumifer	*Paratriatoma hirsuta*	Newell & Ryckman 1966
BAJA CALIFORNIA SUR		
Pimeliaphilus peninsularis	*Triatoma peninsularis* (from nest of *Neotoma* sp.)	Newell & Ryckman 1966
Pimeliaphilus plumifer	*Triatoma rubida* or *T. peninsularis* (from nest of *Neotoma* sp.) and *T. rubida*	Newell & Ryckman 1966
CAMPECHE		
Geckobia bataviensis	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia keegani	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
CHIAPAS		
Geckobia keegani	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia leonilae	*Phyllodactylus tuberculosus*	Hoffmann & Morales-Malacara 1986; Paredes-León *et al*. (in press)
Geckobia sp. A	*Coleonyx elegans*	Paredes-León 2006
Geckobia sp.	*Phyllodactylus tuberculosus*	Paredes-León *et al*. (in press)
Hirstiella sp. B	*Phyllodactylus tuberculosus*	Paredes-León 2006
Hirstiella sp. C	*Phyllodactylus tuberculosus*	Paredes-León 2006
CHIHUAHUA		
Geckobiella texana	*Sceloporus ornatus*	Jack 1959
	Sceloporus sp.	Hoffmann 1969
COAHUILA		
Hirstiella otophila	*Coleonyx brevis*	Paredes-León *et al*. (in press)
COLIMA		
Geckobia keegani	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobiella texana	*Sceloporus pyrocephalus*	Jack 1959
DISTRITO FEDERAL		
Geckobiella texana	*Sceloporus torquatus*	Jack 1959
	Sceloporus sp.	Hoffmann 1969; Hoffmann & López-Campos 2000
Hirstiella pelaezi	*Sceloporus torquatus*	Montiel-Parra *et al*. 2007
	Sceloporus grammicus	Paredes-León *et al*. (in press)
	Sceloporus torquatus	Cunliffe 1949; Montiel-Parra *et al*. 2007
	Sceloporus palaciosi	Gadsden 1988
	Sceloporus microlepidotus	Hoffmann 1969
DURANGO		
Geckobiella texana	*Sceloporus poinsetti*	Jack 1959
ESTADO DE MEXICO		
Geckobiella texana	*Sceloporus* sp.	Hoffmann 1969
	Sceloporus torquatus	Hoffmann & López-Campos 2000
Hirstiella sp.	*Sceloporus microlepidotus*	Hoffmann & López-Campos 2000
Pimeliaphilus rapax	*Vaejovis nitidulus*	Hoffmann & López-Campos 2000
GUANAJUATO		
Hirstiella trombidiformis	Unknown	Berlese 1920 in Hirst 1926
	Sceloporus torquatus	Hoffmann & López-Campos 2000
Pimeliaphilus rapax	*Vaejovis intrepidus*	Hoffmann & López-Campos 2000
GUERRERO		
Hirstiella sp. A	*Phyllodactylus bordai* and *P. tuberculosus*	Paredes-León 2006
Hirstiella sp. B	*Phyllodactylus lanei* and *P. tuberculosus*	Paredes-León 2006
Geckobia bataviensis	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia keegani	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)

Geckobia leonilae	*Phyllodactylus lanei* and *P. tuberculosus*	Paredes-León *et al*. (in press)
Geckobia sp. C	*Phyllodactylus lanei*	Paredes-León 2006
Geckobia sp. D	*Phyllodactylus tuberculosus*	Paredes-León 2006
Geckobia sp.	*Hemidactylus frenatus* and *Phyllodactylus tuberculosus*	Hoffmann 1979
	Phyllodactylus lanei and *P. tuberculosus*	Paredes-León *et al*. (in press)
Geckobiella texana	*Sceloporus horridus*	Jack 1959
HIDALGO		
Geckobia keegani	*Hemidactylus frenatus*	
Hirstiella pelaezi	*Crotaphytus collaris*, *Sceloporus microlepidotus* and *S. torquatus*	Paredes-León *et al*. (in press) Hoffmann 1969
JALISCO		
Geckobia keegani	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia leonilae	*Phyllodactylus lanei*	Hoffmann & Morales-Malacara 1986; Paredes-León *et al*. (in press)
Geckobiella texana	*Sceloporus horridus*	Jack 1959
Geckobiella sp. A	*Sceloporus* sp	This study
MICHOACAN		
Geckobia keegani	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia leonilae	*Phyllodactylus lanei*	Paredes-León *et al*. (in press)
Geckobia sp.	*Phyllodactylus lanei*	Paredes-León *et al*. (in press)
Geckobiella texana	*Sceloporus horridus*	Jack 1959
	Sceloporus torquatus	Hoffmann 1969
Hirstiella pelaezi	*Sceloporus microlepidotus* and *S. torquatus*	Hoffmann 1969
Hirstiella sp. B	*Phyllodactylus lanei*	Paredes-León 2006
MORELOS		
Geckobia leonilae	*Phyllodactylus lanei*	Paredes-León *et al*. (in press)
Geckobiella texana	*Sceloporus* sp.	Hoffmann & López-Campos 2000
	Iguana iguana	Hoffmann 1969
	Sceloporus grammicus	Gadsden 1988
	Undetermined cockroach	Hoffmann & López-Campos 2000
Pimeliaphilus podapolipophagus	*Triatoma* sp.	Hoffmann & López-Campos 2000
Pimeliaphilus triatomae		
NAYARIT	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia keegani	*Phyllodactylus tuberculosus*	Paredes-León *et al*. (in press)
Geckobia leonilae	*Sceloporus utiformis*	Jack 1959
Geckobiella texana	*Phyllodactylus tuberculosus*	Paredes-León 2006
Hirstiella sp. C		
NUEVO LEÓN	Unknown	Cunliffe 1952
Hirstiella trombidiformis		
OAXACA	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia bataviensis	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia keegani	*Phyllodactylus muralis* and *P. tuberculosus*	Paredes-León *et al*. (in press)
Geckobia leonilae	*Coleonyx elegans*	Paredes-León 2006
Geckobia sp. A	*Phyllodactylus lanei*	Paredes-León 2006
Geckobia sp. D	*Phyllodactylus muralis* and *P. tuberculosus*	Paredes-León *et al*. (in press)
Geckobia sp.	*Sceloporus siniferus* and *S. spinosus*	Jack 1959
Geckobiella texana	*Sceloporus* sp.	This study
Geckobiella sp. A	*Ctenosaura pectinata*	Paredes-León 2003
Hirstiella bakeri	*Phyllodactylus bordai*	Paredes-León 2006
Hirstiella sp. A	*Phyllodactylus lanei* and *P. tuberculosus*	Paredes-León 2006
Hirstiella sp. B	*Phyllodactylus muralis* and *P. tuberculosus*	Paredes-León 2006
Hirstiella sp. C	*Phyllodactylus tuberculosus*	Paredes-León *et al*. (in press)
Hirstiella sp	*Triatoma barberi*	Newell & Ryckman 1966
Pimeliaphilus gloriosus	*Vaejovis punctatus*	Beer 1960
Pimeliaphilus rapax	*Lepidophyma smithi*	Hoffmann & Sánchez 1980
Tequisistlana oaxacensis		

PUEBLA	*Phyllodactylus bordai*	Paredes-León 2006
Geckobia sp. C	*Sceloporus spinosus*	Jack 1959
Geckobiella texana	*Sceloporus* sp.	Hoffmann 1969
Hirstiella bakeri	*Sceloporus microlepidotus* and *S. torquatus*	Hoffmann 1969
Hirstiella pelaezi	*Phyllodactylus bordai*	Paredes-León 2006
Hirstiella sp. A		
QUERETARO	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia keegani		
QUINTANA ROO	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia keegani	*Aristelliger georgeensis*	Paredes-León 2006
Hirstiella sp. D		
SINALOA	*Coleonyx variegatus*	Hunter & Loomis 1966
Hirstiella otophila	*Triatoma rubida*	Newell & Ryckman 1966
Pimeliaphilus plumifer		
SONORA	*Coleonyx fasciatus*	Hunter & Loomis 1966
Hirstiella otophila	*Triatoma rubida* (from nest of *Neotoma* sp.)	Newell & Ryckman 1966
Pimeliaphilus plumifer		
TABASCO	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia bataviensis	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia keegani		
TAMAULIPAS	*Hemidactylus frenatus* and *H. turcicus*	Paredes-León *et al*. (in press)
Geckobia bataviensis		
TLAXCALA	*Phrynosoma* sp.	Hoffmann 1969
Geckobiella texana		
VERACRUZ	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia bataviensis	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia keegani	*Sceloporus variabilis*	Jack 1959
Geckobiella texana	Undetermined lizard	Hoffmann 1969
	Sceloporus sp.	Hoffmann & López-Campos 2000
YUCATÁN	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia bataviensis	*Hemidactylus frenatus*	Paredes-León *et al*. (in press)
Geckobia keegani	*Thecadactylus rapicaudus*	Paredes-León 2006
Geckobia sp. B	*Coleonyx elegans*	Paredes-León *et al*. (in press)
Geckobiella texana		

Conclusions

A large number of undescribed species is predicted because pterygosomatids have high host specificity and some of them have as hosts animal groups that have great diversity in Mexico (*e.g.* vaejovid scorpions and phrynosomatid lizards) (Francke com. pers.; Sites *et al*. 1992).

The pterygosomatid fauna is far from being completely described. We consider that the total species of Mexican pterygosomatid fauna is probably much higher that currently known. For example, one of us, collected 4,146 pterygosomatid mites from ten different host species of lizards Gekkonidae in the Neotropical Region of Mexico (Paredes-León 2006).

As pterygosomatid mites depend on reptiles and arthropods as hosts, the future research of Mexican Pterygosomatidae will focus on more families of these hosts, and should include the northern states (Neartic Region) to complete the checklist and to carry out another kind of research (*e.g.* biogeographical, cospeciation, phylogeny, etc.).

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ORIBATID MITES IN ELEVEN DIFFERENT HABITATS IN FINLAND

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Abstract

Oribatid mite communities were studied in eleven different habitats; four forest types, two bog types, ant hills (*Formica* spp.), decaying wood, moist meadow, dry meadow, and seashore. Nine sampling areas were selected with geographical coverage of the country; three in southern Finland, three in central Finland and three in northern Finland. Ca. 350 samples were collected, yielding a total of 70376 oribatid mites. 31544 specimens were identified and included 165 species, of which 17 were new to Finland. 12 species were found in all 11 habitats. Species richness was highest in decaying wood and lowest on seashores. The present article gives the proportions of the most abundant species in each habitat, lists the recorded species and discusses the distribution of some rare species.

Key-words

Oribatid mites, community, species diversity, microhabitats, distribution

Introduction

Oribatid communities of different forests and bogs have been much studied in Finland, but almost all studies are local or cover only limited areas (Huhta et al., 1986, 2005, Huhta and Niemi, 2003, Karppinen, 1958 a,b, 1972, 1977). Country-wide studies have been carried out only on Camisiidae (Karppinen, 1955). A study on the oribatids of seashores was performed by Karppinen (1966). Investigations on the communities of meadows and decaying wood are few, and knowledge on the oribatid fauna of ant nests is based on occasional records only.

This study is part of the programme "Deficiently known and threatened forest species" supported by the Finnish Ministry of Environment (2003-07). Our project "Occurrence and distribution of poorly known soil animal groups" covers Acarina, Collembola and Enchytraeidae in 15 habitat types. The present paper reports the results on Oribatida in eleven of these habitats.

Material and methods

Eleven different habitats were chosen; dry coniferous forest (Scots pine), mesic coniferous forest (Norway spruce), mesic deciduous forest, marsh forest (usually spruce), pine bog, open bog, ant hills (*Formica* spp.), decaying wood, moist meadow, dry meadow, and seashore. Nine sampling areas were selected with geographical coverage of the country; three areas in southern Finland, three in central Finland and three in northern Finland. In each sampling area, 4 or 3 replicates of each habitat type were chosen, and three subsamples of 25 cm^2 (100 cm^3) were taken in each. About 350 such samples (3x25 cm^2) were collected during 2004 and 2005. The mites were extracted in the laboratory using a modified high

gradient apparatus (Macfadyen, 1961).

Adult oribatids were identified using Weigmann (2006), Gilyarov (1975), Niedbala (1992) and some selected articles (Bayartogtokh and Aoki, 1997, Behan-Pelletier and Norton, 1983). The families Suctobelbidae and Brachychthoniidae were not identified, and hence were not included in estimates of species richness. The numbers of species and specimens in each habitat were counted. Total species number and the relative abundance of dominant species in each habitat are displayed by charts. As dominants we considered the 3 to 6 most abundant species in each habitat that together exceeded 50% of total number of specimens.

Results

Altogether 31544 specimens of a total of 70376 oribatid mites were identified. The total number of species was 165, of which twelve (*Phthiracarus longulus, P. boresetosus, Atropacarus striculus, Euphthiracarus monodactylus, Hemileius initialis, Tectocepheus velatus, Oppiella nova, Medioppia subpectinata, Congochneta traegardhi, Chamobates borealis, Eupelops torulosus* and *Pergalumna nervosus)* occurred in all 11 habitats. Seventeen species were found the first time in Finland (see Annexe, Table 1). The majority of them have been reported in Central Europe or Sweden, or in the western part of Russia, whereas three, *Maerkelotritia cryptopa* (Banks, 1904), *Epidamaeus fortispinosus* Hammer, 1967, and *Nortonella mongolica* Bayartogtokh & Aoki, 1997, have only been found outside Europe. *Maerkelotritia cryptopa* was collected on seashore in southern Finland (Niedbała and Penttinen, 2006); it was previously reported only in the nearctic region (Niedbała 2002), where it has been found in several localities on the western coast of the USA and Canada. *Epidamaeus fortispinosus* was found in a marsh forest in eastern Finland. Its area of distribution is mainly in western North America and northeast Russia (Behan-Pelletier and Norton, 1983, Golosova et al., 1983). The range of the species *Nortonella mongolica* was to date restricted to Mongolia and Kazakhstan (Bayartogtokh & Smelyansky, 2002); our specimen was collected on a dry meadow in southern Finland.

The numbers of species in different environments

Differences in the number of species recorded in the forest habitats were low. The lowest number of species (74) was found in dry coniferous forests and pine bogs, and the highest in mesic deciduous forests (82). The most diverse communities occurred in decaying wood (95 species) and in ant hills (85). 38 species were common to all seven forest habitats or subhabitats. The highest number of unique species (13) was found in decaying wood, whereas mesic coniferous forests showed only one unique species (*Ceratozetes mediocris*).

Among the open environments, dry meadows harboured the most diverse community (74 species), followed rather evenly by moist meadows (55), open bogs (54) and seashores (52).

Community structure

Forest and bog habitats (Fig. 1)

Abundance of oribatids was highest in bogs and mesic spruce forests, and lowest in marsh and deciduous forests. *Oppiella nova* was the most abundant species in most forest and bog habitats, with the exception of marsh forests and open bogs. In particular, it represented a high proportion of species in dry and mesic coniferous forests and pine bogs (23-27 %). The proportion of Suctobelbidae was almost the same (10%) in each forest type, while it was not among the dominant species in the bog habitats. *Tectocepheus velatus* was abundant in dry coniferous and marsh forests (12-14 %). *Medioppia subpectinata* and *Congochneta traegardhi* occurred only in mesic coniferous and mesic deciduous forests (6 %). Species of Ptyctima were found in all habitats. *Phthiracarus longulus* dominated only in dry coniferous forests, and *Stegenacarus carinatus* only in mesic coniferous forests. *Atropacarus striculus* clearly favoured mesic deciduous forests, pine bogs and especially marsh forests, where it was the most abundant species (22 %). The species *Hoplophthiracarus pavidus* favoured bog habitats; its proportion was 10 % in pine bogs and 28 % in open bogs. *Nanhermannia nana* dominated in open bogs only (16%).

Ant hills and decaying wood (Fig. 2)

The abundance of oribatids in ant hills and decaying wood is comparable with the average in forest floor (per unit volume). The proportion of Oppioidea (30 %) in ant hills exceeded that of the other dominants, but in decaying wood it was only half this value. *Oppiella nova* was especially abundant. The proportion of *Scheloribates pallidulus* in ant hills was the same as the combined abundance of *S. pallidulus* and *S. latipes* in decaying wood. *T. velatus* preferred decaying wood (12 %) over ant hills (4 %), while *Steganacarus carinatus* occurred only in ant hills (8%) and *Euphthiracarus cribrarius* dominated only in decaying wood. The dominant species of the

family Carabodidae were absent in ant hills, whereas their proportion was 13 % in decaying wood.

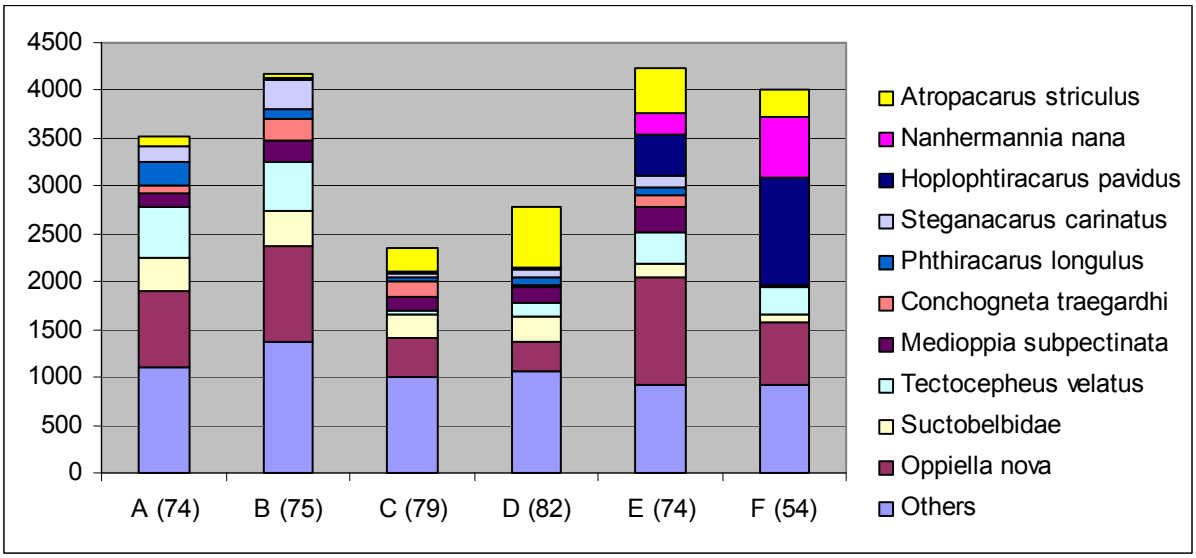

Figure 1. Abundance of different species in forest and bog habitats. A= dry coniferous forest, B=mesic coniferous forest, C=marsh forest, D=mesic deciduous forest, E=pine bog and F=open bog (species richness in parenthesis).

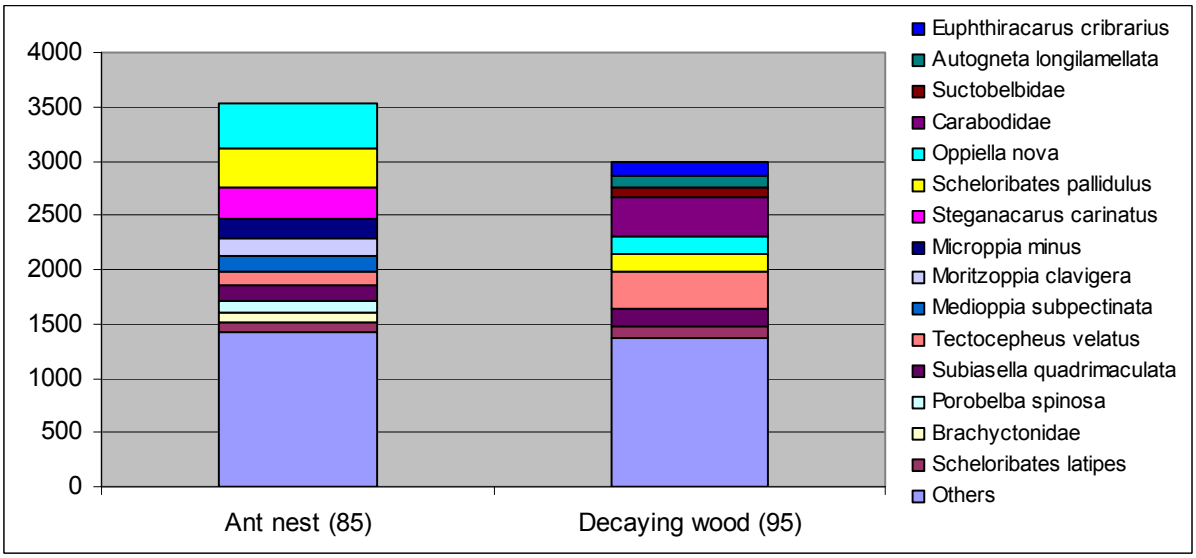

Figure 2. Abundance of different species in ant hills and decaying wood (species richness in parenthesis).

Grassland habitats (Fig. 3)

Oribatids were much more abundant and diverse in dry meadows than in moist meadows, though even in the former the total numbers were low when compared with forest habitats (Figs. 1 and 3). Three species, Scheloribates laevigatus, Tectocepheus velatus and Oppiella nova dominated both kinds of meadows. The abundance of T. velatus, O. nova, Medioppia subpectinata and Liebstadia similis were similar in moist meadows, only the proportion of S. laevigatus was somewhat higher (13 %). The share of T. velatus (20 %) was very high in dry meadows, but S. laevigatus and O. nova were relatively less abundant in moist meadows. The shares of Ceratozetella thienemanni, Nanhermannia nana and Microppia minus remained below 5% of the total specimens.

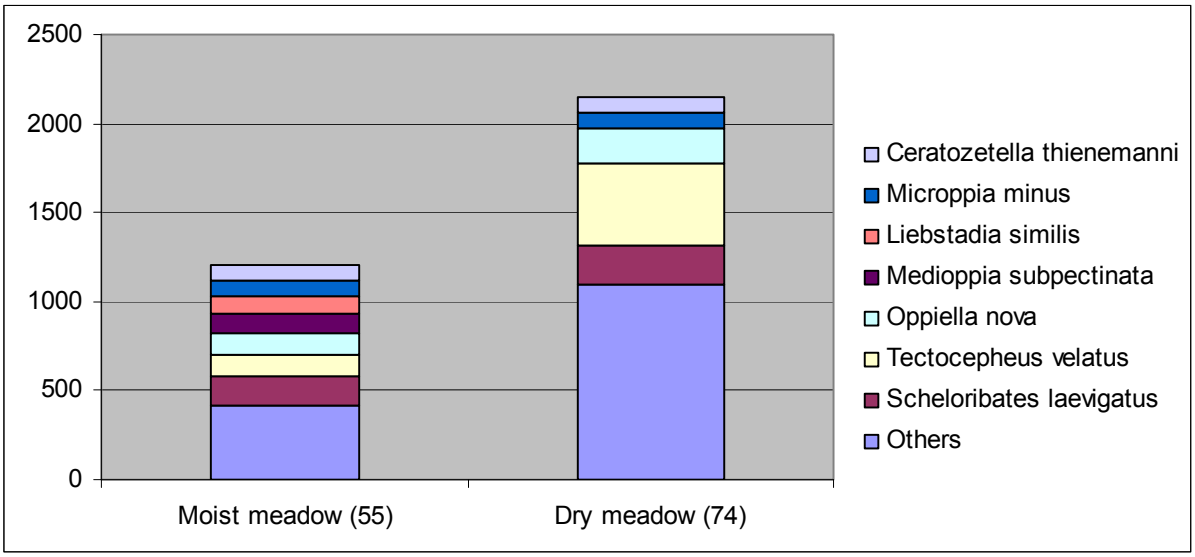

Figure 3. Abundance of species in moist and dry meadows (species richness in parenthesis).

Seashores (Fig. 4)

The lowest numbers of both species and specimens were found on seashores. Six species of a total of 52 dominated these habitats. The high proportion of *Atropacaras striculus* (26 %) was conspicuous, followed by *Scheloribates laevigatus* and *Fuscozetes fuscipes; O. nova*, *Phthiracarus golobosus* and *Punctoribates punctum* reached 5%.

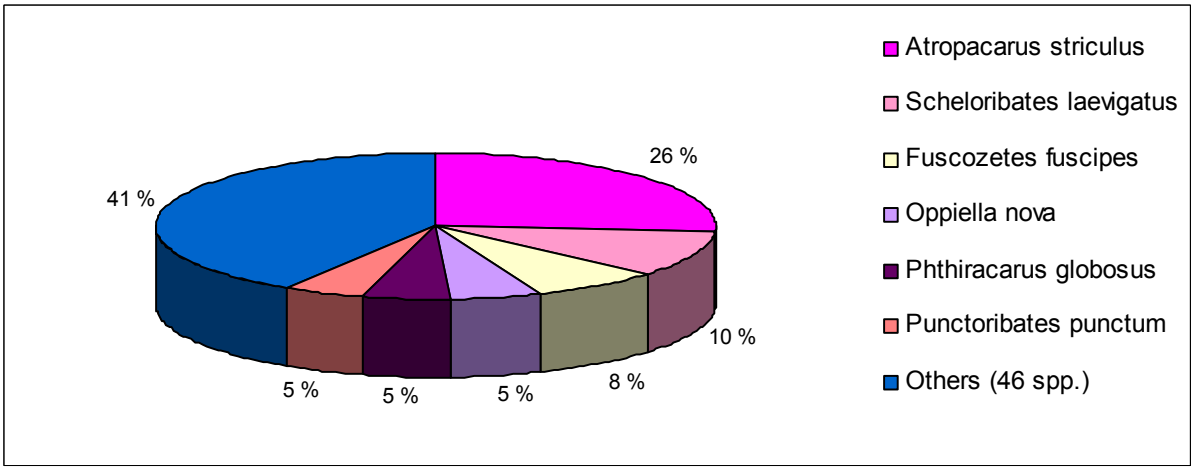

Figure 4. Proportions of oribatid species found on seashores.

Discussion and conclusion

The forest habitats—including dry coniferous forest, mesic coniferous forest, mesic deciduous forest, marsh forest and pine bog—did not differ much in species composition, having 42 species (60%) in common. However, our results differed from those reported by Karppinen (1958a), according to whom the numbers of species in dry coniferous forest and mesic coniferous forest were higher than those in mesic deciduous forest. In Karppinen's study also the abundances of oribatid

mites were much higher in deciduous forests than in the coniferous forests. In contrast to that, Huhta et al. (2005) gave higher abundances of specimens in spruce forest than in deciduous forests.

In our study the open bogs shared only nineteen common species with both seashores and meadows, but twenty-nine species with seashores alone. Hence their oribatid communities were closer to those communities living in the seashores than to those found in grassland habitats. The number of the oribatid species found on seashores was the lowest (52) in our study. In contrast to this, Karppinen (1966) has reported 125 oribatid species on seashores and archipelago. However, these numbers are not comparable with our results, because his samples were collected from various kinds of micro-habitats e.g. from breeding colonies of sea-birds.

In addition to this, our study indicated that ant hills and decaying wood had 38 species in common with forest habitats. Thus their communities were closest to the community in forest habitat rather than to any others. From all studied habitats the decaying wood clearly offered the best environment for oribatids; the number of species (95) exceeded the amount of other habitats. Similarly to our study, Skubała and Sokołowska (2006) have also found a rich number of oribatid species (80) in decaying wood of downed logs.

The large number of new species (17) found in Finland was surprising. These species were found in all the habitats, but most of them occurred in meadow types, ant hills, decaying wood and on seashores, which were less studied environments. Therefore the results indicate that more studies are required in general. In addition to that, the less studied habitats should be more thoroughly examined.

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Annexe

Table. 1 The oribatid mites in eleven habitats. A= dry coniferous forest, B=mesic coniferous forest, C= mesic deciduous forest, D=, marsh forest, E=pine bog, F=open bog, G=meadow, H=ant nest, I=dry meadow, J=seashore, K=decaying wood. The new species with red colour.

SPECIES	A	B	C	D	E	F	G	H	I	J	K	SPECIES	A	B	C	D	E	F	G	H	I	J	K
Achipteria coleopterata		*	*	*			*	*	*	*	*	*Cepheus cepheiformis*					*						*
Acrogalumna longipluma		*	*					*				*Ceratoppia bibilis*	*		*		*		*				
Adoristes poppei	*	*	*	*	*	*		*	*		*	*Ceratozetella minimus*	*	*									
Ameronothrus dubinini	*											*Ceratozetella thienemanni*	*	*	*	*	*	*		*	*		*
Anachipteria deficiens						*						*Ceratozetes gracilis*		*	*				*				
Astegistes pilosus										*		*Ceratozetes mediocris*		*					*		*	*	
Atropacarus striculus	*	*	*	*	*	*	*	*	*	*	*	*Ceratozetes parvulus*						*					
Autogneta longilamellata											*	*Ceratozetes sellnicki*							*	*	*	*	
Autogneta parva	*	*		*			*	*			*	*Chamobates borealis*	*	*	*	*	*	*	*	*	*	*	*
Banksinoma lanceolata	*			*	*	*	*	*	*	*	*	*Chamobates cuspidatus*		*	*					*	*		*
Belba compta	*	*	*	*	*				*			*Chamobates spinosus*								*			*
Belba corynopus							*					*Chamobates voigtsi*		*									*
Caenobelba montana				*					*			*Congochneta traegardhi*	*	*	*	*	*	*	*	*	*	*	*
Caleremaeus monilipes	*							*			*	*Cultoribula dentata*											*
Camisia biurus	*	*			*						*	*Damaeus auritus*			*								
Camisia biverrucata								*				*Damaeus boreus*	*	*	*	*	*		*	*	*	*	*
Camisia solhoeyi				*		*						*Damaeus onustus*		*	*	*	*				*		*
Camisia spinifer	*											*Dameus riparius*			*		*				*		
Carabodes areolatus											*	*Diapterobates humeralis*	*				*	*		*			*
Carabodes femoralis	*	*	*	*	*			*			*	*Dissorhina ornata*	*	*	*	*	*		*	*	*		*
Carabodes forsslundi	*		*		*			*	*		*	*Edwardzetes edwarsii*				*							
Carabodes labyrinticus	*	*	*	*	*			*			*	*Eniochtonius minutissimus*					*		*		*	*	*
Carabodes marginatus	*				*			*			*	*Epidamaeus bituberculatus*								*			*
Carabodes minusculus	*								*			*Epidamaeus fortispinosus*				*							
Carabodes rugosior											*	*Epidameus affinis*	*	*	*	*	*		*	*	*		
Carabodes subarcticus	*	*		*	*			*			*	*Epidameus brevitibialis*		*	*		*				*		*
Carabodes tenuis	*							*	*		*	*Eueremaeus silvestris*	*	*						*	*		*
Carabodes willmanni	*											*Eulohmannia ribagai*	*	*	*	*							

SPECIES	A	B	C	D	E	F	G	H	I	J	K	SPECIES	A	B	C	D	E	F	G	H	I	J	K
Eupelops acronomios	*	*			*							*Maerkelotritia cryptopa*										*	
Eupelops torulosus	*	*	*	*	*	*	*	*	*	*	*	*Medioppia subpectinata*	*	*	*	*	*	*	*	*	*	*	*
Euphthiracarus cribrarius			*	*							*	*Melanozetes mollicomus*	*	*		*		*					*
Euphthiracarus monodactylus	*	*	*	*	*	*	*	*	*	*	*	*Mesotritia flagelliformis*	*	*	*	*	*			*			*
Euzetes seminulum		*	*	*	*		*		*			*Mesotritia nuda*	*		*	*	*			*			*
Furcoribula furcillata	*							*	*		*	*Metabelba pulverulenta*		*	*	*	*		*	*	*		*
Fuscozetes fuscipes			*	*		*				*		*Microppia minus*	*	*	*		*		*	*	*	*	*
Galumna dimorpha			*					*				*Microtritia minima*	*			*							*
Galumna elimata						*	*		*	*	*	*Minunthozetes pseudofusicer*			*								
Galumna lanceata	*		*				*	*	*			*Minuntozetes semirufus*				*			*		*	*	
Gustavia microcephala			*	*			*	*	*	*		*Moritzoppia clavigera*	*	*			*			*			*
Gymnodamaeus bicostatus					*			*				*Moritzoppiella translamellata*	*	*	*	*	*	*	*	*			*
Hafenrefferia gilvipes		*									*	*Mucronothrus nasalis*				*							
Hemileius initialis	*	*	*	*	*	*	*	*	*	*	*	*Multioppia gabra*		*	*	*	*	*		*	*	*	
Heminothrus longisetosus	*	*	*	*	*	*		*			*	*Multioppia laniseta*											
Heminothrus paolianus	*	*	*	*	*			*		*	*	*Nanhermannia nana*	*	*	*	*	*	*		*	*	*	*
Hermanniella dolosa			*					*	*			*Nanhermannia sellnicki*	*	*	*	*	*			*			*
Hoplophthiracarus pavidus		*		*	*	*		*	*	*	*	*Neonothrus humicola*		*		*							
Hydrozetes lacustris										*		*Neoribates aurantiacus*											*
Hydrozetes lemnae										*		*Nortonella mongolica*									*		
Hypochtonius rufulus		*	*	*	*	*	*	*	*	*	*	*Nothrus palustris*		*	*	*	*		*	*	*		
Latilamellobates inicellus							*		*		*	*Nothrus pratensis*	*	*	*	*	*	*		*	*		*
Lauroppia maritima	*	*	*	*	*	*		*	*		*	*Nothrus silvestris*	*	*	*	*	*	*	*	*	*		*
Lauroppia neerlandica											*	*Ophidiotrichus tectus*											*
Liacarus coracinus	*	*	*	*	*		*	*	*		*	*Oppia nitens*								*			
Liacarus subterraneus		*	*						*			*Oppia splendes*	*			*				*	*		*
Licneremaeus licnophorus								*			*	*Oppiella nova*	*	*	*	*	*	*	*	*	*	*	*
Liebstadia similis			*			*	*	*	*	*		*Oribatella calcarata*			*				*				*
Limnozetes ciliatus					*	*						*Oribatella sexdentata*										*	*
Limnozetes rugosus						*						*Oribatula pannonica*											*
Malaconothrus egregius	*	*	*	*	*	*			*	*		*Oribatula tibialis*	*	*	*	*	*	*		*		*	*

SPECIES	A	B	C	D	E	F	G	H	I	J	K	SPECIES	A	B	C	D	E	F	G	H	I	J	K
Oribella pectinata												*Protoribotritia oligotricha*	*		*								*
Oribotritia fennica										*		*Punctoribates hexagonus*	*			*			*			*	
Pantelozetes paolii			*				*					*Punctoribates punctum*		*	*				*	*	*	*	*
Parachiptera punctata	*	*	*	*	*	*	*	*	*		*	*Punctoribates sellnicki*						*					
Paradamaeus clavipes	*	*	*		*			*			*	*Quadroppia quadricarinata*	*	*	*	*	*	*	*	*	*		*
Passalozetes intermedius									*			*Rhysotritia ardua*	*	*	*	*	*	*		*	*	*	*
Pergalumna altera			*			*	*	*	*		*	*Scheloribates laevigatus*	*	*	*	*	*	*	*	*	*	*	
Pergalumna longior						*						*Scheloribates latipes*	*	*	*	*	*	*	*	*	*		*
Pergalumna nervosus	*	*	*	*	*	*	*	*	*	*	*	*Scheloribates pallidulus*	*			*	*			*			*
Phauloppia coineaui								*			*	*Steganacarus applicatus*		*	*	*		*		*			*
Phauloppia lucorum			*								*	*Steganacarus carinatus*	*	*	*	*	*	*		*	*	*	*
Phthiracarus boresetosus	*	*	*	*	*	*	*	*	*	*	*	*Subiasella quadrimaculata*		*		*				*		*	*
Phthiracarus bryobius	*	*	*	*	*			*			*	*Tectocepheus velatus*	*	*	*	*	*	*	*	*	*	*	*
Phthiracarus crinitus			*	*							*	*Tegoribates latirostris*							*				
Phthiracarus ferrugineus			*	*		*				*		*Trhypochthoniellus badius*					*	*					
Phthiracarus globosus	*	*	*	*	*		*	*	*	*	*	*Trhypochthonius tectorum*	*			*		*				*	
Phthiracarus lentulus				*				*		*		*Trichoribates myrica*						*					
Phthiracarus longulus	*	*	*	*	*	*	*	*	*	*	*	*Trichoribates novus*					*		*		*		
Phthiracarus nitens	*	*	*	*	*			*	*		*	*Trichoribates trimaculatus*					*		*		*		
Pilogalumna tenuiclavus			*		*	*			*			*Trimalaconothrus novus*	*			*	*	*	*		*	*	
Platynothrus peltifer		*	*	*	*	*	*	*	*	*		*Tritegeus bisulcatus*								*			
Podoribates longipes										*		*Xenillus tegeocranus*		*	*	*			*	*		*	*
Porobelba spinosa	*	*	*	*	*			*	*		*	*Zetomimus furcatus*						*	*				
Protoribates lagenula									*	*	*	*Zygoribatula exilis*											*

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FAUNA OF ASCID MITES (ACARI: MESOSTIGMATA) IN DAMGHAN REGION, SEMNAN PROVINCE, IRAN

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Abstract

During 2006-2007, in a faunistic survey of ascid mites in Damghan region, a total of 21 species belonging to seven genera and three subfamilies were collected and identified. All of the species are new records for Semnan Province, six species are new records to the mite fauna of Iran and 6 species are new to science.

Keywords

Acari, Ascidae, fauna, new species, new records, Damghan region

Introduction

Members of the family Ascidae Voigts and Oudemans, 1905 are important due to their occurrence in all locations. A few species are parasites of cockroaches and moths (Egan & Moss 1969; Treat 1975). Some species are fungivorous, pollen feeders, predators on young stages of mites, insects and nematodes (Moser 1975). Ascid mites are also phoretic on insects, Myriapoda and even hummingbirds (Lindquist and Evans 1965). Some ascid mites are found in humid or extremely humid habitats such as marshes, alder swamps, banks of rivers and streams, arable soils especially in black and clay soils (Karg 1981), decaying plant material in wet meadows, litter between tree roots and moss (Kaluz & Fenda 2005). The others, as predators, hunt and feed on nematodes and Collembola in moist soils, which also show a liking for humid habitats (Halliday *et al.* 1998; Karg 1979). Ascid mite fauna of Iran was poorly known especially in Semnan Province. The aim of the present study was a faunistic investigation of the ascid mites in Damghan region, Semnan Province, Iran.

Material and methods

Mites extracted from samples of soils, plants (leaves, tree trunks,…), manures, litter, nest of birds and rodents, decaying trunk of trees, galleries of buprestid and scolytid in trunk and twigs of trees and beehives using Berlese's funnel. Specimens were cleared in a mixture of lactophenol and Nesbitt solution (1:1) at common condition of the laboratory. Mites were then directly mounted into faure's medium on microscope slides. Setal notation proposed by (Lindquist & Evans 1965) is followed.

For each species, the number of specimens collected, gender, date of collecting, sampling location, and habitat are listed. Species marked with an asterisk are new records for the mite fauna of Iran.

Results

Subfamily Arctoseiinae

1. *Arctoseius cetratus* (Sellnick, 1940)

107 females, 7 April 2006 in Damghan, soil of alfalfa farm, 14 April 2006 in Kalatehroudbar village, soil of barley farm, in Cheshmeh-Ali, on *Cardaria draba* near the pool of a spring, 19 May 2006 in Dibaj, soil near bay willow trunk, 26 September 2006 in Dibaj, decaying matter of a poplar trunk, in Astaneh village, soil near a pear trunk at the margin of a sike, in Dibaj, soil at the margin of a sike, 26 May 2006 in Tooyehdarvar village, soil of an alfalfa farm, wheat farm and decaying leaves of walnut tree, 18 August 2006 in Haji Abad village, litter in pistachio orchard, 28 August 2006 in Amiriyyeh, soil of corn farm, 7 September 2006 in Naim Abad village, soil mixed with manure in pistachio orchard, 8 September 2006 in Dashtebou village, soil mixed with weeds in a walnut orchard, soil mixed with weeds in an apple orchard, soil mixed with decaying leaves, 12 September 2006 in Fouladmahalleh triway, sawdust mixed manure, 6 October 2006 in Shams Abad Village, Soil near a grapevine trunk, twigs with long horn beetle galleries of fruit tree in apple and pear orchards, decaying crown of apricot with woodborer galleries, sawdust near a decaying trunk of black mulberry, in Abbas Abad village, soil of alfalfa farm, 18 October 2006 in Tooyehdarvar village, chicken manure, 27 June 2007 in Bakhsh Abad village, decaying trunk of a willow tree, 29 June 2007 in Hadadeh village, soil mixed with weeds near a grapevine and pistachio trees, in Kalatehmolla, soil of a grapevine orchard, 7 October 2007 in Cheshmeh-Ali, soil mixed with weeds near the pool of a spring, in Darvar village, litter at the margin of a sike.

Distribution: Europe: (Karg 1993; Kaluz & Fenda 2005; Ignatowicz 2000; Salmane & Heldt 2001). Far North Russia: (Makarova 2000). North America: (Chant 1963). Australian: (Halliday *et al.* 1998). Iran: (Kamali *et al.* 2001).

2. *Arctoseius semiscissus* (Berlese, 1892)

20 females, 26 May 2006 in Tooyehdarvar village, decaying matter of oleaster trunk, decaying leaves of walnut tree, 4 June 2006 in Dibaj, soil mixed with weeds near an almond tree, 9 May 2006 in Berom village, soil of pistachio orchard, 14 April 2006 in Cheshmeh-Ali, on twigs of apricot.

Distribution

Europe: (Karg 1993; Lundqvist *et al.* 1999; Ignatowicz 2000; Kaluz & Fenda 2005). Australia: (Halliday *et al.* 1998). Iran: (Kamali *et al.* 2001).

3. *Arctoseius venustulus (Berlese, 1916)**

13 females, 26 May 2006 in Tooyehdarvar village, soil with ant nest at a wheat farm, 26 September 2006 in Dibaj, soil mixed with weeds near a pear tree, 2 November 2006 in Tooyeh village, soil mixed with litter near a cherry tree.

Distribution: Europe: (Karg 1993; Kaluz & Fenda 2005)

4. *Arctoseius* sp. nov.

2 females, 2 November 2006 in Tooyeh village, soil mixed with roots of weeds near mulberry tree.

5. *Blattisocius keegani* Fox, 1947

10 females, 6 October 2006 in Mehmandust village, from decaying hay.

Distribution: Puerto Rico: (Fox 1947). Hawaii: (Prasad 1968). Malaya: (Evans 1958). Iran: (Kamali *et al.* 2001). North America: (Chant 1963). Western Australia: (Halliday *et al.* 1998). Egypt: (Al-Badry *et al.* 1980).

6. *Blattisocius* sp. nov.

4 females, 12 September 2006 in Agareh village, decaying sawdust left for more than a year.

7. *Cheiroseius cascadensis (De Leon, 1964)**

6 females, 26 May 2006 in Tooyehdarvar village, moss at the floor of a dry sike, decaying matter of a poplar trunk, 26 September 2006 in Dibaj, soil mixed with weeds at the margin of a sike, 18 October 2006 in Tooyehdarvar village, soil mixed with decaying matter of a fallen tree trunk.

Distribution: United states of America: (De Leon 1964).

8. *Cheiroseius curtipes (Halbert, 1923)**

16 females, 26 May 2006 in Tooyehdarvar village, moss at the floor of a dry sike, 11 August 2006 in Cheshmeh-Ali way, soil at the margin of Cheshmeh-Ali river near straw, 8 September 2006 in Cheshmeh-gholghol spring, soil mixed with weeds at the margin of a spring, 26 September

2006 in Dibaj, soil close to Imamzadeh, soil mixed with weeds near a pear tree.

Distribution: India: (Pramanik & Raychaudhuri 1977). Philippines: (Corpuz-Raros & Raros 1999). Kazakhstan: (Chelebiev 1988). Europe, Central and North America: (Karg 1993; Kaluz & Fenda 2005).

9. *Cheiroseius necorniger (Oudemans, 1903)**

4 females, 26 May 2006 in Tooyehdarvar village, moss at the floor of a dry sike, 18 October 2006 in Tooyehdarvar village, soil mixed with weeds.

Distribution: Europe and Africa: (Karg 1993; Lundqvist *et al.* 1999; Lundqvist 1998; Salmane 2001; Salmane & Heldt 2001; Kaluz & Fenda 2005).

10. *Cheiroseius serratus (Halbert, 1915)**

4 females, 8 September 2006 in Cheshmeh-gholghol spring, soil mixed with weeds at the margin of spring, 26 September 2006 in Dibaj, soil at the margin of a sike.

Distribution: Europe: (Karg 1993; Fenda 1999; Kaluz & Fenda 2005). Philippines: (Corpuz-Raros, & Raros 1999).

11. *Cheiroseius* sp. nov.

16 females, 12 September 2006 in Agareh village, soil mixed with hairy roots of a willow tree.

12. *Gamasellodes bicolor* (Berlese, 1918)

44 females, 26 May 2006 in Tooyehdarvar, soil of alfalfa farm, 28 July 2006 in Tazareh village, decaying underbark matter of an alive poplar, 18 August 2006 in Haji Abad village, litter in a pistachio orchard, 7 September 2006 in Naim Abad village, soil of a pistachio orchard, 13 September 2006 in Forat village, soil at the margin of a sike, 26 September 2006 in Astaneh, soil at the margin of a sike near a pear tree, in Damghan, soil near an olive tree, 13 October 2006 in Shams Abad village, decaying crown of an apricot tree along the margin of a sike, 26 June 2997 in Kalatehmolla, soil of a pistachio orchard, 8 July 2007 in Damghan, soil on an apricot orchard.

Distribution: Europe: (Karg 1993; Salmane & Heldt 2001). North and South America, South Africa, Israel and India (Bhattacharyya & Sanyal 2002). Tanzania: (Hurlbutt 1971). Iran: (Kamali *et al.* 2001).

13. *Lasioseius* sp. nov.

43 females, 3 males specimens, 14 Aprile 2006 in Cheshmeh-Ali, decaying matter of a white alive poplar, 26 May 2006 in Tooyehdarvar village, decaying leaves of walnut, 26 September 2006 in Dibaj, underbark matter of an alive poplar.

14. *Lasioseius youcefi* Athias-Henriot, 1959

108 females, 14 April 2006 in Dibaj, moss at the margin of a spring, in Cheshmeh-Ali spring, on the *Lepidium draba*, 28 April 2006 in Tooyehdarvar village, soil near a pear tree, 19 May 2006 in Dibaj, soil near a willow tree, 26 May 2006 in Tooyehdarvar village, decaying trunk contents of poplar tree, soil of alfalfa farm, soil mixed with weeds at the margin of a sike, soil mixed with decaying trunk of oleaster, chicken manure, 12 September 2006 in Agareh village (Chambari`s orchard), soil of alfalfa farm, soil mixed hairy roots of a willow near a pool of spring, 15 September 2006 in Tazareh, decaying wood of oleaster trunk with woodborer galleries, 26 September 2006 in Astaneh village, soil near a pear tree at the margin of a sike, in Damghan, soil near an olive tree, in Dibaj, soil mixed with weeds at the margin of a sike, in Dibaj, soil near a pear tree, in Dibaj, soil in alfalfa farm, 6 October 2006 in Jafar Abad village, soil in Mehmandust village, soil mixed with weeds at the margin of a sike, 17 June 2007 in Astaneh village, soil near a willow tree at the margin of a sike, 27 June 2007 in Cheshmeh-gholghol spring, soil near a medlar tree with chicory weed, in Cheshmeh-gholghol spring, soil mixed with the roots of a willow tree, 8 July 2007, soil in an apricot orchard, in Damghan, soil mixed with weeds near a walnut tree, 7 October 2007 in Cheshmeh-Ali spring, soil mixed with weeds, in Darvar village, soil at the margin of a sike.

Distribution: Europe, Southern and Northern Africa: (Athias-Henriot 1959; Karg 1993). North America: (Walter & Lindquist 1989). Iran: (Kamali *et al.* 2001).

15. *Proctolaelaps* sp. nov.

1 female, 31 July 2006 in Mohammad Abad village, soil in a pistachio orchard.

16. *Proctolaelaps pygmaeus* (Müller, 1859)

36 females, 26 May 2006 in Tooyadarvar village, decaying leaves of walnut tree, 4 June 2006 in Dibaj, soil mixed with weeds near a Almond tree,

13 September 2006 in Forat village, soil mixed with weeds at the margin of a sike, 26 September 2006 in Dibaj, decaying apple fruit fallen on the ground in an apple orchard, in Dibaj near Imamzadeh, decaying trunk of a willow tree, 8 July 2007 in Damghan, soil mixed with weeds near a walnut tree.

Distribution: Australia: (Halliday *et al.* 1998). Poland: (Haitlinger 1983; Haitlinger 1989a; Haitlinger 1989b). North America: (Chant 1963). Iran: (Kamali *et al.* 2001).

17. *Proctolaelaps scolyti* Evans, 1958

12 females, in Forat village, soil mixed with weeds at the margin of a sike, 15 September 2006 in Tazareh village, decaying matter of an alive poplar with woodborer galleries.

Distribution: England: (Evans 1958). Iran: (Kamali *et al.* 2001).

18. *Protogamasellus massula* (Athias-Henriot, 1961)

23 females, 18 May 2006 in Tooyehdarvar village, soil near an oleaster trunk, 26 May 2006 in Tooyadarvar village, decaying poplar trunk with woodborer galleries, in Tooyehdarvar village, decaying leaves of walnut, litter near a sumac tree, contents of woodborer galleries on yellow plum, soil in wheat farm, decaying matter of an oleaster tree, 8 September 2006 in Cheshmeh-gholghol spring, soil near a berberry, 12 September 2006 in Agareh village (Sattari`s orchard), decaying wood trunk of an alive willow, 26 September 2006 in Dibaj, soil near an almond tree, in Damghan, soil near a walnut tree, 18 October 2006 in Tooyehdarvar village, soil, 15 November 2006 in Rashm village, moss at the margin of a sike.

Distribution

North Africa: (Karg 2007; Evans 1982). Ausralia: (Halliday *et al.* 1998). Iran: (Kamali *et al.* 2001).

19. *Protogamasellus minor (Karg, 1962)**

10 females, 12 September 2006 in Agareh village (Sattari`s orchard), decaying matter of a willow tree.

Distribution

North Africa: (Karg 2007). Germany and Ireland: (Evans 1982).

20. *Protogamasellus* sp. nov.

2 females, 9 May 2006 in Damghan, soil in a pistachio orchard, 26 September 2006 in Damghan, soil near a walnut tree.

21. *Protogamasellus primitivus* Karg, 1962

28 females, 26 May 2006 in Tooyehdarvar village, litter near a sumac tree, 31 July 2006 in Mohammad Abad village, soil mixed with weeds on a pistachio orchard, 11 August 2006 in Cheshmeh-Ali roadside, soil mixed with weeds near a cherry tree, 26 September 2006 in Damghan, soil in a park, in Dibaj, soil in barley farm, 6 October 2006 in Zarrin Abad village, soil near a willow tree at the margin of a sike, 15 November 2006 in Rashm village, moss at the margin of a sike.

Distribution: Europe: (Karg 2007; Karg 1993; Evans 1982). Australia: (Halliday *et al.* 1998). Ethiopia: (Genis *et al.* 1967). Tanzania: (Hurlbutt 1971). Iran: (Karg 2007; Kamali *et al.* 2001).

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ORIBATID FAUNA IN NORWAY SPRUCE STUMPS. ARE THERE SAPROXYLOPHILIC ORIBATID SPECIES?

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Abstract

The study was designed to collect background information about oribatid communities in deadwood of stumps which is a dominant form of CWD (Coarse woody Debris) in managed forests. Four spruce stumps in the mixed forest of the Wapienica Valley (Beskid Śląski Mountains, West Carpathians) were examined for oribatid mites. Samples were also collected from the adjacent forest floor. A total of 2,960 oribatid mites belonging to 92 species were collected in 128 samples. The highest abundance and species richness were recorded in stumps in middle classes of decay. The abundance was significantly higher in all stumps than in the forest floor. Furthermore, the general number of species in stumps (73) was also higher than in neighbouring litter and soil. Some oribatid species may be regarded as saproxylophiles as 29 species were obligate members of deadwood, among other dominants.

Key words

Stump, dead wood, Oribatida

Introduction

Temperate forest soils house a multitude of animal species comparable to tropical rain forests (Anderson 1975). Some reasons for the coexistence and diversity of similar species, despite the same food requirements, may be the high spatial heterogeneity of forest floor (Sulkava & Huhta 1998). Microhabitats present on small scale in the forest floor in the form of moss cushions, fungal mycelium, needles, decaying cones, excrements, significantly increase spatial heterogeneity. Coarse woody debris (CWD – any woody material >2.5 cm in diameter according to Harmon *et al.* 1986) is another important type of microhabitat in forests. The main sources of coarse woody debris are fallen logs in natural forests. However, most of dead wood is systematically removed from managed forests and cut stumps becoming important type of CWD in this type of forests.

Some investigations considered wood of varying stages of decay as a poor substrate with low oribatid densities and a small amount of species (Seastedt et al. 1989, Johnston & Crossley 1993), while others considered decaying wood as a oribatid species-rich microhabitat (Skubała & Sokołowska 2006, Skubała & Duras in press). In this study we compare the fauna of four cut spruce stumps and neighbouring forest floor in the mixed forest of the Wapienica Valley, focusing on the following three points:

1. Do cut spruce stumps house a more diverse oribatid fauna than forest floor?
2. What decay class of cut stumps is settled most abundantly by oribatid mites?
3. Are some oribatid species restricted to cut stumps?

Material and Methods

The investigations were undertaken in "The Wapienica Ecological Park", in the Wapienica Valley which is surrounded by the Beskidy Mountains, Western Carpathians. It represents the nature-landscape complex: a new form of nature and landscape protection in Poland established in 1991. The Wapienica Ecological Park nature-landscape complex has considerable phytosociological diversity and a high proportion of vegetation communities. Other valuable natural features of that area are the presence of huge trees designated as monuments of nature, and many species of vascular plants which are protected in Poland. However, the vegetation is endangered by a high level of pollution originating mainly from Silesia. There is impoverished biodiversity caused by the elimination of deadwood as a result of forest management practices (Orczewska & Wilczek 2001). The research was conducted in the mixed forest on slope of the Szyndzielnia Mountain. A 10 x 10 m plot was selected in the best typical fragment of the forest. The study area lies in lower montane belt at the elevation of 700 m a.s.l. ((49^{0}39' N, 18^{0}58' E) and at the northern hillside (10º inclination). The stand is dominated by the Norway spruce *Picea abies* with single individuals of beech *Fagus silvatica*.

Four Norway cut spruce stumps have been selected. Cut stumps were classified into classes I-IV adopting the decay class system proposed by Maser *et al.* 1979 (Table 1). The height of stumps and diameter of cut surface ranged from 30-35 cm and 40-45 cm, respectively. Four samples of decaying wood (~45 g of dry matter each) were taken randomly from each cut stump at four seasons (8th November 2004, 21st February 2005, 23rd May 2005 and 19th September 2005). Spacing between stumps was at least 10 m to ensure spatial independence of the data. Twelve soil and litter samples were additionally collected from the homogenous site in the nearest surroundings of stumps at each sampling date. Samples were taking using a corer of 4.8 cm diameter to a depth of 10 cm. The topsoil layer included the litter layer. The area was comparatively flat. Overall, 2,960 specimens of Oribatida belonging to 92 species were collected in 128 samples. Mites were extracted from the samples using a modified Tullgren extractor, the procedure lasting 6 days or until the wood was thoroughly dried. Adult oribatids were identified to species using a compound microscope. The distribution of the species and the number of individuals collected from stumps and forest floor are listed in Appendix I. The systematic ordination proposed by Subias (2004) was followed.

Table. 1. Decay class system of cut stumps in the mixed forest of the Wapienica Valley

Characteristics of cut stump	Decay class			
	I	II	III	IV
Bark	Intact	Intact	Trace	Absent
Texture	Intact	Hard, large pieces	Soft pieces	Soft and powdery
Cut surface	Original	Original but porous	Start to disintegrate	Almost absent
Color of wood	Original	Becoming brown	Light brown	Red/dark brown

Four univariate measures were used to assess community structure: abundance, total species richness per unit area, Shannon index of diversity (H') and equitability (J). The differences in the abundance of oribatid mites between sites, between collection date (seasons) and site by date interactions were tested by two-dimensional analysis of variance (ANOVA). Data were log transformed to minimize violations of parametric statistics. When a statistically significant difference ($p < 0.05$) was noted differing pairs were identified with the LSD *post-hoc* test. Levene's test was used to verify homogeneity of variance. Correspondence analysis (CA) was chosen as the ordination method to explore the compositional variation between microsites. We restricted the interpretation to the ordination space determined by the first two axes. Rare species (less than 10 individuals) were removed from the analysis because they do not improve the CA analysis and this was confirmed in an initial analysis with all species. The numbers of individuals were log (x+1) transformed and equal weight was applied to all species. All statistical calculations for this research were done in STATISTICA 7.1 software.

Results

In total 2,131 specimens of Oribatida were collected from stumps and 829 individuals originated from forest floor. Table 2 gives the calculated F-values after two-way ANOVA analysis and the confidence level for two sources of variation – site and season. The mean abundance of oribatids in spruce stumps varied from 24.5 individuals per 100 gram of dry weight of substrate (stump I) to 140.7 indiv./100 g d.w. (stump II) (Table 2). In older stumps it was slightly lower, however, the differences were not statistically significant ($p<0.05$, LSD test). With regard to adult and juveniles stages, the tendency was similar. However, the abundance of juveniles was generally low at all sites and reached the highest value at stump III. It is noteworthy that the abundance of oribatids from the top 10 cm of litter and soil of the adjacent forest floor (14.7 indiv./100 g d.w.) was significantly lower than the abundance in all stumps ($F=15.648$, $p=0.0000$). The two-way ANOVA revealed significant differences in oribatid abundance between seasons ($F=6.741$, $p=0.0003$) and also significant difference in the interaction of these two variables was observed ($F=6.011$, $p=0.0000$). The results of the analysis of variance were similar for adults and juveniles.

Stump II displayed the highest species richness (52 species) and the lowest richness was observed in stump I (34 species) (Table 2). The general number of species recorded from forest floor (62 species) was higher than in one particular stump. It is noteworthy that the total number of species from four stumps (73 species) was higher than the number of species from all the samples from forest floor. Out of a total of 92 species, 44 (47 %) were common to decaying stumps and forest litter and soil. There were 29 and 19 exclusive species collected in stumps and forest floor, respectively. Similarly the Shannon index (H') of oribatids was the highest in stump II, followed by stump III. It was slightly lower in other stumps and of medium value in the forest floor. With regard to the evenness (J), the relationship was more or less similar. In general the value of diversity indices was high and it did not varied between sites (Table 2).

Table 2. General characteristics of oribatid mite communities in dead wood of four stumps and adjacent forest floor in the "Dolina Wapienicy". Mean abundance of oribatids (indiv./100 g d.w. ± S.E.) is tested by two-way analysis of variance. The results are compared by the LSD *post-hoc* test for differences between sites.

	Source of variation	Habitat					ANOVA	
		Stumps				Forest floor		
		I	II	III	IV		F	p
Total Abundance	**H**	24.5 ± 6.3 [a]	140.7 ± 25.2 [b]	117.0 ± 30.9 [b]	89.8 ± 32.6 [ab]	14.7 ± 2.7 [a]	**15.648**	**0.0000**
	S						**6.741**	**0.0003**
	H x S						**6.0112**	**0.0000**
Abundance of adults	**H**	22,3 ± 5.5 [a]	132.5 ± 23.7 [b]	104.7 ± 27.0 [b]	82.8 ± 31.2 [ab]	11.8 ± 2.2 [a]	**16.587**	**0,000**
	S						**6.495**	**0,0004**
	H x S						**6.4259**	**0,0000**
Abundance of juveniles	**H**	2.1 ± 1.1 [a]	8.2 ± 2.3 [a]	12.3 ± 5.1 [b]	5.9 ± 2.8 [a]	2.9 ± 0.6 [a]	**3.739**	**0.0068**
	S						**5.271**	**0.0019**
	H x S						**2.154**	**0.0190**
Shannon index (H')		**2.391**	**2.921**	**2.885**	**2.228**	**2.697**		
Evenness index (J)		**0.678**	**0.739**	**0.762**	**0.627**	**0.654**		
Total species richness		**34**	**52**	**44**	**35**	**62**		
Number of exclusive species		**4**	**8**	**5**	**6**	**17**		

H – Habitat; S – Seasons; H x S: Habitat x Seasons

Bold typed values denote significant differences between abundances at the 0.05 and lower probability level. The results of the LSD test are given by letters. Means sharing a common letter (a or b) do not differ significantly from other means at the 5% level.

The proportion of oribatid mites collected from decaying stumps (total number of mites) varied from 77.3% (stump IV) to 86.6% (stump II). It is noteworthy, that the proportion of oribatid mites in the total number of mites was slightly lower in the forest floor (72.5%) than in each of the stumps.

Ordination by Correspondence Analysis (CA) was used to assess community similarities and relations between species and communities in stumps and litter and soil. The scores for the first 2 axes are shown in Fig. 1. The eigenvalue (the dispersion of the sites/species distribution along the ordination

axis) was significant for axis 1 (λ_1 = 0.467) and axis 2 (λ_2 =0.317). Ordination axes are considered as significant when their eigenvalue is higher than 0.3 (Dekkers *et al.* 1994). Over 63% of the variance was explained by the first two axes. Overall, oribatid mites from stumps and forest floor are well separated on the ordination plot (Fig. 1). Both axes (1 and 2) appear to differentiated between the oribatid fauna in forest floor from the fauna in cut stumps. The oribatid species of stumps, especially those associated with stump I, II and III, were closely clumped together. Species associated with forest floor are ordinated along the negative part of axis 1 and the positive part of axis 2. They were species of five different families: Oppiidae (*Rhinoppia subpectinata*), Autognetidae (*Conchogneta willmannii*), Suctobelbidae (*Suctobelbella (S.) subtrigona*), Oribellidae (*Pantelozetes paolli*), and Euphthiracaridae (*Acrotritia duplicata*).

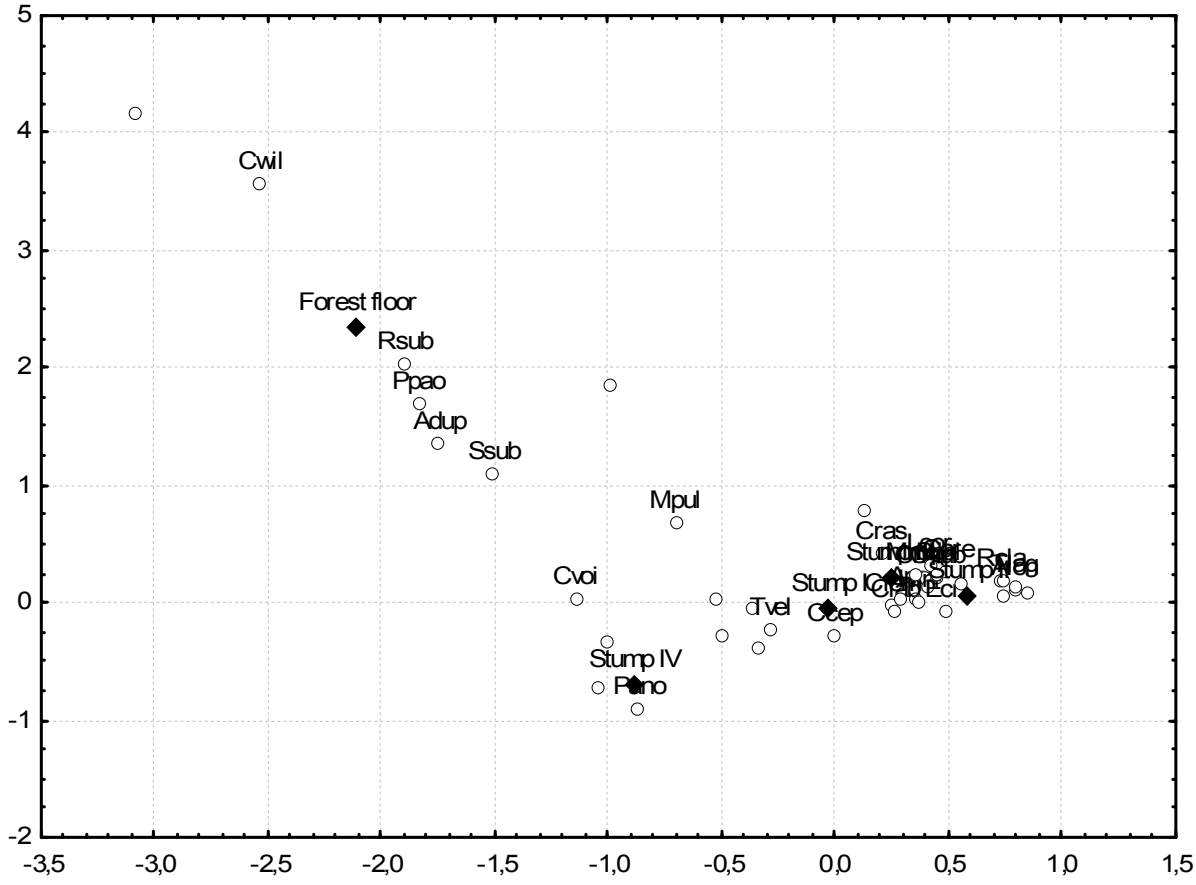

Figure 1. Correspondence analysis (CA) biplot showing 40 oribatid species and arrangements of 5 plots in the "Dolina Wapienicy". Codes: see Appendix I

In order to evaluate relationships between species abundances and four cut stumps a correspondence analysis (CA) was performed omitting data from forest floor (Fig. 2). Eigenvalues of axes 1 and 2 were λ_1 = 0.430 and λ_2 =0.284, respectively. Over 80% of the variance was explained by the first two axes, so there is little need to bother about further axes. Overall, axis 1 appears to differentiate between stumps, however, a gradient from young to older stumps was not reflected. The species and sites are grouped into 4 clusters, however, some species show no preference for any site and were distributed among those clusters. Species associated with the oldest stump were grouped on the right side of axis 1. They were the same species which were found characteristic of forest floor (besides *C. willmannii*) and *Chamobates (Xiphobates) voigtsi* and *Phthiracarus (Archiphthiracarus) anonymus*. Species associated with stump II and III were distributed along the negative part of axis 1. Axis II well differentiated between fauna of these two stumps. Species associated with stump II were distributed in the upper part of axis II. They were representatives of

four families: Oppiidae (*Ramusella (R.) clavipectinata)*, Autognetidae (*Autogneta (A.) longilamellata)*, Protoribatidae (*Transoribates lagenula*), Euphthiracaridae (*Euphthiracarus (E.) cribrarius*). Species associated with stump III were located in the negative part of axis II. They were namely: *C. willmannii* (species characteristic of forest floor) and *Carabodes (C.) areolatus* (Carabodidae), (Carabodidae), *Caleremaeus monilipes* (Platyameridae), *Liacarus (L.) coracinus* (Gustaviidae) and *Melanozetes mollicomus* (Ceratozetidae). Many species do not show significant preference for any stump, among them dominants species, e.g. *Adoristes poppei* (Liacaridae), *Hemileius (H.) initialis* (Hemileiidae), *Scheloribates (S.) pallidulus* (Scheloribatidae) (Fig. 2).

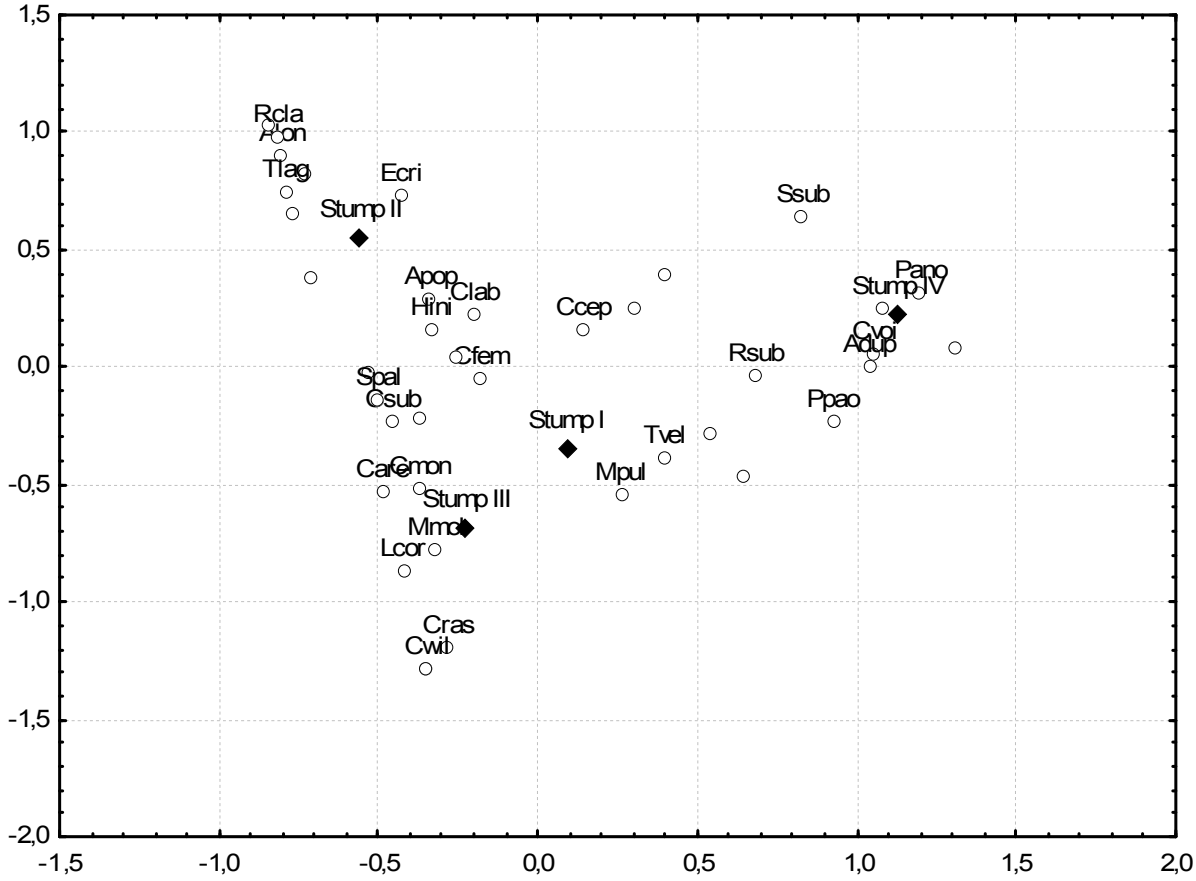

Figure 2. A plot of the first two axes of correspondence analysis (CA) of the 39 species and 4 stumps in the "Dolina Wapienicy". Codes: see Appendix I

Discussion

Oribatids are among the most characteristic elements of soil fauna. Possibly they play an important role in microsites related to the ground, e.g. decaying stumps. The food base for most oribatid mites consists of fungal hyphae (abundant in decaying wood), although some species appear to use leaf litter or woody materials directly. It is worth mentioning that the calcium-rich bark of decaying wood may be especially important for oribatids, which use calcium oxalate or calcium carbonate as cuticular hardening agents (Norton & Behan-Pelletier 1991).

The oribatid mite fauna from spruce cut stumps was quantitatively and qualitatively studied and compared with oribatid populations in the adjacent forest floor. The results indicate that decaying wood of stumps is not a poor substrate for oribatid mites. The average abundance of oribatids in stumps was almost two times (the youngest stump) to even ten times higher than in nearby litter and soil (14.7 ind./100 g d.w.). Furthermore, eleven more species were recorded from four decaying stumps (73 species) than from the top 10 cm of litter and soil of adjacent forest floor.

Some authors found the mite fauna in dead wood to be impoverished in comparison with that found in forest litter and soil, e.g. Seastedt *et al.* (1989) or Johnston & Crossley (1993). Seastedt *et al.* (1989) recorded 2-10 times lower abundance of microarthropods in woody debris forest litter and soil than in equivalent amount of forest litter and soil woody debris. However, our findings are in line with studies on mite fauna in dead spruce or beech logs by Skubała & Sokołowska (2006) or Skubała & Duras (in press), respectively. The average abundance of oribatids in forest floor was lower than the number of oribatids in log IV and only slightly higher than in other decaying spruce logs, however, the differences were not significant (Skubała & Sokołowska 2006). Similarly, the abundance of oribatids in beech logs (III class of decay) was two times higher than from the top 10 cm of litter and soil (Skubała & Duras in press). Many authors, whose studied similar habitats, recorded lower number of species, e.g. Travè (2003) for dead beech wood (68), Seastedt *et al.* (1989) for tree trunks at various stages of decay (22), Johnston & Crossley (1993) and Fager (1968) for coarse woody debris on forest floors (60 and 26, respectively) or Wunderle (1992) for dead wood on the beech forest floor (50). However, Skubała & Sokołowska (2006) recorded 80 oribatid species for spruce logs and Skubała & Duras (in press) found 86 species in beech logs.

Oribatids made up a slightly higher proportion of the acarofauna in cut stumps than in forest floor. Previously I have found similar proportion of oribatids in beech logs (Skubała & Duras in press) or spruce logs (Skubała & Sokołowska 2006) and in adjacent forest floor. It may imply that oribatids in decaying stumps are subjected to similar microclimatic conditions and develop at similar rate.

Spruce stumps of the 2nd class of decay represent a most rewarding habitat for oribatid mites. However, the difference in abundance and species richness between stump II, III and IV was not significant. These results may be surprising as, dead wood in the middle of a decay sequence (class III) is characterised by the greatest heterogeneity (Pyle & Brown 1998). Decaying wood in the third class of decay is characterised by comparatively high moisture. The number of fungi (many oribatids are fungivorous) increased during decomposition of wood and the highest diversity of fungi is observed in logs III and IV (Maser & Trappe 1984, Bader *et al.* 1995). And most authors recorded the most diverse mite fauna in the III or IV class of decay, e.g. Skubała & Sokołowska (2006) for spruce logs or Seastedt *et al.* (1989) for woody debris. Maser & Trappe (1984) stressed that the mite fauna began to flourish as a fallen tree approached class IV. Setäla *et al.* (1995) found moderately decayed stumps as the most collembolan species-rich microhabitat. Why the most abundant and diverse oribatid fauna was recorded in comparatively less decayed spruce stumps in the study? It may be caused by the presence of both arboreal oribatid mites and mites associated with decaying organic matter. Arboreal oribatid mites have been studied intensively during last decade (Erdmann *et al.* 2006). And it was proved that the oribatid fauna differs markedly in canopy/ground comparison studies (Lindo & Winchester 2006).

As regards the species composition of oribatid communities from spruce stumps, it differs markedly from that on the forest floor (indicated by the correspondence analysis). Stump specificity was approximately 32% and stumps held in common only 44 species with the forest floor. The dominant species found in stumps were generally different than those recorded in forest floor. Twenty-nine species, some of them dominant ones (e.g. *Carabodes (C.) areolatus*, *Melanozetes mollicomus*, *Transoribates lagenula*) were unique to stumps. *Carabodes (C.) areolatus* is known from forests, preferring dry habitats, *M. mollicomus* is described from forests and bogs and *T. lagenula* is known from acid soils (Weigmann 2006). As regards *T. lagenula*, the species was previously recorded only twice from Poland, from a rocky site (Olszanowski *et al.* 2006) and spruce logs (Sokołowska & Skubała 2006)

There were several other dominant species from spruce stumps, which were recorded rarely outside decaying stumps, e.g. *Adoristes poppei*, *Caleremaeus monilipes*, *Hemileius (H.) initialis*, *Liacarus (L.) coracinus*, *Scheloribates (S.) pallidulus*, *Euphthiracarus (E.) cribrarius* and *Phthiracarus (Archiphthiracarus) anonymus*. All these species are characteristic of forest ecosystems (Weigmann 2006). *C.monilipes* is considered as an arboricol species (Weigmann 2006). It was previously recorded only in three regions in Poland (Olszanowski *et al.* 1996, Skubała & Sokołowska 2006). Only three species known as macrophytophages or herbivorous grazers, e.g. *Phthiracarus anonymus*, *Euphthiracarus cribrarius* and *Caleremaeus monilipes* were represented in this group (Siepel & Ruiter-Dijkman 1993). These species are known as having cellulase and can feed on living plants as well as on litter and are in that case important as comminutors in the decomposer community. Skubała & Duras (in press) observed that macrophytophages / herbivorous grazers

comprised a small minority of the fauna of decaying beech logs. And the dominant species in logs appeared to be mycophagous species. Some preferences of most oribatid species for a stump in a particular stage of decay were detected, e.g. *Transoribates lagenula* – stump II or *Phthiracarus (A.) anonymus* – stump IV. However, they were not strong. On the other hand, out of nineteen species (e.g. *Acrotritia ardua*, *Metabelba (Parametabelba) italica*, *Suctobelbella (S.) similis*, *Suctobelbella (S.) vera*) did not emigrate from the adjacent forest floor into the spruce stumps. However, all these species were recorded in low density.

Johnston & Crossley (1993) also observed that some species of mites use CWD exclusively. However, they considered that fallen logs in forest floor habitats play a role as a refuge for mite species normally occurring in forest soil. Seastedt *et al.* (1989) found only a few species restricted to decaying wood, e.g. *Microtritia*, *Gehypochthonius* and *Epilohmannia*. On the other hand Skubała & Sokołowska (2006) and Skubała & Duras (in press) recorded thirty-two (40%) and forty-nine (44%) species as restricted to dead wood of spruce and beech logs, respectively. Skubała & Sokołowska (in press) recorded fifty-five species (of 131 in total) as obligate members of intra-log community. Even some dominant species in wood, e.g. *Anachipteria deficiens*, *C. monilipes*, *Lauroppia maritima* and *Melanozetes meridianus* were not generally the same species that dominate the fauna of litter and soil.

In conclusion, oribatid fauna of spruce stumps contributes significantly to overall forest biodiversity. If we want to assess overall biodiversity of mites in a forest ecosystem we need to sample multiple microhabitats. If we exclude decaying stumps from the analysis almost 1/3 of oribatid species would not be recorded. Some oribatid species may be described as saproxylophiles, forms which prefer decaying wood instead of organic dead material in the top 10 cm in forest floor. However, it is noteworthy to remember that species, which were confined to decaying stumps in the present study are known from other habitats. Therefore, they cannot be considered as restricted to dead wood in general.

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Appendix I

Check-list of oribatid species recorded in dead wood of spruce stumps and in the litter and forest floor in the "Dolina Wapienicy". Numbers indicate the absolute number of individuals found at each site.

Species	Codes	Stumps				Stumps Total	Forest floor
		I	II	III	IV		
Acrogalumna longipluma (Berlese)		-	-	-	-	-	2
Acrotritia ardua (Koch)		-	-	-	-	-	11
Acrotritia duplicata (Grandjean)	Adup	1	2	9	15	27	248
Adoristes poppei (Oudemans)	Apop	15	25	5	2	47	5
Allosuctobelba grandis (Paoli)		-	1	-	-	1	1
Atropacarus striculus (Koch)		-	1	-	-	1	-
Autogneta (A.) longilamellata (Michael)	Alon	1	27	1	-	29	1
Autogneta (A.) parva Forsslund		-	-	1	1	2	-
Berniniella (B.) sigma (Strenzke)		-	-	-	-	-	1
Caleremaeus monilipes (Michael)	Cmon	5	40	77	4	126	2
Camisia (C.) biurus (Koch)		-	-	-	-	-	2
Camisia (C.) spinifer (Koch)		1	-	-	-	1	1
Carabodes (C.) areolatus Berlese	Care	2	64	114	1	181	-
Carabodes (C.) femoralis (Nicolet)	Cfem	5	19	15	4	43	1
Carabodes (C.) labyrinthicus (Michael)	Clab	18	17	2	2	39	1
Carabodes (C.) ornatus Štorkan		-	9	10	1	20	-
Carabodes (C.) reticulatus Berlese		-	1	-	-	1	-
Carabodes (C.) subarcticus Trägårdh	Csub	1	18	19	1	39	3
Carabodes (C.) tenuis Forsslund		-	11	8	2	21	2
Cepheus cepheiformis (Nicolet)	Ccep	5	18	10	8	41	-
Cepheus dentatus (Michael)	Cden	6	2	2	-	10	-
Cepheus latus Koch		1	-	-	-	1	-
Ceratoppia bipilis (Hermann)		-	-	-	1	1	-
Ceratoppia sexpilosa Willmann		-	-	-	3	3	-
Chamobates (C.) cuspidatiformis (Trägårdh)		-	-	7	4	11	3
Chamobates (C.) pusillus (Berlese)		-	-	-	-	-	1
Chamobates (Xiphobates) rastratus (Hull)	Cras	6	-	23	-	29	7
Chamobates (Xiphobates) voigtsi (Oudemans)	Cvoi	1	1	3	6	11	26
Conchogneta willmanni (Dyrdowska)	Cwil	-	-	1	-	1	49
Cosmogneta sp.		-	-	3	-	3	-
Cultroribula bicultrata (Berlese)		-	3	-	-	3	1
Damaeus (Paradamaeus) clavipes (Hermann)		-	1	3	4	8	-
Dameobelba minutissima (Sellnick)		-	-	-	-	-	1
Dissorhina ornata (Oudemans)		-	3	1	-	4	6
Dometorina plantivaga (Berlese)		2	1	-	-	3	-
Hypochthoniella minutissima (Berlese)		-	-	1	-	1	-
Eueremaeus foveolatus (Hammer)		-	1	-	1	2	1

Eueremaeus oblongus (Koch)		-	15	-	-	15	-
Eupelops acromios (Hermann)		2	1	-	-	3	-
Eupelops torulosus (Koch)		3	-	1	-	4	-
Euphthiracarus (E.) cribrarius (Berlese)	Ecri	-	204	21	21	246	1
Fuscozetes setosus (Koch)		-	-	-	-	-	1
Galumna (G.) lanceata (Oudemans)		-	-	-	-	-	1
Graptoppia (Apograptoppia) foveolata (Paoli)		1	-	-	-	1	1
Hemileius (H.) initialis (Berlese)	Hini	17	11	-	-	28	1
Heminothrus (Platynothrus) peltifer (Koch)		-	-	-	-	-	2
Hypochthonius rufulus Koch		-	-	2	5	7	4
Lauroppia falcata (Paoli)		-	-	-	4	4	4
Lauroppia maritima maritima (Willmann)		2	-	-	-	2	-
Liacarus (L.) coracinus (Koch)	Lcor	3	12	48	-	63	1
Licneremaeus licnophorus (Michael)		-	-	3	-	3	-
Liochthonius (L.) propinquus Niedbała		3	2	-	1	6	4
Liebstadia longior (Berlese)		-	1	1	-	2	-
Melanzetes meridianus Sellnick		-	-	-	-	-	1
Melanozetes mollicomus (Koch)	Mmol	-	12	44	2	58	-
Metabelba (Parametabelba) italica (Sellnick)		-	-	-	-	-	5
Metabelba (M.) pulverulenta (Koch)	Mpul	1	1	6	2	10	24
Minunthozetes pseudofusiger (Schweizer)		-	1	-	-	1	2
Moritzoppia (M.) unicarinata (Paoli)		4	10	6	-	20	-
Nanhermannia (N.) nana (Nicolet)		-	1	1	3	5	7
Nothrus silvestris Nicolet		-	-	2	-	2	12
Ophidiotrichus vindobonensis Piffl		-	-	-	-	-	1
Oppia *sp.*		-	1	-	-	1	1
Oppiella (O.) nova (Oudemans)		2	5	1	3	11	13
Oribatella (O.) calcarata (Koch)		2	-	-	-	2	-
Oribatella (O.) sexdentata Berlese		2	10	-	-	12	-
Oribatula (O.) tibialis (Nicolet)		-	-	-	-	-	1
Oribatula (Zygoribatula) exilis (Nicolet)		3	5	3	1	-	3
Pantelozetes paolii (Oudemans)	Ppao	-	-	1	1	2	23
Phthiracarus (Archiphthiracarus) anonymus Grandjean,	Pano	2	36	28	128	194	5
Punctoribates (P.) punctum (Koch)		-	-	-	-	-	1
Quadroppia (Coronoquadroppia) monstruosa Hammer,		-	1	5	-	6	-
Quadroppia (Q.) quadricarinata (Michael)		-	-	-	1	1	-
Ramusella (R.) clavipectinata (Michael)	Rcla	1	20	-	-	21	4
Ramusella (Rectoppia) fasciata (Paoli)		-	3	-	-	3	-
Rhinoppia obsoleta (Paoli)		-	-	-	-	-	1
Rhinoppia subpectinata (Oudemans)	Rsub	3	1	1	2	7	78
Scheloribates (S.) pallidulus (Koch)	Spal	23	49	37	-	109	16
Suctobelba discrepans Moritz		-	2	8	5	15	-
Suctobelba trigona (Michael)		-	5	2	-	7	2

Suctobelba sp.		-	1	-	-	1	7
Suctobelbata prelli (Märkel y Meyer)		-	12	2	-	14	1
Suctobelbella (F.) alloenasuta Moritz		1	1	-		2	1
Suctobelbella (S.) longicuspis longicuspis Jacot		-	-	-	1	1	2
Suctobelbella (S.) perforata (Strenzke)		-	1	-	-	1	-
Suctobelbella (S.) sarekensis (Forsslund)		-	-	-	2	2	4
Suctobelbella (S.) similis (Forsslund)		-	-	-	-	-	4
Suctobelbella (S.) subtrigona (Oudemans)	Ssub	-	1	-	1	2	14
Suctobelbella (S.) vera (Moritz)		-	-	-	-	-	3
Suctobelbella sp.		-	-	-	-	-	1
Tectocepheus velatus velatus (Michael)	Tvel	106	22	82	48	258	36
Transoribates lagenula (Berlese)	Tlag	-	92	11	-	103	-

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TAXONOMIC DATABASES AND THEIR USE FOR THE BIODIVERSITY ASSESSMENT OF PHYTOSEIIDAE (ACARI: MESOSTIGMATA)

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Abstract

Phytoseiidae is the most diverse family within the order Mesostigmata, comprising 2,068 valid species recorded from all over the World since 1839. The World Catalogue edited by Moraes *et al.* in 2004 is the most recent and complete data compilation on this family. These data are of critical importance for taxonomists but also for incorporating mites into biodiversity research and conservation decisions. Here, we discuss the use of such a database, explaining methodologies and some significant results on Phytoseiidae diversity. Different aspects were developed: estimating global species diversity, orienting future collections, assessing description rates in relation to geographical factors and body size, studying evolutive historical relationships and assessing biodiversity in hotspot areas. Phytoseiidae have been described from 118 countries of the 192 recognised nowadays. The cumulative number of species descriptions has not yet reached an asymptote; so, no accurate estimation could be made of the number of species to be discovered in the future. The correlation between time and the mean body size of the mites described was significantly positive, emphasing the importance of mite size in their discovery pattern. Studies on Phytoseiidae distribution and endemism in biogeographic regions show large numbers of species and genera were observed in the old Gondwana continent and especially in the Neotropical area. High endemism rates at the species and genus levels were also observed in the Gondwana, especially in the Neotropical, Australasian and Ethiopian regions. Several biogeographical scenarios on the evolution of this group have been proposed from these data. Finally, studies on Phytoseiidae mite diversity in hotspot areas show that these zones are a great reservoir of Phytoseiidae diversity, just as they are for vertebrates and plants. Correlations between plant, vertebrate, mite diversity and endemism, as well as congruence rates between endemism levels of the three groups of organisms suggest that the biodiversity patterns of plants and vertebrates mirror those of the Phytoseiidae (both for endemicity and species richness). Perspectives for other macro-ecological studies are presented.

Key-words

Phytoseiidae, database, taxonomy, hotspot, biogeography

Introduction

The Phytoseiidae Berlese is a mite family belonging to the order Mesostigmata (Chant & McMurtry, 1994). Several species of Phytoseiidae are well known as predators of phytophagous mites (Kostiainen & Hoy, 1996; McMurtry & Croft, 1997) although they share particular and narrow relationships with their plant support (Agrawal & Karban, 1997; Kreiter *et al.*, 2002). More than 1,982 species of Phytoseiidae were described in 2004 (Moraes *et al.*, 2004) and more than 2,068 are recorded today. They are divided into three subfamilies (Amblyseiinae, Phytoseiinae and Typhlodrominae) and 90 genera, defined primarily according to idiosomal chaetotactic patterns (Chant, 1993; Chant & McMurtry, 2003a,b; Moraes *et al.,* 2003; Ragusa, 2003; Chant & McMurtry,

2004a,b; Moraes *et al.,* 2004; Chant & McMurtry, 2005a,b,c; Chant & McMurtry, 2006a,b; Kreiter *et al.,* 2006). Data on Phytoseiidae taxonomy, and biology are widely spread across different sources, except for the paper version of the world catalog published in 2004 by Moraes *et al.* However, some data are lacking from this catalogue, such as host plants and recent modifications in genera and tribe taxonomy (Chant & McMurtry, 2007). Data compilation on biodiversity has various utilities: to help in conservation, to retrieve specimens, to design biodiversity priorities and complete data on sibling groups, to determine factors affecting biodiversity patterns, to apply knowledge in a phylogenetic context, etc. (Gaston *et al.*, 1995; Bisby *et al.*, 2002; Meier & Dikow, 2004, Sihvonen & Siljander, 2005). For these reasons, taxonomy and databases are now subject to international efforts in order to create web-accessible tools that log living diversity and attempt to integrate specimen inventories, morphology, and biogeography. Numerous relational database applications have arisen integrating species, specimen numbers, geographic ranges, literature citations, images and other information (i.e. Fauna Europea, Zoological Record, GBIF: Global Biodiversity and information facilities, BioCase: Biological Collection Access Service for Europe, ENBI: European Network Biodiversity Information, Genbank, Tree of Life, TDWG: Taxonomy Database Working Group, ITIS: Integrated Taxonomic, Information System, Species2000) (Sereno *et al.*, 2005). However, few databases are currently available for invertebrates, despite the fact that these organisms are the most diverse on the planet. We can thus wonder how the development of such databases could contribute to biodiversity research and conservation decisions (Myers *et al*., 2000). In this paper, we discuss the use of a database on Phytoseiidae for (1) estimating global species diversity and orienting future collection efforts, (2) assessing biodiversity hotspots for invertebrates and (3) linking information on biogeography and phylogeny.

Material and methods

Database compilation. Data on the Phytoseiidae were obtained from the most recent and complete world catalog of the group (Moraes *et al.,* 2004), and from more recent papers (Chant & McMurtry, 2003a,b; Moraes *et al.,* 2003; Ragusa, 2003; Chant & McMurtry, 2004a,b; Chant & McMurtry, 2005a,b,c; Chant & McMurtry, 2006a,b; Kreiter *et al.*, 2006). All available data were computerised in an Excel database and transferred to Access software. The information included in the database are genus, species, locality of description, plant of description, year of description, recorded localities (country, region and town) and years. Each record and description locality was assigned (1) to the Zoogeographic provinces defined by Wallace (1876): Nearctic, Neotropical, West Palaearctic, East Palaearctic, Oriental, Australasian and Ethiopian regions; and (2) to the 27 hotspots among the 34 defined by Mittermeier et al. 2005. For each locality, a comparison between maps from google Earth and maps provided at the web site (http://www.biodiversityhotspots.org/Pages/default.aspx) were performed to associate a locality to hotspot areas.

Estimating global species diversity and directing future collections. The cumulative number of species described was plotted for the successive years (from 1839 to 2007) in order to estimate the rate at which new species were added to the list of each area studied (world, biogeographic zones, hotspots areas, countries, etc through continued collecting effort. The asymptote value could be used to estimate the expected total number of species (Cabrero-Sañudo & Lobo, 2003; Thompson & Withers, 2003; Sihvonen & Siljander, 2005; Jimenez-Valverde et al., 2006). Information on the sampling coverage of the different regions could then be used to justify and guide future collection efforts.

The factors that determine the probability of a new species description have recently become an area of interest. The probability to find a new species could be linked to the species richness (favourable habitats) of the locality, to the size of the organisms (easy to see or not) and to the last sampling effort in the area (Allsopp, 2007). The compiled database contains information on the latitude and longitude of collection sites, as well as on the size of the species found. Regression analyses were therefore performed between the years of description, the latitude and the sizes of the phytoseiid mites in order to characterize the factors affecting geographic patterns of species descriptions.

Assessing biodiversity hotspots for invertebrates. Thirty-four biodiversity hotspots have been defined as areas (i) having lost at least 70 % of the original area covered by native vegetation (Myers *et al.*, 2000, Mittermeier *et al*., 2005) and (ii) having a high plant endemism level. Vascular plants and vertebrates are the main biodiversity indicators used in these areas (Myers, 1988; Myers et al., 2000). Indeed, according to this definition, little attention is given to invertebrates, even

though they represent the largest part of the biodiversity (Myers *et al*., 2000). The endemism rate of Phytoseiidae in each hotspot was assessed by dividing the total number of phytoseiid species only recorded for that hotspot by the total number of phytoseiid mites described. Correlation analysis using the Spearman's rank correlation test was carried out to assess relationships (1) between the number of Phytoseiidae species and the remaining vegetation surface, (2) between numbers of species of plants, vertebrates and Phytoseiidae, (3) between Phytoseiidae, plants and vertebrates endemism levels (Statsoft France, 2005). The data used for plants and vertebrates were obtained from Mittermeier *et al*. (2005). Congruence indices between the endemism rates of plant-Phytoseiidae, plant-vertebrates and Phytoseiidae-vertebrates were calculated as proposed by Myers *et al.* (2000).

Linking biogeography and phylogeny. Both species numbers and endemism levels in each biogeographic area were considered in this study. A PAE (parsimony analysis of endemicity) (Rosen, 1988; Crisci, 2001; De Grave, 2001) was carried out to determine relationships between the different regions according to the species shared (PAUP, version 4; Swofford, 1998) both for the entire family Phytoseiidae and for each subfamily. Concurrently Jaccard similarity indices were calculated between the regions, for the whole family and for each subfamily (Brown & Lomolino, 1998).

Results and discussion

Data completeness and directing future collection sites

The first Phytoseiidae species was reported in 1839 from Germany and a total of 2,068 species were discovered through to 2007, with a mean rate of description of 27.56 species per year (SE = 23.82). The description rate remained high until 1990 and then decreased, first between 1991 and 2000 and more drastically afterwards (description rate of new species/year: 26.57 between 2001-2007 vs. 50.5 between 1961-1970) (Figure 1a). However, the cumulative number of species descriptions has not yet reached an asymptote (Figure 1b) for either the whole family or the three sub-families. Phytoseiid mites have been described from 118 of the 192 currently recognised countries. In six countries (South Africa, China, Australia, India, USA, Pakistan), more than 100 species have been described. The highest number of species described is observed from Asia (507 species) and America (606 species). The highest numbers of species were described between the latitudes of 20-40° (38 % of species described up to now) and the lowest for latitudes 60-80° and –60-40°.

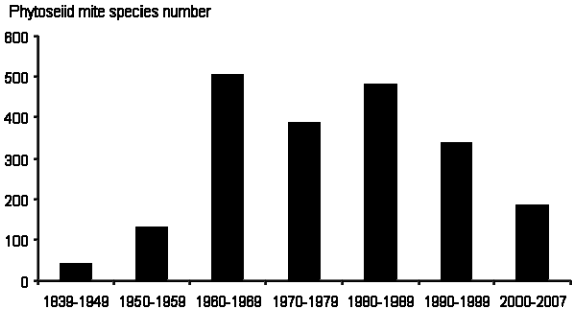

Figure 1a. Number of new species of Phytoseiidae described in each decade.

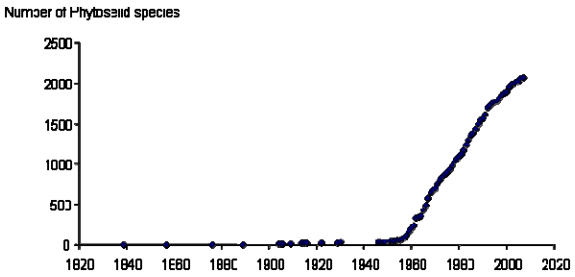

Figure 1b. Cumulative number of Phytoseiidae mite species described over time (1839-2007).

⇨ Therefore, no accurate estimation can be made of the number of species to be discovered in the future. An analysis for each of the seven latitude ranges considered show that the inventory of Phytoseiidae is far from complete, with no asymptoye yet approached. However, the analysis of the country-based distribution shows that some countries have been poorly prospected as well as the latitudes 60-80° and -40-60°.

A very low but significant correlation between mite size (body length) and year of description is observed (R^2=0.008, F(1, 1967)=16.41, P=0.00005). The largest phytoseiid mites were described in the first period (1839-1950) and their size then decreased significantly over time (Figure 2a). The mean size of Phytoseiidae were significantly different between the 7 latitude ranges considered (F(6,1947)=21.06, P=0.00001). The largest phytoseiid mite species were described from the range latitudes 60-80°, -40-60° and 40-60°. On the opposite end, the smallest species were described from the range latitudes -20-0°, 0-20° and 20-40°. (Figure 2b).

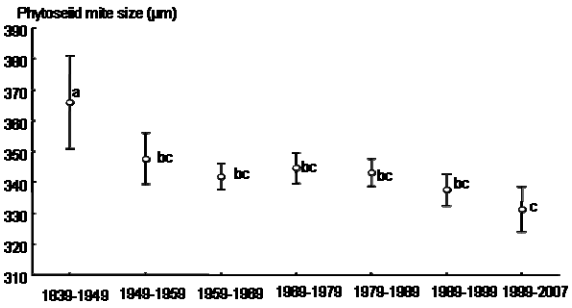

Figure 2a. Size of the Phytoseiidae species described during different decades (1839-2007). Letters show significantly different means (Newman and Keuls mean comparison test).

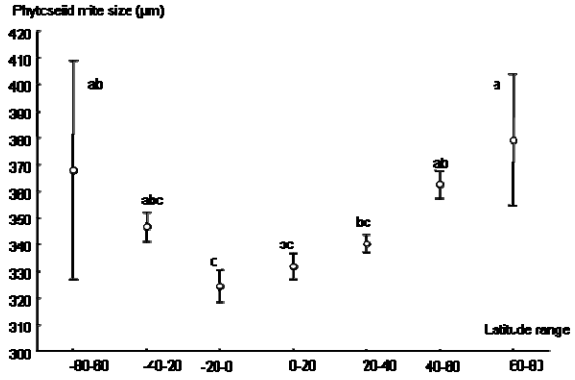

Figure 2b. Size of the Phytoseiidae species described at different latitude ranges. Letters show significantly different means (Newman and Keuls mean comparison test).

⇨ The body size (length) seems thus to affect mite description and we can therefore predict that in the future, mite species to be described will need special attention to find them due to their relatively small size. Furhermore, this special attention will be to be greater in latitude near the equator line.

Data on biogeography and stress in phylogeny

The numbers of species found in each biogeographical region ranges between 231 and 484, the highest values being observed in the Neotropical, Oriental and West Palaearctic regions (Table 1). Thus, the number of species is higher in the old continent Gondwana than in Laurasian regions. Endemic species are observed in all the present biogeographic regions. The highest levels were observed in the Neotropical and Oriental regions and, to a lesser extend, in the West Palaearctic region. In the other areas, the endemism rates are quite similar (Table 1). The number of genera found in each biogeographical region ranges between 26 and 56. Except for the Neotropical region, where the greatest number of genera was reccorded, this number is similar for all the biogeographic regions. Endemic genera are not observed in the East Palaearctic nor in the Nearctic region. The highest rates of endemism are observed for the Neotropical region and, ito a lesser extend, in the Ethiopian area.

⇨ Several evolutionary scenarios for the family, as well as for each sub-family, can be proposed. It will be too long to present all of them in this review (for details, see Tixier *et al.*, 2008). Some important points are however developped. Large numbers of species and genera were observed in Gondwana and especially in the Neotropical region. This could be due to the presence of the group in that area for a longer period than in other parts of the globe (i.e. more time for speciation). Hence, Gondwana could be the region where the family originated and from where it dispersed to other places. This assumes, however, that extant diversity has not moved beyond its place of origin. High endemism rates at the species and the genus levels were also observed in the past Gondwana. This could be explained by a Gondwanian origin of Phytoseiidae, especially in the Neotropics, followed by dispersal (Brown & Lomolino, 1998).

Data on biodiversity and conservation strategies

The cumulative numbers of species' descriptions in hotspot areas has not yet reached an asymptote (Figure 3) and the rate of increase since 1960 has remained steady over time. The cumulative numbers of species' descriptions in non hotspot areas shows the same trend (Figure 3). Our knowledge of Phytoseiidae fauna in hotspot and non hotspot areas could thus be considered as similar.

One thousand seven hundred and eighty five species were encountered in all the hotspots (Table 2). However, a same species was sometimes present in several hotspots. Thus, in total, 1,230 species were reported from at least one hotspot (62 % of the total number of species of Phytoseiidae). In non-hotspot areas, the number of species encountered was 1,319. These numbers are quite similar, despite lower sampling effort in the hotstpot areas and also a lower total surface covered. In addition, 987 species have been described from hotspots (45.2 % of the total diversity of Phytoseiidae), for 1,083 in non-hotspot areas. Some hotspots had particularly high numbers of species (Mediterranean Basin, Meso-America, Atlantic Forest, Caucasus) whereas others had relatively low(i.e., East Melanesian Islands, Southwestern Australia, New-Caledonia); Six hundred and sixty two species (30 % of the total

number of Phytoseiidae) are endemic to the 27 hotspots considered (Table 2), whereas 754 species are endemic to non-hotspot areas. Endemism levels ranged between 0.05 % (New Caledonia and Irano-Anatolian) and 4.03 % (Meso-American forests). The highest endemism levels were observed in the Meso-American forests, Mediterranean Basin, Philippines and Atlantic Forest areas and the lowest (< 0.3 %) in New-Caledonia, Irano-Anatolian region, Brazilian Cerrado and Southwestern Australia. Using the compile database, the same analysis could be performed for assessing genus diversity and distribution.

Table 1. Number of species and genera encountered and endemism levels of species and genera of Phytoseiidae in the different biogeographical areas

Region	Neotropical	Nearctic	Ethiopian	Australasian	West Palaeartic	Oriental	East Palaeactic
Number of species	484	258	253	231	429	465	299
Endemic species	320	116	174	129	264	309	159
% Endemism/ species in the area	66	45	69	56	62	66	53
% Endemism/ total species number	16	6	9	6	13	16	8
Number of genera	56	33	37	39	31	34	26
Endemic genera	17	0	10	7	3	1	0
% Endemism/ genera in the areas	30	0	27	18	10	3	0
% Endemism/ total number of genera	19	0	11	8	3	1	0

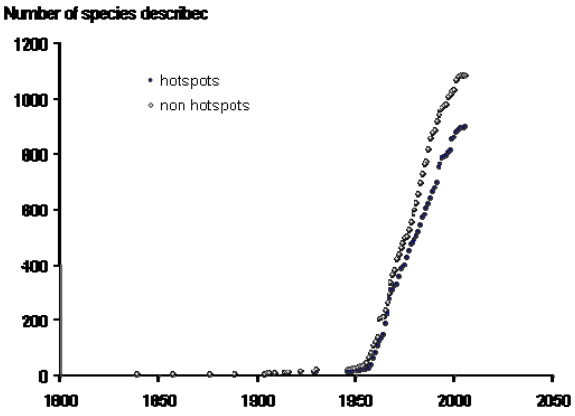

Figure 3. Cumulative numbers of Phytoseiidae species described from 1876 to 2006 in all the 27 hotspots considered and in non hotspots areas.

⇨ The hotspots seem be a great reservoir of Phytoseiid diversity, just as they are for vertebrates and plants (Myers *et al.*, 2000, Mittermeier *et al.*, 2005). Furthermore, even if Phytoseiidae mites are also encountered in non-hotspot areas, the fact that (1) the number of mites reported / publication (sampling effort) is lowest in non-hotspot areas, (2) the surface covered by the non-hotspot areas is substantially larger that the one attribued to hotspot regions, suggesting that non-hotspot zones contain lower diversity than hotspot regions.

A significant positive correlation was observed between the species richness of Phytoseiidae and plants (r= 0.50, P<0.05) and vertebrates (r=0.42, P<0.05). Such correlations have also been observed for the majority of hotspot areas for endemism levels. The mean congruence levels between mites and plants, mites and vertebrates, and vertebrates and plants are, respectively, 49.93 %, 46.63 % and 57.58 % (Table 2). These congruence levels were not significantly different [H(2, 81)=1.65, P=0.44]. There was also no difference in the distributions of the three indices (χ^2=5.41, ddl=8, P>0.05) (Figure 4). These results suggest a good global equivalence between endemism levels in hotspots for the three categories of organisms considered.

Table 2. Data on biodiversity of Phytoseiidae, plants and vertebrates in 27 hotspots of biodiversity

Hot spot	Total number of phytoseiid species	Number of endemic species	Number of species described	Year of first and last description	Description rate per year	% endemism [1]	Congruence Phytoseiidae / plant	Congruence Phytoseiidae / vertebrates
1. Tropical Andes	29	6	8	1979-1991	0.67	0.3	6.1	4.8
2. Mediterranean Basin	238	105	138	1876-2005	1.07	5.3	73.6	15.0
3. Madagascar and the Indian ocean islands	52	14	21	1970-1985	1.40	0.7	18.1	18.9
4. Mesoamerica	188	87	116	1958-1999	2.83	4.4	22.8	98.9
5. Carribean islands	167	56	116	1904-2000	1.21	2.8	77.9	84.9
6. Indo-Burma	71	21	32	1977-1997	1.60	1.1	46.1	27.6
7. Atlantic Forest	124	52	65	1962-2004	1.55	2.6	97.2	98.8
8. Philippines	95	58	66	1966-1995	2.28	2.9	68.3	74.0
9. Cape Floristic region	37	32	35	1964-1990	1.35	1.6	76.9	14.1
10. Himalaya	65	22	25	1981-1997	1.56	1.1	99.1	49.5
11. Sundaland	42	11	18	1914-2002	0.20	0.6	11.1	13.7
12. Cerrado	27	5	5	1997-2002	1.00	0.3	16.8	23.6
13. Southwestern Australia	14	5	5	1986-1987	5.00	0.3	25.2	85.0
14. Polynesia / Micronesia	51	13	19	1968-1984	1.19	0.7	65.6	78.2
15. New Caledonia	15	1	4	1979-1981	2.00	0.1	6.3	13.8
16. Tumbes / Choco / Madgalena	105	37	44	1930-2000	0.63	1.9	48.2	71.4
17. Western Ghats and Sri Lanka	40	12	19	1960-1986	0.73	0.6	60.5	33.3
18. California floristic Province	77	12	34	1954-1997	0.79	0.6	86.5	42.4
20. New Zealand	33	10	15	1962-1989	0.56	0.5	84.1	88.9
21. Chilean winter rainfall – valvidian forests	26	21	22	1962-2004	0.52	1.1	66.1	37.0
22. Guinean Forests of West Africa	44	15	17	1966-2001	0.49	0.8	79.3	49.0
24. Eastern Afromontane	16	7	9	1954-1991	0.24	0.4	44.1	88.3
25. Japan	89	28	62	1958-2002	1.41	1.4	49.6	47.5
26. Caucasus	119	20	53	1958-1994	1.47	1.0	49.6	19.6
27. East Melanesian islands	8	3	4	1981-1984	1.33	0.2	15.1	15.7
28. Mountains of central Asia	13	9	10	1962-2002	0.25	0.5	90.8	12.9
Total	1785	662	962					

[1] in relation to the total number of phytoseiid mites

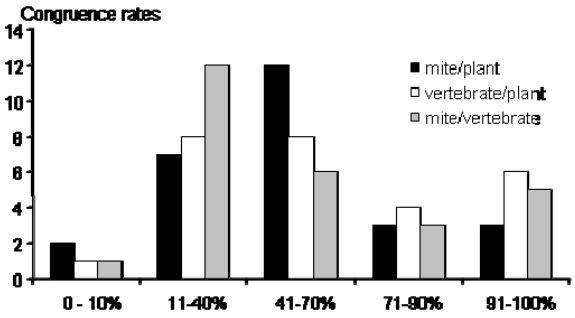

Figure 4. Distribution of the congruence rates in the different hotspot areas calculated between the diversity of mites-plants, plants-vertebrates and mites-vertebrates.

Conclusion

This review shows for some related examples the interest of taxonomic databases for purposes linked to biodiversity, macro-ecology, taxonomy and phylogeny. The analyses presented also emphasize the great field of research opened by the data compilation. In the future, further analysis are planned, including synonymy rates, rarity indices, host plant relationships (incorporating data on plant substrates: host plant, families, genera) and phytoseiid prey relationships especially with Tetranychidae, for whom a very complete database also exists and is available for researchers.

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MICROHABITAT DISTRIBUTION OF OPPIOID MITES IN YOZGAT PINE GROVE NATIONAL PARK, TURKEY

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Abstract

Samples of soil, litter, moss, lichen and fungi from Yozgat Pine Grove National Park for two years beginning from May 2005 to May 2007 were collected and extracted by Berlese funnels. The physico-chemical properties of the soil were analyzed. Vegetation and some climatic features of the investigation area were identified. A total of 28 taxa, in 4 families of oppioid mites were represented in the samples. The species richness was calculated by using Margalef's index (D_{Mg}) and the results are as follows: 3.51 in soil, 3.13 in litter, 0.46 in moss, 0.50 in lichen and 0.00 in fungi. The similarity index between community pairs were computed by using Sørensen's index (C_S) and the highest index value (47 %) was found between soil and litter.

Key-words

Acari, Oribatida, Oppioidea, Ecology, Yozgat Pine Grove National Park, Turkey.

Introduction

The Acari are often the dominant group of arthropods living in soil, and have a world-wide distribution. These organisms, together with the Collembola, make a major contribution to the total faunal diversity in a wide range of soil types (Wallwork 1976). Within this group, oribatid mites are likely the most numerous of the soil mites, with about 10,000 described species world-wide (Subias 2007).

Although the oribatid mites are typical soil-dwelling animals, they also inhabit various habitats such as mosses, lichens, duff, litter, punk, fungi, leaf mats, turf, humus, and on insects and trees (Woolley 1988). In addition, fewer oribatid mites have been found in caves (Mahunka 2001), arid deserts (Wallwork 1972, Wallwork *et al*. 1984), freshwater (Grandjean 1948, Fernández & Athias-Binche 1986, Schatz & Behan-Pelletier 2008) and salt-marshes (Luxton 1964, 1967). The Oppioidea on which this work is based are apterogasterine oribatids. They comprise about 1,100 species and subspecies in 12 families. They live in almost all terrestrial habitats, especially in soil, litter and moss. Members of the Oppioidea are microphytophages and consume fungi, yeasts, bacteria, and algae (Luxton 1972).

Yozgat Pine Grove National Park chosen as the investigation area and located on the 5 km South of Yozgat was established in 1958. The black pine tree (*Pinus nigra*) grove of 265 *ha* is the nucleus of this area; the empty lands surrounding this area have also been added to the National Park by afforesting during the years of 1984–1986. Today, 800 *ha* of lands are under protection and mostly consist of black pine trees and oaks (Anonymous 1988).

Ecological researches are of special importance in protected areas. The variety of microhabitats in these areas creates favorable living conditions to

the diversification of animal communities (Zbikowska-Zdun *et al.* 2006). An ecological research on oribatid mites in this area has never been carried out before, and some information on the systematics of the species found here was available (Toluk *et al*. 2006, 2007). The aim of this study was to describe the microhabitat distribution of the oppioid mites collected during a systematic investigation of the oppioid mites of Yozgat Pine Grove National Park.

Material and methods

Sampling sites

The study was carried out at Yozgat Pine Grove National Park which is 5 km South to Yozgat, in Central Anatolia. The border and coordinates of the investigation area were given in the figure 1. This area has a good coverage of black pine, oak and juniper trees and covers 265 hectares. Five microhabitats were chosen in this study: soil, litter, moss, lichen and fungi. From the five microhabitats within the area studied, samples were collected using the random sampling method.

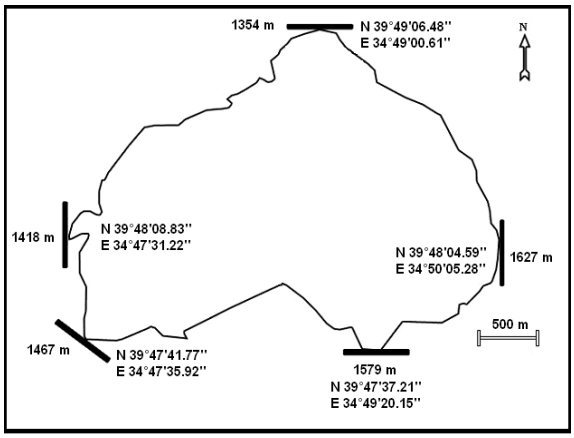

Figure 1. The border and coordinates of the investigation area, Yozgat Pine Grove National Park.

Physico-chemical properties of soil and climate

The physico-chemical properties of the soil in the examined area are given in the table 1. As can be seen from this table, the soil of the investigation area was clay loamy, lacking in lime, saltless, slightly acidic, poor in phosphorus and rich in organic matter.

Table 1. The physico-chemical properties of soil in the investigation areas.

Sample No	Water saturation (%)	Total Salt (%)	pH (soil saturated with water)	Lime ($CaCO_3$) (%)	Phosphorus (P_2O_3) (%)	Organic matter (%)
1	55	0.030	5.9	1.43	2.06	3.76
2	55	0.030	6.0	0.72	3.43	4.78
3	51	0.001	6.1	0.72	1.14	3.36
4	53	0.037	6.0	0.72	5.27	5.41
5	50	0.044	6.5	0.72	1.83	1.40

The air temperature for 2005, 2006, and 2007 averaged 9.6, 9.4 and 10.0°C, and the precipitation was 50.1, 41.9 and 45.4 mm, respectively, to Yozgat Meteorological Observatory. The soil temperature at the depth of 10 cm for 2005, 2006 and 2007 was on average 11.7, 11.9 and 12.3°C, respectively.

Sampling and extraction

Samples were collected from 30 different places in the area studied in every month from May 2005 to May 2007. A total of 702 samples were collected using the random sampling method. 525 of them belonged to soil, 96 litter, 37 lichen, 36 moss, and 8 fungi. Soil samples were taken with a metal sampler (10 cm x 10 cm wide 10 cm deep). Litter samples were collected from an area of 100 cm^2 on the soil surface. Moss and lichen samples were collected from stone, rock and trees. Fungi samples included parasol mushrooms.

Oribatid mites were extracted from all the samples by using Berlese funnels under 60-W electric bulbs for 72 - 96 h. Oppioid mites were sorted and counted under a stereo microscope and mounted on slides in modified Hoyer's medium, and only adults were identified to the species level (Balogh 1983, Woas 1986, Subias & Balogh 1989, Subias & Arillo 2001, Miko 2006, Subias 2007). Names of oribatid species follow Subias (2007).

Table 2. The distribution of the microhabitats of the oppioid species determined from the investigation areas.

Taxa	Soil	Litter	Moss	Lichen	Fungi
AUTOGNETIDAE					
Autogneta (A.) parva Forsslund	51	53		25	
Cosmogneta ozkani Toluk, Ayyıldız & Subias,	130	15			
EPIMERELLIDAE					
Epimerella sp1	2	2			
Epimerella sp2	13	8			
QUADROPPIIDAE					
Quadroppia (Coronoquadroppia) nasalis Gordeeva	164	93			
OPPIIDAE					
Oppiella (O.) nova (Oudemans)	337	105	25	25	14
Berniniella (B.) serratirostris hauseri (Mahunka)	347	169	45		
Berniniella (B.) bicarinata (Paoli, 1908)	74	50			
Moritzoppia (M.) escotata escotata (Subias & Rodriguez)	45	15			
Moritzoppia (M.) unicarinata yozgatensis Toluk, Ayyıldız & Subias	6				
Moritzoppia (M.) problematica Mahunka & Mahunka-Papp	51	51			
Moritzoppia (M.) keilbachi (Moritz)	55	55			
Microppia minus minus (Paoli)	69	16			
Microppia arcuata Gordeeva & Tarba	225	5			
Rhinoppia sp1	185	3			
Rhinoppia trilobata (Khanbekyan & Gordeeva)	38	5			
Rhinoppia sp2	81	34		1	
Rhinoppia obsoleta (Paoli)	75				
Oxyoppia (Dzarogneta) sp1	8				
Oppia denticulata (Canestrini)	1	1			
Ramusella (R.) puertomonttensis Hammer	49	18	8		
Ramusella (R.) sengbuschi sengbuschi Hammer	19				
Ramusella (Insculptoppia) elliptica (Berlese	15	15			
Ramusella (Insculptoppia) sp1	41				
Ramusella (Insculptoppia) luxtoni (Ayyıldız)	28	24			
Ramusella (Insculptoppia) insculpta (Paoli)	7				
Anomaloppia ozkani Ayyıldız	61	81			
Multioppia (M.) sp1	15	1			
Total species number	28	22	3	3	1
Total individual number	2192	819	78	51	14

Species richness and similarity index

The species richness was calculated by using Margalef's index (D_{Mg}):

$D_{Mg} = (S-1)/ \ln N$

where S is the number of species recorded, and N the total number of individuals in the sample. The similarity index between communities was computed by using Sørensen's index (C_S):

$C_S = 2a/ 2a+b+c$

where *a* is the total number of species present in both microhabitats, *b* the number of species

present only in microhabitat 1, and *c* the number of species present only in microhabitat 2 (Magurran 2004).

Results and discussion

The list of the oppioid species determined from five microhabitats at the investigation area is given in the table 2; a total of 28 species belonging to four families were represented. We have examined 3154 specimens of oppioid mites extracted from totally 702 samples. This study also resulted in the discovery of seven species of undescribed oppioid mites, to be discussed in subsequent publications.

Two of the families determined in the area studied, Quadroppiidae and Oppiidae, have a cosmopolitan distribution; the others, Autognetidae and Epimerellidae are distributed in the Holoarctic and Australian, and Palaearctic and Oriental regions, respectively. Almost all genera and species are widespread in the Palaearctic region. So, it is difficult to say that some families, genera, and species could be associated with a microhabitat.

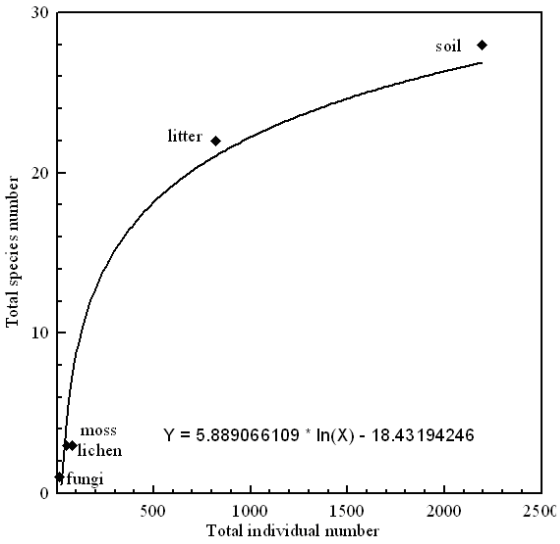

Figure 2. Diagram showing the relationship between the number of species and the number of individuals in relation to each microhabitat studied.

The oppioid species diversity indices were the highest the soil and in the litter (Table 3 and Figure 2). This index gradually decreased drastically in the three other microhabitats considered. Oppiids occur in almost all terrestrial habitats worldwide and they are especially profusely represented in soil, litter and moss samples (Subias & Balogh 1989). Our results are thus consistent with these previous records, and with the fact that oribatid mites are predominantly soil-dwellers (Wallwork 1976). In continuous mainland areas, the species-area relationship has been attributed to sample size effects (large areas contain species that are too rare to be present in small areas), habitat heterogeneity (large areas contain more types of habitat allowing more species to coexist), and population and metapopulation processes causing spatial aggregation (Storch *et al*. 2003). Soil as a microhabitat consists of mineral material, the roots of plants, microbial and animal biomass, and organic matter in various states of decay, as well as water and a gaseous atmosphere. The uneven distribution of these components provides a great variety of conditions at all levels of scale from field to soil micropore (Killham 1996). In this respect, the soil microhabitats have an environmental heterogeneity affecting the species diversity positively. Therefore, it is an expected result existing in soil of higher species richness, compared to litter, moss, lichen and fungi microhabitats. Even though the difference between soil and litter for species richness are not very high (28 / 22 species), their population densities are somewhat different (2192 / 819 individuals). According to Mondchasky, the secondary periodical factors (moisture, precipitation, feeding habit, intraspecific competition, etc.) cause fluctuations in a species' abundance (Kocataş 1992). Accordingly, the difference in the population densities results from the structure and porosity of the soil and litter environment. As can be observed on the table 2 and figure 2, our results also indicate a positive relationship between mite diversity and density. As seen in figure 2, since Y=5.889lnX-18.4319 is a logarithmic function, it can be said that the more individual numbers in the microhabitat implies a higher species number. Among the oppioid mites collected, *Oppiella (O.) nova* (Oudemans) occurred in all the microhabitats considered. As mentioned in previous studies (Schatz 1996, Miko 2006), this result also supports the opinion that it is eurytopic species.

It has been found that the body length to width ratio for the taxa observed in the investigation area, ranges between 1.70 to 2.63 µm in soil microhabitat, 1.70 to 2.37 µm in litter microhabitat, 1.90 to 2.20 µm moss, lichen and fungi microhabitats. These ratios are not significantly different according to the microhabitats considered because the ranges of ratios overlap approximately. Wallwork (1976) reported that vertical stratification of species in the soil profile is related to body size and the ability to resist desiccation. He also pointed out that Cryptostigmatids such as *Oppia* species which live in the soil and deeper layers of the litter have

no water-proofing layer on the surface of the cuticle. The larger-sized species occur mainly in the upper litter layers and in the aerial vegetation (Wallwork 1976). We have no evidence for a close relationship between the body size and the microhabitat preference.

Table 3. Comparison of the species richness in five microhabitat types at the investigation area.

	Soil	Litter	Moss	Lichen	Fungi
D_{Mg}	3.51	3.13	0.46	0.50	0.00

Equitability and Simpson's diversity indices take into account both the abundance patterns and the species number for the microhabitats that have been explored are given in table 4. As seen in tables 3 and 4, diversity indices calculated on the basis of Margalef's index and Simpson's diversity index are similar to each other in the relationship between the microhabitats. However, moss, lichen, and fungi microhabitats are less species-rich, but highly equitable when compared with soil and litter microhabitats.

Table 4. The comparison of Simpson's diversity and equitability indices in five microhabitats. S, The total number of species in the microhabitat; D, Simpson's diversity index; E, Simpson's equitability index.

	Soil	Litter	Moss	Lichen	Fungi
S	28	22	3	3	1
$D = 1 / \Sigma p_i^2$	11.91	9.74	2.24	2.08	1
E = D / S	0.43	0.44	0.75	0.69	1

Sørensen's measure (C_S) is one of the most effective presence or absence similarity measures (Southwood & Henderson 2000). The observed similarity indices between community pairs in five microhabitat types at the investigation area are given in Table 5. Community pairs with a quotient of less than 50% can be considered as distinct (Price 1975). As expected, the most faunal similarity exists between soil and litter microhabitats. As seen in table 2, soil and litter microhabitats have been occupied not discriminating by the most of the species. It is impossible to border these two microhabitats because of the vertical migrations of oribatid mites. The similarities between community pairs were mostly about 16-33%. These results show that moss, lichen and fungi represent different microhabitats for oppioid mites when compared to soil and litter. And also mite communities are different between moss, lichen and fungi.

Table 5. The observed similarity indices between community pairs in five microhabitat types at the investigation area.

	Soil	Litter	Moss	Lichen	Fungi
Soil	100	47	16	16	6
Litter		100	19	19	8
Moss			100	25	33
Lichen				100	33
Fungi					100

In conclusion, it can be said that the size and heterogeneity of the habitat are the important factors affecting oppioid mite distribution, and oppioid mites are most abundantly found in soil and litter compared with moss, lichen and fungi.

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ORIBATID MITE COMMUNITIES IN ATLANTIC SALT MARSHES: AN ECOLOGICAL AND BIOGEOGRAPHICAL COMPARISON BETWEEN GERMAN AND PORTUGUESE SEA SHORES.

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Abstract

This contribution compares oribatid mite communities in salt marshes from Northern Germany (Sylt and Meldorf), Northern Portugal (Aveiro) and Southern Portugal (Faro), regarding the species composition and their dominance structures. Similar to the vertical zonation of the vegetation in the sites, the mite associations show characteristical vertical changes each. Yet, there is a more or less constant halophilous species stock in all regions, with *Ameronothrus schneideri*, *Zachvatkinibates quadrivertex* and *Hermannia pulchella*. Additional species characterize the median or upper salt marsh zones, contrasting between the three regions. The patterns of oribatid mite communities support biogeographical and ecological interpretations. As main factors the regional climate, the regional vegetation structure, the regular marine inundation and the substrate salinity are assumed.

Key-words

Oribatida, ecology, biogeography, Portugal, Germany

Introduction

The purpose of this study is to compare salt marsh communities of oribatid mites from southern and from northern Atlantic coasts of Europe with regard of possible biogeographic and ecological contrasts. From North Europe until the middle of the Portuguese Atlantic coast the vegetation aspect within the upper intertidal zone is more or less uniformly a grassy salt meadow. In the south of Portugal the aspect changes and we find predominantly halophilous scrubs in the respective intertidal zone, as can be observed in the middle-Atlantic and the Mediterranean regions. In all regions oribatid mites inhabit the eulittoral to supralittoral zones with salt vegetation of higher plants in differentiated species compositions and community patterns. The question arises whether the fauna of Oribatida follows the regional contrasts and the vertical vegetation zones of the salt marsh plants in a semiterrestrial to terrestrial sampling catena.

At the coasts of Portugal the oribatid mite communities of several areas with salt marsh vegetation were studied extensively in 1971 and additionally from 2003 to 2007; a publication of all results is in preparation. Within this contribution only the results from the Lagoon of *Faro* and from the Lagoon of *Aveiro* are presented which are supposed representative for the faunistic zonation of mite communities in salt marshes in southern and northern areas of Portugal. In the moderately warm climate of South Portugal the salt marshes are classified as "Mediterranean and thermo-Atlantic halophilous scrubs (*Sarcocornetea fruticosi*)", dominated by perennial vegetation of Chenopodiaceae (*Sarcocornia*, *Arthrocnemum*, *Atriplex, Suaeda*) (European Commission 2003). The studied area in the Lagoon of Faro (called " Ria

Formosa" by local ecologists; Machás & Santos 1999) includes wet salty intertidal plots of lower topographic level, similar plots of higher level, up to a dry adjacent slope of a dike on clay soil. The studied salt marshes in the Lagoon of Aveiro (called "Ria de Aveiro") are classified as „Atlantic salt meadows (*Glauco-Puccinellietalia maritimae*)", dominated by rush and grass species (*Juncus*, *Puccinellia*, *Festuca*) (European Commission 2003). The tidal dynamics and partly the water salinity are reduced in the lagoon (Mahowald et al. 2000).

The substrate of the lower zone of the sampling catena is sandy clay, that of the upper zone is sandy soil. The aspect of the vegetation is very similar to those in salt marsh meadows in northerly regions of Europe (from France to Denmark), but partly the same plant genera are represented by other species.

In salt marshes at the North Sea (west-coast of North Germany), classified as „Atlantic salt meadows", the oribatid mite communities were studied at several sites from 1966 to 1970 (Weigmann 1973). For this comparative analysis two areas in Germany were selected which look representative for two ecological variants of the vegetation zonation: At the shore of the Isle of *Sylt* the sampling catena is on sandy clay in the intertidal lower salt marsh and in a sandy dune area in the upper supralittoral zone; at the sampling catena near *Meldorf* all lower and upper salt marsh zones are characterized by heavy clay soil.

The selected sites give the possibility for biogeographical and ecological comparisons: (1) Portuguese vs. German salt marshes; (2) northern vs.southern salt marsh types in Portugal; (3); clay-dominated sites vs. sandy-clay sites: Faro vs. Aveiro sampling catena; Meldorf vs. Sylt sampling catena.

Material and Methods

Localities

Faro Lagoon (Portugal). Salt marsh areas in the western part of the lagoon, dominated by perennial vegetation of halophilous Chenopodiaceae (*Sarcocornia*, *Arthrocnemum*, *Atriplex, Suaeda*) in the topographical zones 2-4 with low to high level; zone 3 about 30-60 cm, zone 4 about 80 cm higher than zone 2. Zone 4 at an embankment of a moderately dry dam bordering salt marsh areas, with some *Sueda*, but dominated by non-salt-indicating plants. 10 sampling points.

Aveiro Lagoon (Portugal). Shores of the western border of the lagoon in the surrounding of Torreira and Costa Nova; localities with brackish and salty water and with salt marsh vegetation. Zone 2 with *Puccinellia* grass dominating, zone 3-4 with *Festuca rubra maritima* and *Juncus* dominating; zone 5 in the adjacent salt-tolerant meadow, with *Trifolium repens*, *Plantago maior*, *Bellis perennis*, grass species and others. 11 sampling points.

Meldorf (Germany). Salt marsh meadows on clay soil, near Meldorf; zone 2 dominated by *Puccinellia maritima*, zone 3 dominated by *Festuca rubra maritima*, zone 4 dominated by *Juncus gerardi* and *F. rubra maritima*. Zone 3 about 30 cm, zone 4 about 60 cm higher than zone 2 (details in Weigmann 1973). Monthly samples in two years.

Sylt (Germany). At the eastern shore (lee-side) of the Isle of Sylt. Sampling catenas near List and near Kampen from lower salt marsh level up to transition area salt marsh – dune complex; sand rich soils. Vegetation of zones 2-4 similar to that in Meldorf; zone 5 dominated by *Ammophila*, with some *Juncus gerardi, Festuca rubra maritima* and others. 15 sampling points.

Sampling and laboratory methods

The samples at each sampling point in Portugal were taken semi-quantitatively with a special shovel, about 250 cm², 1-2 cm depth. The mites were extracted using a modified Tullgren apparatus. The German samples were treated with a Macfaydyen-extractor. The specimens were stored in ethanol and after clearing they were studied microscopically in lactic acid in open hollow-ground microscope slides.

Oribatid mites determination and data treatment

The oribatid species from Portugal were determined with the Spanish text books of Perez-Iñigo (1993, 1997) and Subias & Arillo (2001) in combination with special literature, as cited in the text books, and with the German text book (Weigmann 2006), which was also thebasis for a redetermination of the German species. All sampling data of the mites were fused together for every locality and littoral zone, each, presented in table 1 as dominance percentages. The cluster analysis in figure 1 compares the communities of all sites after Southwood (1971), based on the dominance identities of each and all sites.

Results

Table 1 in the appendix presents the condensed data on the oribatid mite communities from the littoral zones of the four sites. The lowest zone 1 with pioneer vegetation (*Salicornia* or *Sarcocornia*) was very sparcely inhabited by oribatid mites, with similar species complex to that of zone 2, and these results are omitted. At the site Faro in zone 2 to 4 alltogether 16 species were collected; at the site Aveiro alltogether 18 species in zones 2 to 5, and 9 species from these in the zones 2 to 4. At the site Meldorf in zone 2 to 4 alltogether 13 species were collected; at the site Sylt alltogether 23 species in zones 2 to 5, and 13 species from these in the zones 2 to 4. As a general tendency, the species numbers increase from zone 2 to zone 5 (zone 4 in Faro is not fully representative in this regard because only one sampling point within this zone with about 250 cm² sampling size could be analyzed, including only 50 specimens).

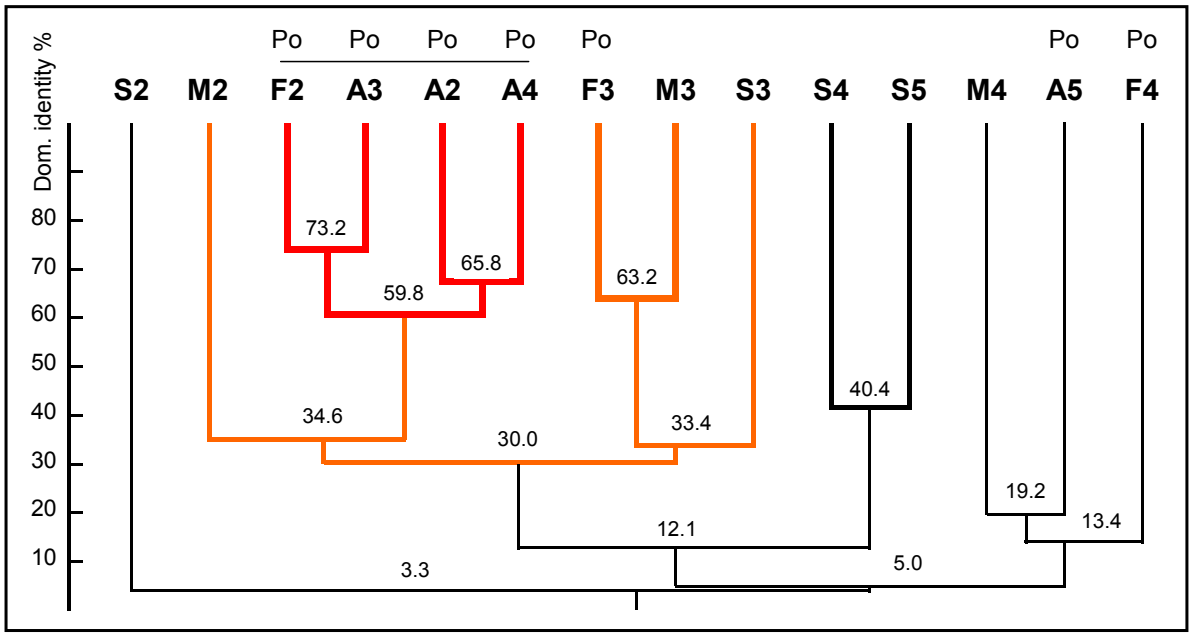

Figure 1. Cluster with the dominance identities (%) of the oribatid mite communities of the sites (S2-F4). Portuese sites indicated with *Po* (**A**: Aveiro; **F**: Faro); German sites (**S**: Sylt; **M**: Meldorf); numbers at the sites refer to the level in littoral zone

The species communities of every zone in the sites in Portugal and in Germany is compared with each other concerning the dominance identity. A high value indicates high sum of the dominance values of common species. The identity values are clustered in figure 1, resulting in small to larger subclusters of site/zone groups with descending species composition similarity.

A pure Portuguese subcluster in fig. 1 with high internal similarity is that formed by F2–A4 (in red). This subcluster is joined with site M2 from Germany; both together are joined with a mixed subcluster (F3–S3 with Portuguese and German sites) to a larger cluster (M2–S3; red and orange). This cluster with more than 30% internal dominance similarity is caused by high dominance values each of *Zachvatkinibates quadrivertex*, *Ameronothus schneideri* and partly of *Hermannia pulchella*. The first two species are dominant especially in the littoral zones 2 and 3, as indicated in figure 2 (cf. in detail in table 1), wereas *H. pulchella* prefers zones 3 and 4. Obviously, the clusters depend more on the ecological preferences of the dominant species than on the geographical position of the sites.

S2 fom the lower littoral level of the salt meadows in Sylt (Germany) is separated from all other site/zones whether being in low or high topographic level. This effect is caused by the extreme dominance of the halobiont species *Ameronothrus nigrofemoratus*, which occurs in Germany at sandy littoral salt marshes only, and which were not found in Portugal (cf. figure 2B).

A subcluster with S4–S5 from Sylt (Germany) is caused by common dominance of some salt tolerant and more or less eurytopic species (cf. figure 2B, second group of species).

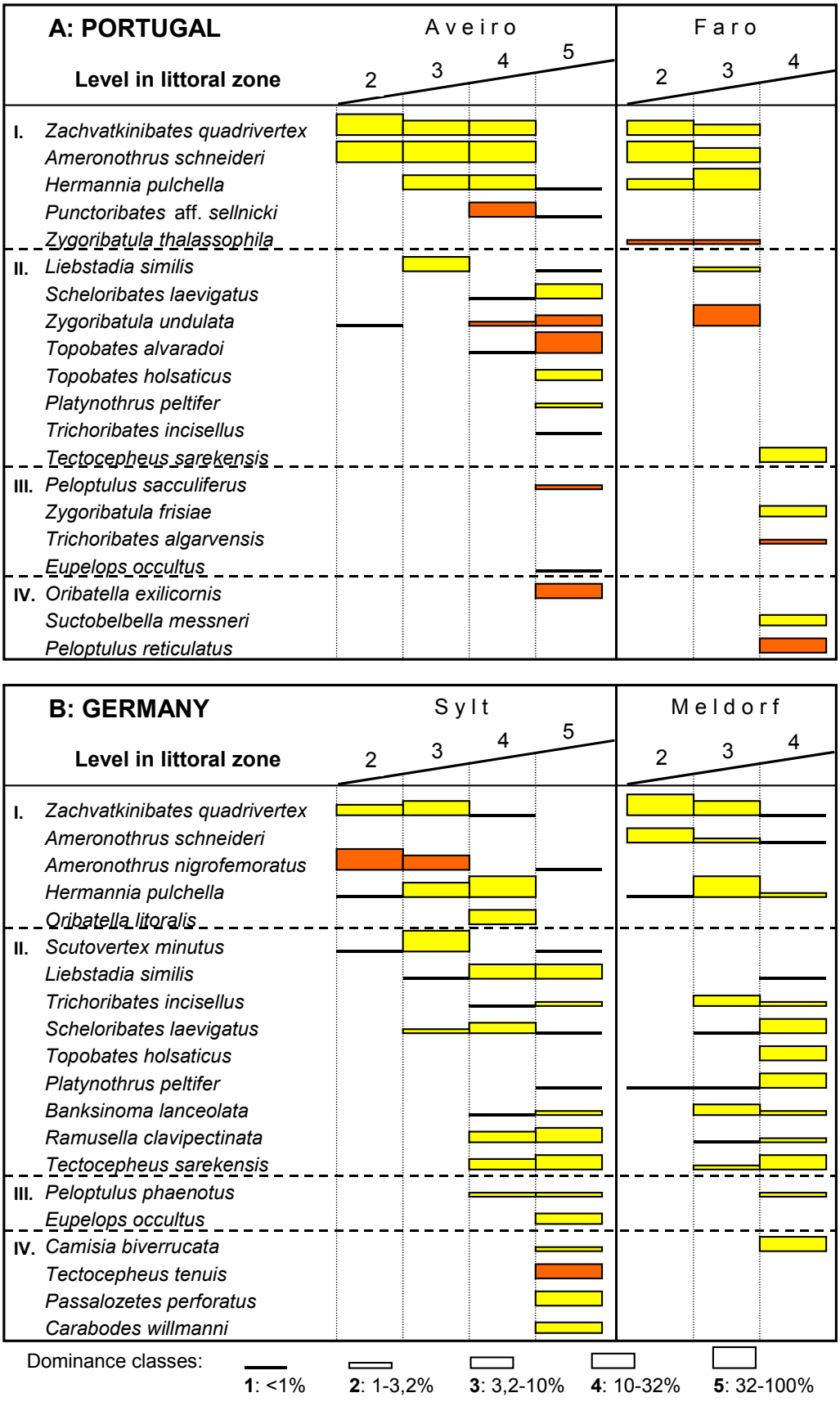

Figure 2. Occurrence of important species in the littoral zones in Portugal and in Germany (dominance classes). In yellow: species in both countries; in orange: in one only.

Figure 2 includes the most dominant 20 species each from Portugal and from Germany, with more than 2 % dominancy at least in one site (exceptionally *Oribatula tibialis* and *Dissorhina ornata* not included; see Table 1). The species of both regions are grouped together in four groups with different ecological preferences. Species in common in the Portuguese (resp. Iberian) and German region are coloured yellow, the species with ocurrence restricted to Portugal or Germany are marked in orange.

The first group of species in figure 2A and 2B consists of halobiont species, which occur in salty habitats only. In the lower littoral zone 2 of all sites in Portugal and Germany, where the salt marsh is flooded nearly daily during tidal high waters the halobiont species *Zachvatkinibates quadrivertex* and *Ameronothrus schneideri* are dominant; yet on the sandy substate of Sylt in Germany *A. schneideri* is substituted by *A. nigrofemoratus*. *Hermannia pulchella* has relevant dominance values in zones 2 to 4, mostly with optimum in zone 3, where the marine water inundation is less regular. *Oribatella litoralis* is a characteristic species in dryer salt marsh meadows of the zone 4 of the Sylt site (the species occurs in the upper salt marsh also in Portugal at the shore of the Minho River (own unpublished records) and in other German dry littoral sites). *Puncoribates* aff. *sellnicki* seems to prefer the upper zone 4 in the lagoon of Aveiro; *Zygoribatula thalassophila* is less dominant in zone 2-3 of the Faro sites, a halobiont species descibed for the French Atlantic coast (Grandjean, 1935); both species do not occur in Germany. Summed up, even in the group of halobiont species the zones 2 to 4 are differently inhabited, probably following the decreasing salt and wetness status of the salt marsh zones from low to high topographic level.

The second group of species in figure 2A and 2B includes halotolerant species which are found as well in salty as in salt free habitats and which are not restricted to dry supralittoral zones; these species are more or less frequent "attendents in salt marsh communities" (Weigmann 1973), and some species are common for both studied regions. Most of these species prefer the upper topographic levels of zones 4 to 5. All species in the German sites inhabit Iberian localities also (Subias & Gil-Martin 1997). But in the Portuguese sites, there are two species which were not found in Germany, up to now: *Zygoribatula undulata* and *Topobates alvaradoi*.

The third group of species in figure 2A and 2B includes species with supposed optimum in salt-free habitats which may be regarded as "guests" in the uppermost salt marsh zone 4 and the adjacent supralittoral zone 5. *Peloptulus sacculiferus* and *Trichoribates algarvensis* are found in Portugal only (Gil & Subias 1990).

The fourth group of species in figure 2A and 2B avoids the salt marsh zones 2-4 with regular marine influence and occurs only in adjacent dry habitats, as coastal dunes are (supralittoral zone 5 in figure 2). *Oribatula exilicornis* and *Peloptulus reticulatus* are not represented in Germany, whereas *Tectocepheus tenuis* is not recorded on the Iberian Peninsula.

Discussion

Most of all, the distribution of the halophilous oribatid mites is important in the scope of this study. Generally, there is present a constant and dominant complex of halophilous species in the salt marsh zones from North Germany to South Portugal including *Zachvatkinibates quadrivertex*, *Ameronothrus schneideri* and *Hermannia pulchella*. (In the Sylt site from Germany *A. schneideri* is substituted by *A. nigrofemoratus*, which is most probably caused by the sandy substrate in the Sylt littoral zone; cf. Weigmann 1973). These "indicator species" for littoral salt marshes do not discriminate between the Southern Portuguese salt vegetation with scrub plants and the Northern Portuguese and German salt vegetation of the grassy type. A similar ecological preference of low salt marsh zones by these species was described by Luxton (1964, 1967) for British coasts and by Weigmann (1973) for some more German sea shore sites.

Zachvatkinibates quadrivertex is most abundant in the lower littoral zones in all studied sites in Germany and Portugal, whereas *Hermannia pulchella* prefers the medium and upper salt marsh zones in all sites and therefore is less dominant in the lower zone 2. In the site Meldorf, Weigmann (1973) recorded some ecological key data over two years: the zone 2 with highest abundance of *Z. quadrivertex* and *Ameronothrus schneideri* is characterized by about 220 marine tidal inundations per year and regularly by high soil wetness. Zone 3 with highest abundance of *Hermannia pulchella* (at that time determined as *H. subglabra*) is characterized by about 90 inundations per year and by moderate soil water content, in mean. Both zones do not differ significantly in the fluctuating soil salt contents (zone 2 with 0,7-3,0 %; zone 3 with 0,3-2,8 % salinity).

Other halophilous oribatid species, which are included in the first group of species in figure 2, were found only at one site each. *Punctoribates* aff. *sellnicki* is a species new for science, which was recorded only at the Aveiro site with more than 70 specimens, mostly in the topographical zone 4, which is characterized by salt meadow vegetation in the comparatively seldom inundated level. *Zygoribatula thalassophila* was described from the shore of the Bretagne in France (Grandjean 1935); this new finding in the Lagoon of Faro is the second one (as far as I know) and confirms the assumed ecological preference for salty habitats. The biogeographical preference is incertain.

Ameronothrus nigrofemoratus was found at the Sylt site only; it is a holarctic species with a distribution in arctic to moderately cold climates, occuring in Europe in Atlantic salt marshes northern than France (Schulte 1975). It has been recorded in sandy salt marshes in Germany and Denmark (Weigmann 1973; Koehler et al. 2008). *Oribatella litoralis* inhabits moderately dry and salty zones in coastal meadows at some German localities (Strenzke 1952; Weigmann 1973; Koehler et al. 2008). It was declared as nominal species of the "*Oribatella arctica litoralis* synusia of salt meadows" at the German Atlantic coast by Strenzke. Within this study it occurs at Meldorf only; yet, a new record in Northern Portugal (salt marsh at the Rio Minho: Weigmann, unpublished) expands the geographical range to South Europe.

Within the species group II in figure 2, there are some widely distributed "meadow species" in common at the Aveiro site in Portugal and the German sites, as *Liebstadia similis*, *Scheloribates laevigatus*, *Topobates holsaticus*, *Platynothrus peltifer* and *Trichoribates incisellus.* This may be due to the vegetation similarity within these sites contrasting to the Faro site. Besides *Ameronothrus nigrofemoratus* In figure 2, there is only *Tectocepheus tenuis* restricted to a German site. In contrast, there are several species restricted to Portuguese sites: *Punctoribates* aff. *sellnicki*, *Zygoribatula thalassophila, Zygoribatula undulata, Topobates alvaradoi, Peloptulus sacculiferus, P. reticulatus, Oribatella exilicornis* and other seldom species (see table 1). All these species (except *P. reticulatus*) were never recorded in Central or Northern Europe and indicate a biogeographical differentiation.

Summed up, the community pattern similarities and contrasts between the studied sites can explained partly by biogeographical distributions of species, partly by their ecological reactions. The zonation patterns within each site show obvious similarities between the localities in both countries, at least regarding the lower zones of the littoral habitats.

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Appendix

Table 1. Dominance values (%) of all oribatid mite species

	PORTUGAL							GERMANY						
	AVEIRO				FARO			SYLT				MELDORF		
Site code and littoral zone	**A2**	**A3**	**A4**	**A5**	**F2**	**F3**	**F4**	**S2**	**S3**	**S4**	**S5**	**M2**	**M3**	**M4**
Ameronothrus nigrofemoratus (L. Koch, 1879)								95,8	18,4		0,2			
Ameronothrus schneideri (Oudemans, 1903)	49,7	53,4	34		73,6	21,8						15,9	1,3	0,2
Astegistes pilosus (C. L. Koch, 1841)										0,3				
Banksinoma lanceolata (Michael, 1885)										0,3	1		6,7	2
Camisia biverrucata (C. L. Koch, 1839)											1,9			13,7
Camisia spinifer (C. L. Koch, 1835)											0,2			
Carabodes marginatus (Michael, 1884)											0,8			
Carabodes willmanni Bernini, 1975											8,1			
Dissorhina ornata (Oudemans, 1900)											2,1			
Eupelops occultus (C.L. Koch, 1835)				0,4							8,5			
Eupelops sp.				0,4										
Galumna obvia (Berlese, 1915)				0,4										
Hermannia pulchella Willmann, 1952		22,5	19,9	0,4	9,7	68,2		0,2	20,1	36,4		0,1	58,7	1,3
Humerobates rostrolamellatus Grandj., 1936			0,4			0,4								
Liebstadia similis (Michael, 1888)		13,5		0,4		3,1			0,3	18,5	17,2			0,8
Malaconothrus monodactylus (Michael 1888)				0,4										
Oribatella exilicornis Berlese, 1910				6,3										
Oribatella litoralis Strenzke, 1950										16,4				
Oribatula tibialis (Nicolet, 1855)					2,4	0,4								
Passalozetes perforatus (Berlese, 1910)											11			
Peloptulus phaenotus (C.L. Koch, 1844)										2,4	2,7			1,8
Peloptulus reticulatus Mihelcic, 1957							30							
Peloptulus sacculiferus Weigmann, 2008				1,1										

Table 1 ff

	AVEIRO				FARO			SYLT				MELDORF		
Site code and littoral zone	**A2**	**A3**	**A4**	**A5**	**F2**	**F3**	**F4**	**S2**	**S3**	**S4**	**S5**	**M2**	**M3**	**M4**
Platynothrus peltifer (C.L. Koch 1839)				2,9							0,2	0	0,6	21,1
Pulchroppiella plurisetosa (Mihelcic, 1956)						0,4								
Punctoribates aff *sellnicki* (Willmann, 1928)	0,4	0,6	31,7	0,8										
Quadroppia quadricarinata (Michael, 1885)											0,2			
Ramusella clavipectinata (Michael, 1885)										9,6	19,7		0,2	1,6
Scheloribates laevigatus (C.L. Koch, 1835)			0,4	11,3					2,3	8,1	0,2		0,1	10,6
Scheloribates sp. 7					0,3									
Scutovertex minutus (C. L. Koch, 1835)								0,3	35,2		0,4			
Suctobelbella messneri Moritz, 1971							4							
Tectocepheus sarekensis Trägardh, 1910							18			7,2	10,4		2	23,4
Tectocepheus tenuis Knülle, 1954											13			
Topobates alvaradoi (Perez-Inigo, 1969)			0,4	61,4										
Topobates holsaticus Weigmann, 1969				4,5										20,8
Trichoribates algarvensis (Subias & Gil, 1990)							2							
Trichoribates incisellus (Kramer, 1897)				0,4						0,3	2,1		5,8	2,5
Xenillus tegeocranus (Hermann, 1804)						0,6								
Zachvatkinibates quadrivertex (Halbert, 1920)	49,7	10,1	11,3		11,2	3,2		3,7	23	0,6		84	24,6	0,3
Zygoribatula friesiae (Oudemans, 1900							6							
Zygoribatula thalassophila Grandjean, 1935					2,8	1,8								
Zygoribatula undulata Berlese, 1916	0,2		1,8	8,8			40							
species	**4**	**5**	**8**	**15**	**6**	**9**	**6**	**4**	**6**	**11**	**19**	**4**	**9**	**13**
Sum of all species at a site		**18**				**16**				**23**			**13**	
Sum of specimens	304	178	274	342	223	406	50	574	304	335	517	4716	849	1743

INTEGRATIVE APPROACH OF ERIOPHYOIDEA

ERIOPHYOIDS WORKING GROUP GENERAL ADDRESS

When talking with several eriophyoidologists, the need to plan a session entirely dedicated to Eriophyoid Mites at the VI Eur.A.Ac. Symposium came up.

The session was proposed and promoted after a questionnaire sent to about 50 worldwide researchers working on these mites. There was definitely a strong interest and initially a lot of researchers were going to attend the meeting. Unfortunately, financial resources have limited their presence and we won't have the pleasure of Jim Amrine's presence, who is surely one of the leaders on this taxon. Among other researchers who couldn't make the trip, are Jan Boczek, Evert Lindquist, George Oldfield and Valery Shevtchenko.

Finally, about 30 researchers were involved in this project and aggregated to prepare presentations in which their experience and competence in different fields of research were joined together. In addition, Boczek and Shevtchenko, who could be remembered for this taxon along with Carlos Flechtmann and still active in research, wanted to take part in this event in some way.

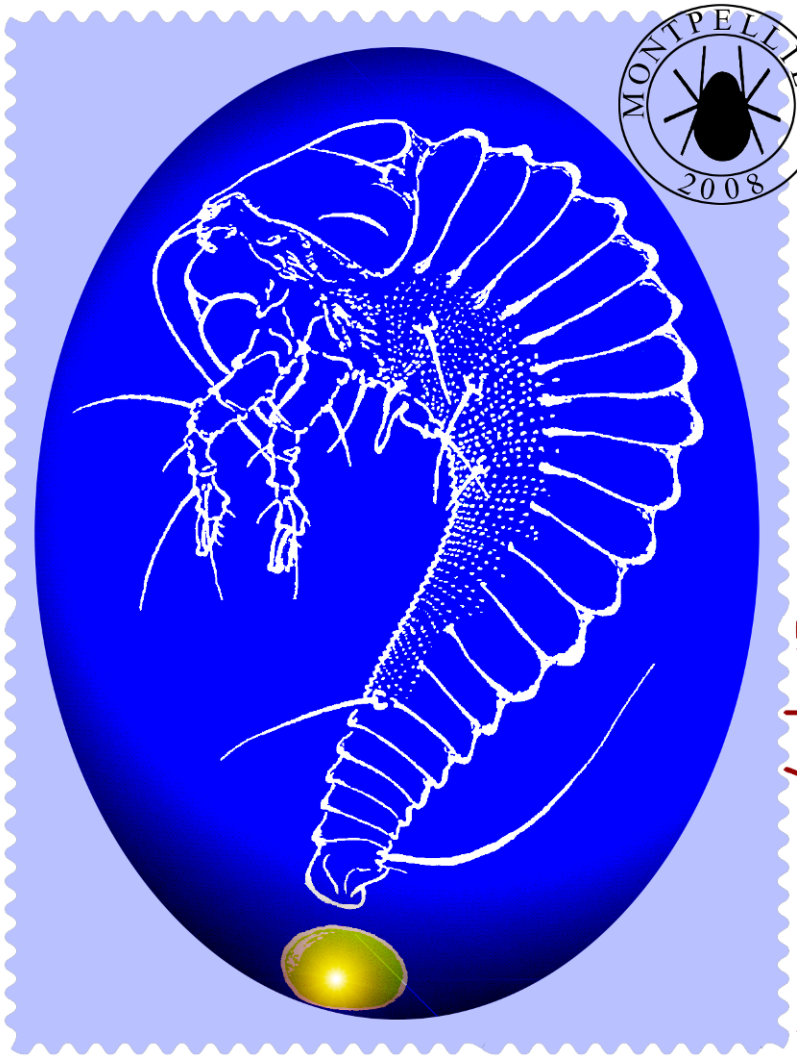
So, everything was and is great!

The session has the scientific aim to make the state of the art about 12 years after the Red Book of the World Crop Series (Editors: Lindquist, Sabelis and Bruin, 1996) on these tiny mites, and to understand if something new came out and which way we should address our research. We also intend to define the place of Eriophyoidology in the future of Science and Society, and to show the need of common projects involving the greatest cooperation of people interested in Eriophyoid Mites.

In addition, it is the first time, as far as we know, that the Eriophyoid Mites are at the centre of the attention in a congress and it is the first time that so many researchers tried to manage this topic all together in one place and at the same time.

To conclude, as Shevtchenko wrote in one of his letter to us, one of the main results of the session might be the establishment of closer working contacts and research collaborations between colleagues who are studying and will study Eriophyoid Mites in different countries in the future.

Let's hope for the best.



6th Symposium of European Association of Acarologists

Eriophyoid session

Contributions to Eriophyoids session

Integrative Acarology. Proceedings of the 6th European Congress
M. Bertrand, S. Kreiter, K.D. McCoy, A. Migeon, M. Navajas, M.-S. Tixier, L. Vial (Eds.)
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WHY SHOULD WE TALK ABOUT ERIOPHYOID MITES?

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Abstract

The economic importance of Eriophyoidea as pests, quarantine organisms, weed control agents, virus vectors, gall-making mites, and the literature produced on them after the book edited by Lindquist *et al.* (1996) suggest the need to focus consideration on the current Eiophyoid Mites knowledge. We are going to introduce very briefly the most recent achievements on these phytophagous mites, pointing out their relevant aspects and giving reasons of the thematic lectures planned on the Eriophyoidea session.
This meeting might provide a chance of comparison among researchers involved on investigations about gall-making and vagrant eriophyoid mites throughout the world and aiming the promotion of a future scientific progress on specific and required topics, and a larger international cooperation among researchers with complementary competence and expertise.

Keywords

Acari, Eriophyoidea, research lines

Introduction

Mites belonging to the superfamily Eriophyoidea can be considered unique among the Acari. From a morphological viewpoint, eriophyoid mites (hereafter EMs) are the smallest phytophagous arthropods, along with tarsonemids. They are highly specialized, displaying a remarkable morphological reduction and adaptation of the body structures to their ecological niches as demonstrated by their size and shape, missing appendages, unusual mouthparts complex, integument respiration, chetotaxy, sensillar dislocation and arrangement, etc. (Lindquist 2001). Although EMs are generally mild plant parasites, they injure plants of agricultural, forestry, ornamental, and medicinal value, causing quantitative and qualitative losses. The plant growth deformations induced by numerous species are so typical as to often be diagnostic. EMs are frequently referred to as "gall-making mites" (Oldfield 2005). In addition, several species are known to vector plant pathogens (Hong & Cheng 1999). Interest in EMs is often centered on their host specificity, which is often strictly intimate to a single plant species (about three fourths of the known species are related only to one host species), and on their effects on the plant reproductive fitness, which makes them greatly suitable for weed biological control (Briese & Cullen 2001).

Brief literature review

By literature examination, since the first ancient papers in which an eriophyoid mite (EM) was distinctly recognized, a clear constant increasing interest in this taxon can be appreciated, mainly in

the last three decades.

A milestone for eriophyoidologists has surely been the volume "*Eriophyoid Mites – Their Biology, Natural Enemies and Control*" edited by Lindquist, Sabelis & Bruin (1996). After this review, EMs have been the object of many new studies and the amount of printed papers about them was second only to tetranychids, among the phytophagous mites.

These papers present much of exciting data and cover a large range of topics with the main interest on the pests of the agricultural crops, on the control strategies, and on the species surveys including new taxon description.

Remarks and conclusions

At the XX International Congress of Entomology in Florence (Italy), in August 1996, Nuzzaci and de Lillo dealt briefly with the status of knowledge of EMs focusing their lecture on an array of enquiries for future studies. Coming back to those recommendations, many aspects still appear to be poorly understood and should stimulate basic and applied research.

Table 1. Topic preferences of 44 researchers involved on Eriophyoidea studies on the basis of a submitted questionnaire. Additional topics proposed by researches were: 1) impact of EMs on agricultural crops (2.3%); 2) host range and speciation (4.0%); 3) biogeographical study or hot spots of EMs (2.3%); 4) EMs in tropical areas (2.3%); 5) set up a globe database (2.3%).

Topics	**Interest [%]**
Consideration on EM detection (symptoms, collecting techniques, development of micro-array techniques)	45.5
Behaviour of EMs	40.9
Critical aspects for EM identification by means of biomolecular techniques	34.1
EMs and transmitted pathogens	34.1
Weed control in Europe: critical points, state of the art, future perspectives	29.5
Unanswered questions on EMs for weed control	27.3
Nalepa's species: problems	22.7
Critical aspects, tips and tricks for a correct morphological and morphometrical description of EMs	25.0
Pathogens attacking eriophyoids (like fungal diseases)	20.5
EMs inhabiting coniferous trees and their host range	11.4
Aceria drabae and its host range	4.5

The VI Symposium of European Association of Acarologists in Montpellier (France), in July 2008, provides an opportunity to talk extensively about gall-making and vagrant EMs, for the first time on the scene of an international meeting. In the attempt to engage the attention of the specialists throughout the world, a participated discussion of possible lecture topics has produced strong interest in the taxon, as demonstrated by the results of a submitted questionnaire (Table I) and by the development of spontaneous aggregations in the lecture authorships. These facts have partially satisfied our desire to exchange information about EMs and to have a session dedicated to them, the ultimate goals of which are the promotion of future scientific advances on specific and required topics and a large international cooperation among researchers with complementary competence and expertise.Among many other aspects, some of which pertain to reviews in the current session on the EMs, some will be stressed here while a few other topics also deserve particular attention.

A strong concern should be paid to further development of rearing techniques, to which several authors have recently paid more attention (*e.g*. Gispert *et al.* 1997, Courtin *et al.* 2000, Schwoebel & Beiderbeck 2000, Haq 2001).

Successful rearing of EMs is needed for progress in investigations regarding host specificity (*e.g.* for measuring mites capacity for host colonization, host preference and acceptance), population development, mating and feeding behaviours, distribution on host plants, plant pathogens vectoring, dispersal, and many other studies.

Only a small fraction of total EM diversity is thought to be described up to now (Amrine *et al.* 2003) and a strong impulse toward discovering new taxa during the last decade has yielded a considerable increase in new taxon descriptions. A DNA-based identification system can provide a useful tool for species identification and discovery (Navajas & Fenton 2000), as well for invasive pests which may be introduced through international trade. Investigations on the genetic variation at the intra- and inter-specific levels will assist in detecting polymorphism, cryptic species, geo-grahical races and other biologically significant groupings.

In addition, more complete and detailed alpha-level taxonomic descriptions and revisions of the EMs should be strongly encouraged and produced. Often, morphometric characterizations appear to be quite poor from an iconographic viewpoint without giving a clear delineation of the precise features of the species which could support further studies.

Furthermore, internal morphological knowledge has not been advanced enough. This fascinating and fertile area of research may be difficult to be fund in institutions of applied interest and the EMSs' tiny size and body complexity could represent another hurdle to wider study.

In conclusion, another point to stress is the urgent need to make all data available worldwide for translation, abstract indexing and paper accessible. Looking through the Abstract Reviews, many papers printed in Arabian, Chinese, Indian and Russian countries are lost. Similarly, papers still written in Arabian, Chinese and Russian are not easy to manage due to translation costs. The arrangement of a collaborative website could foster progress in the EM research community.

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CONSIDERATION ON ERIOPHYOID DETECTION

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Abstract

Eriophyoids represent a group of mites of major economical and ecological importance. Their small dimensions, specific host plant interaction and hidden life style make them difficult for routine detection. Symptoms induced by eriophyoids on host plants are considered as preliminary detection signs. Past methods included searching for abnormal plants, recognizing typical symptoms and detecting eriophyioids from detached organs and were the only known methods for finding these mites.
In the past few decades, searching for routine detection methods of these animals, many techniques have been developed to assist the operator to detect or measure eriophyoid populations. In a few cases procedures using sticky tape, ultrasonic baths, centrifugal flotation or brushing machines have been used. Most of the applied methods utilize a shake-and-wash technique to dislodge mites from plant organs by means of water (with or without detergent) or ethanol, propanol or liquid paraffin. For aerial trapping, sticky-coated glass slides, silicone grease-coated slides, petroleum jelly, vaseline or detergent-water pan traps were used.
Considering the importance of eriophyoids as pests of cultivated plants, vectors of viruses or useful agents in biological control of weeds, new efficient methods should be found. Developing immune enzyme assays and molecular methods for detection of eriophyoids will become very useful and possibly necessary for competent quarantine services and for other purposes.
Characteristics of different applied methods of eriophyoid detection, in respect to information available and needed for specific research purposes are described.

Key-words

monitoring, host-specificity, taxonomy, routine procedure.

Introduction

Eriophyoid mites represent a major, agriculturally important group of mites. They require considerable attention due to the damage caused to plants and their role in the transmission of plant pathogens. Besides *Tetranychus telarius* (Linnaeus) transmitting *Potato virus Y* (Schulz 1963), eriophyoids are the only mites transmitting plant viruses. Among the Acari, they are second only to the spider mites (Tetranychidae) in their economic importance as plant pests in the world (Lindquist *et al*., 1996).

Furthermore, these mites can be efficiently applied as biological control agents against weeds (Rosenthal 1996).

Specific research purposes (assessment of

population size and composition, pest surveys, taxonomy, rearing and mass release, etc.) require often a careful and standardized detection of the eriophyoids.

The small dimensions of these mites, the specific host/plant interactions and the hidden life-style make their population density difficult to estimate accurately and rapidly.

In the last two decades, many techniques have been developed to assist the operator in searching a method for the routinely detection of the eriophyoid populations.

Usually, specimens are collected directly from symptomatic plant material under a dissecting microscope. Mobility and tiny size of the mites beside the plant architecture complexity are responsible for the scarce efficacy and accuracy of this method. In addition, asymptomatic plant organs can host eriophyoid populations. Finally, the direct observation of infested plant material is time-consuming, tedious and often inaccurate.

The main eriophyoid detection methods currently used in specific research fields are discussed.

Review of detection techniques

A few developed procedures have used sticky tape or other glued traps, ultrasonic baths, centrifugal flotation or brushing machines.

Sternlicht (1966) modified a centrifugal flotation method to extract mites from buds of citrus infested by *Aceria sheldoni* (Ewing). A combination of alcohol and ultrasonic vibration was used firstly by Gibson (1975) who estimated the density of *Aculodes dubius* (Nalepa) on rye grass, and then by Ramarethinam *et al.* (2000) for sampling the coconut eriophyoid mite *Aceria guerreronis* Keifer.

David and Varadarajan (2001) applied the glycerine drop trap to assess populations of *A. guerreronis*: a drop of glycerine was placed on a fixed unit area using a steel pin-head, and the trapped mites were counted under a dissecting microscope. This method was compared to the counting template method proposed by Youthers and Miller (1934), who placed a fixed unit area cutting windows over a mite infested surface where eriophyoids were counted. The glycerine drop method was easier, more accurate and reliable to apply than the counting template procedure. In fact, mites in the cutting windows continued to move and hide on plant refuges, while mites inside glycerine drop were motionless and easier to count.

Harvey & Martin (1988) developed a sticky-tape method to evaluate the numbers of wheat curl mite *Aceria tosichella* Keifer by placing immature spikes on the sticky side of a strip of transparent tape. As the spikes dried, the mites crawled from them and became stuck to the tape. At the end of mite emergence, they were counted with the aid of a microscope at 15x magnification. Davis *et al*. (2002) applied a synthetic pyrethroid spray to the sticky-tape for estimating the critical abundance of *Acalitus essigi* (Hassan) from complex blackberry structure. As for spike studies, blackberry fruits at the red stage were placed on the centre of the sticky side, and located in a plastic pot to prevent mite air dispersal. After berry drying, the fruit residues were removed and the mites were counted at 20x magnification, showing that the insecticide application to the tape increased significantly the detection of the eriophyoids. In addition, the visual fruit receptacle count was less efficient than these two sticky-tape (spray and no spray treatments) methods.

Bernard *et al.* (2005) used a sticky-method, developed by Duffner & Schruft (1998) and Duffner (1999), to trap *Calepitrimerus vitis* (Nalepa) from buds during the spring migration period. Double side sticky-tape trap was placed above the base of each grape spurs and below node-1 bud, and replaced every 3 days. Mite numbers on tapes were counted through plastic sheets, at 50-70x magnification, to assess the population density.

The sticky-tape methods mentioned above are useful for the assessment of population density in architecturally complex plant structures, where the visual count under a dissecting microscope is difficult, and when the collection of live mites is not required. Moreover, mites collected from sticky tape could be deformed and, often, difficult to detach from the glue without destroying them. These methods appear to be not reliable for taxonomic study.

Most of the recent applied techniques have used a shake and wash method to detect mites from infested plant material.

Zacharda *et al*. (1988) described a shake and wash technique for monitoring mites in apple orchards, useful also to *Aculus schlechtendali* (Nalepa). Plant material (leaves, spurs and shoots with undeveloped leaves) were covered by 80-90% ethanol and were shaken for 5-10 seconds. Subsequently, the plant material was removed and the alcohol containing the preserved mites was poured into a separating funnel. The mites, settled on the bottom, were counted under a dissecting microscope after the alcohol evaporation. The authors compared this method with a direct mite

count on leaves under a dissecting microscope. Shake and wash technique was more efficient by 10-20%.

Pérez-Moreno & Moraza-Zorrilla (1998) developed a similar technique in investigations on the biology of *C. vitis*. Grapevine leaf samples were submerged in a 70% ethanol solution for 5 minutes in order to kill the mites. The leaves were individually washed under tap water and mites collected using a 25-µm sieve, and thus transferred in a Petri dish using a solution of 70% ethanol and 5% glycerine. After ethanol evaporation and glycerine mite embedding, eriophyoids were counted under a dissecting microscope at 70x magnification by placing a sheet of millimeter graph paper under the dish.

Gabi and Mészáros (2001) developed their washing technique to estimate the population of *C. vitis* on grapevine leaves which were cut up in small pieces and washed with tap water. Subsequently, the shaking solution was coloured with Azur II eosin and the leaf suspension was sieved through a two-phase vacuum sieving. Mites were counted at 50x magnification on the sieving dish.

Faraji *et al.* (2004) developed an elaborate method for the extraction of mites from leaf samples, in which the mite cuticles adhere to liquid paraffin. Fresh apple leaves were collected in bottles filled with tap water and 3 ml of detergent. Bottles were shaken three times for a few seconds during a period of 1-2 h. The suspension was poured onto a 45 µm- meshed sieve. Mites and debris were washed with 70% ethanol and stored in centrifuge tubes. The sediment of the tube was poured in a mite-counting channel and ethanol was added to fill the channel. A few droplets of methylene blue were added to stain the ethanol and the solid material, but not the mites. Subsequently, liquid paraffin was added to embed mites at the ethanol/paraffin interface, allowing an easy count of mites under a dissecting microscope.

In all the wash methods described above, a destructive chemical (usually ethanol), that immediately kills eriophyoids, is used. Nevertheless in some studies such as evaluations of toxic effects of chemical applications on plant, rearing and mass release, etc., researchers need to have live specimens from infested plant extraction. Moreover, the sieve size is decisive in eriophyoid collection: in 45 µm-meshed sieves, most adults are caught, but the smallest species, mites at juvenile stages, and eggs are lost. To avoid these undesired losses, 20-25 µm- meshed sieves are recommended.

Siriwardena *et al*. (2005) developed an accurate method to estimate *A. geurreronis* population density on an infested coconut. A simple equipment, composed of a translucent silicone tube, a conical plain funnel and a Mohr's clip, was used to wash plant material. A solution of 8-10 drops of a detergent (Tween 80) in 250 ml of tap water was used to wash mites from coconut. Counting of eriophyoids was made on only 1 ml of washing solution collected from the bottom of the tube, than the population density was proportionally estimated. The main advantage of this method is the homogeneity of mite distribution in the wash solution immediately after the shaking, which thus allow to count only 1 ml of the wash solution per each extraction.

Recently, Monfreda *et al.* (2007) described methods for routine detection, collection and extraction of eriophyoid mites and their eggs. The protocols described modify methods presented by de Lillo (2001) and de Lillo & Monfreda (2004). Infested plant material was placed in a container and covered with a washing solution (0.2% household detergent or Tween 80 and 1–2% bleach in tap water). The suspension was stirred for 5-10 minutes manually or by using a magnetic stirrer. Then, mites were collected by pouring the suspension onto four ASTM-stainless-steel sieves (mesh size: 850, 180, 53 and 25 µm). The sediments of each sieve were poured in a Petri dish and a few droplets of detergent were added to the suspension to allow mites to sink to the dish bottom. Mites were counted under a dissecting microscope at 20-25x magnification. Motile eriophyoids typically remain live and active under these conditions.

The authors compared this washing and sieving technique with the direct counting of mites on infested plant material. This method was more accurate and less subjective than the direct counting on plant material under a dissection microscope, and its efficiency probably increased with the complexity of plant structures, and the plant trichome density, which can hide small aggregated colonies.

The authors also described a procedure to concentrate mites and eggs for rearing and mass release.

This procedure offers some improvements to the older methods and simplifies mite extraction. Mite counting were not affected by plant debris because they are collected in the fraction containing larger mites (180 µm mesh size). Tap water, bleach and domestic detergent are highly effective, readily available and an economic alternative to ethanol

or other chemicals. Moreover, the chemicals and the technique used did not affect both slide-mounting procedure and viability of mites, which could be used for taxonomical and biological studies, rearing, mass release, host specificity tests and biological assays.

For aerial eriophyoids trapping, most researchers have used sticky glass slides or plates coated with grease (silicone grease, petroleum jelly or vaseline) (Perring *et al*., 1996; Duffner *et al*., 2001). Zhao & Amrine (1997) developed a new method for studying aerial dispersal of eriophyoid mites, based on detergent-water pan traps. Aluminum pans were filled 3/4 full with soapy water (2 ml dishwashing detergent) and were placed horizontally on a 15 cm-high support. Twenty-four hours after their exposition to the air, the soapy water was collected and vacuum-filtered on micro-filter papers. Mites trapped on the filter papers were examined under a dissecting microscope at 20-80x magnification. The efficiency of detergent-water pan traps was compared with that of conventional sticky plates. The new method was superior to the sticky plate one in the detection of eriophyoids from the air, for the considerable time required to screen grease-coated plates under a dissecting microscope and manage mites contaminated by grease which may be deformed and difficult to identify. This method is more efficient for investigation of airborne activity and taxonomic studies on eriophyoids.

Concluding remarks

In the last two decades, eriophyoid detection has been the object of many studies, some of that are here cited, which have appreciably improved the knowledge in this matter. Due to the importance of eriophyoids as crop pests, virus vectors and weed control agents, new and more efficient detection methods should be found.

Immunochemical and molecular methods have assumed an increasing importance in pest detection on plant and stored products (Rotundo *et al*., 2000; Brader *et al*., 2002; Phillips & Zhao, 2003; Krizkova-Kudlikova & Hubert, 2007; Krizkova-Kudlikova *et al*., 2007). Being cheap, practicable, highly specific and sensitive, these techniques can also be very useful to determine and quantify mite infestations. However until now, no applications of sierological and molecular techniques on eriophyoid detection have been reported in literature.

Serological methods are based on the recognition of pest's antigens by antibodies, while molecular ones are based on the recognition of pest's genomic sequences by nucleic acid probes. Also, specific pest products such as toxins or allergens can be seeked.

Nowadays, both serological and molecular techniques can be integrated into micro-arrays, that allow the simultaneous analysis of many thousands of single tests.

Developing immune enzyme assays and molecular methods for detection of eriophyoids could be very useful and possibly necessary for competent quarantine services and other purposes such as resolving the separation of overlapping symptoms of eriophyoids with phytoplasma diseases.

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BEHAVIOUR OF ERIOPHYOID MITES (ACARI: ERIOPHYOIDEA)

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Abstract

In our review we present the actual state of investigations on eriophyoid behaviour. The studies to date are fragmentary and refer mainly to reproduction, feeding and dispersal. We suggest that more stress should be put on the ecological and evolutionary aspects of eriophyoid behaviour. Further research should include reproductive behaviour, host acceptance, avoidance of predation and dispersal behaviour of eriophyoid mites.

Key-words

dispersal, antipredatory behaviour, feeding and host-acceptance behaviour, mating, sex dissociation, guarding

Introduction

Eriophyoid mites are highly specialised plant parasites, and many species are serious pests of crop plants (Lindquist *et al*. 1996). A knowledge of the behavioural interactions in these mites can be extremely helpful for a better understanding of their ecology and evolution, as well as to resolve many problems of a phylogenetic and applied nature. The purpose of this paper is to give a brief review of what has been done so far in respect of eriophyoid' behaviour as well as to present developing research topics. Much attention has been given to the following topics: mating, feeding and host acceptance, dispersal and avoidance of predators. We hope that our discussion will stimulate further development of behavioural studies on this fascinating group of mites.

Feeding and host acceptance behaviour

As summarized by Westphal & Manson (1996), most information concerns the feeding behaviour of free-living eriophyoids mites (Krantz 1973, Gibson 1974) or gall mites studied in artificial conditions outside their galls (Westphal *et al*. 1981). These observations have shown many interesting details regarding the position of the body and mouthparts during feeding as well as movement of mites in order to find a suitable feeding place. Strict preferences for host plants have not been observed. However no-choice experiments testing the host acceptance of the cereal rust mite *Abacarus hystrix* (Nal.) have been carried out. This experiment has supported the hypothesis of narrow host specialization of host-associated populations of this species (Skoracka *et al*. 2007). Using such behavioural data the host specificity of eriophyoids can be tested. This is especially important with respect to invasive species which are able to extend their host range and become a problem in agroecosystems, as for

example *Aceria guerreronis* Keif. (Navia *et al*. 2005), and *Aculops lycopersici* (Massee) (Oldfield 1996).

Reproductive behaviour

Eriophyoid mites reproduce by sex dissociation (non-pairing). Males deposit spermatophores regardless of the presence of females, while females seek spermatophores and pick them up on their own (Oldfield *et al*. 1970, Sternlicht & Goldenberg 1971, rev. Thomas & Zeh 1986, Oldfield & Michalska 1996). Behavioural observations made so far indicate that the dissociation between eriophyid male and female is complete, i.e. a male does not require the presence of a female or female's chemical cues in order to deposit a spermatophore. Males emit spermatophores at some intervals preferentially placing them in aggregations with previously deposited ejaculates (Oldfield & Michalska 1996). Males of some eriophyoid species showed interest in quiescent female nymphs (QFN), encircled them with spermatophores, and even guarded the QFNs for many hours until female emergence (Michalska1999, rev. Michalska & Mańkowski 2006). Several factors can influence spermatophore deposition rate of eriophyoids, e. g. the time of day, the presence of conspecifics, leaf age, and the injury to the leaf (Michalska 2000, Michalska & Shi 2004, Michalska 2005).

There are still some fundamental questions regarding eriophyoid reproductive behaviour still remain to be answered: What is the effect of external sperm competition on spermatophore deposition by an individual male? What is the role of sex attractants in communication between males and females? Do eriophyid females choose spermatophores of specific males? What are the mating systems of eriophyoids and what could be the impact of a host plant on the evolution of these systems?

Dispersal

Eriophyoid mites are known generally to be dispersed between plants passively by wind currents (Nault & Styer 1969, Zhao & Amrine 1997a). Many authors have reported eriophyoids in the air by inference from catches in sticky traps or on plates. Some behavioural adaptations to facilitate aerial dispersal have also been observed (rev. Sabelis & Bruin 1996). The other way of eriophyoid mite dispersal is phoresy. Several cases of eriophyoid mites holding on to other arthropods have been reported (e.g. Shvanderov 1975, Waite & McAlpine 1992, Zhao & Amrine 1997b). Which of these two modes plays the more significant role in eriophyoid mite dispersal? Such knowledge is needed to define strategies for management of eriophyoid crop pests, to estimate the risk posed by pest species to a new area, and also to estimate and optimize spread of weed biocontrol agents.

Avoidance of predation

The use of refuges appears to be a common phenomenon in eriophyoid mites. It refers not only to gall formers or the eriophyoids inhabiting the narrow spaces of plants (e.g. acarodomatia, spaces under scales of buds, bulbs and fruits) (Sabelis & Bruin 1996, O'Down & Willson 1997, Kasai *et al*. 2002, Leśna *et al*. 2004, Aratchige *et al*. 2007, Lawson-Balagbo *et al*. 2007) but also to free-living species (Kranz 1973, Michalska 2003). An experiment with the vagrant *Rhinophytoptus concinnus* has confirmed that "perching" on leaf tips enables the quiescent nymphs to avoid predation by phytoseiid mites. Climbing trichomes by the eriophyids was independent of the actual presence of predators on leaves (Michalska 2003). Still, there are no data on the individual flexible behaviour of eriophyoid mites. It is urgently needed to determine whether these mites can respond to the actual risk of predation and, if so, how it can influence their feeding, reproduction and dispersal behaviour.

Conclusions and future perspectives

There are still many gaps in our knowledge about eriophyoid behaviour. It concerns especially the host acceptance behaviours, avoidance of predators and dispersal behaviour. Continuation and deeper investigation of the above-mentioned aspects is needed from both ecological and evolutionary points of view. The development of research programs focused on eriophyoid mites' behaviour can have ramifications for management of pest or invasive mite outbreaks and perhaps also for the spread of associated plant pathogens.

Many other important problems have still not been touched. Such issues regard e.g. social behaviour of some nest building eriophyoid species, behaviours connected with parental care as well as inter-specific competition. It is also important to recognize internal mechanisms explaining how eriophyoid mite behaviour is elicited and coordinated and what the physiological and anatomical mechanisms that underlie the behaviour are. The impact of genetic components, physiological mechanisms, environmental

conditions needed and hormones on eriophyoids' behaviour should be studied.

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CRITICAL ASPECTS OF DNA-BASED METHODS FOR ERIOPHYOID MITE DIAGNOSTICS AND GENETIC STUDIES: REVIEW, PROSPECTS AND CHALLENGES

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Abstract

DNA-based methods have revolutionized the field of species diagnostics, and today it has applications for an increasingly number of taxa including several Acari. The media excitement around the international Barcode project is an example. Besides their potential for species identification, DNA marker techniques are nowadays routinely being also used for addressing ecological, evolutionary, phylogenetic and genetic questions. In contrast to other groups of plant mites and despite the economical relevance of many species of Eriophyoidea, very few scientists have dared so far to use DNA methods for the study of this group of mites; their very small size being certainly the major cause. However, DNA-based techniques are now well established and their advantages as well as limitations have been realized. We review here the main techniques used for identification and discus on their applicability in eriophyoids. Main results from the literature will be examined. We will emphasize prospects and challenges of the molecular genetics approach to study several essential issues of the eriophyoid biology to: clarify suspect synonymies, test hypothesis of cryptic species; examine the occurrence of biotypes, especially in rapport to virus ability; understand colonization patterns of invasive species; and use of biological control agents against invasive plants. We will discuss these questions that can be link to economical issues, together with more fundamental aspects as reviewing the phylogeny of the Eriophyoidea. Much is now expected from molecular techniques in many fields of biology. Eriophyoids should not be the exception.

Keywords

Molecular markers, species diagnostics, phylogeny, Eriophyoidea

Introduction

DNA-based techniques are increasingly used in Acarology studies, particularly for systematic and population biology, having contributed to explore some questions that were difficult to answer some years. The field of biological diagnostics, for instance, was revolutionized with the advent of the polymerase chain reaction (PCR). The ability to amplify numerous copies of a gene or genomic region of interest opened up a world of possibilities in terms of identification of organisms, genes, genotypes, mutations and populations. Hence, besides providing solid taxonomic criteria, data obtained through DNA based analyses can aid in testing phylogenetic hypotheses and gain in understanding the partition of the variability within a species.

Although efforts in using molecular biology techniques have been also made on Eriophyidae, the attempts are still scarce compared to other plant mite families. This is a regrettable observation, considering that among plant feeding

mites, Eriophyidae represent the second group in economic importance as pests after the Tetranychidae (Lindquist and Amrine 1996) and that is one with the higher number of taxa (3.440 species from 301 genera) (Amrine Jr. and De Lillo 2003). Although Eriophyoidea is an extensively studied group, studies have mainly focused on the biology and control of a limited number of species basically based on observational data (Lindquist et al 1996; Davies et al 2001). In the last few years, the wealth of DNA-based resources has started to be used in eriophyoids. This paper presents a review of the advances made using molecular techniques and gives prospects and challenges to be addressed in the near future.

Which molecular genetic marker to use?

In the last two decades, several key advances in molecular genetics appeared which have greatly increased the impact of molecular techniques on biology. Most important have been: (1) the development of PCR, which amplifies specified stretches of DNA to useable concentrations; (2) the application of evolutionarily conserved sets of PCR primers (Simon et al. 1994); (3) the advent of hypervariable microsatellite loci (Goldstein and Schlötterer 1999); and (4) the advent of routine DNA sequencing in biology laboratories. These innovations, coupled with the recent explosion of powerful analyses and relatively user-friendly computer programs (Excoffier and Heckel 2006), made that much of the power inherent in molecular genetic data can be useable for biological studies.

After the invention of the PCR technology, a large number of approaches to generate molecular markers have been created (Behura 2006). These techniques are well established and their advantages as well as limitation have been realized. All genetic markers reflect differences in DNA sequences, usually with a trade-off between precision and convenience, and then in addition to technical details, focusing on important properties helps to make sense of the methods. In addition, separate loci can provide independent test of hypothesis, thus using several together can increase sensitivity. Table 1 summarizes attributes of markers commonly used. Among them, the media excitement around the international Barcode project is an example of the power and limitations of using molecular techniques, in this case for species diagnostics purposes (Savolainen et al. 2005).

The Barcode initiative

The concept of a DNA barcode has recently been proposed as a method of diagnosing species both known and unknown. The DNA barcode approach uses nucleotide sequences consisting of unique combinations of bases occurring in conserved regions of genes that are easily amplified with PCR and direct sequencing. It uses short DNA sequence from a standardized and agreed-upon position in the genome for molecular diagnosis and identification at the species level. For most animals, including the Acari, the Cytochrome C Oxidase subunit 1 (*COI*) mitochondrial gene has become the standard barcode region.

When eriophyid biology and molecular techniques meet:

As for other Acari, the Eriophyidae have beneficiate of the rapid development of molecular methods that measure genetic variation. Although the palette of technical approaches used for this group is still limited, important advances could be done by using DNA-based techniques.

The nuclear regions used in eriophyoids include the ribosomal Internal Transcribed Spacer (ITSI and ITS2) and associated genes (18S, 5.8 and 28S). Results obtained with *Cecidophypsis* mites (Kumar et al. 1999; Lemmetty et al. 2001) have indicated that the ITSI was more useful than ITS2 to distinguish closely related species. In addition, microsatellite loci have been used by (Carew et al. 2004) to evaluate population structure of a grapevine pest *Colomerus vitis* (Pagenstecher). Among the mitochondrial genes, the mitochondrial 16S was used (Navia et al., 2005), and the COI of four eriophyid – *Aceria tulipae* (Keifer), *Aceria eximia* Sukhareva, *Eriophyes pyri* (Pagenstecher) and *Floracarus perrepae* Knihinicki & Boczek– has been already sequenced (data published on data bases only; source www.ncbi.nlm.nih.gov, on May, 18th 2008).

Identification of species

Molecular tools can be extremely useful in Eriophyoidea systematics considering that using exclusively morphological characters to their identification present several limitations. Because of the considerable reduction and simplification in the body plan of eriophyoids, the structures that can be used for eriophyoid systematics are scarce, compared to most of other mites. Another limitation of some species is their lack of ontogenetic diversity as well as the lack of useful

characters peculiar to the adult male (Lindquist and Amrine 1996). Advances in molecular biology have provided data on nucleotide variation that added to more traditional morphological features help in establishing reliable criteria to determine species.

Table 1. Comparison of features of frequently used molecular marker techniques. Abbreviations of each marker appear in the table and the corresponding full name is indicated at the bottom.

	Abundance	Reprodu-cibility	Single locus	Degree of polymorphism	Codominant	Technical requirement	Tissue required	PCR assay
Mitochondrial								
RFLP	high	straight	yes	low to high	yes	high	high	yes
Sequences	high	straight	yes	medium	yes	medium	low	no***
Multilocus nuclear								
RAPD	high	limited	no	high	no	low	low	yes
AFLP	high	limited	no	high	no	medium	medium	yes
Single-locus nuclear								
Allozymes	low	straight	yes	low	infrequent	medium	high**	no
Microsatellites	high	indirect	yes	high	yes	high	low	yes
Anonymous scn	high	indirect	yes	medium	yes	medium	low	yes
Specific scn	medium	straight	yes	low	yes	medium	low	yes
ribosomal DNA	low	straight	*	medium	yes	medium	low	yes

Full name of markers: RFLP restriction fragment length polymorphism; RAPD random amplified polymorphic DNA; AFLP amplified fragment length polymorphism; *scn* single copy nuclear.

* ribosomal DNA consists of tandem arrays of a few regions. In some taxa the arrays are effectively identical and regions act as single loci, but in some taxa there can be many different sequences within individuals, in which case rDNA acts more like a multilocus system
** Fresh or frozen material is needed for allozymes. By contrast, all other techniques allow using ethanol preserved samples
***Sequencing it self do not use PCR techniques but sequences are usually obtained after PCR amplification of the targeted DNA fragment.

A series of studies on species identification, phylogeny and intraspecific variability in the *Cecidophyopsis* Keifer genus, have been conducted since 1995 (Fenton et al. 1995; Fenton et al. 1996; Fenton et al. 1997; Fenton et al. 2000). This group includes mite species known to occur on twelve *Ribes* species and several of them are serious agricultural pest(De Lillo and Duso 1996). Kumar and co-workers (1999) developed a PCR multiplex technique for identifying *Cecidophyopsis* mites using species-specific differences in ITS-1 sequences. The PCR multiplex technique presented in Kumar and coworkers (2001) was used by (Lemmetty et al. 2001) to conduct a detailed study on the identification of *Cecidophypsis* mites on *Ribes* in Finland.

Grapevine eriophyoid mites – *Colomerus vitis* Pagenstecher and *Calepitrimerus vitis* Nalepa – are recognized pests. The identity of these mites has recently been investigated using molecular markers - PCR-RFLP of the ITS-1 and microsatellite (Carew et al. 2004). Authors concluded that PCR-RFLP of the ITS-1 region could be routinely used as a rapid diagnostic tool for confirming the species of mite present in a vineyard.

Plant-mite interaction

One very useful application of genetic data in pest management is to investigate specialisation of mites to their host plant, which in some cases have uncovered host races. In eriophyoids, some economically important issues could be addressed by using DNA markers as illustrated below.

The eriophyid mite *Aceria cajani* (Channabasavanna) is the vector of the agent of pigeonpea sterility mosaic disease (PSMD).

Integrated management of PSMD includes the development of resistant cultivars. However, pigeonpea genotypes resistance was found to be location specific. It is possible that the breakdown in PSMD resistance at various locations is due to the occurrence of different *Aceria* species or biotypes of *A. cajani*. Aiming to test this hypothesis, the variation of *A. cajani* was assessed using the ITS region and associated rDNA genes, by analyzing nucleotide sequences and patterns of restriction enzymes (Kumar et al. 2001). Results strongly suggested that *A. cajani* on pigeonpea across the Indian subcontinent constitutes a single species.

The phylogenetic relationship of seven species of *Cecidophyopsis* mites with its *Ribes* hosts was inferred from ribosomal sequences of the ITS region and surrounding regions (18S, 5.8S and 28S) (Fenton et al. 2000). The comparison of two phylogenetic trees (mites versus hosts) showed clear differences of structure, implying that the mite speciation did not closely follow speciation events in the plant hosts.

Pest-movements, colonisation patterns and bioinvasions: the coconut mite *Aceria guerreronis*, a case study

The coconut mite, *Aceria guerreronis* Keifer, has recently spread and rapidly established in the main coconut production areas worldwide, being considered as an invasive species. The mite has not been recorded in the Indo-Pacific region, the area of origin of coconut, suggesting that it has infested coconut only recently. To investigate the geographical origin, ancestral host associations, and colonization history of the mite (Navia et al. 2005) conducted a phylogeography study, using DNA sequence data from two mitochondrial (16 S) and one nuclear region (ITS1 and ITS2) from samples from the Americas, Africa and the Indo-ocean region. The results suggest that the mite originates from the Americas and not from the ancestral region of coconut in South East Asia and lend evidence to a previous hypothesis that the original host of the mite is a non-coconut palm (Fig 1).

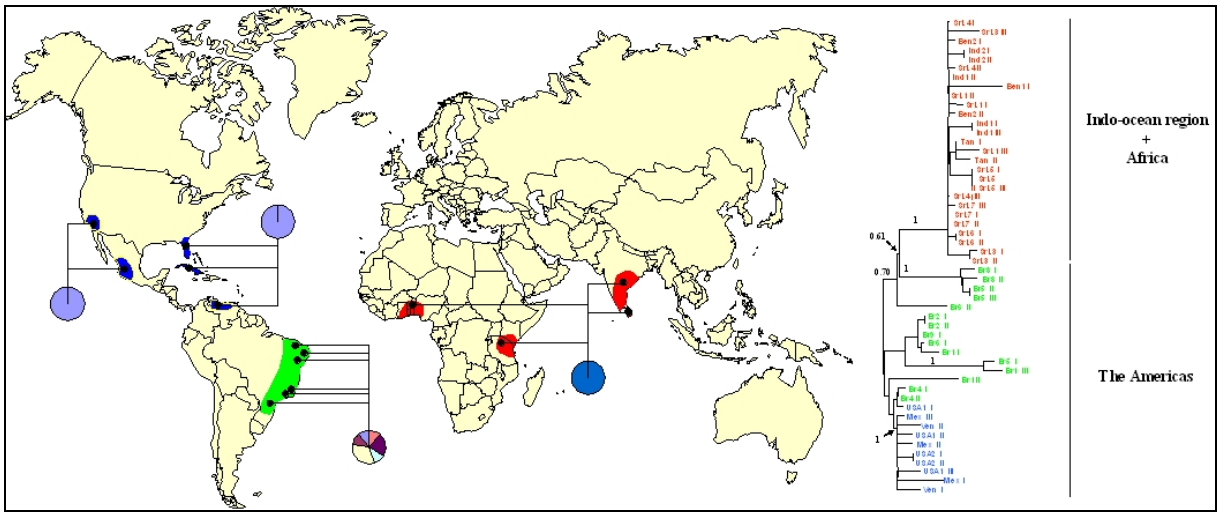

Figure 1. Phylogeographical history of the coconut mite, *Aceria guerreronis*. The three geographical regions sampled are indicated in red (Indo-ocean region), green (Brazil) and blue (other American countries) (a) different mitochondrial haplotypes detected and their frequency in the different sampled localities (black dots) are indicated by the pie charts. The highest nucleotide diversity was found in Brazil where six out of the seven haplotypes were present. By contrasts one haplotype (here in pink) was found in Central and North America and a single one (here in yellow) was shared by non-American mites from Africa and the Indo-ocean region (India and Sri-Lanka). (b) Congruently, the tree constructed with the nuclear ITS sequences revealed that all non-American samples (in red) are very little diversified and cluster together, whereas the Brazilian (in green) are represented in several branches of the tree. The rest of the American samples (in blue) are gathered in a single cluster.

Molecular techniques to study eriophyids: challenges and new avenues

The field of molecular marker technology is fast progressing by adopting new forms and innovative approaches of the existing genetic principles in detecting DNA polymorphism and the minute eriophyoids in increasingly beneficiating of all this progress. Much is now expected of the molecular techniques on eriophyoids biology. We discuss some of the major issues that we think might take advantage of molecular approaches in the next future. Much progress might be expected for systematis. The majority of taxonomic groupings of eriophyoid species are artificial. As a result, the current classification has little predictive power (Lindquist and Amrine 1996). The lack of information on the Eriophyoidea phylogeny has been an important limitation on the progress of systematic and biology of the group. For lower taxonomic levels, uncertainties on Eriophyoidea systematics are also numerous and molecular techniques can answer questions on synonymies and cryptic species. For more applied areas, molecular studies can significantly contribute to define pest management strategies in eriophyoid, as for example by using them as weed natural control agents or for they relevance as phytovirus vectors. Among phytophagous mites, eriophyoid are becoming increasingly recognized for their potential as invasive. Molecular data can provide information on the routes of colonization or pathways of invasive eriphyoid, required to guide adoption of quarantine measures.

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ERIOPHYOID AND TRANSMITTED PATHOGENS: THE ROLE OF THE MANGO BUD MITE IN MANGO MALFORMATION EPIDEMIOLOGY, PRESENTED AS A CASE STUDY

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Abstract

Mango malformation caused by the fungus *Fusarium mangiferae* is one of the most destructive mango diseases. Numerous reports suggest an association between the mango bud mite *Aceria mangiferae* and the pathogen and maintain that the mite plays an important role in disease epidemiology. However, those claims were mainly based on circumstantial evidence and it is still not clear that the two organisms actually interact, partially due to a lack of suitable tools for tracking the pathogen. To elucidate the role of the mite in the disease epidemiology we studied the possible interactions with *F. mangiferae.* Following the mite's exposure to a gfp-marked isolate, conidia were observed clinging to the mite's body. Conidia were found in bud bracts only when both mites and conidia were co-inoculated on the plant, demonstrating that the mite vectored the conidia into the apical bud. Frequency and severity of infected buds were significantly higher in the presence of mites, demonstrating its relevant role in the fungal infection process. No windborne bud mites bearing conidia were found when monitoring for the organisms in an infected orchard, however, high numbers of windborne conidia were detected in the traps. These results suggest that *A. mangiferae* can vector the pathogen, assist in fungal penetration, but does not appear to play a role in the aerial dissemination of conidia. The results of this case study are discussed in the context of similar bud mite related plant disorders, proposing that future research take into account potential mite fungal interactions.

Key-words

Aceria mangiferae, *Fusarium mangiferae*, Eriophyidae, *Mangifera indica* L., bud proliferations

Introduction

Mango malformation disease is one of the most destructive diseases of mango and is prevalent in most of the mango production areas worldwide (Kumar *et al.* 1993; Ploetz *et al.* 2002). The disease causes malformation of vegetative growth and inflorescence, and results in serious yield loss since malformed panicles do not bear fruit (Kumar *et al.* 1993; Majumder & Sinha 1972).

Fusarium mangiferae Britz, Wingfield & Marasas is

the causal agent of mango malformation disease (Chakrabarti & Ghosal 1989; Freeman *et al.* 1999; Noriega Cantu *et al.* 1999). Little is known about the epidemiology of the disease, location of penetration sites, modes of infection and colonization of the plant tissue. One work suggests a wind dispersal mechanism of fungal conidia (Noriega Cantu *et al.* 1999), but since trapped airborne conidia were not exclusively attributed to this species, more evidence needs to be provided.

Aceria mangiferae Sayed was first recorded and described in Egypt (Hassan 1944; Sayed 1946). It is commonly found inside generative and vegetative closed mango buds, in both malformed and non-malformed trees (Sternlicht & Goldenberg 1976). Many reports suggest the involvement of *A. mangiferae* in mango malformation as both the mite and the fungus are found together within the bud, however it is still not clear that these two organisms actually interact in the epidemiology of this disease (Ploetz 2001). Possible reasons for the lack of information can be related to problems with identification of the pathogen and the lack of molecular tools for tracking the fungus.

With the intention of elucidating the role of the mite in mango malformation disease epidemiology we explored four possible interactions between the mango bud mite and the pathogen: 1- carrying *F. mangiferae* conidia on its body; 2- vectoring the pathogen's conidia into the apical buds; 3- assisting conidial penetration to plant tissue; 4- aerial dissemination of conidia. In this proceedings paper we concisely present the methods and results of this study, for a more detailed account please see Gamliel-Atinsky *et al.* (in press).

Materials and methods

Mites bearing the pathogen

Mites collected from infested buds in the orchard were exposed to a gfp (green fluorescent protein) - marked isolate, using two different methods. In the first method 20 mango bud bracts bearing approximately 100 mites were dipped in a gfp-marked *F. mangiferae* suspension of 10^6 conidia per ml for 5 seconds. After allowing the bud bracts to dry, mites were removed from the bracts with an ultra fine paint brush and mounted on double-sided sticky tape. In the second method 30 mites were placed on an agar plug (5mm^2) which was inoculated 48 hours beforehand with the gfp-marked isolate. After 24 hours the mites were removed and mounted for microscopic observation. Images were acquired using a confocal laser-scanning microscope system OLYMPUS IX81. Confocal images were obtained via a PLAPO 40X WLSM immersion objective lens at an excitation wavelength of 488nm (Argon laser), a BA515-525 emission filter for gfp and BA660IF emission filter for auto-fluorescence. Transmitted-light images were acquired using Nomarski differential interference contrast.

Vectoring the pathogen into apical buds

The experiment was performed on potted mango plants, in a controlled environment growth chamber with 25C°±2 C° and 14:10 L:D. The plants were fumigated twice with dichlorvos (30ml/100m^2; Makhteshim Chemical Works Ltd, Beer Sheva, Israel), two weeks before the beginning of the experiment to ensure that they were void of mites or insects. The base of the stem was ringed with a sticky barrier to prevent infestation by ambulant arthropods. Each plant was placed in a disinfected plastic cage and was treated with one of the following four treatments: treatment 1- 100 mites were placed on two 5mm^2 agar plugs with the gfp-marked isolate. The agar plugs bearing bud mites and the gfp-marked pathogen were then placed on a leaf, approximately 5cm away from an apical bud; treatment 2- 100 mites were placed on two 5mm^2 agar plugs without the fungus and then placed near an apical bud as described above; treatment 3- two 5mm^2 agar plugs with the gfp-marked isolate were placed near an apical bud; treatment 4- untreated control. Four apical buds were inoculated in each treatment and the experiment was repeated 5 times. Two days following inoculation the apical buds were inspected under a stereomicroscope and the bud mites were counted. Then, the gfp-marked conidia (if present) were washed from the bud bracts and plated on a potato dextrose agar (PDA) selective media amended with 50 μg/ml hygromycin, allowing selective isolation of the transgenic ungal strain. After 5 days, the gfp-marked colonies were enumerated on the plates.

Assisting fungal penetration

Quantitative evaluation – The experiment was performed on potted plants as described above. Each plant was placed in a disinfected plastic cage and was treated with one of the following two treatments: treatment 1- 40 apical buds were inoculated with a 10^6 gfp-marked conidial suspension; treatment 2– 40 apical buds were inoculated as in treatment 1 and also with bud mites that were collected from the orchard (50 mites per bud). Three weeks post-inoculation apical buds were separated into bracts, surface-sterilized 10 seconds in 70% ethanol and 3.5

minutes in 0.1% $NaClO_3$, and placed on Nash-selective medium (Nash & Snyder 1962). Fungal growth was evaluated after 5 days and two parameters were measured: 1. Frequency of infected buds was evaluated calculating the percentage of infected buds in the treatment, using a Pearson statistical test to compare the two treatments ($P<0.05$); 2. Severity of infection was measured by calculating the average number of infected bracts per bud and the means of the two treatments were compared using Tukey-Kramer analysis ($P<0.05$).

Qualitative evaluation. – Apical buds that were inoculated with both the pathogen and the mites, were separated into bracts and inspected with a confocal microscope (as described above) and Jeol (Tokyo, Japan) scanning microscope 5410 LV.

Aerial conidial dissemination

Both fungal conidia and mango bud mites were trapped using the following trapping methods: 1. Spore dissemination in the Volcani mango orchard was monitored using a Burkard™ volumetric spore trap (Burkard Manufacturing Co Ltd, Rickmansworth, England) placed in the orchard, sucking in air at a speed of 10 liters per minute on adhesive coated transparent plastic, continuously, for periods of seven days. The adhesive tape was then washed and mounted on the selective Nash medium for enumerating *F. mangiferae* colonies. 2. Petri dishes containing the selective Nash medium for *F. mangiferae* were opened and exposed to the orchard's environment overnight, then incubated in the lab and examined for the presence of *F. mangiferae* colonies. 3. For monitoring wind blown mites in the orchard, a freely rotatable wind trap made of a PVC pipe (9-cm in diameter) was constructed and mounted on a pole attached to a wind vane (Duffner *et al.* 2001). The pipe's floor was covered with 70 sticky slides that were replaced monthly, then inspected under a stereo-microscope for the presence of mites and placed on Nash selective media for pathogen detection.

Results and Discussion

Mites bearing the pathogen

Mites from bracts removed from the conidial suspension did not bear conidia. However, gfp-marked conidia were observed on mites that were placed on agar plugs bearing the marked strain (Fig. 1). Conidia of the pathogen did not seem to cling to any particular part of the mite's body.

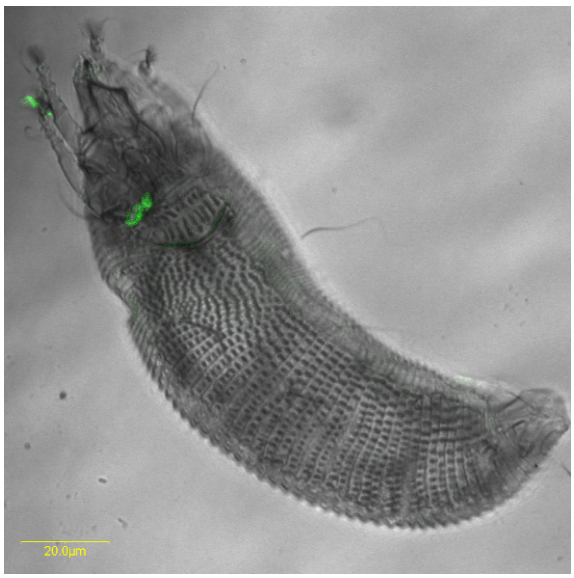

Figure 1. Mango bud mite, *Aceria mangiferae*, bearing gfp-marked conidia (in green) of *Fusarium mangiferae*, the causal agent of mango malformation disease.

Vectoring the pathogen into apical buds

Bud mites were found in 25 % and 35 % of the inoculated buds of treatments 1 (inoculation with bud mite and with gfp-marked conidia) and 2 (inoculation with bud mites), respectively, showing clearly that the bud mites could orientate themselves from the adjacent leaves to the apical bud. No bud mite was found in treatments 3 (inoculation with gfp-marked conidia) and 4 (untreated control) which confirms that the plants used in these experiments were not infested prior to the initiation of the experiment. The gfp-marked conidia were found in bud bracts of 25% of the inoculated buds in treatment 1 and not in buds of other treatments. This demonstrates that the mango bud mite is able to carry *F. mangiferae* conidia on its body and transfer them into the apical bud.

Assisting fungal penetration

A significantly higher degree of apical buds were infected in the gfp + mites combined treatment (50 % in the gfp-inoculated treatment compared with 82% infected buds in the combined treatment- Fig 2 A). Moreover, the infection severity was also higher in the combined treatment where more bracts per bud were infected (Fig. 2 B). This suggests that the bud mite may play a role in pathogen penetration of the bud tissue, perhaps through the feeding wounds it creates on the bud bracts, thereby facilitating conidial penetration. Microscopic observations supported our finding by demonstrating the physical proximity and the actual contact between the two organisms. Bud mites were observed touching gfp-glowing hyphae and conidia using a confocal microscope (Fig. 3 A). SEM (scanning electron microscope) observations also illustrated bud mites bearing hyphae and conidia on their body (Fig. 3 B).

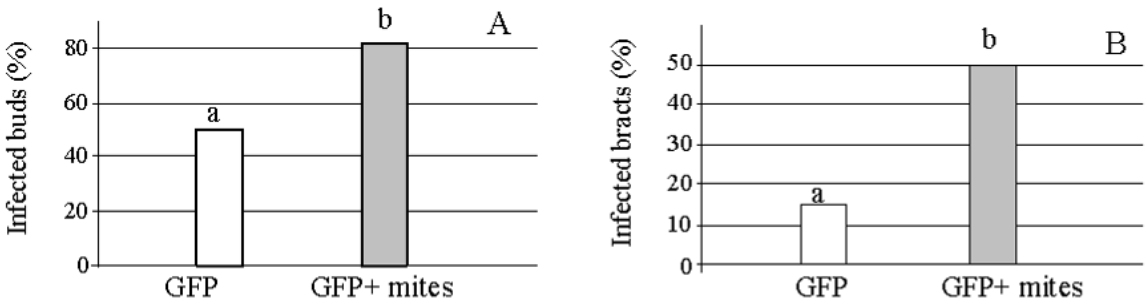

Figure 2. Frequency and severity of infection in mango apical buds inoculated with gfp-marked conidia of *Fusarium mangiferae* (treatment 1-GFP) or with a gfp-marked conidia and with bud mites, *Aceria mangiferae* (treatment 2- GFP + mites). A- Frequency of infected buds with/without the presence of bud mites. B- Frequency of infected bracts per bud with/without the presence of bud mites.

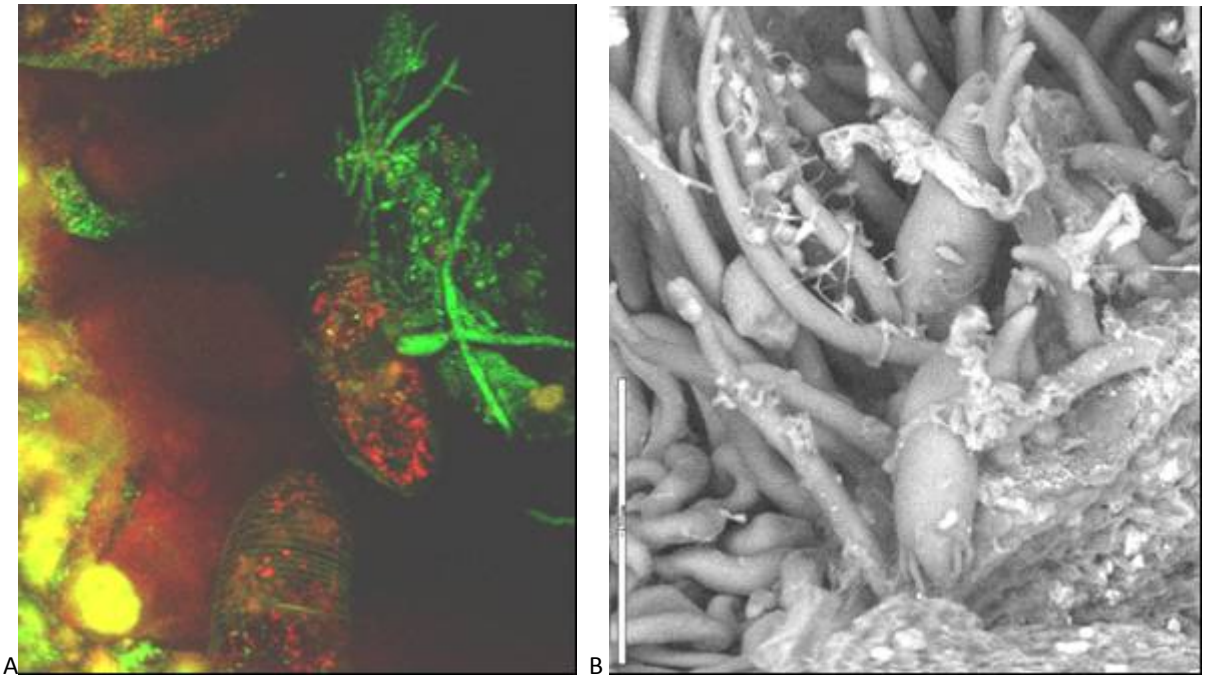

Figure 3. Microscopic observations demonstrating the physical proximity and actual contact between *Aceria mangiferae and Fusarium mangiferae*. A- Confocal microscope image showing bud mites touching gfp-glowing hyphae and conidia. B- SEM image illustrating bud mites bearing hyphae and conidia on their body.

Aerial conidial dissemination

Conidia of F. mangiferae were trapped successfully using both trapping methods. An annual peak of dissemination was found in the spring/early summer months (Fig. 4). Similar results were obtained with the BurkardTM trap where higher numbers of conidia were caught early in the summer months (May/June) declining towards the end of the summer/beginning of autumn. A.

mangiferae was trapped throughout the season, but no fungal growth was detected after placing these mites on selective media. Our findings imply that conidia can reach the bud independently of the bud mite and thus the latter does not seem to play a role in the windborne dissemination of the pathogen.

Conclusion

Results from this study suggest that *A. mangiferae* can carry *F. mangiferae* conidia on its body and vector the pathogen's conidia into apical buds, which serve as penetration sites for the pathogen. The mite can also improve fungal penetration perhaps via it's feeding wounds, and finally, it also appears that the bud mites do not play a role in aerial dissemination of conidia. These results support involvement of *A. mangiferae* in mango malformation epidemiology (Ploetz 2001).

While the transmission of viral pathogens by eriophyoids has been studied (Oldfield & Proeseler 1996) the interaction with other pathogens has received much less attention. Our findings suggest that fungal as well as other pathogens may play an important role in additional cases where plant disorders have been attributed, till now, solely to eriophyoid mites. For instance on lemons and oranges distortion of fruit growth leading to the production of numerous buds and premature fruit drop is associated with the citrus bud mite, *Aceria sheldoni* Ewing (Keifer *et al.* 1982). On *Lantana camara* L. the lantana gall mite *Aceria lantanae* (Cook) causes vegetative deformations appearing as masses of very small leaves (Meyer 1996).

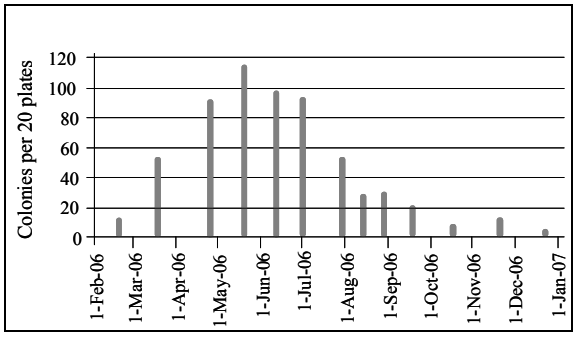

Figure 4. Air-borne conidia of *Fusarium mangiferae* trapped on selective media plates exposed overnight to Volcani experimental orchard conditions.

On Protaceae witches broom has been related to *Aceria proteae* Meyer (Meyer 1996) but recently a spiroplasma has been isolated from the mite and the diseased plant tissue (Wieczorek & Wright 2003).

While these examples of plant disorders do not have the same symptoms to those of mango malformation there does seem to be marked similarities. We propose that future research on eriophyoid related plant disorders, especially those without typical eriophyoid galls and erinea (Westphal & Manson 1996) should consider the evaluation of pathogens as causal agents and the potential interaction with eriophyoid mites.

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CHALLENGES TO EVALUATION OF ERIOPHYID MITES FOR BIOLOGICAL CONTROL OF INVASIVE PLANTS

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Abstract

Eriophyid mites have been considered to have a high potential for use as classical biological control agents of weeds. However, in the past 20 years few species have been authorized for introduction, and few have significantly reduced the target plant's population. Natural enemies, resistant plant genotypes, and adverse abiotic conditions may all reduce the ability of eriophyid mites to control weeds. Furthermore, host specificity experiments conducted under laboratory conditions sometimes indicate a wider host range than that observed in the field, which results in failure to obtain approval for release. We need to know more about the natural behavior, life history and evolutionary stability of eriophyid mites. This is critical for designing and interpreting experiments to measure host plant specificity and potential impact on target and nontarget plants, which must be known before they can be released.

Keywords

Eriophyoidea, biological control, invasive plant, host plant specificity, phytophagous mite.

Introduction

Classical biological control involves the introduction of host-specific natural enemies to help regulate pest populations (Goeden & Andres 1999). It is generally applied to control invasive alien species that lack effective natural enemies in the adventive region. In order to avoid damage to nontarget species, biological control agents must be highly host specific. Because many species of eriophyoid mites (EMs) are highly host specific, as demonstrated by the fact that about two thirds of currently known species have been recorded in association with a single host species (de Lillo & Amrine, personal database), this taxon has been considered to have a high potential as a source for biological control agents of weeds (*e.g.*, Cromroy 1977, Andres 1983, Boczek 1995, Boczek & Petanovic 1996, Rosenthal 1996, Briese & Cullen 2001). However, at present, few species have been introduced to new regions, and few have significantly reduced populations of the target weed. In her 1996 review, Rosenthal discussed eight species of EMs with high potentials. Twelve years later we find that only three of these species have been successfully introduced to other countries for classical biological control: *Aceria malherbae* Nuzzaci, *Aceria chondrillae* (Canestrini), and *Aculus hyperici* (Liro), all of which had been used before 1996. Since then, at least nine other species have undergone some level of pre-release

evaluations, but only two, *Cecidophyes rouhollahi* Craemer and *Floracarus perrepae* Knihinicki & Boczek, have been approved for release. Here we review the status of some active biological control projects and discuss what research needs to be done to help advance the use of EMs for biological control.

Status of introduced EMs

More background information on the following three species can be found in Rosenthal (1996). *Aceria chondrillae* is native to Europe and has been introduced to control *Chondrilla juncea* L. (rush skeletonweed, Asteraceae), in Australia, the USA and Argentina (Cullen & Briese 2001). The plant has at least four different genotypes that vary in resistance to the mite, so it was necessary to find strains of the mite suitable for the various "forms" of the plant. The Greek strain released in Australia induces galls on only two of the three weed forms present, which limits its effectiveness. In California, the mite is widespread, but its effectiveness appears to be limited by predation by *Typhlodromus pyri* Scheuten (Piper & Andres 1995). In Oregon and Washington, *A. chondrillae* is widespread and in some areas it may reduce flowering and seed production by 50-90% (Piper *et al.* 2004, E. Coombs, pers. com.). It does best in dry areas and where deer do not graze the early growth of the host plant. However, In Idaho *A. chondrillae* appears to be limited by the cold winter weather which causes high mortality (Milan *et al.* 2006).

Aculus hyperici is native to Europe and was introduced in 1991 to control *Hypericum perforatum* L. (St. Johnswort, Clusiaceae) in Australia (Briese & Cullen 2001). Although the mite impacts the target weed, reducing shoot and root biomass by 14 to 47%, it has not significantly reduced weed populations. Nevertheless, it is perceived to help reduce reproduction and dispersal of the weed. However, *A. hyperici* can reproduce on a native nontarget species, *H. gramineum* Forst., although populations and impact on this plant are lower than on the target weed, and it does not appear to significantly affect the plant's population in the field (Willis *et al.* 2003). In the USA, this weed was successfully controlled over most of its range by several species of introduced beetles (McCaffrey *et al.* 1995), and the mite was never introduced.

Aceria malherbae is native to Europe and was first released on *Convolvulus arvensis* L. (field bindweed, Convolvulaceae) in the USA in 1987 (Boldt & Sobhian 1993, Rosenthal 1996) and in Canada in 1993 (McClay *et al.* 1999). This species was initially mistaken to be *Aceria convolvuli* Nalepa (Rosenthal 1983), and there is some confusion in the literature regarding *Aculus convolvuli* (Nalepa). All three are recognized species. It was discovered that mowing was an effective way to disperse and multiply the mite. Integrated weed management methods using the mite, herbicides and mowing have been developed (Boydston & Williams 2004, Lauriault *et al.* 2004), and the weed population has been reduced in many parts of Texas and Colorado (G. Michels & D. Bean pers. com.). The mite is widely established in eastern Montana; however, the patchiness of infestations both within and among sites suggests that some plants may be resistant to the mite, or that the mite does not disperse very well. The weed population has not declined at a site in central Montana during the eight years since the mite was first released (J. Littlefield pers. com.). However, the mite has established and is spreading in a dry range area in Tygh Valley, Oregon, where it has reduced biomass 90% (E. Coombs, pers. com.). In South Africa, *A. malherbae* was permitted in 1994 and has been released, but it has not been widely distributed due to lack of resources (Craemer 1995, pers. com.).

In host plant specificity tests, *A. malherbae* caused galling on 3 *Convolvulus* and 12 *Calystegia* species (Clement *et al.* 1984, Rosenthal & Platts 1990, Craemer 1995). The mite is not permitted for release in California because of concern about risk to the 11 native *Calystegia* species. Although galling was observed on two nontarget species of *Convolvulus*, mite reproduction was only observed on *C. arvensis* (Craemer 1995). The other host testing studies reported only damage (gall presence) (Clement *et al.* 1984), or percentage of plants infested (not distinguishing between live mites or damage) (Rosenthal & Platts 1990). In the latter experiment plants were in direct contact with others, so mites could probably migrate between plants, increasing the risk of observing mites on plants that they did not develop on. So, some of these results may over estimate the amount of damage likely under field conditions. Recently conducted field experiments with some of these nontarget species in Colorado showed very little to no galling (D. Bean & R. Hansen pers. com.), so there may be less risk of damage to these species than originally thought. Environmental conditions in Colorado are drier than the coastal regions where many native *Calystegia* plants occur in California, and the effect of these environmental differences on ability to infest plants are not known.

Cecidophyes rouhollahi Craemer was introduced to western Canada from southern France in 2003 to control *Galium spurium* L. (false cleavers, Rubiaceae) (McClay *et al.* 2001). In field experiments in Alberta, Canada, mites caused 30% reductions in seed yield and biomass of false cleavers, growing either alone or with canola. However, despite several years of releasing the mite, it consistently failed to survive the winter (McClay *et al.* 2001, McClay pers. com.). Alberta is at a much higher latitude (50°N) than southern France (44°) and winter temperatures are much lower (minimum of -16°C vs. -2°C), so it may be worth searching for a population of the mite in Russia, which would be better adapted to the Alberta climate.

Floracarus perrepae, which galls *Lygodium microphyllum* (Cav.) R. Br. (climbing fern, Lygodiaceae), was approved for release in Florida (Goolsby *et al.* 2004). Although in general it has been relatively easy to establish EMs in the field by transferring infested plant parts, in the case of *F. perrepae*, the regulatory permit prohibited removal of infested plant material from quarantine. This made it more difficult to move the mite out of quarantine, but the mite is now established on potted plants outside of quarantine, and field releases began in June 2008 (R. Pemberton pers. com.). There is known phenotypic variability among populations of both the plant and the mite, so not all plants in Florida are expected to be susceptible to infestation by this biotype of the mite (Goolsby *et al.* 2006).

Examples of recent research

Aceria salsolae de Lillo & Sobhian is a recently described Eurasian species that has only been found on *Salsola tragus* L. or *Salsola kali* L. (*sensu lato*) (tumbleweed, Chenopodiaceae) (de Lillo & Sobhian 1996). The mite has undergone evaluation as a prospective biological control agent of *S. tragus*, which is invasive in the western USA. During laboratory host plant specificity tests the mite was able to reproduce only on species within the *Salsola* section Kali, which includes *S. tragus*, *S. paulsenii* Litv., *S. australis* R. Br. and *S. collina* Pall. (*S. kali sensu stricto* was not tested) (Smith 2005). However, in subsequent experiments, the mite sometimes multiplied on *Bassia hyssopifolia* (Pall.) Kuntz and *B. scoparia* (L.) A.J. Scott (Smith, unpublished data). This was surprising because these plants are more distantly related to *S. tragus* than other plants, such as *Salsola soda* L. and *Halogeton glomeratus* (M. Bieb.) C.A. Mey., that failed to support mite populations. Because the mite had not been reported previously on *B. hyssopifolia* and *B. scoparia*, which normally occur within the geographic range of the mite, we suspected that the plants may be more susceptible to mite infestation under laboratory conditions than in the field. A field experiment conducted in Italy, in which plants were inoculated with the mite, showed that these plants did not maintain significant mite populations (Smith *et al.* unpubl. data).

Leipothrix dipsacivagus Petanovic is being evaluated as a potential biological control agent of invasive *Dipsacus* spp. (teasel, Dipsacaceae) in the USA (Rector *et al.* 2006). The mite was originally found damaging *D. laciniatus* in the field in Europe and was identified as *Epitrimerus knautiae* Liro (Petanovic 1999). However, it was later determined to be a new species (Petanovic & Rector 2007). This is significant because *L. dipsacivagus* does not colonize *Knautia arvensis*, the known host plant of *E. knautiae* (now called *Leipothrix knautiae*) (Stoeva *et al.* 2008), offering host-range data to support the taxonomic data that was used to describe this new species. Preliminary host-specificity testing has shown it to be highly specific to hosts in the genus *Dipsacus*, only colonizing *D. laciniatus* L. and *D. fullonum* L. in tests that included several other closely related genera (Stoeva *et al.* 2008). Both of these teasel species are invasive in the USA and are targets of biological control, so it is possible that this one EM species could be used for both invasive teasel species. Studies of laboratory-infested and field-collected plants have shown significant damage caused by this mite at the cellular and anatomical levels (Pecinar *et al.* 2007, 2008), although population reduction experiments have not yet been conducted.

Aceria solstitialis de Lillo is a recently described species that has been collected only on *Centaurea solstitialis* L. (yellow starthistle [YST], Asteraceae) (de Lillo *et al.* 2002). The mite has been found in eastern Turkey, southern Bulgaria, Greece and southern Italy, and it causes stunting of the plant and flower heads and an abnormal broom-like shape of the plant. In 2006 a laboratory experiment was conducted to test the host specificity of the mite. A total of 13 species from Asteraceae including YST were used in a choice and a no-choice test. In the no-choice test *A. solstitialis* reproduced and established a population on YST, *Centaurea diffusa* Lam., *Ce. cyanus* L., *Cynara scolymus* L. (artichoke), and *Carthamus tinctorius* L. (safflower), while in the choice test the mite developed only on YST, *Ce. diffusa*, and *Cy. scolymus*. Because the mite had not been reported

previously on these nontarget plants, we suspect that the laboratory experiments may not properly predict the risk of infestation and damage to these nontarget plants. We plan to conduct further tests with these non-target plants under field conditions to compare results to those observed in the laboratory.

In each of the preceding examples it is notable that a new species of EM was discovered during exploration for prospective biological control agents of the target weed.

Conclusions

Given the increasing levels of safety required of classical biological control agents, and our relative lack of experience with and knowledge of EMs, there are many areas of research that can help improve our ability to use EMs as safe effective agents to control invasive plants.

Research needs.

- Molecular genetic methods to help identify species and host specific strains.
- Life history studies. How long can mites survive off the host or on nonhosts? What weather stresses (*e.g.*, temperature, relative humidity, solar radiation) limit EMs?
- Dispersal behavior. What factors influence aerial dispersal and phoresy?
- Physiology and behavior of host plant specificity. What is the mechanism of host plant specificity (*e.g.*, attraction, repellence, shelter, host-mite biochemical interactions related to feeding, gall formation and sensory perception)?
- Evolutionary stability of host plant specificity. How fast can EMs adapt to a new host plant and how far (taxonomically) can they jump?
- Population regulation. Are EM populations regulated by natural enemies in the native region, and by what species?

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THE IMPACT OF ERIOPHYOIDS ON CROPS: NEW AND OLD CASE STUDIES

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Abstract

The potential economic importance of eriophyoid mites continues to increase worldwide. A large number of species have reached permanent pest status in definite crops, while others represent a quarantine threat for several countries. Nevertheless, the nature of the damage caused by eriophyoid mites and its assessment still require detailed studies in order that appropriate control strategies can be planned. Interactions between eriophyoids and host plants (e.g. resistance, varietal susceptibility), or between eriophyoids and pesticides (e.g. impact of fungicides, resistance to acaricides) also require research. We have selected four case-studies related to eriophyoid mites of economic importance in Europe and elsewhere, that have been considered in recent research. Two of these (*Aculus schlechtendali* and *Calepitrimerus vitis*) involve temperate fruits, one (*Aculops lycopersici*) affects vegetables and the last, *Trisetacus juniperinus*, causes considerable damage to forest and ornamental trees in the Mediterranean region. Damage assessment related to *A. schlechtendali* has been evaluated on different apple varieties. Some factors affecting the spread and the economic importance of *C. vitis* have been identified. The complexity and difficulty in controlling *A. lycopersici* by chemicals enhances its interest for biological control. The impact of *T. juniperinus* on evergreen cypress in nurseries and young stands has been evaluated in different conditions.

Key words

pest status, Aculus schlechtendali, Calepitrimerus vitis, *Aculops* lycopersici, *Trisetacus juniperinus*.

Introduction

The economic importance of eriophyoid mites (Acari: Eriophyoidea) is increasing worldwide. A large number of eriophyoid mite species have reached permanent pest status on some crops, while others represent a quarantine threat for several countries. Control strategies (e.g. action thresholds) should be based on a precise evaluation of the impact of a definite species on crop yields. However, the nature of the damage caused by most eriophyoid mites, and its assessment in particular, still require detailed studies. Moreover, interactions between eriophyoids and host plants are mediated by various factors (e.g. plant nutrition, varietal susceptibility) as well as those between eriophyoids and pesticides (e.g. resistance, side-effects of pesticides on predator-prey equilibrium). The last comprehensive contribution to the knowledge of eriophyoids and their pest status was coordinated by Lindquist *et al.* (1996). Since then, a number of papers have furthered knowledge on the economic importance of definite eriophyoid pests. We have selected four case-studies related to eriophyoid pests important in Europe and elsewhere, that have been considered

in recent research. Among these, *Aculus schlechtendali* (Nalepa) and *Calepitrimerus vitis* (Nalepa) occur on apples and grapes, respectively; *Aculops lycopersici* (Tryon) affects tomatoes and other vegetables and *Trisetacus juniperinus* (Nal.), causes considerable damage to forest and ornamental trees. In this paper, recent issues related to the pest status, economic importance and control strategies of these four pests are reviewed with emphasis on the last 15 years.

Aculus schlechtendali (Nalepa)

Aculus schlechtendali is a worldwide apple pest, but severe damage is reported especially from Europe and North-America. Apple leaves injured by *A. schlechtendali* roll up longitudinally, their undersurfaces become rusty and they sometimes fall prematurely. In Spring, mite feeding on developing fruitlets damages epidermal layer cells, causing the subsequent russet on fruits (Easterbrook & Fuller 1986). Russet occurs frequently around the calyx but severe infestations are associated with russet and cracking on the cheek.

Easterbrook (1996) reviewed the most important issues related to *A. schlechtendali*, and summarised significant data on the biology and the behaviour of this pest. He pointed out that little is known about the effects exerted by *A. schlechtendali* on tree physiology and growth parameters. Recently, the impact of *A. schlechtendali* on net carbon dioxide exchange, transpiration rate, and leaf colour has been investigated in Switzerland (Spieser *et al.* 1998). A number of young plants were artificially infested with rust mites and population densities per leaf were estimated to be 4-5000 in June. Infested leaves turned dark brown and the net CO_2 exchange was affected (e.g. a reduction of 65% in severely infested leaves). Highly infested leaves showed partially open stomata, guard cells lacking turgor, epidermal and parenchyma cells desiccated with consequent disorders in the transpiration process. Rust mites cannot feed on the photosynthetically active mesophyll but *A. schlechtendali* may affect photosynthesis indirectly by damaging the epidermis and inducing effects on the mesophyll.

Easterbrook & Palmer (1996) did not find any effects of early *A. schlechtendali* infestation (<100 mites per leaf) on photosynthesis in field-grown apples but populations were probably too low to determine such implications. The same authors evaluated the impact of *A. schlechtendali* on the numbers of fruit sets on individual blossom clusters or trees of various apple cultivars (Bramley's Seedling, Crispin and Cox's Orange Pippin). Mite feeding on the leaves of the blossom clusters did not reduce the initial fruit set, despite rust mite densities reaching several hundred per primary spur leaf.

Several environmental and cultural factors can affect the impact of *A. schlechtendali* on apple crops. This may explain the contrasting opinions on *A. schlechtendali* levels which cause economic damage to apple yield (e.g. Easterbrook 1996). In experiments conducted by Spieser *et al.* (1999) mite infestation caused a decrease in fresh fruit weight, but only for the cultivar Jonagold. Infested fruits of Golden Delicious and Jonagold had a lower soluble sugar content than poorly infested fruits. The colour intensity of fruit skins was reduced on Jonagold but not on Golden Delicious. Flower formation was also negatively affected. This study confirmed that the impact of *A. schlechtendali* on apple yield is influenced by cultivars: Golden Delicious was less affected than Jonagold by rust mites.

However, a recent contribution made in Trentino (North-eastern Italy) has shown that *A. schlechtendali* populations can seriously affect Golden Delicious yield parameters, too (Angeli *et al.*, unpubl. data). The effects of tree mite densities (2399, 14523, and 31750 mite-days/leaf) were compared. Population peaks were reached in July. The two highest density levels negatively affected the size, weight and colour of fruits. In contrast, fruit russeting was not influenced by rust mites, probably because mite colonization of fruitlets in spring was low and the population started to increase more than one month after petal fall. Soluble solids and acid malic content decreased with higher mite levels but these effects were not significant. The study conducted in Trentino showed that the cultivar Golden Delicious may be particularly susceptible, in terms of fruit size and weight, when moderate to high *A. schlechtendali* populations occurred in summer. The reduction in fruit size from the optimal values for the fresh market is the most relevant problem from the economic point of view.

The high susceptibility to *A. schlechtendali* damage by some cultivars can explain why this pest is the object of acaricide applications in some parts of Europe. Meantime, in North America there are few reports of damage to fruits by *A. schlechtendali,* probably due to a low susceptibility to *A. schlechtendali* damage by apple cultivars common in the USA. When *A. schlechtendali* populations are

damaging, abamectin, spirodiclofen and lambda-cyhalothrin are effective pesticides (Raudonis *et al.* 2007). Horticultural mineral oil applications can also reduce *A. schlechtendali* numbers (Fernandez *et al.* 2006).

The biological control of *A. schlechtendali* has been the focus of studies for a long time. Phytoseiids play an important role in maintaining its populations below economic levels. In Europe, *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) are well known predators of *A. schlechtendali* (Easterbrook 1996, Fitzgerald *et al.* 2003, Duso & Pasini 2003). In eastern USA, apple rust mites seemed to be the primary prey for a number of phytoseiids, e.g. *Galendromus flumenis* (Chant), *G. occidentalis* (Nesbitt), *T. caudiglans* Schuster and *Metaseiulus citri* (Garman and McGregor) in untreated orchards (Croft & Luh 2004). Moreover, low *A. schlechtendali* populations can support phytoseiid populations when tetranychids are scarce (Easterbrook 1996, Hill & Foster 1998).

Among predatory mites, *Zetzellia mali* Ewing has been identified as a common predator of rust mites (Walde *et al.* 1995). Phytoseiids and stigmaeids interact in reducing tetranychid and rust mite densities (Slone & Croft 2001). Walde *et al.* (1997) evaluated direct and indirect species interactions affecting the dynamics of *A. schlechtendali* in Canada and showed that *T. pyri* was more important than *Z. mali* in reducing the abundance of *A. schlechtendali*. In Ontario (Canada), *Z. mali* became the dominant predator in pyrethroid sprayed plots in an experimental apple orchard, being fundamental in the control of *Panonychus ulmi* (Koch) and *A. schlechtendali* (Villanueva & Harmsen 1998). The role of another predatory mite, *i.e. Anystis baccarum* (L.) has been evaluated in Ireland. The experimental exclusion of *A. baccarum* caused a significant increase in *A. schlechtendali* in some trials (Cuthbertson *et al.* 2003).

Predatory mite populations should be preserved in orchards to improve the biological control of *A. schlechtendali*. Insecticides and fungicides frequently used to control apple pests can disrupt the beneficial activity of predatory mites. As an example, the use of mancozeb was associated with fewer *A. baccarum* and more *A. schlechtendali* compared to other treatments (Cuthbertson & Murchie 2003). It should be mentioned that some fungicides, e.g. tolylfluanide, can strongly reduce populations and thus knowledge of their effects on predatory mites is a fundamental requirement for IPM (De Maeyer *et al.* 1993).

Calepitrimerus vitis (Nalepa)

The vagrant eriophyoid *Calepitrimerus vitis* has long been considered a grape pest, especially in Europe (Duso & de Lillo 1996), but recently it has also been associated with crop losses in Australia (Bernard *et al.* 2005) and the USA (Prischmann & James 2005). Additional records of this pest come from Brazil (Reis *et al.* 1998).

The most common symptoms associated with *C. vitis* are the death of the growing point of buds, stunted shoot growth, shortened shoot internodes, leaf and cluster deformation, development of lateral shoots and latent buds, reduced cluster size and flower drop (Duso & de Lillo 1996). Symptoms caused by rust mites may be confused with those caused by other events (e.g. thrips, microelement deficiency) and thus misidentified. Recently, Australian vineyards were affected by a syndrome called 'Restricted Spring Growth' (RSG) which was not associated to a clear cause. Bernard *et al.* (2005) showed that most of these symptoms were mostly due to early *C. vitis* infestations. Severe leaf distortion was associated with more than 400 *C. vitis* per spur, while densities higher than 1000 *C. vitis* per spur affected shoot growth. Shoot length reduction (43.0-47.2% for Cabernet Sauvignon, 27.1-32.8% for Sauvignon Blanc) was still evident at flowering. Treatments applied to control *C. vitis* populations did not completely prevent the RSG syndrome since a number of unburst buds were associated with populations of the bud strain of *Colomerus vitis* (Pagenstecher). Bernard *et al.* (2005) also evaluated the effect of *C. vitis* on vine fruitfulness, and yield parameters at fruit set.

Another syndrome, the so-called Short Shoot Syndrome (SSS), similar to the previous one was reported in the Pacific Northwest of the United States. Symptoms were represented by bunch necrosis during early season, malformed leaves, short and angled shoots in spring, scar tissue and bronzed leaves in summer. Walton *et al.* (2007) showed that SSS was associated with high *C. vitis* population densities. Before bud break no evidence of damage from rust mites was found inside undeveloped buds while damage was evident at sprouting. Crop losses at harvest reached 23.7% in some vineyards.

Calepitrimerus vitis is still an important pest in European vineyards, in particular in 1-2 year-old vineyards. The role of nurseries in the dispersal of mite populations has been discussed in Italy (Zandigiacomo & Frausin 1998). It has been shown that wind, rain and human practices can increase

the dispersal of *C. vitis* (Duffner *et al.* 2001). Factors promoting *C. vitis* outbreaks are under study in a number of countries. In Germany, the increase in summer temperatures has been positively related with infestations because of a higher number of generations (Kast *et al.* 2004). The effect of pesticides (e.g. sulphur) toward natural enemies of *C. vitis* has also been suggested as a factor involved in rust mite outbreaks (Bernard *et al.* 2005, Walton *et al.* 2007).

A better knowledge of the behaviour of *C. vitis* could improve the effectiveness of control measures. Recent investigations have added new findings on the phenology and the behaviour of this pest in different geographic areas (Perez-Moreno & Moraza 1998, Gabi & Meszaros 2001).

Regarding natural control, the predatory mite *Typhlodromus pyri* is considered an important antagonist of *C. vitis* in various parts of Europe (Wegner-Kib 2003, Perez-Moreno & Moraza 1997). *Euseius victoriensis* (Womersley) and *Typhlodromus doreenae* (Schicha) were reported associated with *C. vitis* in Australia (Bernard *et al.* 2005). Conservation biological control tactics have an important role in preventing rust mite outbreaks.

Young vineyards can be more exposed to *C. vitis* infestation since predatory mite populations are often low during this phase. Chemical control has been largely based on the use of bromopropylate but new EU rules have removed this acaricide from practical use in Europe. Other acaricides (e.g., spirodiclofen, fenpyroximate, fenazaquin) and fungicides (e.g., sulphur, tolylfluanide and dichlofluanide) seem to be effective (Wegner-Kib 2003, De Lillo *et al.* 2004, Bernard *et al.* 2005).

Aculops lycopersici (Tryon)

Aculops lycopersici, the tomato russet mite, is a worldwide key pest on the cultivated tomato, *Lycopersicon esculentum* L. the most important vegetable in the European Union. *Aculops lycopersici,* differently from most eriophyoids (Lindquist & Oldfield 1996), has a broad host range, mainly Solanaceae (e.g. tomato, tomatillo, potato, eggplant, peppers) (Perring 1996). This frequently implies diversified intimate interactions with plants; furthermore, competition for food, as happens for other strictly monophagous eriophyoid species, is reduced for *A. lycopersici.*

Due to its broad distribution, data concerning *A. lycopersici* biology are often ascribable to a wide range of climatic or laboratory conditions. As many as several generations can be produced per growing season and its population can double in less than 3 days at 25°C. Moreover, it seems able to tolerate considerable variations in temperature and relative humidity (Fischer & Mourrut-Salesse 2005).

The distribution, intrinsic dispersal and survival abilities of *A. lycopersici* determine serious harm to the tomato as well as economic drawbacks. Silvering of the undersurface of lower leaves is an early symptom. The lower parts of the stems lose hairs, become dusty brown in colour and sometimes develop small cracks. A sure and early identification of this mite damage can crucially address control as some of *A. lycopersici* damage symptoms are also similar to those of broad mite and thrips (Royalty & Perring 1988).

Generally, in northwestern Europe, climatic conditions allow its control through biologically-based Integrated Pest Management (Khromova 2001). The efficiency of this strategy in southern Europe is reduced because mild climatic conditions favour a wider spectrum of alternative host plants for the eriophyoid mite as well as a higher number of pests (Yoldas *et al.* 1999). Currently chemical control (e.g. abamectin and sulphur) is mainly used to limit *A. lycopersici* but its dispersal and the high chance of possible alternative host plants lower the efficacy of acaricides.

The monitoring of *A. lycopersici* is often problematic and the population may be undetected or underestimated (Hirata *et al.* 2007). Furthermore, high temperatures and low relative humidity in tomato greenhouses favour fast pest growth, but are suboptimal for natural enemies (Drukker *et al.* 2000).

Crucially, to better evaluate new control strategies, in the last decade much research has been addressed at furthering the knowledge of the tomato/*A. lycopersici* system and at how host properties could affect the dynamics of this pest. The intensity of damage can depend on the tomato varieties. The different levels of resistance of different varieties of tomato to several polyphagous arthropods have been related to the presence of high contents of 2-tridecanone and other methyl ketones in the leaves (Goncalves *et al.* 1998). This was also the case for *A. lycopersici*: its distribution on *L. hirsutum* and *L. esculentum* significantly varies according to the different levels of tridecan-2-one and undecan-2-one, the density and trichome types and the size of leaves (Leite *et al.* 1999). Furthermore, the tomato trichomes vesture and

this trichome-based host-plant resistance mechanically affects natural enemies more than pests (Simmons & Gurr 2005), and toxic secondary metabolites (additively in plants and prey) can further intoxicate predators (Roddick 1974). These experiments could lead to genetic improvement in the direction of increased levels of mite resistance in the tomato.

Very frequently, the morphological and chemical traits of the tomato make the biological control of *A. lycopersici* ineffective and/or very expensive. There are a few predators that feed on the tomato russet mite, but most of them do not seem feasible for a biological control program. *Aculops lycopersici* can live in different natural habitats and hosts so the search for biocontrol candidates must be screened and tested. Frequently, predator effectiveness can be limited by the presence of tetranychids (i.e. *Tetranychus evansi* (Baker and Pritchard)) in particular by their webbing that hinders the activity of phytoseiids. Considering phytoseiids tested, currently none (*Neoseiulus californicus* (McGregor), *N. fallacis* (Garman) *N. cucumeris* (Oud.), *Euseius concordis* (Chant), *Amblyseius victoriensis* (Womersley), *Typhlodromalus lailae* (Schicha)) have proved to be successful in controlling *A. lycopersici* (Gerson & Weintraub 2007). It seems crucial to investigate whether promising predators can increase their efficiency after some generations/adaptation time to the prey and/or to host (Castagnoli *et al.* 2003).

Trisetacus juniperinus (Nalepa)

The evergreen cypress (*Cupressus sempervirens L.*) is one of the most important tree species for landscape and forestry in the Mediterranean region. It is widely cultivated for its ornamental value, capacity to colonize even the most arid soil, and the high quality of its wood. In this area, the major pests of young cultivated plants are the eriophyoid mite *Trisetacus juniperinus* and the fungal cypress canker disease *Seiridium cardinale* (Wag) Sutton & Gibson. Regarding the latter, the best control strategy seems to be the selection of resistant varieties (Panconesi & Raddi 1998). This leads us to reconsider the increasing problems with *T. juniperinus,* evaluating its control strategies by also taking into account the possible difference in susceptibility to this pest of new selected cypress cultivars.

Trisetacus juniperinus is a bud mite which causes quite localized injury in natural stands of evergreen cypress, but it is able to become a key pest in nurseries and young stands. The eriophyoid mites live in apical and subapical buds and in young reproductive organs (Castagnoli & Simoni 2000, Guido *et al.* 1995). They develop continuously through the year until the colonized parts of the plant become dry, after which they emigrate towards new buds. Massive emigration does not appear to take place at a particular time of year. As it is very difficult to monitor its population, the best approach for increasing knowledge on this mite would appear to focus on induced symptoms.The symptoms, however, range enormously in severity and more than one type of damage can coexist in the same plant: a rating system taking account of the kind and the timetable evolution of damage proved to be useful for description purposes and data comparison at the same time (Table 1). The severity of each symptom is graduated from 0 (absence) to 4 (highest intensity). A global damage index per plant (GDI, i.e. the sum of means of four types of damage recorded) was calculated (Castagnoli *et al.* 2002). By means of this rating system, it was possible to investigate the susceptibility of cypress genotypes. The first damage that appears is damage A which, after some time, evolves into B. These are the most common and can be found in high levels in nurseries and immediately after transplant.

Table 1. Rating system of symptoms caused by *T. juniperinus* on *C. sempervirens* (modified after Castagnoli *et al.* 2002).

A	B	C	D	E
Buds enlarged, deformed, russet and/or branch apex fold	Buds more or less dried out	Brachyblasts and/or part of branch dried out	Irregular proliferations of axillary buds, blastomania, witches' brooms	Cones deformed producing few seeds

A five-year study on seedlings belonging to 15 different families, obtained by self-crossing or crossing with a single heterologous pollen, highlighted a great variation in susceptibility to mite attack and better clarified damage evolution (Castagnoli *et al.* 2002). In the nursery, it was already possible to allocate the cypress family to at least two different levels of susceptibility which, on the whole, transplant in the field did not change. However, in the field stand where higher humidity was registered, the damage was significantly higher; we can thus conclude that the environmental conditions of transplanting localities could in some way affect the susceptibility of plants. Nevertheless, the general trend in the most susceptible family remained the considerable increase of damage types C and D which are mainly responsible for the loss of the aesthetic value of cypress trees, which were restored to partial health only with extreme difficulty. Some practical results derived from the study of symptoms based on the rating system. The assessment of damage A only, the earliest symptom of mite attack, is highly indicative, giving the same evaluation of cypress susceptibility as if all the damage categories were considered. Furthermore, a negative relationship was found between damage and height increase: more intense damage resulted in a smaller average plant height increase and this contributes considerably to the loss of economic value in reared plants.

Another long-term study focused on evaluating the impact of mite infestation both on survival and on apical growth of two commercial clones (Agrimed and Bolgheri) of cypress and different timed pesticide applications to suppress mite populations on newly grafted trees (Simoni *et al.* 2004). The health of rootstock and graft material is crucial for limiting damage by *T. juniperinus*, because natural infestations rarely occur. The mites from the rootstock previously infested in the nursery can migrate quickly to grafted scions and induce tip deformation. This is connected to growth disturbance and even if the infestation decreased significantly in time, apical growth was significantly lower in the plants from infested rootstocks even two years following grafting. The long-lasting effect of the precocious mite infestations can be interpreted as a trade-off between resources devoted to growth and to the defence reaction of trees in accordance with the GDB (Growth Differentiation Balance) hypothesis (Herms & Mattson 1992). Treatments with bromopropylate reduced the percentage of infested plants in both clones and, when performed on the grafted trees, resulted in a higher growth compared to the control trees. As the use of bromopropylate and endosulfan has been limited by recent EU rules, the search for acaricides effective in the control of *T. juniperinus* is necessary.

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Free contributions to Eriophyoids knowledge

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DIFFERENCES IN THE LEAF MORPHOLOGY OF SYCAMORE MAPLE INFESTED BY TWO CONGENERIC ERIOPHYID SPECIES AT TARA NATIONAL PARK, IN WESTERN SERBIA

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Abstract

Morphological malformations of maple plant organs induced by eriophyid mites are well known and have been described in detail but information on the anatomical, i.e. cytological and/or histological changes in maple plants infested by these mites is scarce. Studies were performed on micro morphology of bead leaf galls and erinea caused by *Aceria cephalonea* (Nalepa 1922) and *A. pseudoplatanea* (Nalepa 1922) respectively on sycamore maple *Acer pseudoplatanus* L. collected at Tara National Park in Western Serbia. Histological analysis showed that each type of malformations was a result of a specific disorder in the proper growth of leaf tissue. *A. pseudoplatanea* induce erineum on the undersurface of leaves, while *A. cephalonea* induce meristematic activity of epidermal cells and formation of nutritive tissue within the inner part of leaf galls.

Key words

Eriophyoids, morphological malformations, maple, Serbia

Introduction

Eriophyoid mites are normal components of ecosystems in the nature. They are obligate plant feeders and due to ability to form galls or erinea on leaves, they take important nutrients and water from attacked plant tissues causing, therefore, the reduction of leaf surface together with restriction of assimilation processes in attacked plants (Vaneckova-Skuhrava 1996).

The Tara National Park is the most westward of the national parks in Serbia. It is very diverse in habitat and extends over a surface of about 20 000 ha at 1000 m a.s.l. mean altitude. Even today, the park area has mild, humid, continental climate supporting rich vegetation, primarily forests. So far eriophyoid mite fauna of Tara Mountain and its potential harmfulness is poorly known. From 2004 until the present time surveys of eriophyoids were carried out in the Tara National Park. On sycamore maple, *Acer pseudoplatanus* L. (Aceraceae), important deciduous tree, *Aceria cephalonea* (Nalepa 1922) and *A. pseudoplatanea* (Nalepa 1922) (Acari:Eriophyoidea) are for the first time recorded in the Tara National Park. Both species frequently occured on sycamore maple in Serbia (Petanović & Stanković 1999) as well as in many European countries (de Lillo 2004). In the studies of population frequency of insects and mites causing galls on sycamore maple leaves in Southern Poland, *A. cephalonea* was stated to be the most abundant species (Skrzypczynska 2004).

Morphological malformations of maple leaves induced by eriophyid mites are well known and

have been described in detail but information on the anatomical, i.e. cytological and/or histological changes in maple plants infested by these mites is scarce (Westphal 1977, Dulić-Stojanović 2000).

Studies were performed on micro morphology of leaf galls and erinea caused by *A. cephalonea* and *A. pseudoplatanea* respectively on sycamore maple *Acer pseudoplatanus* L. in order to describe and quantify the anatomical alterations provoked by infestation of these mites.

Material and methods

Plant material, non infested leaves of sycamore maple *Acer pseudoplatanus* L. and leaves with pouch leaf galls and erinea were collected at Tara National Park in Western Serbia (GPS: N 43° 58,354' E 19° 19,019') at 1,250m a.s.l.

The preparation of leaves for anatomy measurements was done according to Ruzin 1999. Samples of leaf were fixed in FAA for 24 hours at room temperature and post-fixed in 70% ethanol. Dehydration of tissue samples was performed in LEICA TP1020-Automatic Tissue Processor through a gradual series of ethanol (80%, 96% and absolute ethanol), xylene and melted paraffin embedding medium (Histowax, 56-58°C). After impregnating, tissue samples were embedded by EG1120 Paraffin Dispenser with integrated Hot Plate into a solid paraffin block. After cooling and hardening on the Leica EG1130 cold plate, paraffin blocks were cut (thickness of 3-6 μm) on a LEICA SM 2000 R microtome. Paraffin was removed from the sections by deparaffinisation procedure passing through the series of ethanol solutions (absolute, 95%, 70%, 50% and 30%) and tissues were stained in 0.5% safranine and alcian blue. After staining, the tissues were dehydrated rapidly through the absolute alcohol and xylol. This was done by LEICA ST4040 Linear stainer and finally, slides were mounted with the cover slip in Canada balsam for microscopic examination. The sections were examined using a light microscope LEICA DMLS and documented by digital camera LEICA DC 300. Measurements of section were done using image analysis system connected to the microscope (LEICA IM 1000). Comparison between means of anatomical parameters infested and non infested leaves were discriminated using Student's unpaired T-test (SigmaPlot for Windows 2.01, Jandel Scientific, Erkrath, Germany).

Results and discussion

Uninfested sycamore maple leaves show dorsoventral anatomy with the upper (adaxial) and lower (abaxial) surfaces (Figure 1a, b). Most of the interior of the leaf between the upper and lower layers of epidermis is a parenchyma tissue - mesophyll. The mesophyll is divided into two layers: An upper palisade layer of tightly packed, vertically elongated cells, one to two cells thick, directly beneath the adaxial epidermis. Beneath the palisade layer is the spongy layer. The cells of the spongy layer are more rounded and not so tightly packed. There are large intercellular air spaces. These cells contain fewer chloroplasts than those of the palisade layer.

Infestation of *A. pseudoplatanea* induces the development of large irregular erinea on underside of the leaves of sycamore maple. The erinea commence as whitish patches which later become purple. Transverse sections through the erineum on sycamore leaf (Figure 2a, b) showed papilar masses on the underside and thick layer of compactly arranged cells under the lower and upper epidermis. Upper epidermis is thinner (12±3μm comparing with 14 ± 4μm in uninfested leaves), and also the palisade layer (29±4μm comparing with 53±14μm in uninfested leaves) which contribute to the smaller mesophyll and consequently the smaller leaf thickness. Lower epidermal cells in infested leaves are larger (11±3μm) comparing with uninfested leaves (9±3μm) and most of them are strongly elongated (310±124μm) into papilar, nutritive hairs. The mites are causing densely curled leaf hairs on the lower surface, in which they feed and reproduce.

Pimples like bead galls caused by *Aceria cephalonea* in the leaves of sycamore are often present in very large numbers. The galls are results of both lower epidermal and mesophyll cell changes. Mesophyll tissue of the gall didn't differentiate into palisade and spongy tissue, and repeated aniclinal and periclinal divisions of lower epidermal cells lead to the formation of nutritive tissue (Figure 3a, b). Galled leaves are characterized by thinner upper epidermis (12±4μm comparing with 14 ± 4μm in uninfested leaves), mesophyll of the same thickness as in part of leaves between the galls, but the differentiation of mesophyll cells into palisade and a spongy parenchyma is lost in these structures. The mesophyle leaf gall cells are isodiametric, closely arranged with inconspicuous intercellular spaces and these cells lacked well-developed chloroplasts. Lower epidermal cells of healthy leaves are 9±3μm thick. The cells coresponding lower epidermis in

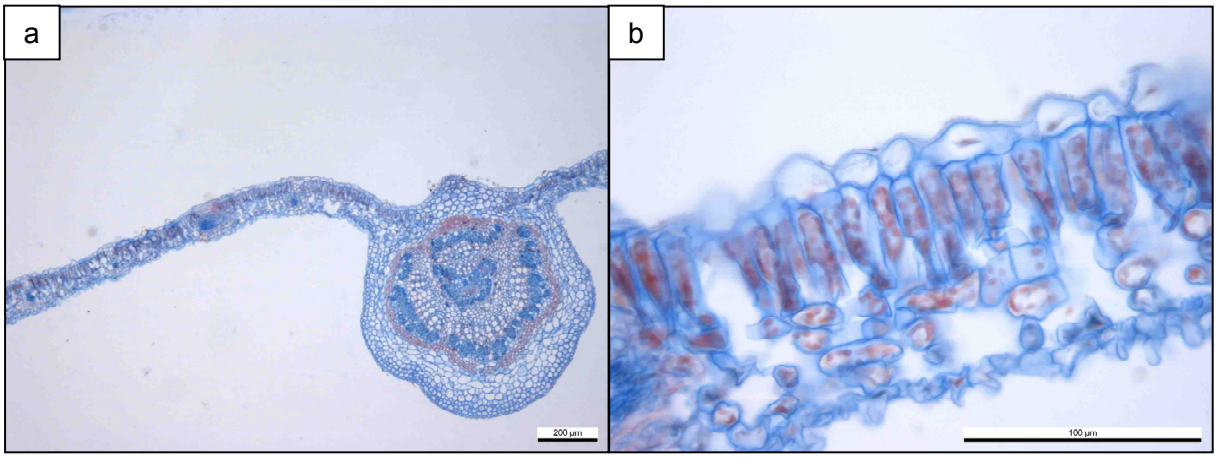

Figure 1. Cross section of uninfested sycamore leaf (a-x50, b-x400).

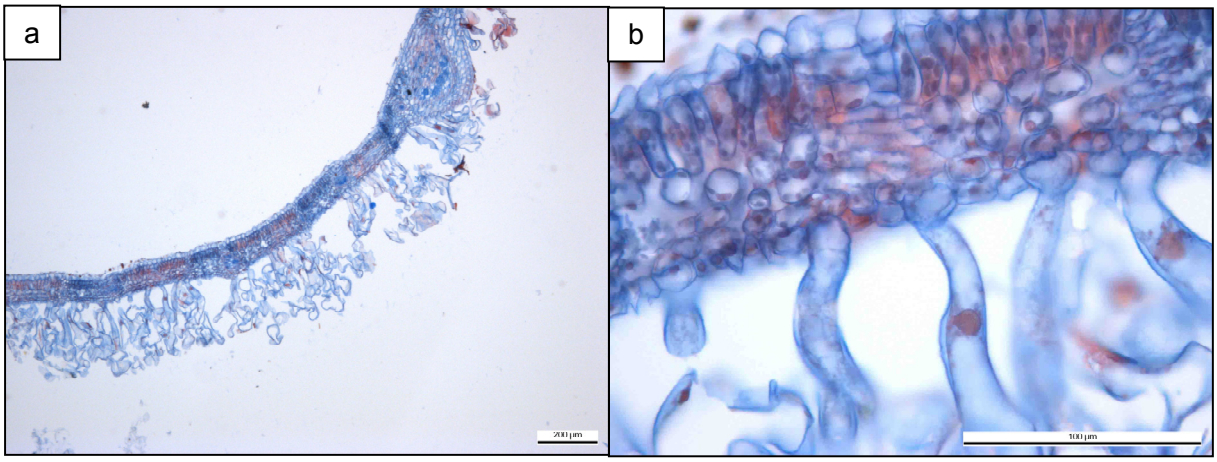

Figure 2 Cross section of sycamore leaf infested by erineum mite *A. pseudoplatanea* (a-x50, b-x400) (notice long unicellular hairs with large prominent nuclei on underside of the leaf).

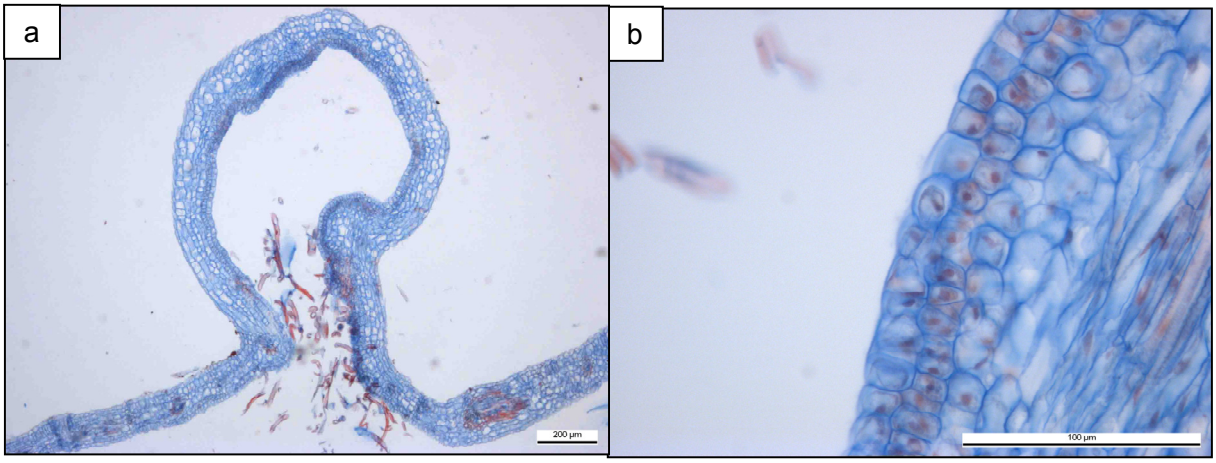

Figure 3 Cross section of sycamore leaf infested by gall mite *Aceria cephalonea* (a-x50, b-x400)(notice nutritive tissue in the inner part if the gall).

the inner side of galls are significantly bigger (16±3µm). Typical lower epidermal cells in the galls are not observed. Instead, another type of cells is developed. Two or three layers of compact cells, with large, round, purple nucleus and dense cytoplasm. These cells undergo transverse and longitudinal division and suppose to be nutritive tissue. The growth pattern of eriphyid mite-affected tissues is usually related to both mechanical injury made by mite chelicerae and specific salivary compounds injected into host cells (Westphal and Manson 1996). Cells of host plant are damaged by mite release signal(s) inducing changes like dense cytoplasm, small vacuoles, and enlarged nuclei. After the subsequent division of underlying cells, they differentiate into nutritive cells. Finally, our transverse sections showed the numerous nutritive cells in the inner part of the gall, in which mites live, feed and reproduce. Also, our histological analyses on the effect of *Aceria cephalonea* on leaf anatomy demonstrated the hairy entrance hole which was seen on the lower side of the leaf.

Comparative data of anatomical analysis infested and uninfested leaves are given on the Figure 4, 5 and 6.

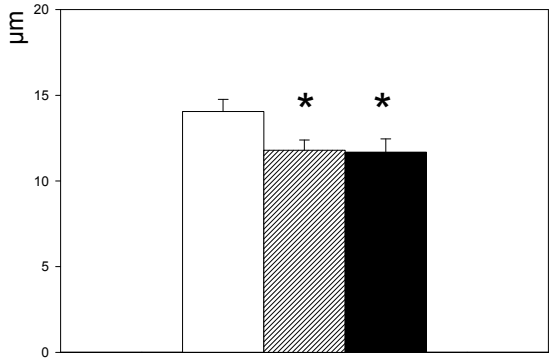

Figure 4. Thickness of upper epidermal cells in uninfested (white), erineum leaves (striate) and gall leaves (black). *, ** and *** indicated differences between healthy and infested leaves significant at p≤0.05, p≤0.01and p≤0.001, respectively.

Comparison of detailed description of galls of sycamore maple and *Acer intermedium* Panč. caused by *Aceria macrorhyncha* (Nal.) and *A.opulifolii* (Nal.) respectively (Westphal 1977, Dulić-Stojanović 2000), leads us to conclude that the anatomy of galls is similar in spite of the fact that different species are causative agents.

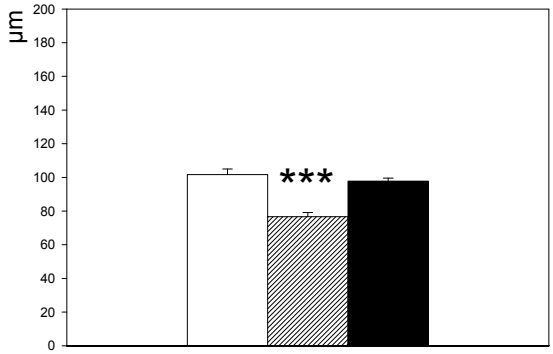

Figure 5. Thickness of mesophyll in uninfested (white), erineum leaves (striate) and gall leaves (black). *, ** and *** indicated differences between healthy and infested leaves significant at p≤0.05, p≤0.01and p≤0.001, respectively.

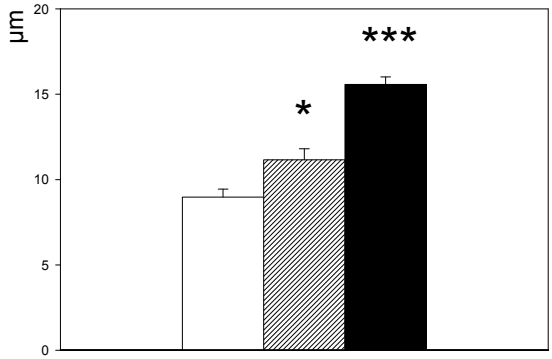

Figure 6. Thickness of lower epidermal cells in uninfested (white), erineum leaves (striate) and gall leaves (black). *, ** and *** indicated differences between healthy and infested leaves significant at p≤0.05, p≤0.01and p≤0.001, respectively.

Conclusion

Histological analysis has shown that each type of malformations was a result of a specific disorder in the proper growth of leaf tissue. *Aceria pseudoplatanea* induces erineum on the undersurface of leaves, while *A. cephalonea* induces meristematic activity of epidermal cells and formation of nutritive tissue within the inner part of leaf galls.

The studies of morphology showed clearly that these two mite species caused different changes on the sycamore maple leaves.

Harmfulness of two *Aceria* species to sycamore maple leaves in conditions of Tara National Park has not been studied yet.

However, as it was stated by Vaneckova-Skuhrava (1996), plant tissues forming eriophyoid galls are changed into tissues of lower quality which are not able to fulfill their assimilate function because of the partial loss of assimilate layers. The leaves of the forest trees, which are strongly attacked by eriophyoid mites, develop and grow more slowly than the unattacked ones.

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MORPHOLOGICAL VARIATION OF *ACERIA* SPP. (ACARI: ERIOPHYOIDEA) INHABITING *CIRSIUM* SPECIES (ASTERACEAE) IN SERBIA

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Abstract

From *Cirsium* spp., only two *Aceria* spp. (Acari: Eriophyoidea) were described, *Aceria anthocoptes* and *A. cirsii*. Studies on host related variability in morphology of *Aceria* mites are generally lacking. The purpose of this study was to investigate quantitative morphological traits of four *Aceria* populations inhabiting four different *Cirsium* spp. MANOVA analysis revealed significant differences in 23 commonly used morphological traits as well as additional four traits related to the shield design. Discriminant analysis yielded 10 traits that significantly differentiate four populations. UPGMA cluster analysis of the squared Mahalanobis distances indicate that *A. cirsii* was the most divergent population, while *A. anthocoptes* populations from *C. arvense* and *C. heterophyllum* were isolated from the branch clustering *Aceria* sp. population from *C. eriophorum.*

Key words

Aceria spp., *Cirsium* spp., morphometry, Serbia

Introduction

The genus *Aceria* includes over 900 species and is known to be a taxonomically difficult group (Amrine *et al.* 2003). Despite the description of so many species of *Aceria,* few have been published on relationships of congeneric taxa inhabiting closely related host plants. According to the World catalogue of eriophyid mites and Fauna Europaea (Amrine & Stasny 1994; de Lillo 2004) a total of 20 *Aceria* species have been recorded on Cardue plants and 10 out of them are known from Serbia (Petanović & Stanković 1999). Most of *Aceria* spp. inhabiting Carduae plant taxa are poorly known. From *Cirsium* spp., only two species were described, *Aceria anthocoptes* (Nalepa) and *A. cirsii* Petanović,Boczek & Shi.

Aceria anthocoptes has been the only known species of the genus *Aceria* inhabiting *Cirsium* spp. until recently. It has been recorded on *C. arvense* (L.) Scopoli*, C. heterophyllum* (L.) Hill and *C. vulgare* (Savi) Tenore (Amrine & Stasny 1994) so far, although its presence on *C. vulgare* was not confirmed (Ochoa *et al.* 2001). Another species, *A. leontodontis* (Lindroth) was also described from *C. arvense* and *C. heterophyllum* in Finland and later it was recorded in Finland again and in Bulgaria (Roivinen 1951; Nachev 1981). Recently, Petanović *et al.* (1997) proposed that these two species are synonymous, concluding that *A. leontodontis* is deutogyne form of *A. anthocoptes*. *Aceria anthocoptes* has been noted in several European countries (Davis *et al.* 1982) and in seven states in the USA (Ochoa *et al.* 2001). *Aceria cirsii* was described from *C. rivulare* (Jacquemin) Allioni and is known only from the type locality in Serbia

(Petanović *et al.* 2000).

First data concerning *Aceria* mites inhabiting *C. eriophorum* could be found in CABI Bioscience annual report (Gasman *et al.* 2004). The taxon identified as *Aceria* sp. near *anthocoptes* was recorded on *C. eriophorum*. Within formerly mentioned study host specificity tests with *A. anthocoptes* protogyne and deutogyne females from *C. arvense* were carried out. Results have shown that *A.anhocoptes* did not develop on *C. eriophourm,* which suggests the field collected *Aceria* sp. from *C. eriophorum* as a different species.

The purpose of this study was to investigate quantitative morphological traits of four *Aceria* populations inhabiting four different *Cirsium* spp. bearing in mind the importance of *Aceria* species as biological control agents and the necessity of their correct characterization and identification. As it was assumed by Skoracka *et al.* (2002) quantitative description of host related morphological variation can provide the basic information needed to improve the eriophyoid taxonomic system and enhance our understanding of mechanisms generating this variation.

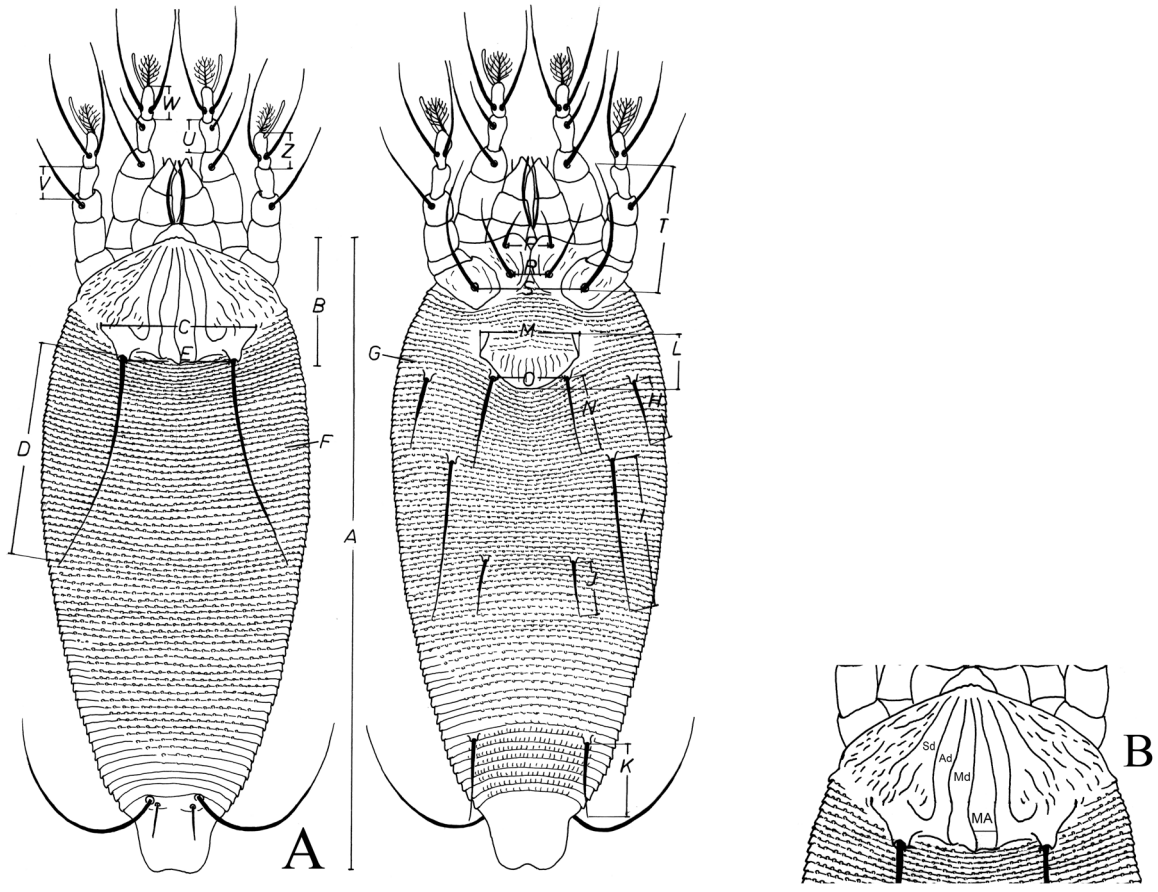

Figure 1. Measurements of A. anthocoptes female morphology used in statistical analysis. Explanation of abbreviations: (A) A-length of body, B- length of prodorsal shield, C- width of prodorsal shield, D- length of scapular setae sc, E- scapular tubercles apart from sc, F- no. of dorsal annuli, G- no. of ventral annuli, H- length of lateral setae c2, I- length of I ventral setae d, J- length of II ventral setae e, K- length of III ventral setae f, L- length of genitalia, M- width of genitalia, N- length of setae 3a, O- spacing of tubercles 3a, P-spacing of tubercles 1b of coxa I, R- spacing of tubercles 1a of coxa I, S- spacing of tubercles 2a of coxa I, T- length of setae 2a, U- length of tibia I, W- length of tarsus I, Y- length of tibia II, Z-length of tarsus II. (B) Md- length of median line, Ad- length of admedian line, Sd- length of submedian line, MA- distance between Md and Ad line.

Material and methods

The main criteria for the selection of samples were different host plants of the genus *Cirsium*. Samples of four *Cirsium* species were collected in Serbia at the following localities:

- *C. arvense* (Ca) and *C. heterophyllum* (Ch): mountain Vlasina (altitude 1200m),

- *C. rivulare* (Cr): mountain Tara (altitude 1100m),

- *C. eriophorum* (Ce): mountain Maljen (altitude 1000m).

Mites were collected using extracting methods described by de Lillo (2001). Twenty-five to 30 mites from each sample were mounted in dorso-ventral position on slides in Kiefer's F medium and identified (Amrine & Manson 1996). Protogyne females randomly selected from each population were examined with a phase-contrast microscope (LEICA DMLS). Twenty-seven traits were measured on each individual with the IM 1000 (Leica, Wetzlar, Germany) software package (Figure 1). Twenty–three of these morphometric traits (Figure 1A) were commonly used, and the four others (Figure 1B) were related with shield design, equally important for eriophyoid mite identification.

All variables that entered the analyses presented normal distribution, as well as homogeneity of variance. The data were tested for normality using Shapiro–Wilk tests. A multivariate analysis of variance (MANOVA) allows for the comparison of the population means of all variables of interest at the same time (multivariate response), rather than considering multiple responses as a suite of univariate responses (Zar 1999). The statistical significance of the MANOVA can be determined in a variety of ways. The most often used statistic test Wilks' Lambda was applied (Zar 1999). A one-way MANOVA was used to examine the differences in morphological variation among *Aceria* spp. populations inhabiting different *Cirsium* species.To describe and interpret effects from MANOVA, a multivariate discriminant analysis (DA) was used as a useful post method to employ following a MANOVA. Discriminant analysis was employed on commonly used 23 morphological traits and separately on four traits related with shield design, in order to determine the relative importance of characters as discriminators between a priori groups and the relative positions of the centroids of those groups (Manly 1986). In addition, canonical variables were computed. All statistical analyses were conducted using the Statistica 6 software package (StatSoft 2001).

Finally, a UPGMA (Unweighted Pair Group Method with Arithmetic mean) dendrogram, based on squared Mahalanobis distance between species centroids, was generated using 23 commonly used quantitative traits. This was used to evaluate the phenetic relationships between species.

Results

Descriptive statistics of the quantitative traits of *Aceria* species are given in Table 1. The one-way MANOVA of four *Aceria* spp. populations revealed significant differences in morphological variation of 23 commonly used morphological traits (Wilks' Lambda = 0.00116; F (69, 254) = 31.812; P<0.001), and in four traits related with shield design.(Wilks' Lambda = 0.05918; F (12, 275) = 43.868; P<0.001).

The result of the discriminant analysis, of 23 and four traits, showed the most important and distinct discrimination to be between the population from *Cirsium rivulare* and the three others, i.e. *C. eriophorum*, *C. heterophyllum* and *C. arvense* based on the first canonical axis (function) (Figures 2 & 3). The total correct percent of Classification matrix of all four groups was very high, on 23 traits (99.099 %) and high on four traits (78.378 %).

From the standardized canonical discriminant function coefficients (Table 2A) it is evident that the first canonical function describes 83.63 % of the total variability; the first and second together, 98.57 %; and all three roots with 100 % of the total variability. It should be stressed that the first and the second canonical functions describe most of the variability. Length of the second ventral (e) setae and length of the first ventral (d) setae contributes most to this discrimination.

The following three characters contribute, but to a lesser extent: P-distance between the first tubercles (1b) of coxae, F- number of dorsal annuli and C – width oh prodorsal shield, based on the first canonical function. Length of the lateral (c2) setae, width of prodorsal shield, length of the first ventral (d) setae, distance between (sc) tubercles and number of ventral annuli has the most distinct discriminative power based on the second canonical function. Bearing in mind that the second canonical function describes only 14.94 % of the total variability, it could be inferred that its discriminative power is significantly lower in comparison with the first canonical function. It should be stressed that this function evidently separates populations from *C. eriophorum, C. arvense* and *C. heterophyllum* (Figure 2).

Table 1. Basic statistical data for 27 morphological traits of four Aceria spp. populations from different Cirsium species. A - commonly used morphological traits, B - traits related with shield design, Ca–C. arvense, Ce–C. eriophorum, Ch–C. heterophyllum, Cr–C. rivulare n = number of specimens, SD = standard deviation.

Populations	Ca (n=30)		Ce (n=26)		Ch (n=25)		Cr (n=30)	
A Traits	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A - body length	196.76	9.22	186.86	16.31	191.57	18.53	169.34	22.87
B - prodorsal shield length	29.80	1.36	28.01	1.44	29.86	1.70	27.94	1.62
C - prodorsal shield width	35.31	1.42	36.17	1.45	34.00	1.69	26.37	1.78
D - setae sc length	61.03	4.55	56.84	5.96	52.78	4.44	55.53	4.29
E - tubercles sc apart	22.11	1.29	24.14	0.79	21.15	1.17	16.69	0.86
F - no. of dorsal annuli	75.87	4.52	67.46	5.21	78.44	5.38	85.03	5.01
G - no. of ventral annuli	86.33	4.82	75.00	4.13	85.36	6.82	85.10	4.06
H - setae c2 length	23.79	1.70	14.94	0.85	22.07	3.77	12.96	1.35
I - setae d length	66.52	3.88	53.53	4.18	54.77	5.36	31.36	1.60
J - setae e length	21.44	1.75	16.58	1.35	19.68	1.45	9.38	0.92
K - setae f length	26.47	1.78	22.79	1.85	25.06	1.66	17.50	0.82
L - genitalia length	11.78	0.79	10.70	0.97	11.60	1.32	10.08	0.73
M - genitalia width	21.95	1.31	21.60	0.96	20.95	1.08	17.24	0.80
N - setae 3a length	17.80	1.73	15.62	1.34	15.12	1.19	8.45	0.64
O - tubercles 3a apart	17.04	0.97	17.25	0.57	15.87	1.13	13.35	0.75
P - tubercles 1b apart	10.41	0.55	10.61	0.64	10.19	1.09	10.04	0.62
R - tubercles 1a apart	7.62	0.60	6.92	0.49	6.79	0.49	5.01	0.49
S - tubercles 2a apart	22.57	1.31	21.37	1.21	20.43	1.59	16.32	1.04
T - setae 2a length	50.03	5.25	44.07	4.77	40.73	5.22	31.11	5.01
U - tibia I length	7.95	0.38	7.47	0.58	7.11	0.88	6.77	0.58
W - tarsus I length	7.37	0.46	6.83	0.46	6.72	0.47	5.74	0.46
V - tibia II length	6.93	0.44	6.83	0.46	6.33	0.60	6.08	0.52
Z - tarsus II length	6.96	0.44	6.61	0.36	6.42	0.50	5.49	0.31
B Traits								
Md – length of median line	27.30	1.46	26.10	1.53	27.45	1.51	22.98	2.71
Ad - length of admedian line	26.88	1.63	26.20	1.30	27.36	1.38	25.90	1.79
Sd - length of submedian line	27.34	4.13	30.10	3.18	31.81	2.76	17.57	1.89
MA – distanca between Md and Ad	4.52	0.50	5.03	0.34	4.20	0.47	2.57	0.22

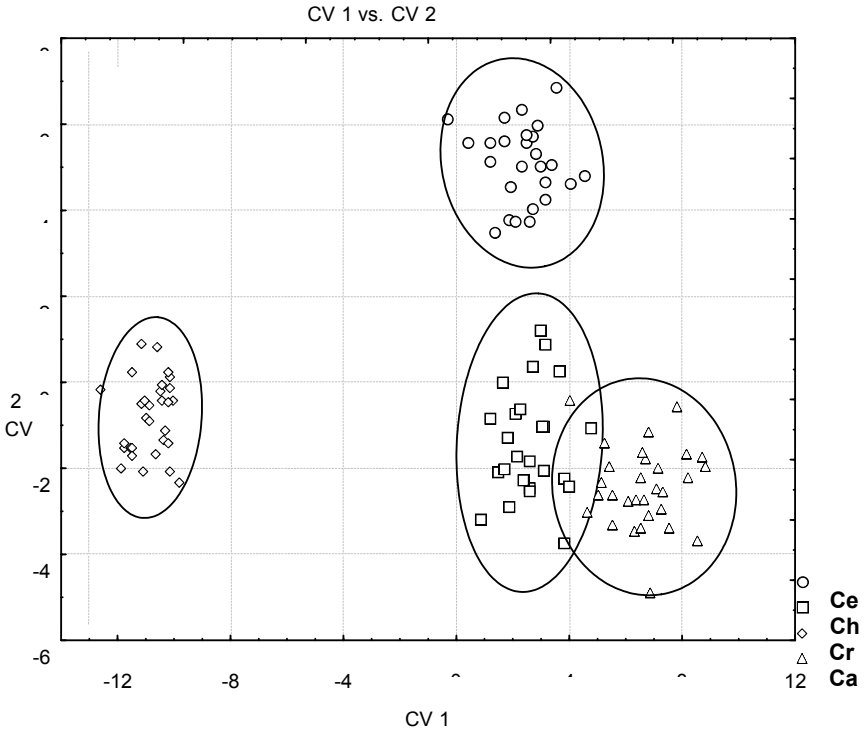

Figure 2. Plots of scores of the first two canonical axes (CV 1 and CV 2) of four populations of Aceria spp. from 23 commonly used morphological traits. Abbreviations of populations are described in «Materials and Methods» section

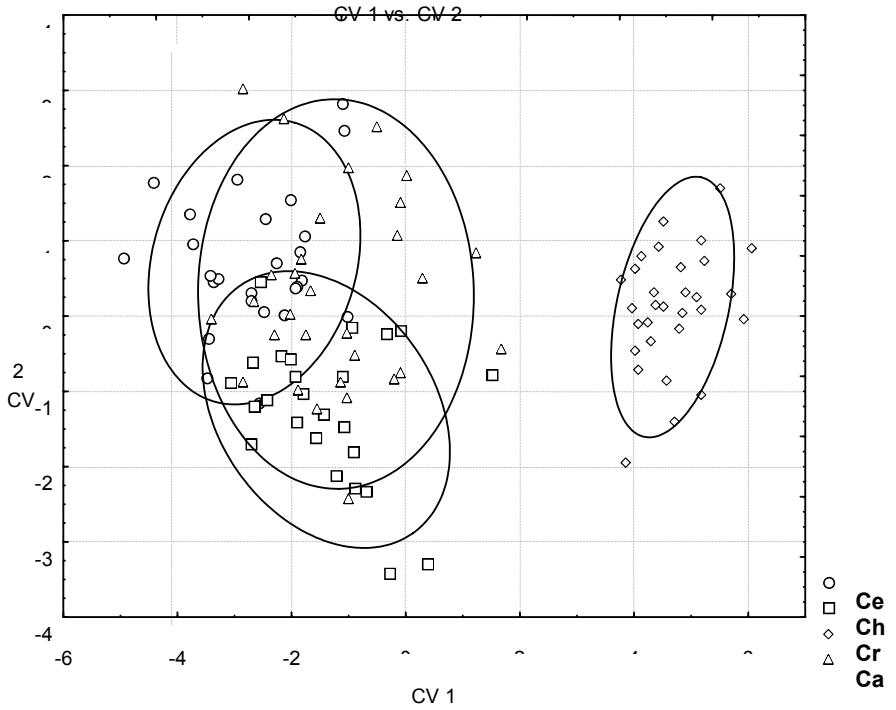

Figure 3. Plots of scores of the first two canonical axes (CV 1 and CV 2) of four populations of Aceria spp. populations from four traits related with shield design. Abbreviations of populations are described in «Materials and Methods» section

Table 2 Standardized coefficients for canonical variables on all three (CV1 - CV3) canonical axes in discriminant function analysis: on 23 commonly used morphological traits (A) and on four traits related with shield design (B)

A	Traits	CV 1	CV 2	CV 3
	A - body length	-0.277	-0.155	-0.083
	B - prodorsal shield length	-0.034	0.049	-0.229
	C - prodorsal shield width	0.345	0.603	-0.130
	D - setae sc length	-0.036	-0.047	0.544
	E - tubercles sc apart	0.182	0.491	-0.143
	F - no. of dorsal annuli	-0.347	-0.152	-0.056
	G - no. of ventral annuli	0.201	-0.454	-0.042
	H - setae c2 length	0.097	-0.707	0.051
	I - setae d length	0.456	-0.525	0.244
	J - setae e length	0.490	-0.073	-0.388
	K - setae f length	0.230	0.108	-0.182
	L - genitalia length	0.091	-0.159	0.209
	M - genitalia width	0.079	-0.034	-0.086
	N - setae 3a length	0.222	0.167	-0.032
	O - tubercles 3a apart	0.164	0.269	0.015
	P - tubercles 1b apart	-0.349	-0.227	-0.092
	R - tubercles 1a apart	0.198	-0.085	-0.082
	S - tubercles 2a apart	-0.036	-0.021	0.295
	T - setae 2a length	0.046	0.155	0.315
	U - tibia I length	0.017	-0.149	0.115
	W - tarsus I length	0.113	-0.174	0.208
	V - tibia II length	-0.039	0.193	0.132
	Z - tarsus II length	0.091	-0.126	0.164
	Eigenvalues	48.252	8.626	0.822
	Cumulative proportions	0.836	0.986	1.000
B	Traits			
	Md- length of median line	-0.247	-0.309	0.871
	Ad- length of admedian line	0.236	-0.067	0.176
	Sd- length of submedian line	-0.568	-0.692	-0.537
	MA- distance between Md and Ad line	-0.757	0.633	0.058
	Eigenvalues	8.713	0.514	0.149
	Cumulative proportions	0.929	0.984	1.000

From the standardized canonical discriminant function coefficients (Table 2B) is evident the first canonical function describes 92.92 % of the total variability; the first and second together, 98.41 %; and all three roots with 100 % of the total variability. It should be stressed that the first and the second canonical functions describe most of the variability. Distance between median and admedian line (MA) and length of submedian (Sd) line have the most distinct discriminative power based on the both canonical functions. Bearing in mind that the second canonical function describes only 5.50 % of the total variability, it could be inferred that its discriminative power is significantly lower in comparison with the first canonical function (Figure 3).

All pairwise squared Mahalanobis distances between populations were significant at the 99 % level. UPGMA cluster analysis of the squared Mahalanobis distances (Figure 4) clustered all

Aceria populations, except from *Cirsium rivulare*, in the same branch, indicating that *A. cirsii* from *C. rivulare* was the most divergent population. Within this main branch, the populations from *C. arvense* and *C. heterophyllum* were isolated from the branch clustering the remaining *Aceria* population from *C. eriophorum*.

Aceria cirsii mites from the host plants *C. rivulare* are characterized by narrower prodorsal shield, larger number of dorsal annuli, shorter length of first and second ventral setae, smaller distance between first coxal setae, compared with three other populations.

Aceria sp. mites from host plant *Cirsium eriophorum* are characterized by wider prodorsal shield, larger distance between scapular setae, smaller number of ventral annuli, shorter length of lateral setae and shorter length of the first ventral setae. The results obtained in this work indicate that the *Aceria* sp. from *Cirsium eriophorum* differs from *Aceria anthocoptes* from *C. arvense* and *C. heterophyllum.*

Morphological differences exist among *A.anthocoptes* from *C. arvense* and *C. heterophylum. Aceria anthocoptes* from *C. heterophyllum* is characterized by narrower prodorsal shield, larger number of dorsal annuli, shorter length of the first ventral setae, shorter length of the second ventral setae and smaller distance between first coxal (1b) setae, compared with population *A. anthocoptes* from *C. arvenese.*

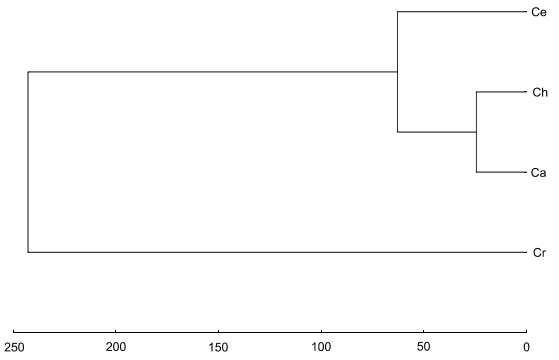

Figure 4 – UPGMA tree diagram (dendrogram) of four Aceria spp.populations from different Cirsium species based on squared Mahalanobis distances (scale showed) obtained from commonly used 23 morphological traits. Abbreviations of populations are described in «Materials and Methods» section.

Regarding traits which characterize shield design *A. cirsii* mites in comparison with other three species have smaller submedian line and smaller distance between median and admedian line. Results obtained in this study indicate that there are no differences between the length of shield lines and the distance among median and admedian lines regarding populations from *C. arvense, C. heterophyllum and C. eriophorum.*

Discussion

The results of our study show that *A. cirsii* is clearly separate from other three *Aceria* populations from *C. arvense, C. heterophyllum* and *C. eriophorum* host plants. According to Petanović *et al.* (2000), *Aceria cirsii* is morphologically close to *Aceria anthocoptes*, but it can be distinguished on the basis of differences in the following characteristics: it has smaller number of empodial rays, more dorsal opisthosomal annuli, shorter length of first ventral setae (*d*), and smaller number of genital striae. Our investigations confirm results presented in the original description showing also that *A. cirsii* mites are characterized by narrower prodorsal shield, shorter length of second ventral setae and smaller distance between first coxal setae. Beside that, comparing shield design it is obvious that *A. cirsii* mites have shorter median line, significantly shorter submedian line and twice shorter distance between median and admedian lines. Sukhareva (2001) analyzing 15 morphological features of 35 *Aceria* species living on Asteraceae stated that the principal differences between species are the length of median line and the length and form of submedian lines.

Original description of *A. anthocoptes,* (Nalepa, 1892) and other old date publications (Roivainen 1950; Farkas 1965) included a relatively low number of quantitative characteristics, without a set of comparative data from other specimens. Petanovic *et al.* (1997) supplemented the description of this species from *C. arvense* host plant adding more quantitative traits. Magud *et al.* (2007) compared morphological variation in different populations of *A. anthocoptes* inhabiting two infra-specific host plant taxa of *C. arvense.*

In this study for the first time, significantly large number of individuals of *Aceria anthocoptes* protogyne females from *Cirsium heterophyllum* were found and investigated in details. Our results show that, although *Aceria* mites from two populations (these inhabiting *C. arvense* and *C. heterophyllum,* respectively) are very similar, there are still characters in which they differ. *A. anthocoptes* from *C. heterophyllum* is characterized by narrower prodorsal shield, larger number of dorsal annuli, shorter length of the first ventral setae, shorter length of the second ventral setae and smaller distance between first coxal (1b)

setae. Shield design of these two populations is similar. The only difference is in the length of submedian line which is a little bit longer in *A. anthocoptes* inhabiting *C. heterophylum.* Bearing in mind that samples were collected on the same day and from the same locality it excludes the explanation regarding seasonal variability (seasonal dimorphism) or geographical races. We may presume the hypothesis of host plant impact on intraspecific phenotypic differences.

Results of our studies show clear separation between *Aceria* sp.from *C. eriophorum* on one hand and *A. anthocoptes* from *C. arvense* and *C. heterophyllum* on the other. Characteristics which separate *Aceria* sp. from *C. eriophorum* are wider prodorsal shield, larger distance between scapular setae, smaller number of ventral annuli, shorter length of lateral setae and shorter length of first ventral setae. On the other hand, shield design is pretty similar in this case and can not be concerned as a distinct character for species characterization.

Phenogram supplied in this study confirms that *Aceria cirsii* is the most divergent in comparison with three others. On the other hand populations *A. anthocoptes* from *C. arvense* and *C. heterophyllum* were isolated from *Aceria* sp. from *C. eriophorum.* Results suggest that on *Cirsium* spp. investigated in this study at least three morphologically different taxa, and one race or ecological host related biotype exist. According to Skoracka *et al.* (2002) variability in phenotypic traits between populations of mites living on different host plants may originate from several causes: total separation of gene pools – different mite species, partial differentiation of gene pools – host races and no separation of gene pool – phenotypic plasticity.

For the time being, it is impossible to understand the origin of phenetic variability between investigated taxa.In addition to classical taxonomy, molecular typing should be applied to help in clarifying the taxonomic status of *Aceria* spp. inhabiting *Cirsium* spp.

Acknowledgements

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POPULATION DYNAMICS OF THE TWO-SPOTTED SPIDER MITE: AN AGE-STRUCTURED MODEL

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Abstarct

It is well known that the age structure of a founding population of spider mites can highly influence the subsequent growth of the colony. In order to study this topic, a mathematical model for the population growth of *Tetranychus urticae* is presented. Existing age-structured models are based on life tables, which are heavy to use. Our aim is to synthesize the information contained in life tables, in order to make it more suitable for theoretical research. The experimental data used to parameterize the model was obtained from 214 mites, living on bean plants at 24°C. Age-specific mortality and fertility rates were fitted to curves. This allowed them to be incorporated into the model as mathematical functions of age, instead of tabulated values. Computation of the model gives an insight on the effect that initial conditions have on population growth. Furthermore, the simplicity of the model makes it an appropriate theoretical tool to study the consequences of phenomena affecting age-structure such as migration or the Allee effect.

Key words

exponential growth, *Tetranychus urticae*, mathematical modelling, fertility, mortality

Introduction

Since the use of insecticides and fungicides became a common practice in agricultural systems, cultivators have had to deal with ravaging outbreaks of spider mites (Acari: Tetranychidae). These phytophagous mites that feed by sucking out the content of leave cells, can destroy vast cultures in just one summer (Walter and Proctor 1999). Their success is mainly due to their varied and efficient dispersal behaviours (Hussey and Parr 1963) and to their outstanding population growth potential (Sabelis, 1981). In this paper, the latter is studied through the approach of mathematical modelling. The species chosen for this study is Tetranychus urticae, the two-spotted spider mite, as it is among the most economically important and documented spider mite pests.

At the beginning of a ravaging process, quantity of food can be seen as unlimited and the population can be considered to grow exponentially. The well-known model for exponential growth (Turchin 2003) includes a constant growth rate r, defined as the intrinsic rate of natural increase (Birch 1948).

$$\frac{dN}{dt} = rN$$

In a population composed of individuals of different ages (hence, of different fertility and mortality potentials), the intrinsic rate of natural increase can only be constant over time if the age

structure of the population is also constant over time (Birch 1948). This stable age distribution (hereafter referred to as SAD) can be calculated from the life tables. In spider mite species the stable age distributions are quite similar and roughly average 66% of eggs, 26% immature and 8% adult (Carey 1982).

In the particular case of *Tetranychus urticae* during an outbreak, the assumption of stable age distribution is hardly ever verified. Besides the fact that founding colonies are too small to have a SAD, their composition is determined by processes that affect age-classes differently. The large part of individuals alive in early summer is wintering individuals (diapause). Diapause is only induced on adult females (Veerman 1985), particularly at very young age (Raworth 2007). Founding mites can also be migrants and it has been shown that preovipositing females have a more pronounced tendency to migrate than other stages (Hussey and Parr 1963, Yano 2008). Such founding populations, mostly composed of adult females, are thus far from the SAD.

Over time, any population eventually converges to its stable age structure. However, at local population level, this situation is never encountered because spider mite populations grow so rapidly that the host plant is killed before SAD can be reached (Hance and Van Impe 1999). Furthermore, age-structure is constantly altered by events that affect only certain age-classes (Carey 1983). A typical example is natural acarine predator Phytoseiulus persimilis that shows a clear preference for eggs (Blackwood et al. 2001).

The exponential growth model is therefore not sufficient to describe population growth of Tetranychus urticae under the conditions that concern us, and age-structure must be taken into account.

Our major objective is to formulate a mathematical model that describes the evolution of an age-structured population in time, starting from a given age distribution, so as to give basic output like the intrinsic growth rate, the SAD, or the amount of time needed to reach it. Moreover, it should give insight into less trivial questions such as what are necessary initial conditions to ensure growth or to quantify the effects of different founding age-structures. Finally, we intend to complete the understanding of population dynamics of *Tetranychus urticae*, as it is fundamental to study topics such as collective choices and Allee effect.

Experimental data

Data used to parameterise the model was gathered from Van Impe's previous work (1985), where a more detailed description of the methods can be found. Experiments were carried out under constant temperature (24°C) and constant relative humidity (85%).

Two hundred fourteen female deutonymphs of same age (± 4 hours) were placed individually on bean leaf discs (4 cm2). The following day, they entered their final quiescent stage (theleiochrysalis) and 4 males were placed on each disc in order to ensure mating at eclosion. One day after, all female adults had emerged and it is considered day one of the experiment. From this point onwards, every day at the same time, each living female was transferred to a new leaf disc. Eggs on the old disc were counted. The offspring of 101 females (a total of 5760 larvae) was kept for further observations on immature development.

Longevity, oviposition period and daily number of eggs were collected for each female. Offspring observations provided data on immature survival and age-specific sex ratios.

Formulation of the model

Few simplifications are made. First of all, only the female population is modelled. In terms of birth rate, the number of males can be ignored if we consider that all females are fertilised (Hance and Van Impe 1998). Indeed, although females usually outnumber males (Krainacker and Carey 1991), occurrence of virgin females is very low (Potter, 1978). Secondly, only three events of life history are taken into account: birth, oviposition, and death.

The first step was to find the mathematical expressions that best described fertility and mortality as functions of age. Experimental data was fitted to its corresponding curve with the least squares non-linear method (MATLAB). Mortality rates at late ages were calculated on a small number of individuals and can present aberrant values. We avoided that problem by fitting the survival curve rather than the mortality curve. Statistics of the fits are summarised in Table 1. Mortality m(a) was deduced from survival s(a) according to the following relation (Gross & Clarke 1976), with a = age.

$$\frac{ds}{da} = m(a)s(a)$$

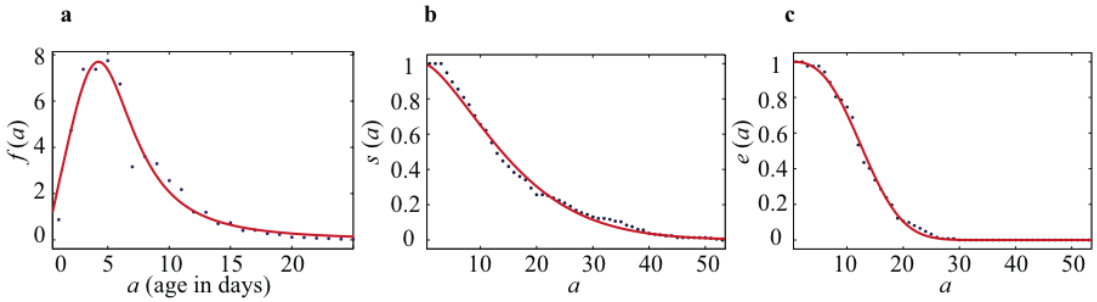

Figure 1. Experimental data (blue dots) and fitted curves (red) for the 3 life events considered. (a) f is Daily number of female eggs per female as a function of adult age a (in days). (b) s is the proportion of individuals reaching adult age a. (c) e is the proportion on females still ovipositing at adult age a.

Table 1. Summary of the curve fits.

Fertility	**Survival**	**Oviposition**
(a)	(b)	(c)
$f(a)=\frac{\alpha \cdot a}{\beta + a^{\sigma}}$	$s(a)=\exp(-\gamma \cdot a^{\mu})$ (survival) $m(a)=\gamma \cdot \mu \cdot a^{(\mu-1)}$ (mortality)	$e(a)=\exp(-\lambda \cdot a^{\theta})$ $o(a)=\lambda \cdot \vartheta \cdot a^{(\vartheta-1)}$
Parameters (with 95% confidence intervals)		
= 2591 (426.1, 4755) = 1075 (106.8, 2043) = 4.057 (3.659, 4.455)	= 0.0145 (0.0115, 0.0175) = 1.471 (1.401, 1.541)	= 6.984 . 10^4 (9.149 , 4.819).10^4 =2.697 (2.582, 2.813)
Statistics		
SSE = 7.281 Adjusted R^2 = 0.9668	*SSE* = 0.03638 Adjusted R^2 = 0.9929	*SSE* = 0.016 Adjusted R^2 = 0.9976

One way to model age-structured populations is with partial differential equations defining the evolution of the number of individuals of continuous ages in continuous time (McKendrick 1926). It is an elegant formulation because with just one equation (equ. 1) and the set of initial conditions (equ. 2), it can account for complex dynamics of an age-structured population.

$$\frac{\partial N(t,a)}{\partial t} = -m(a)N(t,a) - \frac{\partial N(t,a)}{\partial a} \quad (1)$$

with N(t,a) = number of individuals of age a at time t, and m(a) = mortality function. The initial population structure is N(0,a), and the number of births is given by

$$N(t,0) = \int_{1}^{a_{max}} f(a)N(t,a) \quad (2)$$

However, this formulation entails technical difficulties surrounding partial differential equations such as i.e. heavy numerical resolutions or existence and uniqueness of solutions (Cushing1998). Therefore we will use the corresponding discrete equation (equs.3 and 4).

Nt,a is the number of individuals of adult age a at time t. It is given by the number of individuals of age a-1, at time t-1 minus those that died.

$$N_{t,a} = N_{t-1,a-1} \cdot (1 - m(a-1)) \quad (3)$$

The number of individuals of age 1 (births), is given by

$$N_{t,1} = \sum_i N_{t-1,i} \cdot f(i) \quad (4)$$

Before adulthood (the first 10 days), mortality and fertility are considered null. Survival of immature stages is implicit in fertility rates as fertility rates represent female offspring that actually reached adulthood. From age 11 onwards, mortality and fertility are given in table 1.

The total population at time t is given by equation (5).

$$N_t = \sum_a N_{t,a} \quad (5)$$

Most females (57%) stopped laying eggs because they entered senescence, and not because they died. We test to what extent senescence affects the population dynamics by incorporating it into the model. We fitted the proportion of females still able to lay eggs as a function of age (e(a), Table 1). The number of births in this model becomes:

$$N_{t,1} = \sum_i N_{t-1,i} \cdot e(i) \cdot f(i) \quad (6)$$

Finally, a Monte-Carlo simulation of the model is computed. At every time step, each individual of the population has a probability of dying given by the mortality function. Offspring is given by the value of the fertility function rounded up to the nearest integer. We make stochasticity intervene only in deaths, and use mean values for fertility. The reason for this is that our experimental data did not reveal a clear pattern of deviation from the mean, it was impossible to make an objective choice on what distribution to chose.

Results

Regardless of initials conditions, population structure undergoes damped oscillations for a certain amount of time (Fig. 2). It exponentially converges to stable age distribution. It is impossible to tell at which point it reaches SAD, because in theory the oscillations continue indefinitely, and their perception only depends on the scale of the observations. It is very clear nevertheless that if a population is founded by one female (as in Fig. 2), the population remains far from SAD for at least the first 50 days. This is true whether we incorporate senescence into the model or not.

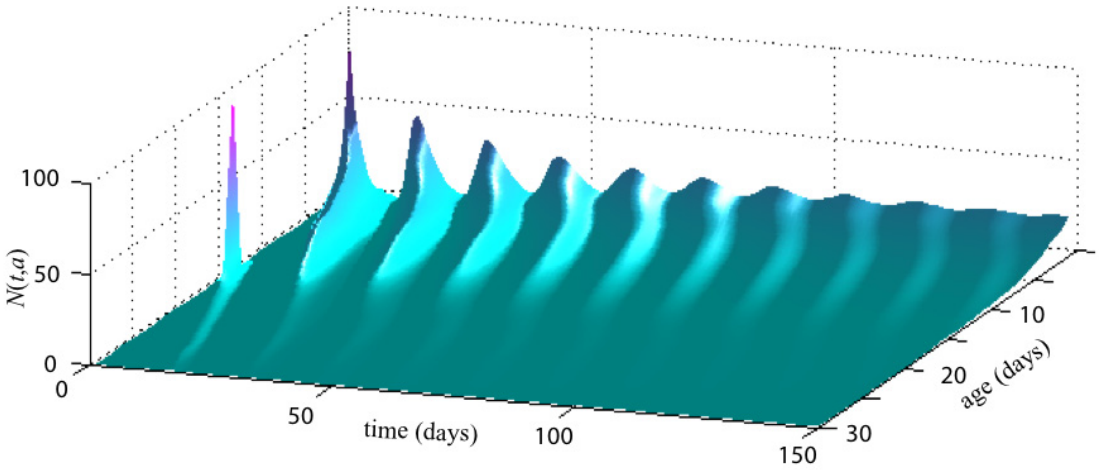

Figure 2. Evolution of the age-structure of a population with time. In this case, the founding female is 14 day old.

Stable age distribution is usually given in percentage of eggs, immatures and adults. In the case without senescence, it is 61.7 % of eggs, 29.9 % of immatures and 8.4 % of adults (Table 2). The rate of increase of the population also oscillates as it converges to the intrinsic rate of natural increase, here r = 0.265.

When it is far from SAD, population grows in pulses corresponding to peaks of oscillations in the growth rate. The evolution of population size greatly depends on initial conditions of age structure. We compared the growth of populations founded by one single female of different ages (Fig. 2). It appears that there is an optimal age to start a population, but it depends on the day when the size of the population is measured. For example, the largest size on day 13 is reached by a 11 day-

old founding female. On day 15 however, the largest size is obtained by a 14 day-old founder. From there onwards, the largest population is always the one founded by a 14 year old.

Table 2. Comparison of the SAD and the intrinsic rate of natural increase obtained with the general model, and the senescence model.

General model	With senescence
r = 0.265	*r* = 0.261
SAD	*SAD*
eggs 61.69	eggs 61.27
immatures 29.88	immatures 30.07
adults 8.43	adults 8.66

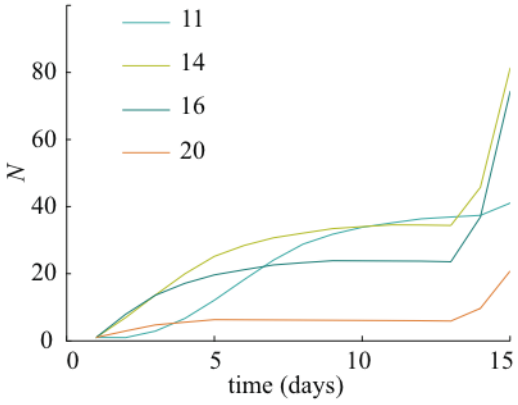

Figure 3. Sizes of populations founded by one female over time. Different colours correspond to different ages of the founders.

Growth by pulses is faster than the exponential growth of SAD populations. Figure 3(a) shows that the population size of a colony founded by 14 days old females is always larger than the size of a population founded by the same number of females, distributed according to SAD. Fig 3b shows that the size of the population growing by pulses can be up to ten times higher than the population with an exponential growth.

If we consider senescence in the model, the value of r slightly decreases and the composition of SAD weakly changes (Table 2). Consequently, colony size reaches lower numbers when senescence is integrated in the model. The greater the age of the founders, the bigger the difference between the results of the two models. Nonetheless, in the first 30 days, the ratio between the two is never higher than 1.25 (Fig. 5).

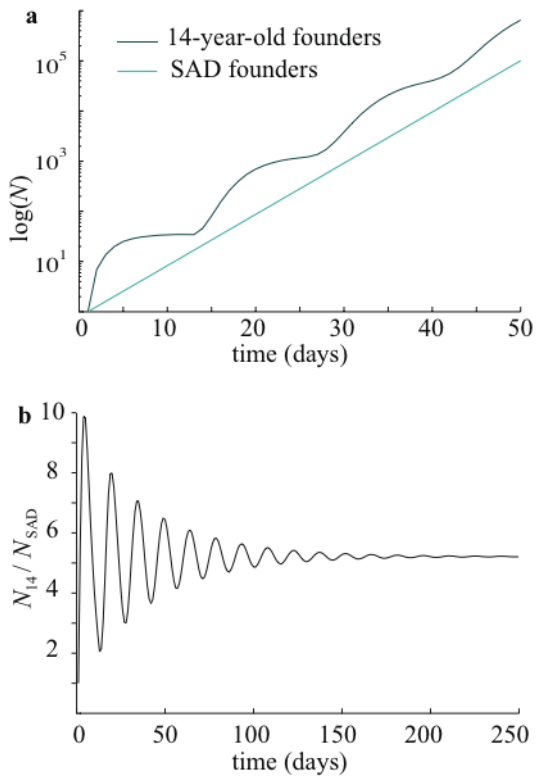

Figure 4. Comparison of populations founded by a stable-age-distributed colony and by 14 day-old. (a) Population size of the two populations (in logarithmic scale) (b) The ratio between the two populations.

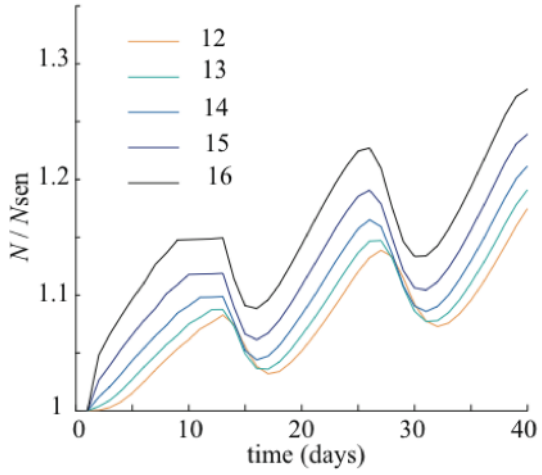

Figure 5. Ratio between the population size calculated with the general model and the population size calculated with the senescence model. Different colours represent different ages of the female founder.

Monte-Carlo simulations show that as long as there is an ovipositing female among the founders of a colony, it will almost inevitably grow. It is easy to understand from a probabilistic point of view.

Even the oldest ovipositing female (24 days old) has a probability of dying of P=0.074. The condition for extinction is that she dies before laying an egg. Otherwise, the condition is that the egg dies during preoviposition (P=0.021). As the number of conditions for extinction increases, the probability decreases. Therefore, even the most likely scenario for extinction has a very low probability (P=0.074).

Discussion

We present mathematical functions that describe the pattern of age-specific mortality and fertility rates of *T. urticae*. However, the relevance of the model is not restricted to *Tetranychus urticae* under our experimental conditions. We tested the goodness of fit of these functions on other spider mite life tables (Gutierrez & Chazeau 1972, Hamilton *et al* 1986, Laing 1969) and obtained very good results (R^2 always above 0.9). Moreover, our equations fit well (R^2>0.9) to life tables of other taxa, like the plant louse *Aphis fabae* (Hance 1990). Life tables represent a key tool in pest control. Synthesising the information they contain by modelling the fertility and survival data could improve and facilitate their use in pest management.

This study confirms that the outbreak potential of *Tetranychus urticae* can be explained by its population dynamics. First of all, the composition of founding populations (in the field as in greenhouses) is mostly composed of young females (Parr and Hussey 1966, Yano 2008). Our model corroborates that females in their first days of oviposition are precisely optimal founders, in terms of ensuring rapid growth. As a consequence of the initial composition of populations, these populations remain far from SAD (Carey 1983). We showed that even in conditions where nothing interferes with age-structure besides deaths and births, SAD is not reached in the first two months. This deviation from SAD allows populations to increase by pulses, which guarantees faster growth than with the classical exponential law. Finally, we pointed out that the stochastic aspect of death is not sufficient to cause extinction of a founding population.

Age-structure and its dynamics have been included in various growth models. Lewis (1942) and Leslie (1945) formulated a discrete matrix model that set the grounds for theoretical and applied research on a large number of species (Cushing 1998, Dennis 1991).

$$N_{t+1} = T \cdot N_t$$

N is the vector containing the number of individuals in each age class, and *T* the transformation matrix derived from life tables. The first row and the diagonal components of *T* are respectively the age-specific fertility and mortality rates.

Such models have been used on *Tetranychus urticae* to study predator-prey dynamics (Hance and Van Impe 1999, Selhorst et al 1991), or for calculation of stable age distributions and their corresponding intrinsic rate of natural increase (Laing 1969, Carey and Bradley 1982). The results of our model are in line with those found in previous studies in terms of SAD and r (for a review see Carey 1982).

Matrix models are fairly simple to formulate and to compute. However, their disadvantage lies in the fact that they are based on life tables, which are heavy to use: they contain as many parameters as entries on the table, which makes them inflexible and unsuitable for theoretical research.

Another way to incorporate variations of age-structure into a colonizing population model was proposed by Hance (1990). The model is based on Lotka's equation. Growth rate *r* is replaced by a damped sinusoidal function of time. This function imitates the damped oscillations of growth rate as the population converges to SAD. The conclusion of his study agrees with ours in that growth by pulses is faster than exponential growth.

The model that we present here can be defined as a combination of discrete and continuous modelling techniques. On one hand, it is discrete in time age, which avoids the complications of partial differential equations. On the other hand, age-specific mortality and fertility are defined as continuous functions of age, like in continuous models. This major difference with the matrix models, allows us to reduce the number of parameters to only 5. This makes it an adequate starting point for theoretical research.

More particularly, it would be interesting to study the role of growth rate oscillations in collective choices. Collective migration of spider mites, for instance, can result from the amplification of trail following (Yano 2008). Oscillations in population size typically play a role in the triggering of such amplifications by provoking the exceedance of thresholds. It also seems that spider mites are subject to Allee effects (Van Impe 1985). Such positive feedback loops can stabilise the oscillations and prevent them from converging into their stationary values.

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EFFECT OF LOW TEMPERATURES ON TWO GENERALIST PHYTOSEIID SPECIES

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Abstract

The delay in phytoseiid ontogeny could enhance the possibility of mass egg storage for commercial and research purposes. The effect of temperature slightly below the developmental threshold for phytoseiids in temperate areas was evaluated in eggs of *Neoseiulus californicus* and *Typhlodromus exhilaratus* originating from central Italy. One-day-old eggs of both phytoseiids were maintained at 5±1°C for different times (5, 10, 20, 30, 40, 50 days) and the subsequent egg viability and progeny survival were recorded. Periods longer than 10 days were detrimental for both *N. californicus* and *T. exhilaratus.* Shorter storage periods allowed 40-84% egg hatching; the highest mortality was always observed in *T. exhilaratus.* Juvenile mortality, sex ratio and escape rate were determined until the third generation. Differences in biological parameters and the possibility to increase cold hardiness of the eggs are discussed.

Key-words

Neoseiulus californicus, *Typhlodromus exhilaratus*, cold hardiness, egg hatching, progeny survival

Introduction

For poikilothermic animals, temperature is one of the most important factors affecting the survival, developmental and reproductive rates. Studies on the effect of low temperatures on predators to be used in biological control, such as phytoseiid mites, are crucial to evaluate the potential establishment of a species in new environments and to furnish data for risk assessment (Hatherly *et al.* 2005).

Among predatory mites, the generalist phytoseiid *Neoseiulus californicus* (McGregor) is one of the most promising and widely used species for biological control of tetranychid mites. Therefore, much information is available on different aspects of its biology, including the influence of climatic conditions on its reproductive parameters (see Castagnoli & Simoni 2004 for a review). Studies on low temperatures have dealt mainly with the effects on females and on their supercooling points (Bale 1987; Gotoh *et al*. 2005; Hart *et al*. 2002).

Data on the influence of cold exposure are also available for other phytoseiid species such as *Typhlodromus pyr*i (Scheuten), *Euseius finlandicus* (Oud.), *T. montdorensis* (Schicha), *N. womersleyi* (Schicha), *Phytoseiulus persimilis* (Athias-Henriot), *Amblyseius andersoni* (Chant) and *A. cucumeris* (Oud.) (Broufas & Koveos 2001; Broufas *et al*. 2006; Gotoh *et al*. 2005; Hatherly *et al*. 2004; Morewood 1992; Moreau *et al.* 2000, Van der Geest *et al*. 1991)). However, they are lacking for *T. exhilaratus* Ragusa, another generalist phytoseiid widespread in the Mediterranean area. It is found especially on vines, often associated with *Eotetranychus carpini* (Oud.) (Castagnoli & Liguori 1986a; Castagnoli *et al*. 1991; Tixier *et al*. 2006).

Some aspects of its biology are known (Castagnoli & Liguori 1986b; 1986c; 1994; Castagnoli *et al*. 1989), but information on diapause and the effect of temperature on its biological features is limited (Liguori & Guidi 1995; Castagnoli *et al*. 1996).

Early releases of our laboratory strains of *N. californicus* and *T. exhilaratus* in vineyards in Tuscany were unsuccessful. Supposing that low minimum temperatures at the start of spring were one of the causes, we decided to investigate the effects on these species of temperatures that often occur in temperate areas in spring and are slightly lower than the known phytoseiid developmental threshold (Castagnoli & Simoni 1991). Laboratory studies could help to explain some aspects related to the low efficiency in field of lab populations usually maintained at higher constant temperatures. Hatherly *et al.* (2005) suggested that LT_{50} at 5°C was indicative of the possibility of survival in the wild in the UK and suggested using this temperature for a first screening of establishment potential for non-native biological control agents in that country.

In this study, we evaluated the effects on egg hatchability and progeny survival of different storage periods at 5°C in eggs of *N. californicus* and *T. exhilaratus.* The egg was chosen since this stage never overwinters, is static and is most vulnerable. From a practical point of view, the possible delay in phytoseiid ontogeny could enhance the possibility of mass egg storage for commercial and research purposes.

Materials and methods

Origin and mass-rearing of the mites

Both *N. californicus* and *T. exhilaratus* came from our laboratory, where they were mass-reared for 5 years on artificial arenas in climatic cabinets at 25±1°C, 75±5% RH and 16-hour photoperiod. Pollen of *Quercus ilex*, supplied twice a week, was used as food.

Experimental set-up

Females randomly selected from mass-rearing units were isolated on artificial arenas and allowed to lay eggs for 24 hours. Groups of 0-24-hour-old eggs were kept in a climatic cabinet at 5±1°C in both dry and humid conditions for 5, 10, 20, 30, 40, 50 days to assess egg hatchability. The experimental units consisted of small plastic discs (diameter 5 cm), floating on water in the case of the humid condition. After the established time at low temperature, the viable eggs were transferred to a climatic cabinet at 25±1°C, always on the plastic discs floating on water. The development of progeny was followed until adulthood, with *Quercus ilex* pollen as food. Egg shrivelling, juvenile mortality, escape and sex ratio were recorded. Mating was allowed between males and females and the resulting eggs were again kept at 5±1°C for different times to assess the above-mentioned parameters. This procedure was repeated until the third generation.

Statistical analysis

A multivariate approach was used to evaluate if and how the species of phytoseiid, cold storage period, dry/humid condition, generation and phytoseiid stage affected the biological parameters of mortality, sex ratio and escape. The General Linear Model Multivariate Analysis Procedure was run in SPSS (1999). This procedure was performed after arcsine transformation of the square root of the proportions of eggs and immature motile stages (Snedecor & Cochran 1989). Within the significant factors, means were separated by *t*-test or one-way analysis of variance (ANOVA).

Logarithmic regression and probits analysis were used to calculate the time (in days) to 50% mortality of the population at this temperature for each phytoseiid species in the dry and humid conditions.

All statistics, post hoc comparisons and regressions were calculated with SPSS (1999).

Results

On the whole, the main general model adopted was highly significant (Wilks' λ = 0.000), with the exception of generation. The species of phytoseiid and the time of exposure to the low temperature were the factors that most affected the response. The species highly affected the mortality (*P*=0.000), sex ratio of progeny (*P*=0.042) and escape rate (*P*=0.000). The different periods of cold treatment affected mortality (*P*=0.004) and escape (*P*=0.000) but not the sex ratio (*P*=0.912) (Table 1 and 2).

Very high egg shrivelling (from 98.57 to 100%) was recorded in the humid condition and with cold storage periods longer than 10 days. Therefore, for subsequent analyses, only data from the dry condition and times of 5 and 10 days were considered (see Table I). When the effects on the progeny were considered independently of the stage, *N. californicus* showed lower mortality (*t*-test=2.08, df=265, *P*=0.038) and escape (*t*-test=2.02, df=96, *P*=0.046) than *T. exhilaratus*. In both species, the mortality rate increased

Table 1. Effects of 5- and 10-day storage of 24-hour-old eggs at 5±1°C on mortality of three successive generations of *N. californicus* and *T. exhilaratus*. For phytoseiid species, storage period and generation, mortality percentages followed by the same letter are not significantly different (Tukey test, P=0.05).

		5 days at 5°C		10 days at 5°C	
phytoseiid	generation	eggs	juveniles	eggs	juveniles
N. californicus	I	15.67a	0.79A	41.50a	7.39A
		(n=300)	(n=253)	(n=347)	(n=203)
	II	2.36 b	4.47B	62.93b	0B
		(n=550)	(n=537)	(n=297)	(n=21
	III	2.97 b	1.42A	25.29a	8.42A
		(n=437	(n=424)	(n=257)	(n=190)
T. exhilaratus	I	30.82a	7.17A	59.54ab	9.02A
		(n=464)	(n=321)	(n=959)	(n=388)
	II	21.51ab	4.70A	67.51a	8.97A
		(n=465)	(n=362)	(n=437)	(n=145)
	III	18.60a	8.87A	56.56b	22.64B
		(n=457)	(n=372)	(n=122)	(n=53)

Table 2. Effects of 5- and 10-day storage of 24-hour-old eggs at 5±1°C on the sex ratio and escape of three successive generations of N. californicus and T. exhilaratus progeny. In the columns, values followed by the same letter are not significantly different (Tukey test, P=0.05); the asterisk shows the significance within the generation ($\chi 2$ test, P=0.05)

	5 days at 5°C		10 days at 5°C	
sex ratio				
	N. californicus	*T. exhilaratus*	*N. californicus*	*T. exhilaratus*
I gen	60.00a	59.92a	66.67a *	43.93a
	(230)	(257)	(78)	(239)
II gen	61.87a *	48.51b	50.00a	60.34b
	(459)	(235)	(12)	m(58)
III gen	62.18a	61.54a	54.74a	55.56a
	(386)	(260)	(137)	(18)
escape				
I gen	8.37a	13.76a	58.51a *	32.29a
	(253)	(321)	(203)	(388)
II gen	10.53a *	31.88b	42.86a	56.06b
	(537)	(362)	(21)	(145)
III gen	7.66a *	23.30b	22.16b *	56.10b
	(424)	(372)	(190)	(53)

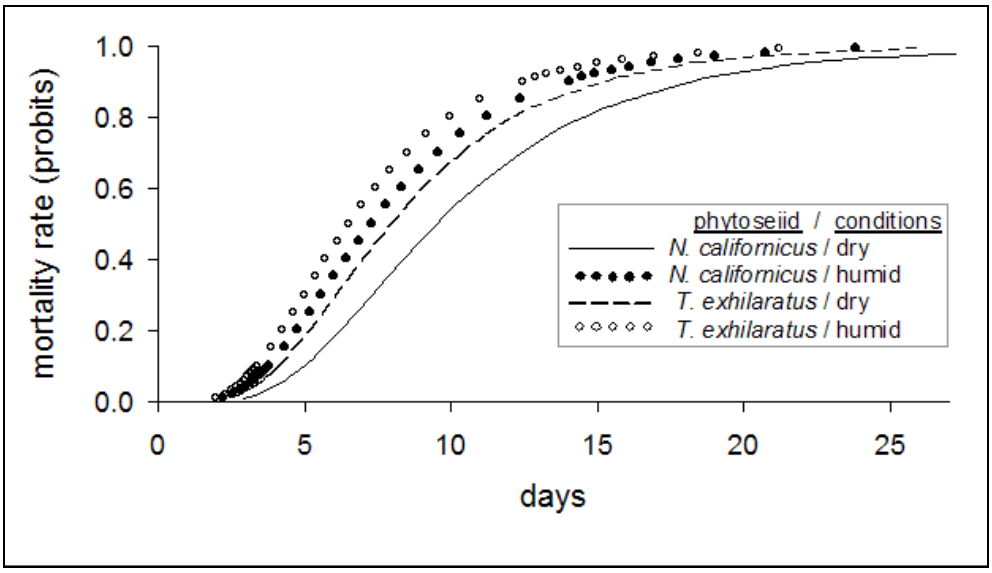

Figure 1. Plot of observed probits against exposure times for the eggs of *N. californicus* and *T. exhilaratus* in dry and humid conditions.

significative with the length of cold treatment. Egg mortality was always higher in *T. exhilaratus* and was highly affected by humidity (*P*=0.000) during storage. After 5 days of low temperature, *N. californicus* showed low egg mortality already at the 2nd generation, while the highest mortality was recorded at the 2nd generation after 10 days of treatment. In *T. exhilaratus,* the rate of egg shrivelling decreased with successive generations in the group treated for 5 days and remained almost constant in the one treated for 10 days. For both phytoseiids, juvenile mortality was much lower than egg mortality, and the highest value was at the 3rd generation after 10 days of treatment.

The increase of mortality with exposure time showed a logarithmic trend and the calculated LT_{50} was higher than 10 days for *N. californicus* eggs and slightly higher than 7 days for *T. exhilaratus* in dry conditions; in humid conditions, the value did not exceed 7 days for either phytoseiid (Figure 1). The analysis of probit regression (Pearson Goodness-of-Fit, Chi Square = 186.2, DF = 19, P = 0.000; Parallelism Test Chi Square = 99,2, DF = 3, P = 0.000) showed significance between the LT_{50} values calculated for the *N. californicus* eggs in dry conditions and for the *T. exhilaratus* eggs in humid conditions (Figure 1).

Discussion and conclusion

The first result of our experiment is that the phytoseiid eggs did not support long times at 5°C, since their LT_{50} ranged from about 6 to 10 days. The time greatly increased when eggs of both species were kept in the dry condition. In phytoseiids, egg hatching is usually favoured by high humidity when the temperature is high (Castagnoli & Simoni 1994; Liguori & Guidi, 1995, Dinh *et al.* 1988, Walzer *et al*. 2006). However, when the temperature is suddenly lowered from the standard value for mass-rearing (25°C, high RH), the free water surrounding the experimental unit probably increased water condensation on the eggs and enhanced the negative effect of low temperature. In previous studies, almost all the eggs hatched in the same strains of *N. californicus* (98%) and *T. exhilaratus* (96%) continuously reared at 25°C and high RH, the same conditions applied to the eggs after the 5°C exposure in the present experiment (Castagnoli & Simoni 1994; Liguori & Guidi 1995).

The second result is that *N. californicus* and *T. exhilaratus* reacted differently to cold storage. *N. californicus* seems to show lower sensitivity to low temperature. For the Italian strain of this species, the egg-adult development threshold was 8.99°C and the threshold was only slightly lower (8.31°C) for the egg (Castagnoli & Simoni 1991). These values are among the lowest calculated for phytoseiid species (Gotoh *et al*. 2005). The LT_{50} at 5°C for the females of a Californian strain was 38.6 days (Hatherly *et a*l. 2005), and the value increased to 3 months for females adapted to conditions near the diapause induction (Hart et al. 2002). Although in the present experiment the eggs seemed to be more sensitive than the females, our data confirm the relative cold hardiness of this species, which is distributed in temperate zones throughout the world (see Castagnoli & Simoni

2003). In contrast, there is no previous information on tolerance of low temperatures in *T. exhilaratus*. Its greater sensitivity to cold is in accordance with its distribution in the Mediterranean area, and in Italy the northernmost record is in Tuscan vineyards (about 43°N) (Castagnoli & Liguori 1986a). However, in both species there was a clear improvement in cold hardiness of the eggs in successive generations after the 5-day treatment. After the 10-day treatment, the second generation was the most sensitive, as has frequently been observed during adaptation to new conditions in *N. californicus* (Castagnoli & Simoni 2004).

On the whole, low-temperature storage of the eggs did not seem to have a strong effect on the mortality of *N. californicus* progeny, compared with the approximately 7% pre-adult mortality (egg plus immature) with the same strain and food at 25°C (Sabbatini Peverieri *et al*. 2006). The same was true for *T. exhilaratus,* whose pre-adult mortality is about 20% (Castagnoli & Liguori 1991).

In *N. californicus* raised on pollen at 25°C, the proportion of females ranged between 55 and 64% and increased with pollen adaptation (Castagnoli & Liguori, 1991; Sabbatini Peverieri *et al.* 2006); for *T. exhilaratus,* it was about 51% in a strain not adapted for a long time (Castagnoli & Liguori 1991). In our experiment, we did not find a sex difference in mortality of eggs and juveniles in *N. californicus*, but there were significant differences in *T. exhilaratus* (Table 2).

Surprisingly, there was an effect of cold storage on escape by the progeny. The escape rate is a typical factor of laboratory trials but is usually not considered since it can be strongly affected by the experimental method. However, using the same the method, we have found that this rate indicates difficulty in accepting new conditions (personal observations). In fact, the escape rate was always higher after the longest period of egg exposure for both species and tended to be lower with adaptation to cold only in *N. californicus*. In summary, both phytoseiids, but especially *N. californicus*, showed some potential for adaptation to cold exposure at 5°C.

Our results could explain the unsuccessful early releases of females not adapted to pre-diapausing conditions and then in active oviposition. Low temperatures could stop oviposition or, more likely, lower the survival of progeny. In addition, other unfavourable conditions (different microhabitat, presence of predators and antagonists) could have contributed to the lack of immediate establishment of these species in the vineyard.

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FUNCTIONAL RESPONSE OF PREDATORY MITE: *PHYTOSEIUS PLUMIFER* (CANESTRINI & FRANZAGO) ON DIFFERENT DENSITIES OF *AMPHITETRANYCHUS VIENNENSIS* (ZACHER) AND *TETRANYCHUS URTICAE* (KOCH) ON APPLE

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Abstract

Phytoseiulus plumifer (Acari:Phytoseiidae), is one of the most important natural enemies of spider mites in Iran. This investigation was employed to examine the effects of the prey species and their different densities on functional response of *P. plumifer*. A leaf disc bioassay conducted in laboratory condition (27±1ºC, 50±10% RH, 16:8 L:D photoperiod condition) on apple leaf disc as host plant. The larva of *Tetranychus urticae* and *Amphitetranychus viennensis* (Acari: Tetranychidae) were offered as prey The results indicated that the predatory mite exhibited type III and II functional response against the *T. urticae* and *A. viennensis* respectively. After determining the type of functional response parameters must be estimated. The attack coefficient (*a*) and the handling time (T_h) for *A. viennensis* were *0.0638 ± 0.0164* and *0.9841±0.1494*, respectively. For *T. urticae*, *b* was *0.0021±0.0003* and *0.9696±0.1185* respectively. Maximum predation on *T. urticae* and *A. viennensis* were estimated 24.75 and 24.39.

Key-words

Phytoseiulus plumifer, *Tetranychus urticae*, *Amphitetranychus viennensis*, Functional response, Apple

Introduction

Spider mites (Acari: Tetranychidae) are important pests for many agricultural crops (Helle & Sabelis 1985). Spider mites are the most common mites attacking woody plants and the two spotted spider mite is considered to be one of the most economically important spider mite. Continued feeding causes a stippled-bleached effect and later the leaves turn yellow, gray or bronze. Complete defoliation may occur if the mites are not controlled (Johnson & Lyon 1991). Two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the principal arthropod pests of eggplant (Bostanian *et al*. 2003). Hawthorn spider mite (*Amphitetranychus viennensis* (Zacher)) is an important pest in Europe, Asia and Japan especially on apple, stone fruit and woody ornamental plants. This specie causes yellow spots on the leaves by sucking, and also indirect damages, by laying eggs on the leaf surface and covering them individually with silky thread (Gotoh & Takayama 1992). Biological control of insect and mite pests is increasingly used in the ornamentals and hardly in nursery stock industry (Skirvin & Fenlon 2001). Insect predators are well known as effective regulators of insect pest populations (Debach & Rosen 1991). The predatory mite species *Phytoseius plumifer* (Canestrini & Franzago) (Acari: Phytoseiidae) is one of the most abundant natural enemy after *Transeius caspiansis* (Denmark & Daneshvar) of phytophagous pests and mites especially in north of Iran (Hajizadeh *et al*. 2002). These phytoseiids that mostly occur on fig trees

are generalist predators. Functional response curves refer to the change in the number of prey eaten per predator per time unit, as a function of prey density (Gitonga *et al*. 2002). Such curves uncover prey-predator interactions, allowing forecasting the suitability of a predator as a biocontrol agent (Wiedenmann & O'Neil 1991).

This paper presents the results of a set of experiments examining how functional response of *P. plumifer* feeding on larvae of *T. urticae* and *A. viennensis* was affected by by prey species.

Material and methods

Mite colonies

Phytoseius plumifer

T The predatory mite *P. plumifer was* collected on fig trees grown in the Botanic Garden, Agriculture Campus, University of Tehran. The predator was not exposed to pesticides previously. The collected individuals were placed in the rearing units which contained a detached fig leaf. The leaf was placed under side up on a water-soaked cotton pad in a cut-out Petri dish (8 cm diameter of cut-out circle) and the dish set into another bigger one (10 cm diameter) filled with water, to water supply the first one. A roll of water-soaked cotton was placed around the leaf to prevent mite escape. Rearing units were kept under laboratory conditions at 27±2°C, 50±5% RH and photoperiod of 16L: 8D. Juvenile stages (mostly larval stage) of the two-spotted spider mite, maize pollen and diluted solution of sugar were added to rearing units as daily food.

Tetranychus urticae

Two-spotted spider mites were collected from infested beans in the green house (mass rearing). Each rearing unit consisted of a detached bean leaf placed upside down on water-saturated cotton wool in a 8-cm diameter Petri dish. Rearing units were kept under laboratory conditions at 27±2°C, 50±5% RH and photoperiod of 16L: 8D.

Amphitetranychus viennensis

Apple (*Malus domestica* Borkh. Cv. Shafi Abadi) leaves infested by hawthorn spider mite were collected from old orchads in the Baraghan region of Karaj, Iran. Leaves alone on cotton were used for rearing. All experimental colonies were kept in a growth chamber maintained at 25±1 ºC, 70±10% RH and 16L: 8D photoperiod.

Functional response experiments

Seven densities (2, 4, 6, 8, 16, 25, 35 larva per adult predator) for *T. urticae* and two more densities (45, 50) for *A.viennensis* and 10 replicates of each density were set up. A three-day-old mated female predator was used for the experiments each testing unit consisted of a detached apple leaf, water saturated cotton wool in a 6 cm diameter Petri dish. Dishes were kept in the growth chamber in the laboratory condition mentioned before. After 24h predatory mites were removed and numbers of alive larvae were counted.

Statistical analysis

Data analysis for functional response includes two steps (Juliano. 2001). In the first step the shape of functional response must be determined by using logistic regression analysis of the proportion of prey killed in relation to the initial density (Trexler & Travis 1993).

In the logistic regression a cubic model (Equation. (1)) was incorporated:

$$\frac{N_a}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3 + P_4 N_0^4)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3 + P_4 N_0^4)} \quad (1)$$

Where N_a denotes the number of prey consumed, N_0 the initial prey density and P_0-P_4 the parameters to be estimated. A negative linear parameter P_1 indicates a type II functional response, while a positive linear parameter indicates density-dependent predation and thus a type III functional response (Juliano. 2001). After determining the type of functional response parameters [T_h, and either *a* (for type II) or *b*, *c* and *d* (for type III)] must be estimated. We used nonlinear least square regression (NLIN procedure in SAS) to estimate the parameters of the Holling's disc equation(1959) (2) and Roger's(1972) random predator equation (3). Holling's disc equation can be used only when Rogers' model does not enable the researcher to estimate valid parameters (Allahyari *et al*. 2004).

$$N_a = aTN_0 / 1 + aT_h N_0 \quad (2)$$

$$N_a = N_0\left(1 - \exp\left[a\left(T_h N_a - T\right)\right]\right) \quad (3)$$

Where *T* denotes the total time available (24 h) and T_h the handling time *a* the rate of successful attack and *b*, *c*, *d* are constant from the function that relate the attack coefficient (*a*) and N_0 in type III functional response

$$a = \frac{d + bN_0}{1 + cN_0} \quad (4)$$

Results and discussion

The functional responses of *P. plumifer* on the two prey species are different. Results of logistic regression indicated that the functional responses were type II and III against *A. viennensis* and *T. urticae,* respectively (Figure 1 and Table1).

Table 1. Results of logistic regression analysis of the proportion of killed preys by *P. plumifer* against two prey species.

Prey species	Coefficient	Estimate	SE	Chi-squared Value
T. urticae	Constant	-1.5773	0.628	6.31
	Linear	0.1946	0.1557	1.56
	Quadratic	-0.0071	0.0098	0.52
A. viennensis	Constant	2.0857	0.3716	31.51
	Linear	-0.1891	0.01	13.46
	Quadratic	0.00581	0.002	8.31

That the predatory mite *P. plumifer* showed two responses can get that the prey species (i.e. its quality and characteristics) play an important role.

The attack coefficient, *a,* (0.0638±0.0164) and the handling time T_h (0.9840±0.1494) were estimated from Roger's random predator equation when *A. viennensis* was as prey.

For *T. urticae*, Holling's disk equation using a nonlinear least square regression has been used in data analysis. Estimated *b* value and handling time (T_h) were 0.0021±0.0003 and 0.9696±0.1185, respectively (Table2).

Plant species had a significant effect on the functional response of phytoseiid mites essentially because of two possible reasons. Firstly, the prey could have sequestered chemical toxins produced by the host plant, thus making the prey less appetant for the predator. Secondly, the morphological structure of the plant might affect the searching behavior of the predator. It is well known that features such as leaf hairs and trichomes can hinder the searching of predators and parasites (Sabelis *et al*., 1999). In the present experiment, the leaf support was the same for the two prey conditions tested (apple). It thus seems that leaf plant would not the key factor that explain the differences of functional responses of *P. plumifer* on the two prey species.

Table 2. Parameters estimated for different data set: functional response of *P. plumifer* against two prey species.

			Asymptotic 95% CI	
Parameter	Estimate	Asymptotic SE	Lower	Upper
T. urticae				
b	0.0021	0.0003	0.00271	0.0014
T_h	0.9696	0.1185	1.2066	0.7325
A. viennensis				
a	0.0638	0.0164	0.0964	0.0312
T_h	0.9841	0.1494	1.2816	0.6866

b: constant must be estimated, T_h: handling time

Reis *et al*. (2003) investigated the potential predation success of *Iphiseiodes zuluagai* Denmark & Muma and *Euseius alatus* Deleon (Acari: Phytoseiidae) on *Brevipalpus phoenicis* (Geijskes)(Acari: Tenuipalpidae), vector of *citrus leprosies* and of the coffee ringspot viruses. In their work, the functional response to prey density by *I. zuluagai* was type II, where the number of killed prey grows with the prey density but begins to decrease in reaching maximum point.

Thus changing the amount of prey density was one of the most important and effective factor on predators behavior.

Santos (1975), showed that the response depends on prey stage. So, for eggs and males of *T. urticae* as food for the phytoseiid, *Neoseiulus fallacis* (Garman), the corresponding curve was type I and for females as food, it was type II. Gotoh *et al*.(2004), showed that the functional response data of *N. Californicus* (McGregor), fitted nicely to a type-II response (Holling 1966), when approximated by the disc equation.

The other researches have assessed the influence of different factors on the functional response of predatory mite of this family (Sabelis & Vand der Meer 1986, Zhang *et al*. 1999).

According to the results of this study we can get that the predator behavior changed with change of prey species, therefore, two spotted spider mite can be preferred to hawthorn spider mite as food. It is necessary to mention that no more researches have been done to determine functional response of *P. plumifer* at relative parameters.

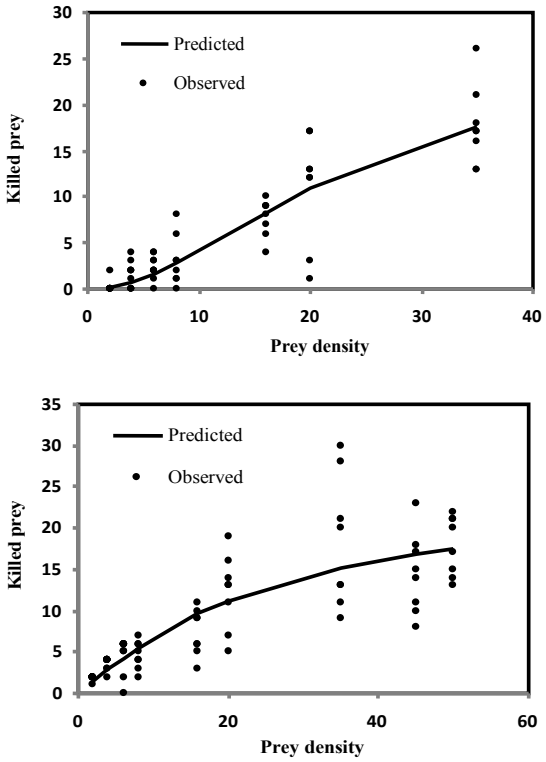

Figure 1. Functional response of *P. plumifer* to densities of *T. urticae* and *A. viennensis*

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TRANSPORT OF ORIBATID MITES TO THE POLAR AREAS BY BIRDS

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Abstract

Researches focused on the study of distribution of oribatid mites (Acariformes, Oribatei) in connection with birds' habitats and their migratory ways. Specimens of oribatid mites were collected in 2001-2007 in the Russian part of the Arctic (the Barents Sea coast of the Kola Peninsula and coastal islands, Novaya Zemlya Archipelago) and on the Spitsbergen. We investigated plumage of 241 birds, 32 species, and also nests, soils and vegetation cover from the places of concentration of birds. In nests and in plumage of birds of the Kola coast 47 and 59 species of oribatid mites were found accordingly, of the Spitsbergen – 6 and 17 species. Diversity and quantity of oribatid mites in plumage of birds were rather low. We added 22 taxons to the oribatid mite fauna list of the Spitsbergen. Distributions of vital forms of oribatid mites in soil and moss-lichen cover, nests, and plumage of birds were significantly different. Hydro-bionts of oribatid mites were absent in nests and plumage of birds. The birds, probably, to greater degree distribute the oribatid mites steadier to drying. Primitive oribatid mites were very useful for plumage of marine and waterfowl birds. Species *Minunthozetes semirufulus*, *Caleremaeus monilipes* collected on birds on the Kola coast, rare in the European part of Russia, but typical of Central and Western Europe, can be brought by birds from the places of wintering. Larvae, nymphs, and adults of oribatid mites were registered in bird plumage. Studying diversity of soil fauna of the arctic habitats it is necessary to focus researches on bird's colonies, resting sites etc. It will allow to understand the ways of invasion of soil mites to the remote and isolate islands and to get a more complete overview about oribatid mite fauna of the slightly investigated territories in the Arctic regions.

Key-words

Oribatid mites (Acari: Oribatei), bird plumage, Spitsbergen, polar terrestrial ecosystems

Introduction

Our knowledge about soil mites of the Russian high-latitude Arctic regions, including species diversity of oribatid mites (Acari: Oribatei) are still not complete enough, Studies of microarthropods in soils of this area began at the end of the XIXth century and actively proceeded in the first half of the XXth century (Kulczynski 1908 a, b, Trägårdh 1900 1904 1928, Thor 1930). The sampling on the Franz Josef Land, Spitsbergen, Novaya Zemlya, and Novosibirsk Islands Archipelagoes refer to that period. Publications on oribatid mites of the Spitsbergen (Karppinen 1967, Niedbała 1971, Solhöy 1976, Seniczak & Plichta 1978) appeared in the second half of the XXth century. European and American researchers discovered only 28 species of oribatid mites in total for 100 years of researches in the high-latitude Arctic regions (Danks 1981). Active researches in the high-latitude Arctic regions were renewed only 25 years ago. New data on oribatid mites of islands and archipelagoes of the Russian Arctic were received only relatively recently (Krivolutsky & Kalyakin1993, Krivolutsky & al. 2003, Lebedeva & al. 2006).

The studying of local fauna needs the understanding of main factors active species diversity. Researches on the drift of soil microarthropods onto the remote Arctic islands by wind and oceanic waters (Coulson & al. 2002 a, b, 2003) were undertaken. The human being is the powerful contemporary factor for dissemination, without limitation by geographical barrier. Our researches were based on the assumption that, right after the warming of climate and the retreat of a glacier about 12-10 thousand years ago, the birds began to visit the Arctic islands and have transported edaphic organisms (Lebedeva & Krivolutsky 2003). Fourty four of the 74 species of oribatid mites in the Russian Arctic were found out on birds (Krivolutsky & al. 2003). The main objective of the present research was to analyze the diversity oribatid mites in the Arctic, in substratum connected with birds, to find out new facts estimating the role played by birds in oribatid mites' diversity in Arctic Islands.

Materials and methods, study areas

Soil microarthropods were sampled in plumage of birds, nests and ornithogeneous soils from sites with concentration of individuals and colonial nesting of birds.

Researches were carried out in August 2000-2005 in the vicinity of a biological research station of Murmansk Marine Biological Institute (MMBI) (a settlement of Dalniye Zelentsy, coast of the Barents' Sea, coastal islands in the Zelenetskaya Fjord, Kola Peninsula, 69°N 36°E). We collected 24 soil-vegetative samples and 13 nests of five birds' species: Common eider *Somateria mollissima* (n=6), Short-eared owl *Asio flammeus* (n=1), Snowy owl *Nyctea scandica* (n=1), House sparrow *Passer domesticus* (n=2), Herring gull *Larus argentatus* (n=2), Great black-backed gull *Larus marinus* (n=2). For the collection of soil microarthropods we observed plumage of 186 individuals of 25 species: Bean goose *Ancer fabalis* (n=1), Common eider (n=11); Ringed plover *Charadrius hiaticula* (n=10); Oystercatcher *Haematopus ostralegus* (n=1); Green-shank *Tringa nebularia* (n=2); Red shank *T. totanus* (n=2); Ruff *Philomachus pugnax* (n=1); Little stint *Calidris minuta* (n=8); Dunlin *C. alpina* (n=18); Arctic skua *Stercorarius parasiticus* (n=1); Herring gull (n=7); Great black-backed gull (n=5); Common gull *Larus canus* (n=1); Black-legged kittiwake *Rissa tridactyla* (n=12); Black guillemot *Cepphus grylle* (n=4); Short-eared owl (n=1); Meadow pipit *Anthus pratensis* (n=7); Red-throated pipit *A. cervinus* (n=6); Pied Wagtail *Motacilla alba* (n=10); Wheatear *Oenanthe oenanthe* (n=2); House sparrow (n=1); Bluethroat *Luscinia svecica* (n=4); Reed bunting *Emberiza schoeniclus* (n=2), Redpoll *Acanthis flammea* (n=68), Arctic redpoll *A. hornemanni* (n=1), and Snow bunting *Plectrophenax nivalis* (n=1).

- in June - July 2004 on the Island of West Spitsbergen in the vicinity of settlements of Longyearbyen (78°12'N 15°36'E) and Barentsburg (78°07'N 14°25'E). Soil - vegetative samples (n=4) were taken in places of nesting and rest of birds (ornithogeneous soils), as well as a substratum from nests of the Arctic tern *Sterna paradisaea* (n=3) and Snow bunting (n=5). Microarthropods from the plumage of one adult of the Arctic tern and fledgling of the Snow bunting, accidental deaths, were investigated. We also used the data on microarthropods from the plumage of birds obtained by colleagues from MMBI for helminthes' studies in July-August 2001 on the Spitsbergen Archipelago in the vicinity of settlements of Barentsburg and Pyramid, Gren and Is fjords. Thus, the plumage of 48 individuals of 7 bird species was studied on the Spitsbergen in total: Fulmar *Fulmarus glacialis* (n=10), Little auk *Alle alle* (n=5), Purple sandpiper *Calidris maritima* (n=10), Glaucous gull *Larus hyperboreus* (n=6), Black-legged kittiwake (n=10), Arctic tern (n=6), and Snow bunting (n=1).

- in September 2002 on the Novaya Zemlya archipelago (72°90'N 53°23'E) several birds (3 of the Common eider, 2 of the Steller's eider *Polisticta stelleri*, and 2 of the Barnacle goose *Branta leucopsis*) were obtained by G.I. Ivanov specifically for our research.

Thus, we studies 241 individuals of 32 species of birds in total in the Arctic.

Two different methods for gathering oribatid mites from plumage were applied: bird skins with feathers from the killed birds. Alive birds were sampled with the help of a vacuum cleaner. Microarthropods were extracted from bird skin with feathers or special filters with Berlese funnels under electric lamps. This method of microarthropods' extraction was offered by D. Krivolutsky (Krivolutsky & Lebedeva 1999). A skin with feathers of a small bird, entirely (or the whole body of a bird with feathers) or part of a skin with feathers of a large specimen, was placed on funnels with plumage downwards and left for 2-3 and more days under the influence of electric light. Part of skins with feathers was preliminary stored in a refrigerator. However, even after a long storage we extracted oribatid mites and other representatives of soil micro-fauna that were alive from the skins. It might be explained by high

adaptation abilities of some soil microarthropods species to the polar conditions (Block 1990, Coulson & Birkemoe 2000). Soil microarthropods were identified by D. Krivolutsky or under his supervision.

Results and discussion

The analysis of structure of non-parasitic microarthropods found in plumage of birds from Murmansk coast of the Barents' Sea, West Spitsbergen and Novaya Zemlya was shown that it is possible to find out in feathers not only oribatid mites (about 60 %), but also other soil inhabitants: prostigmatid, gamasid and astigmatic mites, and collembolans (Fig.1).

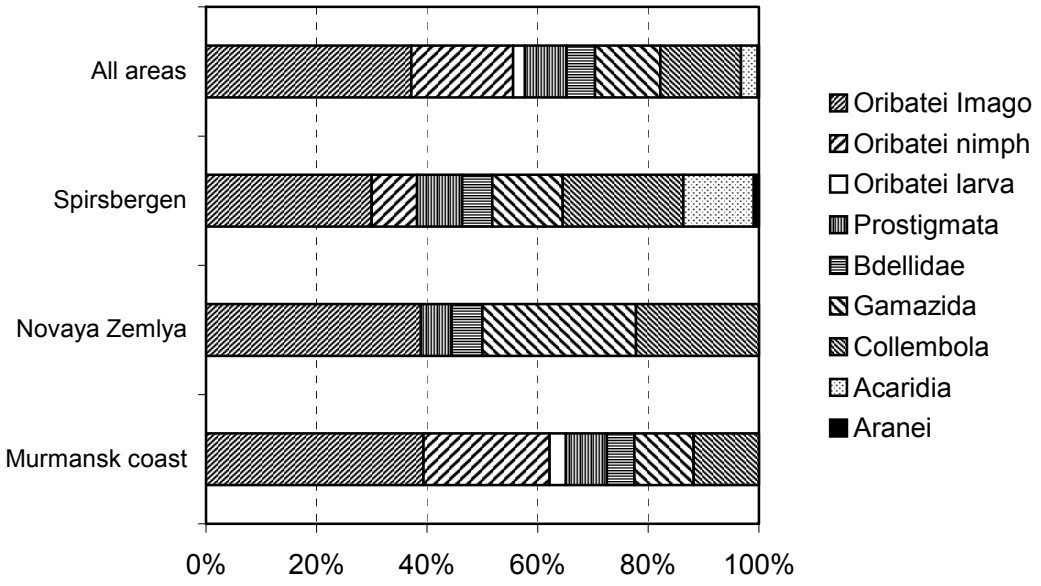

Figure 1. Distribution of soil microarthropods in the feathers of birds from the Barents Sea region.

Collembolans were the second largest group of soil microarthropods registered in the plumage of birds, non-parasitic gamasid mites were also rather numerous (14% and 11% accordingly). We recorded spiders, small insects, and their larvae.

Abundance distribution of the oribatid mites found on one bird, is asymmetric and corresponds to Poisson distribution rarely (Fig.2). Soil microarthropods, including oribatid mites, were absent on third of surveyed birds. But more often 1-2 oribatid mites were registered on one bird. The maximum number (21 specimens) was registered on one of the Common eider nestlings, from the Zelenetskaya Fjord.

Analysis showed that abundance of non-parasitic microarthropods and oribatid mites increases in a sequence of passerines – shorebirds – seabirds – ducks (microarthropods: Kruskal-Wallis test: H (3, N = 221) =49,07 P <0,0001; oribatid mites: H (3, N = 221) =50,92 P <0,0001). That might be correlated to the increasing body size of birds in this sequence (Fig.3).

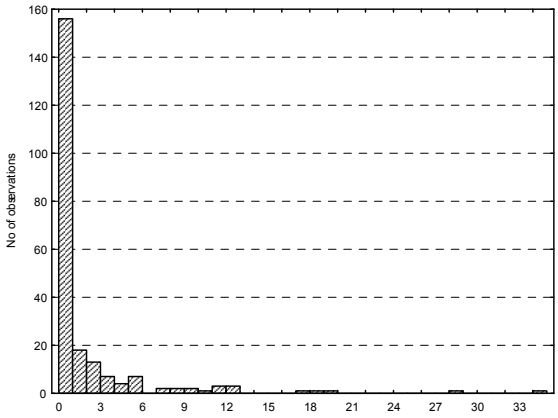

Figure 2. Number distribution of oribatid mites per one bird in the Arctic (Murmansk coast of the Barents Sea, Spitsbergen and Novaya Zemlya).

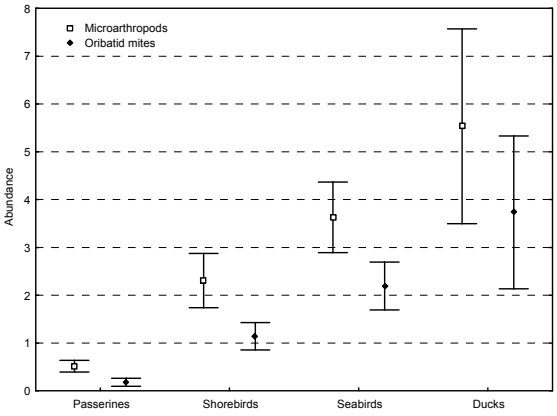

Figure 3. Average (points) and standard errors (whiskers) of microarthropods and oribatid mites' abundance per one bird for different groups of birds.

Notwithstanding, in previous studies on Anseriformes, Lebedeva (2005) showed that the average amount of species and individuals/specimens of oribatid mites in plumage increases in a sequence "swans – geese – ducks".

Oribatid mites were found of the arctic birds at all the stages of life cycle of arctic birds. The ratio of the stages of oribatid mites from the Murmansk coast of the Barents Sea and Spitsbergen did not differ significantly. It is necessary to note that the oribatid larvae in feathers of Spitsbergen birds were not registered. The ratio of oribatid mites at different stages of life cycle (adults, nymphs, larvae) on the birds from the Murmansk coast of the Barents Sea was 60%: 35%: 5% respectively, and nymphs in Spitsbergen samples composed 21%. Only adult oribatid mites were collected from birds at Novaya Zemlya. It might be due to the small amount of samples birds.

Twenty one species birds breeding in the Arctic latitudes were found with oribatid mites (Fig.4).

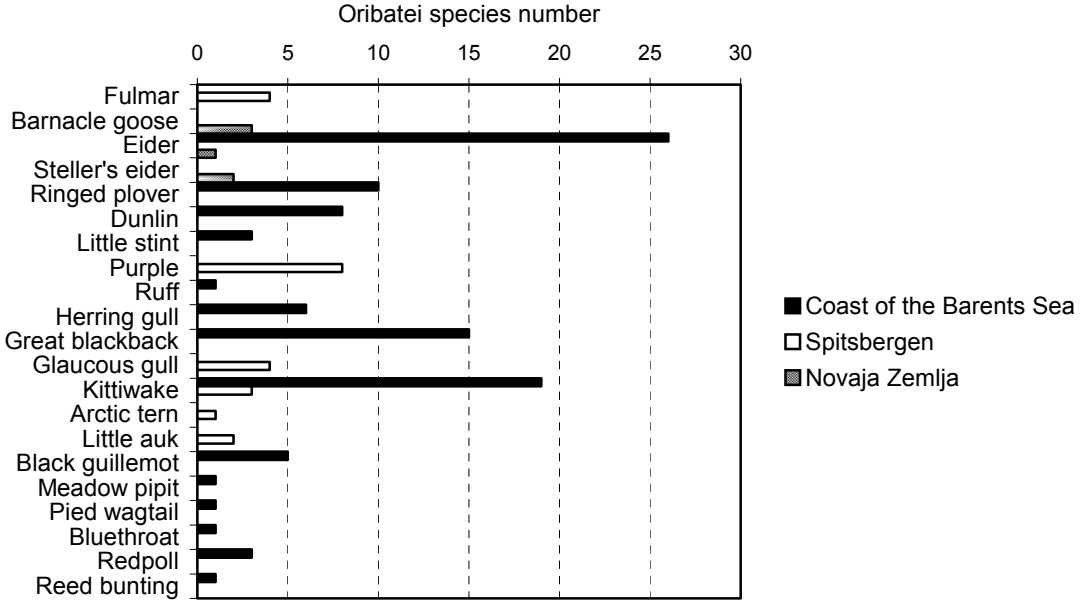

Figure 4. Number of oribatid mite species found in the plumage of different species of the Arctic birds.

Only one species was registered in the plumage of the Steller's eider, Ruff, Arctic tern, Bluethroat, Pied Wagtail, Red-throated pipit, and the Snow bunting. The highest diversity was observed in Black-legged kittiwakes (19 species) and Common eiders (26 species). From 2 up to 15 species of oribatid mites were recorded in the plumage of gulls (Glaucous gull, Great black-backed gull, Herring gull), fulmars, and shorebirds (Purple sandpiper, Dunlin, Ringed plover, Little stint). The unevenness of distribution of species abundance in birds might be partly explained by the sample sizes of the studied birds (fig.5) and partly by the sizes of birds (see below). However, it is possible that preferences of soil microarthropods for humidified habitats (Krivolutsky 1995) explain that some birds where preferred (waterfowl, marine, and shorebirds) notably in the plumage of, birds from coastal and marine habitats (Anseriformes, gulls, and shorebirds).

Carrying out researches on the continental coast of the Barents Sea we registered 53 species of

oribatid mites in the plumage of birds, only 17 species were recorded on the Spitsbergen birds (Lebedeva & Lebedev 2005, Lebedeva & al. 2006). It means that these species of oribatid mites could get onto the archipelago with the near (fulmars, gulls, skuas, auks, eiders) and distant migrants (Snow bunting, Arctic tern). It is obvious that the birds, capable to reach the remote archipelagos can be the basic "suppliers" of soil mites. Migratory ways of some species of birds also pass through Greenland; whence birds could bring soil microarthropods to the Spitsbergen and Novaya Zemlya too.

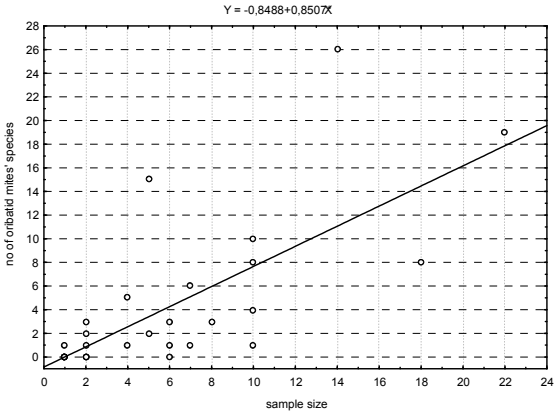

Figure 5. Dependence of number of found species of oribatid mites from sampling value of different species of birds.

Studying oribatid mites from the Spitsbergen archipelago, we focused our attention not only on researching the plumage of birds, but also on studying microarthropods composition in nests and soil in the places of concentration of birds and their breeding. The places enriched with the organic substances most of all (so-called ornithogenius soils) are formed were of the greatest interest.

We registered 35 species of oribatid mites in Spitsbergen samples: in soils 18 species, 16 species from plumage of birds, and in nests only 6 species. The larger fauna of oribatid mites recorded in the plumage of birds may be explained by our purposeful search for soil mites on birds to check a hypothesis about their transportation by birds (see Appendix).

It is interesting to note that concentration of our efforts in the direction of research on the substrata connected with birds allowed to fill up significantly the list of oribatid mites' species of this archipelago. Twenty two new taxa for the Spitsbergen are described, and the largest amount of all new registrations was on birds and from ornithogeneous soils (Lebedeva & al. 2006). These species are included into the list of species of terrestrial and fresh-water invertebrates of Spitsbergen (Coulson 2007). Oribatid mites collected on birds on the Novaya Zemlya: *Suctobelba* sp., *Suctobelbella hammeri*, *Discoppia splendens*, *Moritzoppia unicarinata*, *Quadroppia quadricarinata,* were registered earlier in the samples from the Novaya Zemlya (Karppinen & Krivolutsky 1982, Krivolutsky & Kalyakin 1993).

Distributions of oribatid mites in soil and moss-lichen cover, nests, and plumage of birds were significantly different. Hydrobiont oribatid mites were absent from nests and plumage of birds. The birds, probably, to greater degree distribute the oribatid mites steadier to drying. Primitive oribatid mites were very useful for plumage of marine and waterfowl birds. Species *Minunthozetes semirufulus*, *Caleremaeus monilipes,* rare in the European part of Russia, but typical of Central and Western Europe, can be brought on the Murmansk coast by birds from the wintering places.

Many oribatid species are bacterium and fungi consumers; a fact that lets assume that they can find food resources in the birds' plumage. In the last years, the community of microorganisms in the feathers, bacteria in particular, was studied in details (Bisson & al. 2007).

Oribatid mites during their long stay in the plumage can feed, because it was proved, (at least for species with all stages of their life cycle being recorded on the hosting bird.

In plumage of some Antarctic birds, non-parasitic invertebrates and even on Imperial penguins were found (Krivolutsky & al. 2004). Soil species are also common of the penguins' colonies in the Antarctic area, including the South-polar skua, which plumage was also investigated. This species has a rather large habitat and is capable to migrate far North during the Antarctic winter.

Role played by colonial birds in soil formation, on vegetation cover, structure and shape of vegetative associations, and soil micro-fauna have been studied on the Spitsbergen (Chajkowska 1992, etc.). However, a young volcanic island, formed 30 km off Iceland (Fridriksson 1992) gave an example of what is the bird contribution to the formation of vegetative communities. Only the birds make constant flights from the mainland to islands and reverse flight during seasonal migrations. Birds could be considered and treated as the major alive transport for soil fauna.

The birds' colonies and shifts create soil biota

diversity in the high-latitude Arctic areas. Birds' guano, accumulating in bird's shifts, is favourable substratum for microarthropod naturalization of Artic birds, and they play a active role by transportation of soil organisms (oribatid mites, and non-parasitic edaphic fauna: Mesostigmata (Gamasidae) and Prostigmata (Bdellidae...), Insects (Collembolans) and spiders. However, the question of the way of penetration into the plumage remains? When birds are foraging on the ground or in a plant cover, build nests, tread and form nest holes with the help of the body, during clutching and incubation, warming of offspring in nests, closely contacting either the nest material or the ground, "bathing" in dust?

One of the major research goals not yet completely achieved is still the studying of interrelations of soil microarthropods habitats and migratory ways/routes of birds. It will allow to understand the ways of invasion of soil inhabitants onto the remote islands and to receive better knowledge about geographical tendencies of formation of local fauna of soil invertebrates. We have already managed to show the transport of some species of oribatid mites by birds from the moderate to the high latitudes and vice versa. However these researches demand continuation, as well as many aspects of life of soil mites in the plumage of birds.

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Appendix

List of oribatid mite species founded in different substrates, including bird plumage (B), nests (N) and ornithogenius soils (S) from the Barent's Sea area.

MC – Murmansk coast and island in Zelenetskaya fjord, SP – Spitsbergen, NZ – Novaya Zemlya.

Bird species: 1 – Fulmar, 2 – Barnacle goose, 3 – Common eider, 4 – Steller's eider, 5 – Ringed plower, 6 – Red shank, 7 – Ruff, 8 – Little stint, 9 – Dunlin, 10 – Purple sandpiper, 11 – Herring gull, 12 – Glaucous gull, 13 – Great black-backed gull, 14 – Black-legged kittiwake, 15 – Arctic tern, 16 – Black guillemot, 17 – Little auk, 18 – Snowy owl, 19 – Meadow pipit, 20 – Pied Wagtail, 21 – Bluethroat, 22 – House sparrow, 23 – Reed bunting, 24 – Redpoll, 25 – Snow bunting

Eniochthonius minutissimus (Berlese, 1903) – MC: N(3)

Hypochthonius rufulus C.L.Koch, 1835 – MC: B(9)

Brachychthonius sp. Berlese, 1910 – MC: S

E. borealis Forsslund, 1942 – MC: S

Liochthonius sellnicki (Thor, 1930) – MC: S

Phthiracarus sp. Perty, 1841 – MC: B(14), N(11, 18), S

Ph. borealis (Trägåtdh, 1910) – MC:B(14)

Ph. ligneus Willmann, 1931 – MC:B(13)

Steganacarus striculus C.L.Koch, 1836 – MC: N(18), SP: B(17)

Tropacarus carinatus (C.L.Koch, 1844) – MC: B(3, 5)

Oribotritia loricata Rathke, 1799 – MC: N(18)

Nothrus borussicus Seilnick, 1928 – MC: N(3), S

N. palustris C.L.Koch, 1839 – MC: B(14), SP: B(10)

N. silvestris Nicolet, 1855 – MC: N(3)

Camisia sp. von Heyden, 1826 – MC: B(23), N(18)

C. biurus (C.L.Koch, 1839) – MC: B(11)

C. borealis (Thorell, 1872) – SP: N(25), S

C. segnis (Hermann, 1804) – MC: S

C. spinifer (C.L.Koch, 1835) – MC: B(14)

Platynothrus peltifer (C.L.Koch, 1939) – MC: B(14)

Platynothrus punctatus (C.L.Koch, 1979) – MC: N(13), S, SP: B(1, 10, 12, 17)

Trhypochthonius tectorum (Berlese, 1896) – MC: N(3), S

Malaconothrus egregius Berlese, 1904 – MC: B(3), SP: B(10)

Trimalaconothrus tardus (Michael, 1888) – MC: B(3)

Nanhermannia coronata Berlese, 1913 – MC: B(3), N(3)

N. nana (Nicolet, 1855) – MC:B(3)

Hermannia reticulata Thorell, 1871 – MC: B(9, 13), N(13, 18), S, SP: N(25)

H. scabra (C.L.Koch, 1839) – MC: N(3)

Belba sp. Heyden, 1826 – MC: B(5, 14), SP: N(25)

B. corynopus (Hermann, 1804) – MC: B(3)

Damaeus onustus C.L.Koch, 1841 – SP:N(25)

Epidamaeus kamaensis (Sellnick, 1925) – MC: S

Eremaeus sp. C.L.Koch, 1836 – MC: B(16)

E. foveolatus (Hammer, 1952) – MC: B(14)

E. oblongus (C.L.Koch, 1835) – MC: B(16)

Gustavia microcephala (Nicolet, 1855) – MC: N(11)

Adoristes poppei (Oudemans, 1906) – MC: N(22)

Ceratoppia bipilis (Hermann, 1804) – MC: B(5, 11)

C. sphaerica (C.L.Koch, 1879) – MC: S, SP: S

Carabodes areolatus Berlese, 1916 – MC: B(3, 9, 16), N(3), S

C. forsslundi Sellnick, 1953 – MC: B(3), N(3, 18)

C. labyrinthicus (Michael, 1879) – MC: B(11, 13), N(11, 3), S

C. marginatus (Michael, 1884) – SP: N(25)

C. subarcticus Trägåtdh, 1902 – MC: B(16)

Tectocepheus knullei Vanek, 1960 – SP: B(12), N(25)

T. velatus (Michael, 1880) – MC:B(3, 8, 9, 11, 13, 14), N(3, 11, 18), S, SP: B(15), S

Autogneta longilamellata (Michael, 1885) – MC: MC: N(11)

Conchogneta delacarlica (Forsslund, 1947) – MC:

B(3, 8, 11), N(3)

Caleremaeus monilipes (Michael, 1882) – MC: B(14), N(3)

Discoppia splendens (C.L.Koch, 1840) – MC: B(3, 13, 14), S, SP: B(14) NZ: B(2)

Dissorhina ornata (Oudemans, 1900) – MC: N(13)

Lauroppia neerlandica (Oudemans, 1900) – MC: B(5)

Medioppia falax (Paoli, 1908) – MC: B(3)

Microppia minus (Paoli, 1908) – MC: B(19)

Moritzoppia unicarinata (Paoli, 1908) – MC: B(5) SP:B(10), S, NZ: B(2)

Oppia sp. C.L.Koch, 1836 – SP: B(10, 12)

O. bicarinata (Paoli, 1908) – MC: N(3, 22)

O. translamellata (Willmann, 1923) – MC: B(14), N(3), SP: B(1)

Oppiella nova (Oudemans, 1902) – MC: B(3, 5, 8, 11, 14, 13, 21), N(18) SP: B(1, 10, 14)

Quadroppia quadricarinata (Michael, 1885) – MC: B(3), S, NZ: B(2, 4)

Suctobelba sp. Paoli, 1908 – MC: B(3, 5), N(3); NZ: B(3)

S. trigona (Michael, 1888) – MC: B(7,1 4)

Suctobelbella acutidens (Forsslund, 1941) – MC: B(5)

S. hammeri (Krivolutsky, 1966) – MC: B(3, 9,1 4), N(3), S, SP: N(25), NZ: B(4)

S. subcornigera (Forsslund, 1941) – MC: N(3), SP: B(1)

Suctobelbella sp. Jacot, 1937 – MC:B(3,13) SP:B(10)

Banksinoma lanceolata (Michael, 1885) – MC: S

B. setosa Rjabinin, 1974 – MC: B(3)

Oribella castanea (Hermann, 1804) – MC: N(3), S

Limnozetes sp. Hull, 1916 – MC: B(24)

Ameronothrus lineatus (Thorell, 1871) – MC: N(3)

Micreremus brevipes (Michael, 1888) – MC: B(3)

Licneremaeus licnophorus (Michael, 1882) – MC: N(22)

Scutovertex minutus (C.L.Koch, 1836) – MC: B(3, 13), N(3)

Oribatula tibialis (Nicolet, 1855) – MC: N(3, 11), S

Zygoribatula exilis (Nicolet, 1855) – MC: B(9,1 3, 14, 16), N(18)

Liebstadia similis (Michael, 1888) – MC: B(13, 14, 24), N(3, 11, 18),S, SP:B(10)

Scheloribates laevigatus (C.L.Koch, 1835) – MC: B(13, 14), N(8), S

Sch. latipes (C.L.Koch, 1844) – MC: N(18)

Neoribates roubali (Berlese, 1910) – MC: B(3)

Ceratozetes gracilis (Michael, 1884) – MC: S

C. sp. Berlese, 1908 – MC: N(13)

Diapterobates notatus (Thorell, 1871) – MC: N(3), S, SP: N(25), S

Edwardzetes edwardzii (Nicolet, 1855) – MC: S

Fuscozetes fuscipes (C.L.Koch, 1844) – MC: B(3), N(3)

Fuscozetes sellnicki Hammer, 1952 – MC: S

Svalbardia paludicola Thor, 1930 – MC: S

Chamobates cuspidatus (Michael, 1884) – MC: B(3)

Chamobates cuspidatiformes (Trägärdth, 1906) – MC: S

Chamobates lapidarius (Lucas, 1849) – MC: N(11, 3)

Chamobates schuetzi (Oudemans, 1902) – MC:B(9)

Minunthozetes pseudofusiger (Schweizer, 1922) – MC:B(5,24), N(3)

Minunthozetes semirufus (C.L.Koch, 1835) – MC:B(14),3 (N)

Mycobates parmeliae (Michael, 1884) – SP: B(15)

Punctoribates punctum (C.L.Koch, 1839) – MC: B(13), S, SP: B(12)

Eupelops torulosus (C.L.Koch, 1836) – MC:N(3)

Eupelops plicatus – MC: N(3)

Eupelops subuliger (Berlese, 1917) – MC: S

Parachipteria punctata (Nicolet, 1855) – MC: B(3, 5, 9, 13, 14, 20), N(11, 18) SP: B(14)

Galumna rossica Sellnick, 1926 – MC: S

Galumna sp. Heyden, 1928 – MC: B(3)

Pergalumna nervosa (Berlese, 1914) – MC: B(3), N(3), S

Pilogalumna tenuiclava (Berlese, 1908) – MC:B(13), N(3,13)

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THE INTERACTION OF COMMON BEECH, EUROPEAN LARCH, NORWAY SPRUCE AND SESSILE OAK SILVICULTURED IN A COMMON-GARDEN EXPERIMENT WITH FOUR MITE GENERA OF MESOSTIGMATA

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Abstract

The study were conducted in a common-garden experiment of 9 temperate tree species containing 36-year old monoculture stands on fresh mixed forest site. Every tree species was replicated tree times. On the experimental plots (400 m^2 each), the Mesostigmatid mites=were investigated. Samples were collected three times: twice during spring (2004 and 2005) and in autumn (2004). Altogether 1612 specimens of mites were collected, respectively *Paragamasus* 688 (42, 7%), *Zercon* 650 (40.1%), *Veigaia* 206 (12.9%) and *Trachytes* 68 specimens (4.3%). The average abundances of mites per m^2 are for *Paragamasus* 970, *Zercon* 913, *Veigaia* 294 and *Trachytes* 99 specimens. The average number of analyzed mites per m^2 on explored tree plots was for beech 1940, larch 2131, spruce 2249 and oak 2783 specimens.
The interactions between soil characters and average abundance of all mites of the four genera of Mesostigmata mites have usually no statistical significance on the experimental plots. Average abundance of mites from *Paragamasus* genus is significantly growing with increasing soil characters: Cl, Cu, humus, Hh and with decreasing soil characters: Ca and pH. It shows stronger connection mites of this genus with coniferous trees (larch and spruce) than with broadleaves tree species (oak and beech). Average abundances of mites from *Trachytes* and *Veigaia* genera show reverse than *Pergamasus* correlation with Cl, Cu, humus, Hh and Ca and pH.. It shows stronger connection mites of these genera with broadleaves trees (oak and beech) than with conifers (larch and spruce). Average abundance of mites from *Zercon* genus is correlated significatively with Zn, but correlations are low with other soil characters. In soil, identification of Mesostigmatid mites to genus and species is important, because genera or species can show opposite preferences for the same edaphic factors.

Key words

Acari, forest ecosystem, biodiversity, soil ecology

Introduction

an experiment on the growth in selected more important species of forest trees was established in the Forest Experimental station of Siemianice (51°14.87' N, 18°06.35' E, Poland) by scientists of the Department of Silviculture of the University of Life Sciences in Poznań in 1970 (Szymański 1982). After almost thirty years in the plots under monospecific tree cover different types of forest undergrowth were formed. Such prepared surfaces were used in the initial studies on the Mesostigmatid faunas (Skorupski *et al*. 2003) and on testing preferences for coniferous and broadleaves tree stands of Parasitidae and Uropodina (Skorupski 2007). Since 2002 several

papers describing different elements of the experiment were published (Celka & Kasprowicz 2002, Mroziński 2003, Reich *et al*. 2005, Withington et al. 2003). Results encouraged conducting more advanced surveys on these experimental plots.

Methods

The study were carried on in a common-garden experiment, 9 temperate tree species containing 36-years old monoculture stands on fresh mixed forest site. Every tree species was replicated tree times. On the experimental plots (400 m^2 each) Mesostigmatid mites were investigated. Samples were collected three times: twice during spring (2004 and 2005) and in autumn (2004). Five soil samples were collected with a probe (40 cm^2) from an each plot. These samples (litter, humus and mineral soil) were taken to the depth of 5 cm in mineral soil. The analysis of correlated distribution of four mite genera of Mesostigmata (*Paragamasus*, *Trachytes*, *Veigaia* and *Zercon* with of four tree species (common beech *Fagus sylvatica*, European larch *Larix decidua*, Norway spruce *Picea abies* and Sessile oak *Quercus robur*)Was analyzed. These mites constitute about 80% of collected Mesostigmata mites). Altogether 180 samples were collected and finally 177 were analyzed. Mites were extracted using the Tullgren Berlese apparatus. The analysis of chemicals in the experimental plots (macroelements – Ca, Cl, microelements – Cu, Zn, humus, pH, hydrolytic acidity) and soil adsorptive capacity were already published in Mroziński 2003 (table 1).

Table 1. Soil conditions in experimental plots
– Ca, Cl (mg/100 g soil),
microelements – Cu, Zn, (ppm in dry mass of soil),
pH in H_2O, humus (%), hydrolytic acidity (Hh) (me/100 g soil),
soil absorptive capacity (T) (cmol(+)/kg) for each experimental plot.

plot	tree	Ca	Cl	Zn	Cu	pH	humus	Hh	T
5	beech	110,27	1,54	27,6	2,76	5,30	40,488	17,65	35,35
13	beech	83,27	2,42	31,6	2,99	5,14	53,166	22,85	91,25
22	beech	119,96	4,75	18,1	1,73	5,75	47,038	21,65	89,20
2	spruce	48,08	2,37	28,8	5,13	3,89	51,285	40,35	82,25
12	spruce	69,92	5,74	29,7	3,03	4,22	50,141	39,65	83,50
21	spruce	59,77	4,02	10,8	3,50	4,65	46,229	35,05	85,70
3	oak	108,83	4,87	22,9	3,13	4,72	41,352	23,95	72,60
11	oak	116,86	2,15	25,2	2,61	4,99	31,151	18,65	66,50
19	oak	71,51	3,14	39,3	4,29	5,00	52,154	31,35	86,75
9	larch	32,57	7,29	25,7	4,98	3,87	46,760	31,15	75,95
17	larch	24,91	4,80	21,6	3,06	4,24	60,676	31,05	88,60
25	larch	20,20	4,79	20,2	3,58	4,20	65,584	34,55	93,50

Results

A total number of 1,612 specimens of mites were collected, respectively *Paragamasus* 688 (42,7%), *Zercon* 650 (40,1%), *Veigaia* 206 (12,9%) and *Trachytes* 68 speciemens (4,3%) (figure 1). The average abundances per m^2 were for *Paragamasus* 970, *Zercon* 913, *Veigaia* 294 and *Trachytes* 99 specimens. The average density per m^2 were under beech cover 1,940, under larch cover 2,131, 2,249 under spruce and 2,783 specimens under oak cover (figure 2). Mean number of mites per m^2 was different considering the genera. For *Paragamasus* the lowest number was found on oak plots (678), and was increased under beech (814), spruce (1,025) and maximal under larch (1,365). *Trachytes* and *Veigaia* were low in density on larch plots (33 and 146) and with higher density on oak (67 and 194) and spruce plots (138 and 351). The highest abundance was found on beech plots (157 and 483). *Zercon* genus: the lowest density was found on beech plots (486), upper on larch (586) and spruce plots (735) and the highest was on oak plots (1,844) (figure 3).

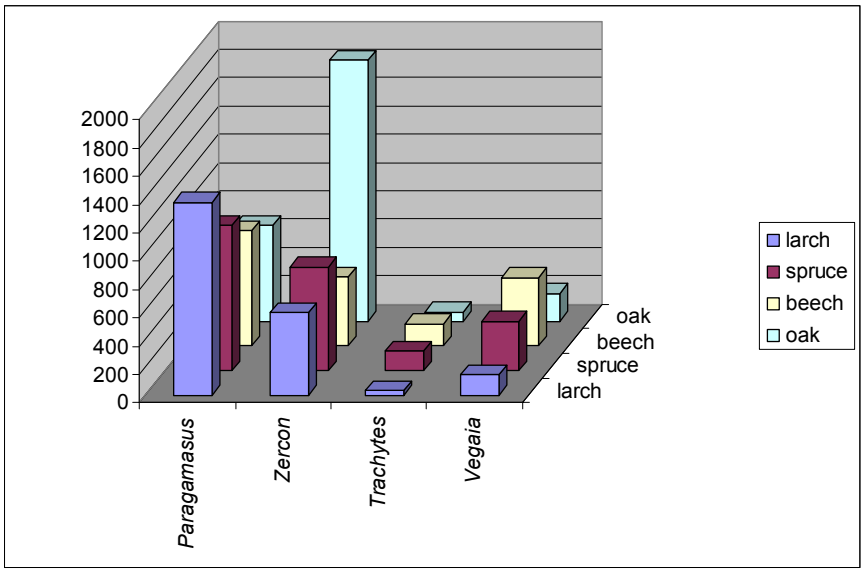

Figure 1. Average density of mite genera (per m^2) under tree covers.

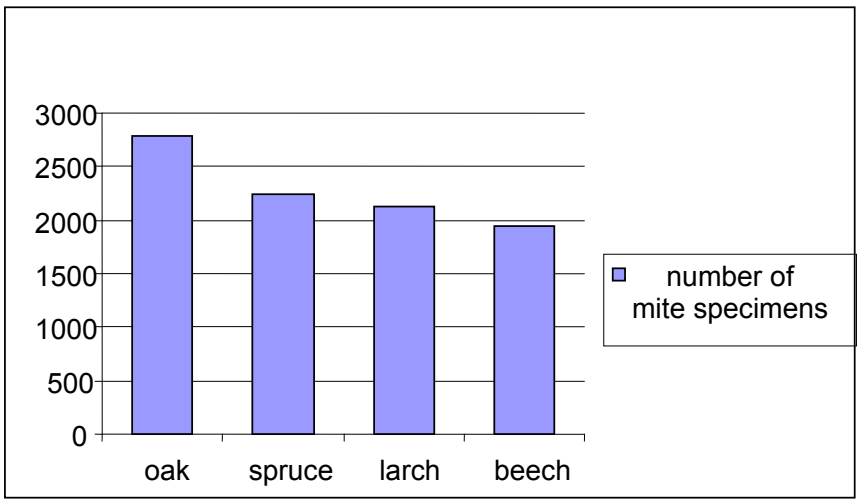

Figure 2. Mean density of four mite genera (nb/per $m^{2)}$ under four tree covers.

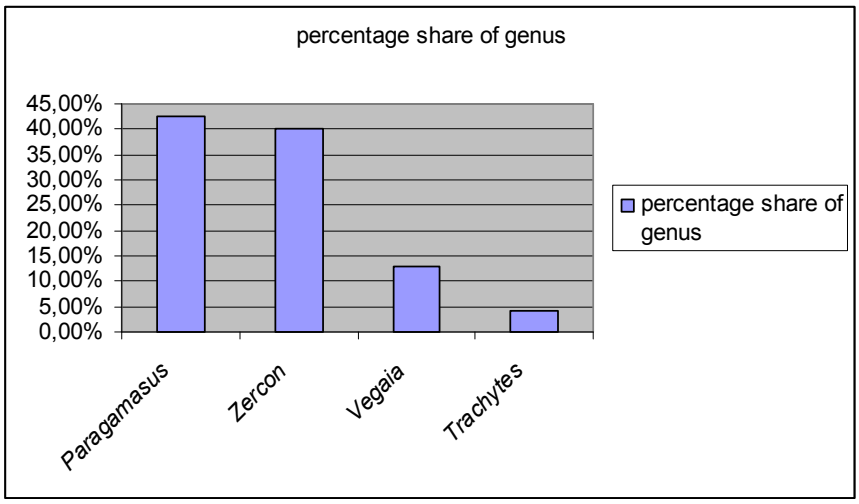

Figure 3. Relative number of the four genera (total number%).

Table 2. Average density of the four genera per m^2 in experimental plots.

plot	tree	Paragamasus	Trachytes	Veigaia	Zercon	Sum
5	beech	800	200	783	200	1983
13	beech	833	50	133	650	1667
22	beech	808	221	533	608	2171
2	spruce	1067	217	617	567	2467
12	spruce	1192	146	254	1254	2846
21	spruce	817	50	183	383	1433
3	oak	750	50	450	150	1400
11	oak	350	67	83	533	1033
19	oak	933	83	50	4850	5917
9	larch	1750	50	117	400	2317
17	larch	533	50	100	250	933
25	larch	1813	0	221	1108	3142

Average abundance of mites per m^2 on each experimental plot is presented table 2. The correlation between selected soil conditions and mean abundance of mite genera is given in table 3.Following results of soil characters and correlation with Mesostigmatid mites, mites preferences can be suspected to particular tree species. What is very interesting is that average abundances of Mesostigmata are not strongly correlated with tested edaphic .

When we compare the ecological preferences of the genus (table 3), there is a negative correlation ($r = -0,61$, $P<0.05$) of *Paragamasus* density with and Ca concentration. *Trachytes* and *Veigaia* showed a reverse reaction,whereas *Zercon* is not affected by Ca concentration. Similar situation is shown with soil acidity: *Paragamasus* abundance is correlated with pH ($r = -0,55$, $P<0.1$) but the three other genera had reverse preferences. These parameters (Ca, pH) were found growing with the following directions of tree species: larch (min.), spruce, oak and beech (max.). Negative correlation was found between *Paragamasus* and amount of humus in soil ($r = 0.50$, $P<0.1$), hydrolytic acidity ($r = 0.51$, $P<0.1$) and Cu concentration ($r = 0.54$, $P<0.1$). *Zercon* has similar preferences but lower correlations. *Trachytes* and *Veigaia* have lower negative correlations everse than *Paragamasus*. These chemicals (Cu and Hh) are growing with the following directions of tree species: beech, oak, larch and spruce and for amount of humus: oak(min), beech, spruce and larch (max).

With Cl., *Paragamasus* has positive and significant correlation ($r = 0.56$, $P<0.1$). The three other genera have negative and low correlations. Cl concentration is growing as the soil absorptive capacity with following tree species directions: beech, oak, spruce and larch.

Correlation for soil absorptive capacity is found with *Veigaia* ($r = 0.55$, $P<0.1$), lower for *Trachytes. Paragamasus* and *Zercon* showed lower but negative correlations.

Significant correlation ($r = 0.63$, $P<0.05$) was solely found with *Zercon* for Zn.

In all combinations we can observe significant correlations with characters which are growing or decreasing from broadleaves tree species (beech and oak) to coniferous tree species (larch and spruce). The reaction of abundance of mites from family *Parasitidae* (growing in plots with coniferous tree species) and *Uropodina* suborder (decreasing in plots with coniferous tree species) was previously presented on the area in research conducting in 1998-1999 (Skorupski 2007). Six years later the tendency still exists (*Paragamasus* is the most abundant genus in Parasitidae family and *Trachytes* is the most abundant among Uropodina in specimens collected from the plots). Nowodays, information on soil parameters modified by each tree species can be connected to the genera of mesostigmatid mites.

The problem of interaction of soil characters and Mesostigmata mites was explored many times, but every time in the context of pollution (Kaczmarek 1998, 2000, Koehler 1990, 1996, Salminen & Haimi 1998, Seniczak *et al*. 1998, 1999). The interactions of soil fauna with Ca and pH of soil was presented in papers of Marshall (1978), Huhta (1984), Straalen & Verhoef (1997) and Kaczmarek (2000).

But in this case this experimental forest is not under influence of any significant pollution. The differences in any edaphic element result from tree species influence and not from strong, unnatural influence of any substance for soil fauna. Any significant correlation can help to explain the choices of mites between different forest types preference being measured by more or less abundance or density per surface unit.

Table 3. Correlation between selected soil characters and average abundance of selected mite genera (red coloured significant correlation, P<0.1, with asterix marked significant correlation, P<0.05).

	Paragamasus	*Trachytes*	*Veigaia*	*Zercon*	Sum
Ca	-0,61*	0,43	0,40	-0,07	-0,17
Cl	0,56	-0,31	-0,32	-0,10	0,02
Zn	0,03	0,15	-0,09	0,63*	0,61*
Cu	0,54	-0,15	-0,15	0,27	0,40
pH	-0,55	0,30	0,24	0,12	-0,01
humus	0,50	-0,27	-0,23	0,22	0,32
Hh	0,51	-0,06	-0,20	0,22	0,34
T	0,25	-0,35	-0,55	0,27	0,22

Conclusions

The interactions between soil characters and average abundance of all mites from four explored genera of Mesostigmata mites have usually no statistical significance on the experimental plots.

Average abundance of mites from *Paragamasus* genus is significantly growing with increasing soil characters: Cl, Cu, humus, Hh and with decreasing soil characters: Ca and pH. It shows stronger connection mites of this genus with coniferous trees (larch and spruce) than with broadleaves tree species (oak and beech).

Average abundances of mites from *Trachytes* and *Veigaia* genera have usually lower correlation with above characters but in reverse direction. It shows stronger connection mites of these genera with broadleaves trees (oak and beech) than with coniferous tree species (larch and spruce).

Average abundance of mites from *Zercon* genus has significant correlation with amount of Zn, but in case of other soil characters the correlations were low.

In soil ecological research, it is very important to determine Mesostigmata mites to genus or species level, because particular mite genus (species) can have opposite interactions for the same soil character.

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MYCOPHAGOUS MITES (ACARI: ORIBATIDA AND ACARIDIDA) AND THEIR COOPERATION WITH CHITINOLYTIC BACTERIA.

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Abstract

The mycophagy of soil saprophagous mites was analyzed by cross tests. Several methods were applied for those purposes: the histology including the fluorescence microscopy, excrement analysis, chitinolytic enzymes tests and bacterial plating respectively from mite homogenate. The actually mycophagous mites evidently consumed the large mass of fungal propagula, which were digested in the mite gut. Those mites, subsequently, produced the great number of bacteria in their excrements. Their homogenate exhibited the clear chitinolytic activity and several bacteria were plated from those. Those bacteria were reported in literature to be chitinolytic. The cooperation of mites and chitinolytic bacteria were supported by the applied cross tests.

Key-words

Soil mycophagy, enzymes, associated bacteria

Introduction

The nitrogen is mobilized continuously from the organic sources in soil. The substantial portion of nitrogen is immobilized in several substances, especially in the fungal cell walls, in chitin. Chitin, however, represents the polysacharid hardly to decompose by animals. The autochthonous chitinolytic ability of animals is considered to be restricted only to the pathogenic stages (Aktjuganov et al. 2004) In soil, indeed, there are many mycophagous animals. Therefore, our searching should be directed to the pattern of consumption of fungi by soil animals. The soil mites have represented the studied animal group in our laboratory. We can introduce these questions which represent the way to the mycophagy explanation:

1. what animals consume fungi?

Many saprophagous mites were tested as regards their food preferences and confirmed in literature to be mycophagous mites. The authors applied several tests for those purposes: the most classical, performed mainly by taxonomists: - only clearing of the fixed mites in lactic acid and recording of their gut content (Kunst 1968);- the morphological view – cheliceral dimensions useful for various types of food (Kaneko 1988); - in many rather experimental papers: one way or cafeteria tests with offered fungi and observation of the mite staying on fungus or motion of chelicerae (Hartenstein 1962, Czajkowska 1970). This observation was supplemented often by the recording of number of the produced excrements, the enzyme tests for the digestion capability (Luxton 1972; Siepel and de Ruiter-Dijkman 1993);

the undirect consideration based on ratio of nitrogen isotopes $^{15}N/^{14}N$ (Schneider et al., 2004) and subsequent niche differentiation in soil animals screening of the gut content in the histological sections (Woodring and Cook 1962a,b, Smrž 1989, 2002a).

2. what is the pattern of such consumption?

The consumption of fungi includes only sucking of cell content or gulping of the fungal propagula?. Again, the answer should result from the view into gut and recording of gulped fungal propagula. The only amorphous mass of food or fragments of plants do not support the fungal feeding (Smrž 2002a).

3. what is the mechanisms of digestion and utilization of fungi? Hence, where is the source of chitinolytic enzymes of mycophagous mites?

The chitinolytic enzymes should be hardly produced by mite (Aktjuganov et al. 2004). There are several groups of chitinolytic bacteria, but the enzyme participation of chitinolytic or cellulolytic bacteria appeared to be controversial in the mite digestion (cf.Luxton 1972, Zinkler et al. 1986).

4. Are the bacteria able to participate in the fungal digestion in mites?

This paper should introduce the group of such bacteria isolated from mycophagous soil mites.

Material and Methods

Methods

The following system of several methods was constructed:

1. gulped and potentially digested, hence, palatability of food.

This problem has been solved by histological way, by looking into alimentary tract. The process has been based on the special fixing of mites (modified Bouin-Dubosque-Brasil: Smrž 1989), following by embedding into paraplast and sectoning in Leica 2155 rotation microtome (5µm thickness of sections). The Masson´s triple stain has been perfectly distinguished microanatomical details including hemocytes and bacteria within the mite body (Smrž 1995, 2002a).

2. particles in rectum and those expelled from gut.

The excrement analysis revealed living and dead propagula in smeared excrements under the fluorescence microscope. Orange G has been applied as dye. (Smrž, 2002b). The histological sections as well as excrements were observed under the AX-70 Provis microscope (Olympus)

3. enzyme activity in the mite body

The mite was washed in ethanol and detergent to remove the surface microorganisms. The mite homogenate was dropped on the thin carboxylmethylchitine cover on the microsllide. All microslide was incubated for 24 hours under the laboratory temperature and subsequentky stained in the basic fuchsin. The blanks indicated the chitinolytic activity (Smrž 2000). The results were strongly uniform, therefore no statistical methods did not need.

4. source of chitinolytic enzymes.

The mite homogenate was plated. The grown bacteria was purified and identified in the certificated laboratory (CCM, Brno, Czech Republic).

All experiments were triplicated.

Tested mites

Several soil saprophagous mites were tested according to literature data and their gut content:

1. the actual, in literature confirmed mycophages: *Damaeus auritus* C.L. Koch, *Damaeus riparius* C.L. Koch, *Tyrophagus putrescentiae* Schrank (Pauly 1956, Czajkowska 1970, Luxton 1972, Schneider et al. 2004, Smrž 2002a, 2003, Smrž and Jungová 1989,);

2. oribatids with reported affinity to fungi as omnivores: *Achipteria coleoptrata* (Linnaeus) , *Archegozetes longisetosus* Aoki, *Chamobates voigtsii* (Oudemans) (Černý 1999, Smrž 2007, Smrž and Norton 2004)

3. oribatid without any affinity to fungi: *Trichoribates trimaculatus* (C.L.Koch) (Smrž 2006).

Results

The spores or fragments of fungal mycelium were gulped by most of tested mites except *Trichoribates trimaculatus*. Therefore, all following characteristics in the microanatomy, excrements, enzyme tests and bacterial plating were observed only in others mites unlike *Trichoribates trimaculatus* in our analysis, hence, in the mites consuming the fungi. In those mycophagous mites, the following digestion was indicated by the activity of their gut wall cells (enzyme granula, vacuoles), but also by the progressive thinning of the fungal mycelium walls from mesenteron to rectum. The fungi were nearly completely digested, as seen in the excrements. The fungal fragments were red in excrements under

fluorescence light, hence, dead. The conspicuous hemocytes ocurred between the internal organs. The chitinolytic activity of the mite homogenate was positive under the test.

Several chitinolytic bacterial genera and species were isolated from the homogenates of the tested mycophagous mites (table 1).

Table 1. Bacteria isolated from mites.

Bacterial species	mite species
Bacillus badius	*DR, A, Ch*
Bacillus cereus	*DR, A, Ch*
Bacillus megaterium	*DR, Ch*
Janthinobacterium lividum	*DR*
Pseudomonas fluorescens	*DA*
Serratia marcescens	*TP*
Serratia liquefaciens	*TP*
Pseudomonas stutzeri	*TP*
Brevundimonas vesicularis	*TP*
Stenotrophomonas maltophilia	*TP*
Serratia rubidea	*AL*
*Alcaligenes fecalis**	*TT*

Abbreviations used: A – *Achipteria coleoptrata*, AL – *Archegozetes longisetosus,* DA – *Damaeus auritus*, DR – *Damaeus riparius,* Ch – *Chamobates voigtsii*, TP – *Tyrophagus putrescentiae*, TT – *Trichoribates trimaculatus, *- chitinolytic activity not found*

Discussion

The most of previously applied methods were able to found some affinity of mites to fungi, but the actual mycophagy remained unclear. Only staying of mites on fungus or moving of chelicerae appears to be insignificant for the real palatability of the fungal food (cf.Hartenstein 1962, Czajkowska 1970). Its digestion and utilization remain enigmatic. Moreover, the gulping of the fungal fragments can be followed by only passing of them through gut, as intact corpuscles and without any digestion (cf. Kunst 1968, Smrž 2002b). The complete mycophagy should be characterized by the actual grazing and subsequent by the digestion of all fungal fragments including the chitinous cell walls. The only cell content utilization does not need to characterize the mycophagy. So-called fungal sugar - trehalose - occurs in many organisms including animals (Wiggleswort 1974). Our tested mites crowded all three parts of thier gut (mesenteron, colon, rectum) by the fungal food. The fungal cell wall becamed thinner caudad, the gut wall exhibited the high activity (enzyme granules, vacuoles) and guanine was deposited into gut walls and mesenchyme tissue (Smrž 2002a, Smrž et al. 1991). The mite homogenate exhibited the chitinolytic activity. The origin of chitinolytic enzymes, however, seemed to be in issue (cf.Luxton 1972, Zinkler et al. 1986). But according to the above mentioned inability of animals to produce chitinolytic enzymes, the production of such enzymes should be related to some species of bacteria.

All bacteria isolated from the homogenate of our mycophagous mites were confirmed to be chitinolytic by test or in literature by other laboratories (Watanabe et al. 1990, Ho-Seong Lim et al. 1991, Molloy and Burke 1997, Zhang and Yuen 2000, Citterio et al. 2001, Wen-Teish Chang et al. 2003, Khatuntseva et al. 2008).

So, we study the mite with the all gut parts crowded by fungal fragments, with the high activity of the gut walls, with the progressive thinning of the fungal cell walls, with the great density of guanine crystals and hemocytes in body, with the great number of the living bacteria in excrements. The chitinolysis test of homogenate is positive. Finally, we isolate the chitinolytic bacteria from that mite. Therefore, the synergetic processes of the internal extraintestinal bacteria and their host mites can be considered for the digestion of chitinous fungal cell walls. The production of the chitinolytic enzymes can be allocated to the isolated bacteria. This synergy, hence, enables mycophagy of such soil mites.

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INTERACTIONS OF HISTIOSTOMATID MITES (ASTIGMATA) AND LEAFCUTTING ANTS

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Abstract

The phoretic Histiostomatidae are assumed branching off basically within the Astigmata. They are associated with arthropods, mainly insects. Often complex ecological interactions can be found with breeding insects such as *Forficula* or *Nicrophorus* and social insects such as ants and bees.
Histiostoma bakeri was found in the nests of *Atta texana* and was successfully cultured under laboratory conditions. The morphology of its mouthparts is introduced. Experiments with leafcutting ants were performed. *Atta sexdens* and *Atta vollenweideri* were used as model ants due to a better availability of their workers within Germany. A conspicuous ant-behaviour was observed, after middle-sized workers were artificially loaded with many deutonymphs of *H. bakeri* and subsequently reintroduced to their fungus garden community: A rhythmical hopping behavior of the loaded workers attracted the attention of minor workers which finally began to clean these deutonymphs. During that procedure the afflicted workers remained completely motionless in a specific posture.

Key words

Atta texana, *Atta sexdens*, *Atta vollenweideri*, Histiostomatidae, *Histiostoma bakeri*, behavior, cleaning, leafcutting ants

Introduction

The Histiostomatidae represents a monophyletic subgroup within the Astigmata (Wirth, 2004; OConnor, 1981), and over 300 species are described. Histiostomatids usually live in bacteria rich habitats that only persist for a short time, and therefore need to be dispersed by a phoresy (Wirth, 2004). Mites are often specific to only certain insect species (Wirth, 2004). Some histiostomatids are specifically associated to hymenopterans such as bees (e.g. Fain & Erteld, 1998) or ants (e.g. Scheucher, 1957).

This paper concentrates on the biological aspects of histiostomatids found in nests of the leafcutting ant *Atta texana*.

Due to their size and complexity the nests of *Atta* offer different habitats for arthropods as e.g. beetles, mites, cockroaches (Moser 1963). Four habitats within nests for these different invasive arthropods are described by Moser (1963, 2006): fungus garden cavities, irregular shaped dormancy cavities, detritus cavities and galleries. Leafcutting ants react to arthropod invaders in different behavioral ways. Hitchhiking behaviour by minor workers prevents ants outside the nest from being attacked by phorid (Diptera: Phoridae) parasitoids (Linksvayer et. al., 2002). Another ant behavior in correlation with arthropod invaders is the “jigging” observed in the fungus-growing ant *Cyphomyrmex costatus* (Kweskin, 2004). It is described as a rhythmic rocking behavior. Jigging was found to be

correlated to the existence of collembolan fungal garden pests and other arthropod invaders. It is assumed to function either to drive these arthropods off from the garden surface or to respond more generally to disturbance. Another possibility to react on disturbances is an acoustical communication. Movements between the postpetiolar tergite and a special area of the first gastral tergite produce stridulation (Markl, 1965).

Experiments with phoretic mites were performed for the first time during the studies introduced here. The behaviors mentioned above were considered to happen during an ant's reaction to numbers of phoretic mite- deutonymphs. These studies were performed with *Histiostoma bakeri*, which was found to occur in the nests of *Atta texana*. *H. bakeri* was also found in rotting logs, woodlawn and dung (Hughes&Jackson, 1958).

Material and Methods

Cultures of *Histiostoma bakeri* were reared from specimens collected in detritus of *Atta texana* from both the field and laboratory colonies in Central Louisiana/USA. Mites were cultured on 1.5 % water- agar in Petri dishes of 5 cm in diameter and potato pieces to stimulate microorganism growth. Mite preparations for scanning electronmicroscopy (SEM) were performed by drying specimens with dimethyldisilazane, fixing them in 99% ethanol, and sputtering with gold.

Workers of leafcutting ants and the fungus cultivar were reared in dishes 10 cm in diameter that were connected to second dishes of the same size, but with fresh air circulation to dry the detritus. The dish bottoms were covered with plaster to regulate the humidity. When workers of *A. texana* were not available, workers of *A. sexdens* and *A. vollenweideri* (given by F. Roces, Würzburg, Germany) were substituted as suitable model ants, because *H. bakeri* could easily become established in their nests too.

The following behavioral experiment with mites and ants was performed, and results were described. Middle sized workers (head width of about 1.7 mm) were placed in mite culture dishes with numbers of deutonymphs. Specimens were covered with 150-200 deutonymphs distributed mostly on the ventral head/mandibles and ventral abdomen, more rarely on legs and the dorsum. These specimens were used for experiments, because this situation was assumed to represent a realistic situation in the ant nests. Due to the unusual growth success of *H. bakeri* compared to other histiostomatid species, large numbers of deutonymphs can easily ascend single ant workers. Covered ants were then transferred to their fungus garden community. Ant behaviors were documented with both a digital video camera of Samsung S850 and a Canon XL-H1 3CCD (30-60 frames per second). Ants were habituated to a cold light lamp, and the experiment was repeated 7 times with both *A. sexdens* and *A. vollenweideri*.

Results

Biology and morphology of H. bakeri (Fig. 1C)

The developmental period of *H. bakeri* lasted about two weeks at room temperature (20° C). Due to the lack of males a thelytokous reproduction of females is assumed. Because of extremely high numbers of specimens in the culture dishes, we assumed that females produced many offspring compared to other histiostomatids. Deutonymphs aggregated in big accumulations and ascended usually ant workers, gamasid mites, and rarely other arthropods with help of their suckerplates (Fig. 1B).
H. bakeri was only found in the detritus of *Atta texana*. The histiostomatid species *Histiostoma myrmicarum* occured in nests of *A. texana* and was originally known from nests of *Myrmica* and *Lasius* (Scheucher 1957). Other histiostomatids were discovered from nests of *A. vollenweideri* and *A. sexdens*. The morphology of the mouthparts of *H. bakeri* was observed with scanning electronmicroscopy (SEM). The digitus fixus (Fig 1 E, F) is complex shaped and consists of differently shaped ventral extensions. The same components are found in the *Histiostoma-feroniarum* group, a small monophyletic subgroup of the Histiostomatidae (Wirth, 2004). The proximal components are relatively large, arranged in one row, and each are slightly bulged posteriorly. The smaller distal extensions are arranged transversely and are rakeshaped. A conspicuous single structure at the distal end of the digitus resembles of a fishhook (Fig. 1 F). It seems that many of the single extensions have eight components (+ 7 (rake) + 1 (fishhook)) and are not variable.

Other distinct structures concern the distal pedipalps (Fig. 1B) and corresponding membraneous structures derived from the coxal endites. The ventral "lobe" (Wirth, 2004) is completely missing. The dorsal part is partly divided into conspicuous fringes (Fig. 1E).

A ventral structure called "vaulting" (presumably homologous to parts of coxal endites) is conspicuously enlarged and its ventral margin is divided into many small fringes (Fig. 1E).

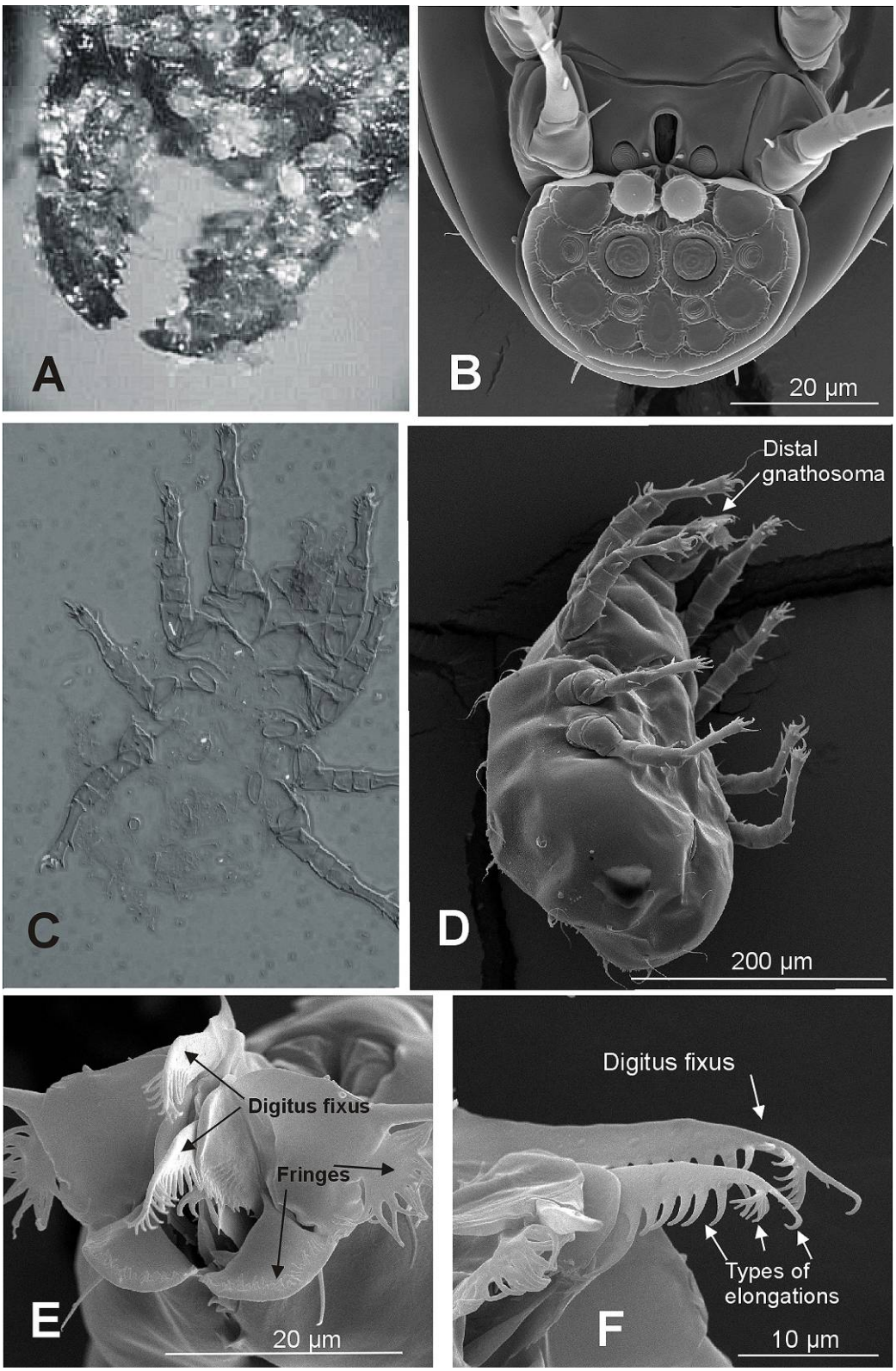

Figure 1. A: Deutonymphs of *Histiostoma bakeri* attached to the mandibles of *Atta sexdens* worker. B-F: *H. bakeri*. B: Suckerplate of the deutonymph (SEM). C: Female in ventral view (light microscope, specimen 14, 854 from the collection of J. Moser, USA). D-F: SEM pictures. D: Female in latero-ventral view. E: Distal gnathosoma of female in frontal view. F: Digitus fixus laterally.

Experiment

The mite may need to be distributed within the ant nest. To understand more about this procedure the following experiments were performed.

Ant workers of both *Atta* species covered with deutonymphs (Fig. 1A) were slightly handicapped in walking. They were observed to walk to the fungus cultivar and to perform a behavior in 3 parts. The described video sequences, showing the activities around one ant worker with deutonymphs, seemed to be representative despite a slight variability in the sequence of movements.

1) First part of the following behaviors (Fig. 2.1.) had duration of 10.27 seconds. Within this time the ant performed hectic movements. It was sitting very close to the fungus, the body stabilized with help of the fore leg, and with the right hind leg extended to its posterior. The left posterior leg first moved up and down twice, in uncertain steps, but afterwards quickly moved up and down 4 times in high steps. During those movements the ant's head turned twice to the right accompanied by alternating movements of the antennae. Several quickly alternating up and down movements of the first pair of legs followed. Then the ant made a short jump up to the margin of the cultivar and groomed its lateral abdomen and lateral thorax with the tibia and tarsus of the hind legs folded upwards and directed to anterior. This behavior was repeated 6 times with both hind legs alternating. These grooming actions suggested a hectic and adumbrated cleaning bahavior. Afterwards the worker jumped back to its original position and performed 3 short and unfinished sidekicks using its left hind leg again. During this series of motions two single small cleaner ants approached to the worker and tried to examine its surface with antennae and mandibles, but were frightened away by the worker's rapid movements.

2) Second part of this behavior had a duration of about 13.95 seconds. After few seconds the ant straightened its body and began cleaning its chelicerae and the dorsal head with the first pair of legs, while pulling legs II alternatively through the mandibles. Then legs I and II performed trembling movements scrubbing against each other. The whole body was meanwhile mostly stabilized by both hind legs, which slightly moved alternatively 4 times; this led to a rhythmical stumbling of the whole upright standing body. On the whole, this second behavioral part (Fig. 2.2.) began as a self-cleaning procedure, but then changed into hectic fidgety, unorganised movements. A few small ants approached, but again were temporarily driven away until the cleaning described under part 3) began.

3) The third bahavior lasted about 3.5 minutes. The ant stopped moving completely and remained near the fungus. That elevated position (Fig. 3, left) can be described as fellows: The left legs II and III were slightly angled and pressed to the surface of the cultivar. The right hind leg was extended posteriorly, while the right leg II being extended laterally while the first legs of both sides were slightly stretched latero-anteriorly. Originating from a nearly horizontal body position, the thorax and head were stretched upwards with the head slightly tipped to the right.

At first only single cleaner ants approached and began cleaning the worker's ventral head, but left after a few seconds. More cleaners then appeared mostly cleaning the ventral and dorsal head including mandibles and legs; only rarely the dorsal abdomen and thorax. Three to 7 ants cleaned at the same time, 2-3 ants often being occupied with the same leg of the "infested" worker.

It was difficult to determine if individual deutonymphs were killed during that procedure. But it was observed, that most survived and climbed the fungus.

Many mites were observed to be removed from the cultivar by large workers using the mandibles to grasp or as shovels, and were discarded in old fungus, which wa**s** carried away as detritus.

After about 1.14 minutes only two cleaners remained, and the soldier began to fidget again for a few seconds, scrubbing its lateral head with the right fore leg, and rubbing left legs I, II and III against each other. It then returned its normal stance, but with the body partially elevated.

Eleven cleaners then arrived, working again mostly on legs and head. The major soldiers were never observed to clean, but often transported hurt and dead bodies as detritus. Sometimes they examined the ants covered with deutonymphs. In this case, one major worker approached and palpated the ant's head with its antennae for a few seconds and left afterwards (Fig. 3, right).

After a few minutes less than a quarter of the deutonymphs remained mostly on dorsal head and dorsal abdomen. The ant became active again and disappeared into fungus cultivar cavities.

This cleaning procedure was similar in all observed experiments.

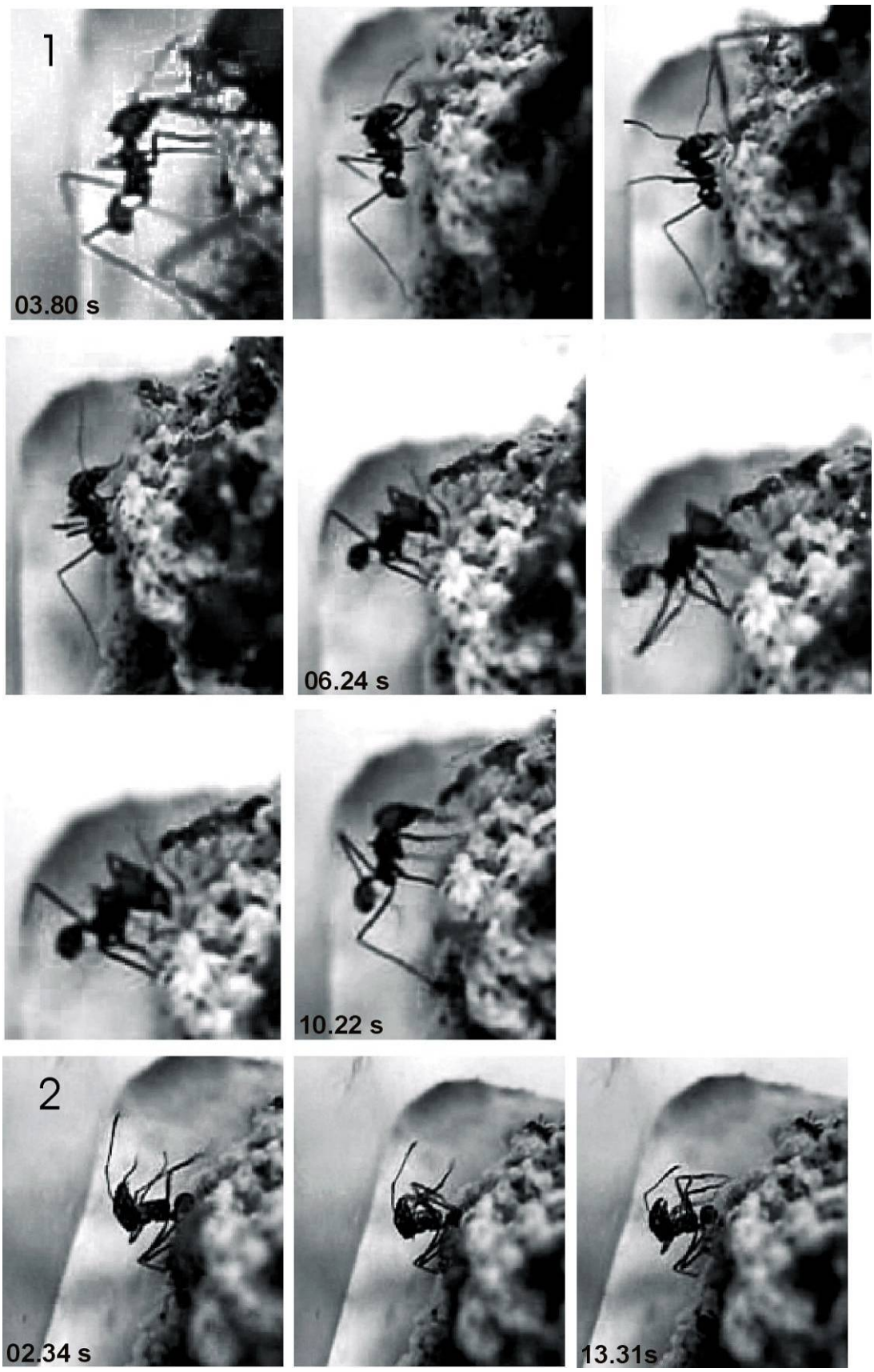

Figure 2. Middle sized worker of *Atta sexdens* covered with deutonymphs of *Histiostoma bakeri*. Single frames taken from video files. 2.1.: beginning fidget behavior, presumably to attract attention of cleaner ants. 2.2.: second part of the behavior, the ant partly cleaning itself, partly performing movements with all legs against each other, while the ant is bent backwards. Periods 2.1. and 2.2. observed separately.

Discussion

Morphology and biology of Histiostoma bakeri

Histiostoma bakeri was not only found in *Atta* nests, but also in other habitats (Hughes & Jackson, 1958). This may be accidental, unspecifity of the mite, or due to a group of morphologically very similar species. Further morphological and biological studies should support a better understanding of the phenomenon.

The morphology of the complex digitus fixus evolved in the stem species of the *Histiostoma-feroniarum*-group (Wirth, 2004). In this group very different kinds of habitats were colonised such as mud around waters, compost, earwig nests, mushrooms and ant nests. It is assumed, that a digitus fixus with the depicted components (Fig. 1F) is a multifunctional instrument that allowed the colonization of all these different habitats within the histiostomatid subgroup. A digitus fixus of that type was observed to be able to dig deeply into a muddy substrate to wrench bacterial substrates from the ground (Wirth, 2004). The fringes at the pedipalps of *H. bakeri* resembled convergently evolved fringes in *Bonomoia opuntiae* and could have a similar function. They are used to rip food material out of the ground (Wirth, 2004).

Many deutonymphs of *H. bakeri* often develop in cultures under laboratory conditions. This may be a useful adaptation when being dispersed by the leafcutting ants within the nest, but the ant's behavior to clean the mites may limit dispersal.

Thelytokous parthenogenetical reproduction is common within the Histiostomatidae, and may enable colonization of new habitats by single female deutonymphs.

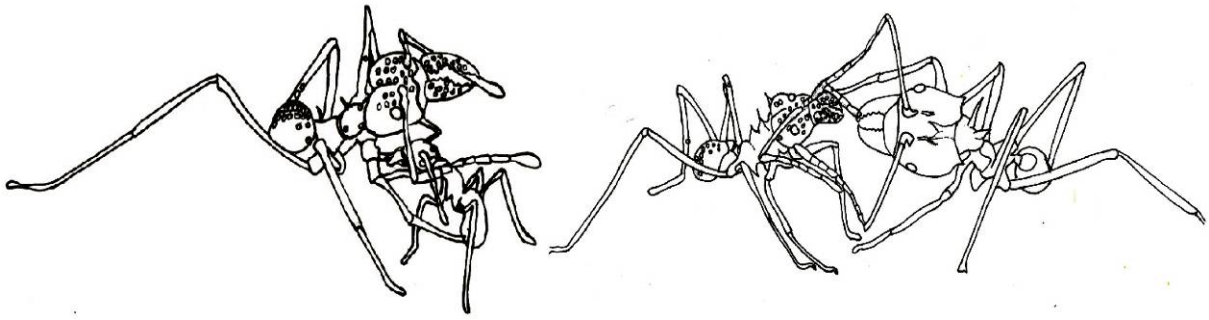

Figure. 3. On the left: worker of *A. sexdens* covered with deutonymphs, remaining in a motionless position and being cleaned by cleaner ants. On the right: The same worker examined by a major worker without a cleaning behavior.

Cleaning behavior of the ants

The behaviors of an ant covered with deutonymphs differ from the usual interactions between ant workers. This behavior is divided into 3 parts and seems similar to the behavior of fishes waiting for cleaner shrimps. The function of the hectic and complex movements in the first part of the ant's behavior remains unclear. The conspicuous rubbing of the hind legs to the lateral body portions does not remove the mites. This may be a signal for other ant workers, but needs to be observed more closely. The second part of the behavior differs from the self cleaning by rubbing movements of legs I and II. It is hypothesized that both behavioral parts could correlate with the third part of the behavior, in which the ant gets cleaned by minor workers. Future observations need to test if behaviors 1 and 2 or at least one of them attracts the cleaner workers or if they determine what kind of problem must be resolved. Another possibility would be to interpret these behaviors as intention- movements just to heighten the activity of other workers to clean this congener. The experiments showed that there must be a stimulus for the ant suddenly to change into an off position. It remains unclear if approaching cleaner ants or the mite deutonymphs stimulate that behavior. The driving away of approaching cleaners could indicate that they don't stimulate that off position. The position was in all experiments similar and differed only slightly. We interpret this to relate to the ventral head and legs, which are usually covered by the deutonymphs that disturb locomotion and orientation.

We conclude that under natural conditions, mites regularly contact ant workers from the detritus, the place where mites develop. This could affect

the mite's dispersal within the nest. The fungus chambers could function as distribution stations distributing deutonymphs from one ant specimen to several others. Thus it would be important that most deutonymphs survive the cleaning procedure and afterwards climb other ants in small numbers. This would permit the ant numbers on the fungus to be more numerous than those on the detritus. Ants with only a few deutonymphs do not provoke that cleaning procedure. The observations that deutonymphs are not allowed to develop on the fungus, could support the idea of a distribution station. Despite the mite obviously acting as a pest for ant workers covered with numerous deutonymphs, the role of the mite in the ant nest is unknown. The mites are bacteria feeders and could prevent bacterial infections of ants coming from the detritus chambers. Such correlations were assumed for histiostomatids in earwig nests (Wirth, 2004). How mites originally arrive at ant nests is unknown, unless transported by queens. But to date we have seen none on alates of *A. texana*. Single components of the observed ant behaviors as e.g. a possible jigging (Kweskin, 2004) could not be distinctly differed from each other due to the complexity of different kinds of movements at the same time. Aspects of stridulation (Markl, 1977) during these behaviors were not observed so far. But it seems certain, that stridulation alone does not need such fidget movements (Markl, 1965). Further studies about a possible sound production are needed.

Acknowledgements

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OLFACTORY RESPONSE OF THE STIGMAEID PREDATOR *ZETZELLIA MALI* (EWING) TO A PREY PATCH OCCUPIED BY CONSPECIFIC OR/AND HETEROSPECIFIC PREDATORS

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Abstract

While searching for food, predators can use volatiles associated not only with their prey, but also with their competitors for prey and their heterospecific predators. *Zetzellia mali* (Ewing) (Acari: Stigmaeidae) is an important predator of the hawthorn spider mite, *Amphitetranychus viennensis* (Zacher) (Acari: Tetranychidae), along with a heterospecific competitor for Predatory thrips (*Scolothrips longicornis* Priesner (Thysanoptera: Thripidae), in black cherry orchards in Baraghan, Iran. Using a Y-tube olfactometer, its response was tested to odours from black cherry seedlings fed upon by this spider mite, either with or without a conspecific and heterospecific predator. Female predators avoided odours from seedlings with both prey and conspecific predators.This avoidance behavior also happened when the heterospecific predator was added to the patch occupied by spider mite. We discuss whether avoidance emerges in response to cues from the competitor/predator, the herbivore/prey or the herbivore-damaged plant.

Key words:

Avoidance behaviour, predator-prey interaction, intraspecific competition, Stigmaeidae, Tetranychidae, Thripidae

Introduction

Herbivorous arthropods use volatile plant chemicals to locate their host from a distance (Bell and Carde 1984, Visser 1986), thus increasing the probability to find a profitable host plant. However, the profitability of this food source is not only determined by the quality and quantity of food, but also by the absence or presence of predators of the herbivore (Dill 1987; Dixon and Baker 1988; Lima and Dill 1990; Ohsaki and Sato, 1994; Lima 1998; Janssen et al. 1998; Faraji et al. 2001; Nomikou et al. 2003). When predation is a strong selective force, herbivorous arthropods are expected to also use volatile chemicals that provide information on the risk that they or their offspring are eaten by predators. There are examples of herbivores that avoid odours coming from plants occupied by both the herbivore and its predators. Two-spotted spider mites, for example, use volatile cues to avoid plants occupied by western flower thrips, which are both competitors and predators (Pallini et al. 1998). The mites also avoid plants occupied by conspecifics and

predatory mites (Pallini et al. 1999). Adult whiteflies have been shown to learn volatile cues to avoid ovipositing on plants with conspecifics and predatory mites, which can attack their young only (crawlers and eggs) (Nomikou et al. 2003). There are also examples of predatory mites avoiding odours that come from plants occupied by both herbivorous mites and conspecific predators that are potential cannibals of juveniles and therefore a threat to any offspring the searching predator might produce on that plant. Females of the phytoseiid mite *Phytoseiulus persimilis* Athias-Henriot avoid odours from leaves occupied by two-spotted spider mites and conspecific female predatory mites (Janssen et al. 1997).

In this article we test such olfactory responses of another predatory mite, *Zetzellia mali* (Ewing) (Acari: Stigmaeidae), towards leaves occupied by the hawthorn spider mite *Amphitetranychus viennensis* Zacher (Acari: Tetranychidae) and a conspecific/heterospecific predator. These mites and thrips are of agricultural importance, as pest and biocontrol agents in fruit orchards in Iran (Khanjani and Ueckermann 2002). The predatory mite *Z. mali* feeds on various phytophagous mites in apple orchards (Jamali et al. 2001; White and Laing 1977ab; Santos 1975). In Iran, it is the only predatory mite in the region where hawthorn spider mites occurs and it is therefore considered as a potential biocontrol agent of this spider mite (Zahedi-Golpayegani et al. 2003). Besides, the predatory thrips, *Scolothrips longicornis* was found as an effective predatory agent fed on not only all stages of spidermite but also destroyed eggs of its heterospecific competitor, *Z. mali*. So one may wonder if its presence would affect the predatory mite olfactory decision toward prey.

To understand the mechanisms by which *Z. mali* predators distribute themselves over leaves, it is important to assess their ability to locate prey and intraspecific/heterospecific competitors from a distance. In this article, we present the results of olfactometer experiments in which adult females of *Z. mali* were offered a choice between leaves with hawthorn spider mites only and leaves with hawthorn spider mites and conspecific/heterospecific predators.

Materials and Methods

In May 2006, hawthorn spider mites and predatory mites and thrips were collected from black-cherry trees in pesticide-free orchards in the Baraghan region, Karaj-Iran. The hawthorn spider mites were reared on black-cherry seedlings in pots in a greenhouse (26 ± 2 °C, 16 : 8hrs, 60 ± 10% RH) (Plant Protection Department, College of Agriculture, University of Tehran, Iran). Care was taken to prevent the seedlings from being infested by other common spider mites by brushing the leaves once per day for the duration of one week preceding the introduction of hawthorn spider mites. The predatory mites were reared on a diet of eriophyoid mites and eggs of hawthorn spider mites on a substrate of detached black-cherry leaves on a tray in a transparent plastic container. The containers were left open to avoid high humidities, detrimental to the survival of *Z. mali* (Zahedi-Golpayegani, *pers. obs.*).

Olfactometer experiments

A Y-tube olfactometer was used for assaying the response of *Z. mali* to odours associated with its prey, the hawthorn spider mite only and along with predatory thrips. The olfactometer consisted of a Y-shaped glass tube (d: 4cm) with a Y-shaped metal wire in the middle of the tube and positioned parallel to the tube walls (Sabelis and van de Baan 1983). The basal end of the Y-tube was connected to a vacuum pump to create air flow from the two arms to the basal tube. Glass tubes containing the odour sources were connected to the end of each of the two arms. These tubes were closed with metal gauze with a mesh width small enough to prevent predatory mites from reaching the odour sources.

Predatory mites were collected from the seedlings and starved for 3 hours in small plastic containers prior to the test (Pallini et al. 1997). These starved predators were transferred with a small brush to the base of the metal wire in the Y-tube, where they initiated upwind movement. Each predator was observed until it passed the junction and moved into one of the arms of the Y-tube. However, if it did not reach the junction within 5 minutes, the experiment was stopped and the outcome of the experiment was scored as a case of non-preference. The criterion of 5 minutes was based on preliminary observations, showing that most predators reached the junction within this time. For each treatment, tests were performed in three independent replicate experiments, each with ca. 15 predators and each with a new set of odour sources consisting of black-cherry leaves with or without herbivorous and predatory mites. The first treatment involved the following two alternative odour sources: (1) leaves infested by a single female of the hawthorn spider mite and (2) leaves infested by one female hawthorn spider mite but in addition occupied by one female of *Z. mali*. The second treatment involved leaves with one female *Z. mali* and leaves infested by one

female hawthorn spider mite as the alternative odour sources. Our third treatment involved leaves with conspecific predators and leaves with both hawthorn spider mite and *S. longicornis*. In all treatments all leaves had an unavoidable infestation of eriophyid mites, a second potential prey for *Z. mali*. However, the density of these mites was very low (2 individuals/leaf) and not different among the leaves. Because the main aim of our experiments was to test whether *Z. mali* females avoided leaves occupied with conspecific predators and not to analyze the source of the odours, we did not test separately for the effect of eriophyid mites alone, nor for the effect of hawthorn spider mites alone.

Under the null hypothesis, we expected 50% of the predators to enter each of the two arms of the Y-tube and we tested to detect significant deviations from this expectation. Statistical analysis was done using a replicated *G*-test, which includes a test for heterogeneity among replicate experiments (Sokal and Rohlf 1997).

Results

When *Z. mali* predators were offered a choice between odours from leaves with hawthorn spider mites and conspecific predators and leaves with only spider mites, 40 predators showed a preference for one of the arms, 77% of which moved into the arm with volatiles emanating from leaves with prey (Table 1a). Fourteen predators did not reach the trifurcation point in the Y-tube within 5 minutes. Thus, 74% of the *Z. mali* females can cover a distance of at least 10 cm within this period. It was noted that the walking speed of the predatory mites on the metal wire was much higher than on a leaf (Zahedi-Golpayegani, *pers. obs.*), but compared to phytoseiid mites their walking speed is much lower. This might have affected the number of non-preference cases within the maximum period of 5 minutes to reach the trifurcation point in the Y-tube.

The overall results deviated significantly from the 50% expected under the null hypothesis (Table 1b). This was not due to heterogeneity among replicate experiments, but due to significance of the *G*-test for pooled results. Two of the three replicate experiments showed a significant deviation from the null hypothesis (not shown in Table 1b). We interpret this result as either a preference for the odours coming from leaves with prey alone or avoidance of the odours coming from leaves with prey and conspecific predator.

Table 1. Results of replicate experiments of olfactometer tests (a) and replicated *G*-test (b) for the response of *Z.mali* to odour from blackcherry leaves with hawthorn spider mites and conspecific predators (+) when offered leaves with hawthorn spider mites only (–) as an alternative.

(a)

Replicate experiments	n (+)	n (-)	n (0)	n (total)
	2	11	2	15
	4	9	5	18
	3	11	7	21

(b)

Replicate G test	df	G-*statistics*	*P*
G_h	2	0.89	0.6401
G_p	1	12.81	0.0003
G_t	3	13.70	0.0034

When one of the arms of the olfactometer was connected to leaves with one female *Z. mali* and the other to the leaves with one female of the hawthorn spider mite alone, 28 out of 37 predators moved into one of the arms, 78% of which selected the arm with odour from leaves with hawthorn spider mites only (Table 2a).

Table 2. Results of replicate experiments of olfactometer tests (a) and replicated *G*-test for the response of *Z. mali* to odour from blackcherry leaves with conspecific predators (+) and leaves with hawthorn spider mites (–).

(a)

Replicate experiments	n (+)	n (-)	n (0)	n (total)
	1	8	2	11
	3	8	2	13
	2	6	5	13

(b)

Replicate G test	df	G-*statistics*	*P*
G_h	2	0.93	0.6280
G_p	1	9.72	0.0016
G_t	3	10.65	0.0137

While one of the arms of the olfactometer was connected to leaves with Z. mali and another one receved odours from leaves contained both spider mite and thrips, 87% of the predators moved toward one of the arms. About 12% of predators showed preference to none of the arms.17 out of 24 mites moved into arms received odours from conspecific alone (table 3a)

The overall results deviated significantly from the 50% expected under the null hypothesis (Table 3b).This was not due to heterogeneity among replicate experiments, but due to significance of the *G*-test for pooled results.

Table 3. Results of replicate experiments of olfactometer tests (a) and replicated *G*-test for the response of *Z. mali* to odour from blackcherry leaves with conspecific predators alone (+) and leaves with hawthorn spider mites and thrips (–).

(a)

Replicate experiments	n (+)	n (-)	n (0)	n (total)
	8	3	2	13
	9	1	1	11

(b)

Replicate G test	df	G-*statistics*	*P*
G_h	1	0.527	0.700
G_p	1	7.93	0.004
G_t	2	5.991	0.010

Discussion

Our olfactometer experiments show that *Z. mali* predators tend not to visit patches in which conspecific predators reside. We hypothesize that this behaviour has several advantages for the searching predator. First, it avoids competition for prey with the resident conspecific and it may find fewer preys, already upon arrival since the resident conspecific has a headstart in exploiting the prey. Second, from the viewpoint of the searching predator, a leaf with alerted prey will become less profitable in the long term. This is because the resident conspecific predator may induce the hawthorn spider mites to lay fewer eggs (Zahedi-Golpayegani, *pers. obs.*) and to release alarm pheromones that cause other spider mites to move or stay away. Thus, for a searching predator, a leaf with prey and no conspecific competitors, harbours prey that continue feeding and ovipositing, thereby creating a more profitable prey patch. As soon as this patch with prey is discovered by a searching predator, other conspecific predators will avoid this patch and the process of finding competitor-free prey patches starts all over again.

More experiments are needed to assess the reasons for the predators to avoid patches with conspecific competitors. Identifying the source of information to the searching predator will also require more experimental work. Because mites have no visual and auditory capacity, the information comes most likely in the form of volatile chemicals and is perceived by means of olfactory sensors. These volatile chemicals may be released by the resident predator, the herbivores (spider mites and/or eriophyid mites) or the plant. If the resident predator is the source, then the volatiles may either be inevitable byproducts of physiological processes or products intended to inform the searching predator that there is a competitor. If the herbivore is the source, then the volatiles are likely to be pheromones that help to alert conspecific herbivores. This possibility has been suggested by Janssen et al. (1997) in the case of two-spotted spider mites and the phytoseiid predator *Phytoseiulus persimilis* Athias-Henriot. If the plant is the source, this requires the plant to somehow recognize the predator or herbivores exposed to predation and to change the released chemical signal accordingly. Such mechanisms have hitherto not been shown, however.

Avoidance behavior is not a fix predictable reaction in response to conspecific occupied patches. Our last olfactometer experiment revealed that the coincident presence of both prey (spider mite) and competitor (thrips) provides more risky situation that makes *Z. mali* prefer staying with conspecific competitors. One may wonder which group of volatiles would play the most important role in such a behavior. This needs additional experiments with separate volatile sources that will come follow up this article.

The consequences of avoidance behaviour for the population dynamics of predatory mites and phytophagous mites are worth to be investigated because the overall impact is not self-evident. Avoidance of conspecifics (this article) may cause stigmaeid predators to space out more evenly over the leaves occupied by phytophagous mites. Moreover, the predators may induce reduced egg-laying in the hawthorn spider mites (Zahedi-Golpayegani, *pers. obs.*), thereby increasing the negative impact of the predators on the prey population. In contrast, the hawthorn spider mites may provide less food to the predators when they reduce their rate of oviposition (Zahedi-Golpayegani, *pers. obs.*) and possibly disperse to predator-free leaves. This would decrease the predator's numerical response and hence decrease the predator's impact on prey populations. Whether these counteracting processes will result in increased or decreased levels of hawthorn spider mites, is a major question for understanding predator-prey dynamics in general and the biological control of hawthorn spider mites in black-cherry orchards in particular.

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HOW DOES *PHYTOSEIULUS PERSIMILIS* FIND ITS PREY WHEN FORAGING WITHIN A BEAN PLANT?

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Abstract

The role of herbivore-induced volatile substances in prey-finding by phytoseiid mites has been repeatedly documented using an olfactometer. The objective of the present paper is to test the hypothesis that movement by *Phytoseiulus persimilis* is affected by these volatiles even on plants. Two series of laboratory experiments were carried out. In the first series we studied searching behavior of *P. persimilis* females on young bean plants in which a single leaf was infested with spider mites. The effect of spider mite colony location on the walking pattern of predatory mites while on a leaf was studied in the second series of experiments. We found that *P. persimilis* individuals were unable to discriminate between infested and uninfested leaves when they walked up the stem of a bean plant. On the other hand, results of the second series of experiments indicate that walk was not random once a predator was on the leaf surface since it was attracted to the spider mite patch, at least over a distance of 1 cm. These results thus demonstrate that herbivore-induced volatiles can be utilized by *P. persimilis* during search for prey also under conditions that mimic natural situations better than an olfactometer does.

Keywords

Acari, Phytoseiidae, Tetranychidae, herbivore-induced volatiles, prey location

Introduction

The role of volatile chemicals emitted from infested leaves and classified as herbivore-induced synomones in finding prey by phytoseiid mites was repeatedly documented using an olfactometer (Sabelis & van de Baan 1983, Dong & Chant 1986, Dicke *et al.* 1993). Under such test conditions predators are provided with a relatively constant and highly directional flow of kairomones and thus locating the volatiles source seems to be easier compared to real situations in the field. Results of our earlier experiments (Zemek & Nachman 1999) which better mimicked natural conditions revealed that *P. persimilis* does not disperse randomly to the surrounding plants in a greenhouse but predominantly moves in the direction of nearby plants infested with two-spotted spider mites, *Tetranychus urticae* Koch. Janssen (1999) also tested whether *P. persimilis* locates spider mite-infested cucumber plants in greenhouse release experiments. His results confirmed that predatory mites released in the center of a hexagon of cucumber plants were indeed guided to the prey infested plants by herbivore-induced plant odors.

The objective of the present paper is to extend the previous studies by testing the hypothesis that predatory mites can use volatile chemicals emitted from infested leaves to localize spider mite

colonies within a plant. The results should contribute to our knowledge about the actual role of volatiles in finding prey by *P. persimilis*.

Materials and Methods

1 Plants and Mites

Lima beans, *P. vulgaris*, var. Katka were used for experiments as well as for rearing of spider mites. The plants were grown in a greenhouse and provided with artificial light when necessary to ensure long day photoperiod (at least 16L:8D). The plants were grown in plastic pots with 10.5 cm top diameter, 8.5 cm bottom diameter and 8 cm height filled with a sphagnum based growth medium. A single seed was sown in each pot to avoid competition for nutrients, space and light. No fertilizers and no pesticides were applied. The plants used in experiments were approximately 2-3 weeks old. Leaves applied for experiments were taken from the second node of the plants.

The culture of two-spotted spider mites originated from a local population collected in České Budějovice, Czech Republic, and have been reared at the Institute of Entomology for several years using beans as host plants. The culture of predatory mite *P. persimilis* was established from mites provided by Biocontrol Vodany, Czech Republic. Predatory mites were reared in a laboratory at temperature 22-24°C with long day photoperiod (18L:6D). They were kept in plastic trays (50x40x6 cm) within which a plastic platform was placed in the center surrounded by water. The water prevented the predatory mites inhabiting the platform from escaping and served to maintain a high humidity necessary for their successful development. The predators were supplied with spider mites via heavily infested detached bean leaves.

2 Design of experiments

Two series of laboratory experiments were carried out. In the first series we studied the searching behavior of *P. persimilis* females on a young bean plant in which one leaf was infested with spider mites.

The plants used were at the stage of the first two leaf triplets. One leaf triplet was infested by at least 40 adult *T. urticae* females and both petioles were treated with petroleum jelly to prevent migration of spider mites from the infested to the uninfested leaf. The plants were used for an experiment when the leaf damage index of the infested leaf reached approximately 3 according to the scale by Hussey & Parr (1963).

The observation of *P. persimilis* behavior was conducted in a metal box (90x60x60 cm) painted black inside and equipped with two fluorescent tubes fixed to the ceiling. A round mirror was placed behind the plant to allow for observations of predatory mites when they walked on back side of the plant. An experiment started by introduction of a single, one-day starved *P. persimilis* female on the plant about 10 cm below the bifurcation point of the petioles. Its behavior was then observed until the mite entered one of the petioles. Each plant and predatory mite were used only once. The position of infested and uninfested leaves in the box alternated to eliminate any potential factors affecting the direction of the predator's walk. The effect of the presence of a spider mite colony on the decision of *P. persimilis* to enter particular petiole was analyzed by a sign test (Siegel & Castellan 1988).

In the second series of experiments we studied the effect of spider mite colony location on the walking pattern of predatory mites while on a leaf. The observation arena was made up by a single bean leaf placed upside down on filter paper which in turn was placed on a water-saturated sponge in a Petri dish. Prey colony was established by introducing 40 adult females of *T. urticae* at one half of the leaf and isolated by covering them with a glass vial with an inner diameter of 2 cm. The leaf was kept for three days at 25^{o}C and a photoperiod of 18L:6D prior to an experiment. After that period, the vial was removed and a thin black entomological pin was inserted at distance of 1 cm from the colony border so that it was perpendicular to the leaf surface. The dish with the leaf was then placed into a climate cabinet (25°C) equipped with a circular fluorescent tube placed horizontally in the center of the cabinet to ensure an even illumination of the observation arena. After 15 minutes which was expected to allow diffusion of kairomones into the surroundings, a single, one-day starved *P. persimilis* female was released on top of the pin. Each leaf and predatory mite were used only once.

The walking path of the mite was recorded by means of the computerized video tracking system EthoVision (Noldus Information Technology 1997). Data acquisition was started manually when the mite moved down, left the pin and started to walk on the leaf surface and finished automatically when the mite left a circular zone with a diameter of 2 cm. A mean heading direction was used as a parameter determining if predator movement was random or directed towards the prey colony. Although light was supposed to have a uniform intensity in all directions thanks to the circular

tube, the position of the prey colony was switched so that it in half of the experiments was oriented towards the back of the cabinet and in the other half towards the cabinet's door.

The obtained data were analyzed by means of circular statistics (Batschelet 1981). First, the mean vector was calculated and then Rayleigh test was applied to test if direction of walk of *P. persimilis* was random or not. Bi-modality of data was tested using the method described by Fisher (1993). A sign test (Siegel & Castellan 1988) was used to reveal whether significantly more mites walked in direction of the prey patch.

Results and Discussion

1 Searching for prey-infested leaves on a plant

The predators either immediately climbed up the plant stem after their release or they walked down first and then soon turned back. In a few cases predatory mites left the plant and entered the soil substrate. Such experiments were omitted from the analysis. In 16 cases out of 30 individual experiments *P. persimilis* walked to the petiole of the infested leaf. The preference was not statistically significant (χ_1^2=0.133, *P*=0.715).

Thus, the results indicate that *P. persimilis* walking on the stem of a bean plant was unable to discriminate between infested and uninfested leaves. The reason might be that the concentration gradient of volatiles was not high enough as uninfested leaves of the spider mite-infested plant also produce predator-attracting infochemicals (Dicke *et al.* 1993). Moreover, the shape of the odor plum does not need to reflect plant morphology and/or could also be affected by the manipulation close to the plant when releasing a predatory mite. Another reason could be that a predator, when moving up the stem had relatively short time to perceive differences in odor concentration at the point of stem bifurcation. The conclusion is that we did not find any evidence of *P. persimilis* being capable of utilizing plant volatiles to improve its likelihood of discovering spider mite infested leaves within a plant.

2 Searching for a prey colony while on a leaf

The average speed of predatory mites was 0.22 cm/s (*n*=58). In most experiments predators usually left the observation zone within a short time as they moved more or less straight. In experiments in which a spider mite colony was turned to the back of the cabinet, the walking pattern of the predatory mites was significantly non-random and oriented towards areas infested with spider mites (Tab. 1, 2). Contrary to this, some predators walked to, and some of them away from spider mite colony in experiments where the spider mite colony was oriented closer to cabinet door resulting in a statistically non-significant mean vector (Tab. 1). Since the latter data indicate that the predators have a symmetric bimodal distribution, we tested the bi-modality by subtracting 180° from data points lying in the arc (180°, 360°), then doubling all the angles and finally calculating the mean vector. The mean vector of the derived data turned out to be statistically significant (Tab. 1), giving an evidence of bi-modality and suggesting that the mean direction of predatory mites is along a 0^o-180^o axis, i.e. the mites moved both towards and away from the colony.

Table 1. Results of circular analysis of directions in which females of *P. persimilis* moved on the leaf arena.

Position	Φ[a]	r[b]	Φ[c]	v[d]	n[e]	P[f]
door	180	0.158	16.365	-0.151	29	0.468
back	0	0.396	28.776	0.347	29	0.007
door[g]	0	0.324	-6.51	0.322	29	0.045

[a]The angle in which the prey colony was located; [b]The length of the mean vector; [c]Mean angle; [d]The component of the mean vector with respect to the position of a prey colony; [e]Number of experiments; [f]Significance - Rayleigh test; [g]Data derived by reversing the angles between 180° and 360°, and doubling all measurements to convert them to vectors.

The sign test revealed that significantly more mites walked in the direction of the prey patch in experiments where the patch turned to the back of the cabinet but not in the experiments where it turned to the door (Tab. 2).

Table 2. Comparison of numbers of *P. persimilis* females walked in direction either towards or away from prey patch.

Position	Towards	Away from	P[a]
door	10	19	0.136
back	22	7	0.008
door[b]	22	7	0.008

[a]Sign test; [b]Data derived by reversing the angles between 180° and 360°, and doubling all measurements to convert them to vectors.

The bioassay used in this series of experiments was much less sensitive than an olfactometer for detecting predator responses to volatile kairomones, but it better mimicked natural conditions. According to Zhang & Sanderson (1992), the odor from a single spider mite colony

may not be strong enough to stimulate a significant response as a large number of spider mite-infested leaves is required for a significant response, even in an olfactometer (Sabelis & van de Baan 1983). Despite of that, our results indicate that the search path of *P. persimilis* once on a leaf was influenced by the location of spider mite colony. There might be two mechanisms underlying predator behavior: (1) predators either utilize volatile kairomones which are produced only at prey patch and then diffused into the air or (2) kairomones are produced by the whole leaf (Dicke *et al.* 1993) but their production decreases with the distance from the patch allowing the predators to follow the concentration gradient. Nevertheless, any air turbulences or wind might strongly affect such a concentration gradient. The results further show that some other factors like e.g. the reflection of the copper wall in the climate cabinet or an uneven temperature distribution may interfere with the effects of volatiles.

We can conclude that the spider mite-induced volatiles affect the searching pattern of predatory mites once they are on the leaf, at least over a distance of 1 cm. This paper thus demonstrates that *P. persimilis* at least to some extent is able to utilize prey-associated volatiles when searching for prey within a bean plant under relatively realistic conditions and not only in the rather artificial situation when studied in an olfactometer.

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ISOLATION AND PROPERTIES OF SIX PEPTIDE ANTICOAGULANTS FROM THE EMBRYOS OF THE CAMEL TICK *HYALOMMA DROMEDARII* (ACARI: IXODIDAE)

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Abstract

Changes in the level of anticoagulants were examined during embryonic development of the camel tick: *H. dromedarii*. However, the specific activity of the anticoagulants in the eggs did not change significantly during embryogenesis. Six multiple forms of anticoagulants were isolated from 24-days-old embryos by chromatography on DEAE-cellulose column. Further purification on Sephadex G-50 column revealed the presence of six peptide anticoagulants ranged from 390 to 790 dalton. All of the six peptide anticoagulants prolonged both the prothrombin time (PT) and the activated partial thromboplastin time (APTT) of the camel plasma in concentration dependent manner indicating their inhibition of the extrinsic and intrinsic coagulation pathways respectively. The presence of NaCl increased the activity of the anticoagulants which prolonged the PT but did not affect the anticoagulants activity which prolonged the APTT. The ultraviolet absorption spectra of the six anticoagulants displayed a maximum between 200 and 220 nm proving the presence of the peptide bond. The multiplicity of these embryonic peptide anticoagulants, their possible role in biological control of tick and their therapeutic application in controlling thrombosis were discussed.

Key words

Anticoagulants, embryogenesis, Purification and characterization, Camel tick, *Hyalomma dromedarii.*

Introduction

Blood coagulation is the culmination of a series of proteolytic reactions which terminate in the thrombin- catalyzed conversion of soluble fibrinogen to an insoluble fibrin clot. Activation of the coagulation cascade through either its extrinsic or intrinsic pathways results in the formation of factor Xa (fXa) which consequently catalyzes the formation of thrombin (Neeper *et al.*, 1990). Thrombin converts fibrinogen to fibrin, which polymerizes to form the clot (Gaspar *et al.,* 1995). The inhibitor of intrinsic and extrinsic coagulation pathways prolongs the activated partial thromboplastin time (APTT) and the prothrombin time (PT) of the plasma respectively (Becker *et al.,* 1984).

The hematophagus arthropods have developed a variety of strategies to facilitate blood meal from their vertebrate hosts. Central to the success of these blood-feeders is their ability to effectively interfere with host coagulation process, thereby allowing for rapid and uninterrupted ingestion of blood from lacerated vessels in the epidermis (Cappello *et al.,* 1996).

Ticks feed for several days and will have to battle continuously against host haemostasis of the wound site (Stark & James, 1996). Inhibitors of specific steps in the blood clotting cascade (anticoagulants) have been characterized from salivary glands and whole body extracts.

In soft tick species, *Ornithodoros moubata* (Waxman *et al.,* 1990) and *Argas persicus* (Markwardt, 1994), the whole body extract presents anticoagulant activity which inhibits factor Xa. In addition, the soft tick *Ornithodoros savignyi,* is reach source of such anti-haemostatics. Two anticoagulant proteins from its salivary glands have been isolated and characterized; they are factor Xa inhibitors (Gaspar *et al.,* 1995, 1996; Joubert *et al.,* 1998) and savignin, a thrombin inhibitor (Nienaber *et al.,* 1999; Mans *et al.,* 2002). Another two inhibitors of the extrinsic blood coagulation pathway were isolated and characterized from the salivary glands of *Ornithodoros savignyi,* (Ehebauer *et al.,* 2002). In hard tick species, *Ixodes ricinus* (Hoffmann *et al.,* 1991), an anticoagulant activity has been isolated from whole body extracts and characterized as antithrombin. The nymph extract of the camel tick, *Hyalomma dromedarii* contained one factor Xa inhibitor and two thrombin inhibitors (Ibrahim *et al.,* 2001 a and b). The saliva of *Dermacentor andersoni* (Gordon & Alien, 1991) has anticoagulant activities directed against both factors V and VII. The salivary glands of the hard tick, *Rhipicephalus appendiculatus* (Limo *et al.,* 1991) and *Hyalomma truncatum* (Joubert *et al.,* 1995), showed anticoagulant activities that inhibit factor Xa. A specific inhibitor of thrombin, americanin, was isolated from the salivary glands of the lone star tick, *Amblyomma americanum* (Zhu *et al.,* 1997). From the haemolymph of the tick *Amblyomma hebraeum,* a thrombin inhibitor, amblin, was identified (Lai *et al.,* 2004). The cattle tick *Boophilus microplus* contained two thrombin inhibitors in its saliva (Ciprandi, *et al.,* 2006) and one thrombin inhibitor in its gut (Ricci *et al.,* 2007). A large variety of tick anti-haemostatics were reviewed with their properties by Maritz-Olivier *et al.,* (2007).

The eggs and unfed larvae of the cattle tick *Boophilus microplus* contain large amounts of a protein protease inhibitor exhibiting, an anticoagulant activity but its concentration falls very rapidly after the start of the parasite stage of the life cycle. The purified trypsin and chymotrypsin inhibitor prolonged both the prothrombin time (PT) and the activated partial thromboplastin time (APTT) clotting assays (Willadsen & Riding, 1979, 1980). However, the developing embryos of the camel tick, *Hyalomma dromedarii,* contain multiple forms of trypsin and chymotrypsin inhibitors (Hamed *et al.,* 1990) and the larval extract of the same tick species contains a protein trypsin and chymotrypsin inhibitor and another separable anticoagulant without overlapping of their activities (Ibrahim, 1998).

The goal of our present research project is to study the embryogenesis of the camel tick *H. dromedarii* at the molecular level. Such study will throw more light on the adaptability of the tick cells as a host for many pathogens and consequently will contribute in the selective biological control of tick by blocking biosynthesis or biodegradation during embryogenesis. Most interestingly, we observed that the tick eggs are rich in peptide anticoagulants in spite of the lack of a direct contact between the eggs and the host. Therefore, this report is the first study which represents data on the purification and characterization of various peptide anticoagulants from tick embryos.

Materials and methods

Tick material:

Engorged tick *H. dromedarii* females were collected from camels market near Cairo and held at 28ºC and 85% relative humidity. Eggs were collected daily from fertilized ovipositing female ticks and either frozen immediately (-40ºC) or incubated under the same condition until the appropriate age and transferred to frozen storage at intervals of three days (3, 6, 9 etc.).

Chemicals:

Trypsin (EC 3.4.21.4) from bovine pancreas type III 2x crystallized, Blue dextran, Cytochrome C, and Diethylaminoethyl-cellulose (DEAE-cellulose) were purchased from Sigma Chemical Co., England. Protamine sulfate was obtained from Merck. Myoglobin from horse heart and Cyanocobalamin were products of BDH Chemicals Ltd., England. Sephadex G-50 was a product of Pharmacia Fine Chemicals, Uppsala, Sweden. Cephalit Kit for activated partial thromboplastin time (APTT) and thromboplastin with calcium Kit for prothrombin time (PT) were purchased from bioMérieux, France. All other chemicals were of analytical grade.

Preparation of crude extract for the developmental profiles:

The crude extract was prepared by homogenizing 100 mg from each embryonic stage in 1 ml of 0.05M sodium phosphate buffer, pH 7.2 (1:10 w/v). The homogenate was centrifuged at 5,000 xg for 15 min at 4ºC and the supernatant was saved and designated crude extract.

Preparation of camel plasma

Plasma was obtained by centrifugation of a mixture of 900 ml camel blood and 100 ml of 0.11

M trisodium citrate solution at 2700 xg for 15 min. at 4°C. If the plasma was not used immediately, it was dispended into Eppendorf tubes and stored at -20°C.

Bio-assay for the inhibition of the intrinsic blood coagulation pathway

The activated partial thromboplastin time (APTT) measures the clotting time of a plasma at 37°C in the presence of a platelet substitute and an activator. This overall test evaluates the entire intrinsic pathway with the exception of the platelet factors (Becker *et al,* 1984). 50 µl sample were added to 50 µl of the camel plasma and incubated for 3 minutes at 37°C. Cephalite APTT from bioMérieux (50 µl*)* were added and the mixture was incubated for another 3 minutes at 37°C. Finally, 50 µl of 0.025 M $CaCl_2$ (pre-warmed at 37°C) were added and the clotting time recorded. For determination of the control time, the experiment was performed by using 0.05M sodium phosphate buffer, pH 7.2 (50 µl*)* instead of tick extracts (Gaspar *et al.,* 1995).

Bio-assay for the inhibition of the extrinsic blood coagulation pathway

Prothrombin time (PT) studies the total extrinsic clotting system. It measures the clotting time of a plasma at 37°C in the presence of excess tissue thromboplastin and calcium **(**Becker *et al.,* 1984). 50 µl sample were incubated with 50 µl of camel plasma for 6 minutes at 37°C. 100 µl of calcium-thromboplastin reagent from bioMérieux (pre-warmed at 37°C), were added to determine clotting time (Gaspar *et al.,* 1995). One unit of anticoagulant activity (APTT or PT) was defined as the amount of the tick extract capable of prolonging the clotting time of 50 µl of citrated camel plasma by one fold over that of control samples.

Purification of H. dromedarii embryonic anticoagulants:

Unless otherwise stated all steps were performed at 4 – 7°C.

Preparation of crude extract:

The crude extract was prepared by homogenizing 2 g egg of 24-days-old in 20 ml of 0.05M sodium phosphate buffer, pH 7.2 (1:10 w/v). The homogenate was centrifuged at 5,000 xg for 15 min at 4°C and the supernatant was saved and designated crude extract.

Chromatography on DEAE-cellulose column:

The crude egg extract was applied on the top of DEAE-cellulose column (65 x 2.6 cm) previously equilibrated with 0.05 M sodium phosphate buffer pH 7.2. The protein fractions were eluted with stepwise NaCl gradient ranging from 0 to 0.5 M prepared in the equilibration buffer. 10 ml fractions were collected at a flow rate of 60 ml / h. The fractions of each peak exhibiting anticoagulant activity were pooled separately and lyophilized.

Chromatography on Sephadex G-50 column:

The lyophilized material obtained from each peak was dissolved in H_2O and applied on the top of Sephadex G-50 column (92 x 1.6 cm) previously equilibrated with 0.05 M sodium phosphate buffer, pH 7.2 containing 0.02 % sodium azide (NaN_3). The proteins were eluted with the same buffer at a flow rate of 20 ml / h. Fractions of 3 ml were collected.

Molecular weight determination:

Sephadex G-50 column was used for molecular weight determination of *H. dromedarii* embryonic anticoagulants according to the method of Andrews (1964). The above described Sephadex G-50 column was calibrated with blue dextran (2000 KDa), carbonic anhydrase (29 kDa), trypsin (23.3 kDa), myoglobin (17.2 kDa), cytochrome C (12.4 kDa), protamine sulfate (5.1 kDa) and cyanocobalamin (1.355 kDa).

Protein determination:

Protein was determined by the dye-binding protein assay method of Bradford (1976). Bovine serum albumin was used as standard protein.

Statistical analysis:

The statistical analyses were performed by using Student *t*-test (Bailey, 1997).

Results

1- Changes in the level of anticoagulants during embryogenesis of the camel tick H. dromedarii.

The changes in the specific activity of the anticoagulants which prolong the activated partial thromboplastin time (APTT) and the prothrombin time (PT) of the camel plasma during the embryonic development are recorded (Fig. 1). All differences in the specific activity of the anticoagulant between the different embryonic stages statistically are insignificant.

2- Purification of anticoagulants from the developing embryos of the camel tick H. dromedarii.

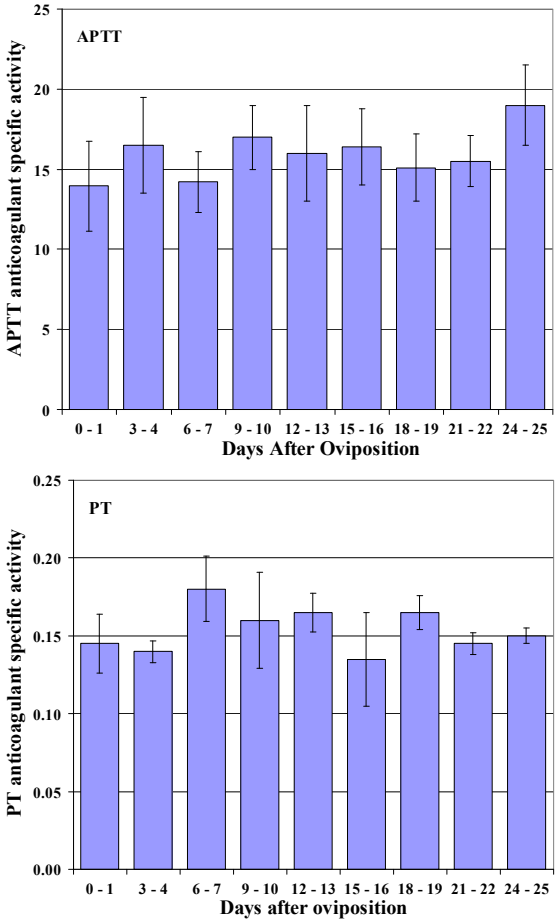

Figure 1. Changes in the anticoagulants specific activity which prolong the activated partial thromboplastin time (APTT) and the prothrombin time (PT) of the camel plasma during the embryonic development of the camel tick H. dromedarii. Each point represents the mean of at least 5 runs for each developmental stage ± S.E.

The purification procedure involved chromatography on DEAE-cellulose column (Fig. 2) and gel filtration on sephadex G-50 column (Fig. 3). The chromatography of crude extract on DEAE-cellulose column (Fig. 2) revealed the presence of six peaks of proteins showing anticoagulant activities and designated F1, F2, F3, F4, F5, and F6. Each fraction was pooled, lyophilized and chromatographed on sephadex G-50 column (Fig. 3). For each fraction one major active peak was resolved on the sephadex G-50 column and also designated F1, F2, F3, F4, F5, and F6. A typical example for the purification steps of tick embryonic anticoagulants from 24-days-old eggs is given in table (1). The increase in the total units of the anticoagulant which prolongs the PT and eluted from the DEAE-cellulose column in comparison to the starting PT units in the crude egg extract led to study the effect of NaCl concentration on the PT anticoagulant activity.

3- Effect of NaCl concentration on the PT anticoagulant activity:

The effect of NaCl on the crude anticoagulant activity which prolongs the prothrombin time (PT) is shown in table (2). A proportional increase in the PT anticoagulant activity was observed with the NaCl concentration up to 0.5 M which increased the activity 10 fold.

4- Molecular weight of the anticoagulants:

The molecular weights of the different APTT and PT anticoagulant activity peaks are summarized in table (3). The molecular weights of the anticoagulants were calculated from their elution volumes from the sephadex G-50 column and its calibration curve (Fig. 4). The void volume (Vo) of the sephadex G-50 column was found to be 85 ml as determined by blue dextran (2000 KDa).

5- Spectral analysis:

The ultraviolet absorption spectra of the pooled six peptide anticoagulant fractions are similar (Fig. 5). They displayed a maximum between 200 and 220 nm which is characteristic of peptide bond.

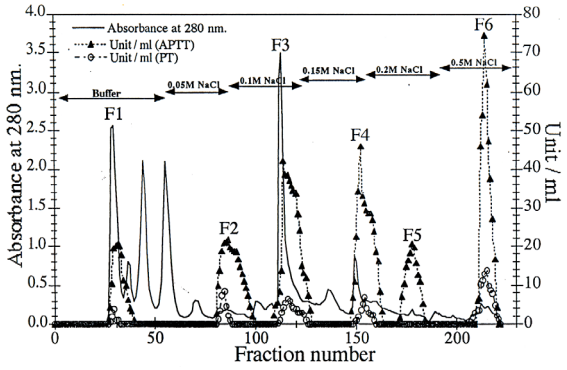

Figure 2. A typical elution profile for the chromatography of the crude extract of 24-days-old eggs of the camel tick *H. dromedarii* on DEAE-cellulose column (65 x 2.6 cm i.d.) as described under the materials and methods section. The anticoagulant prolongs the prothrombin time (PT) and the activated partial thromboplastin time (APTT) of the camel plasma and expressed as unit / ml.

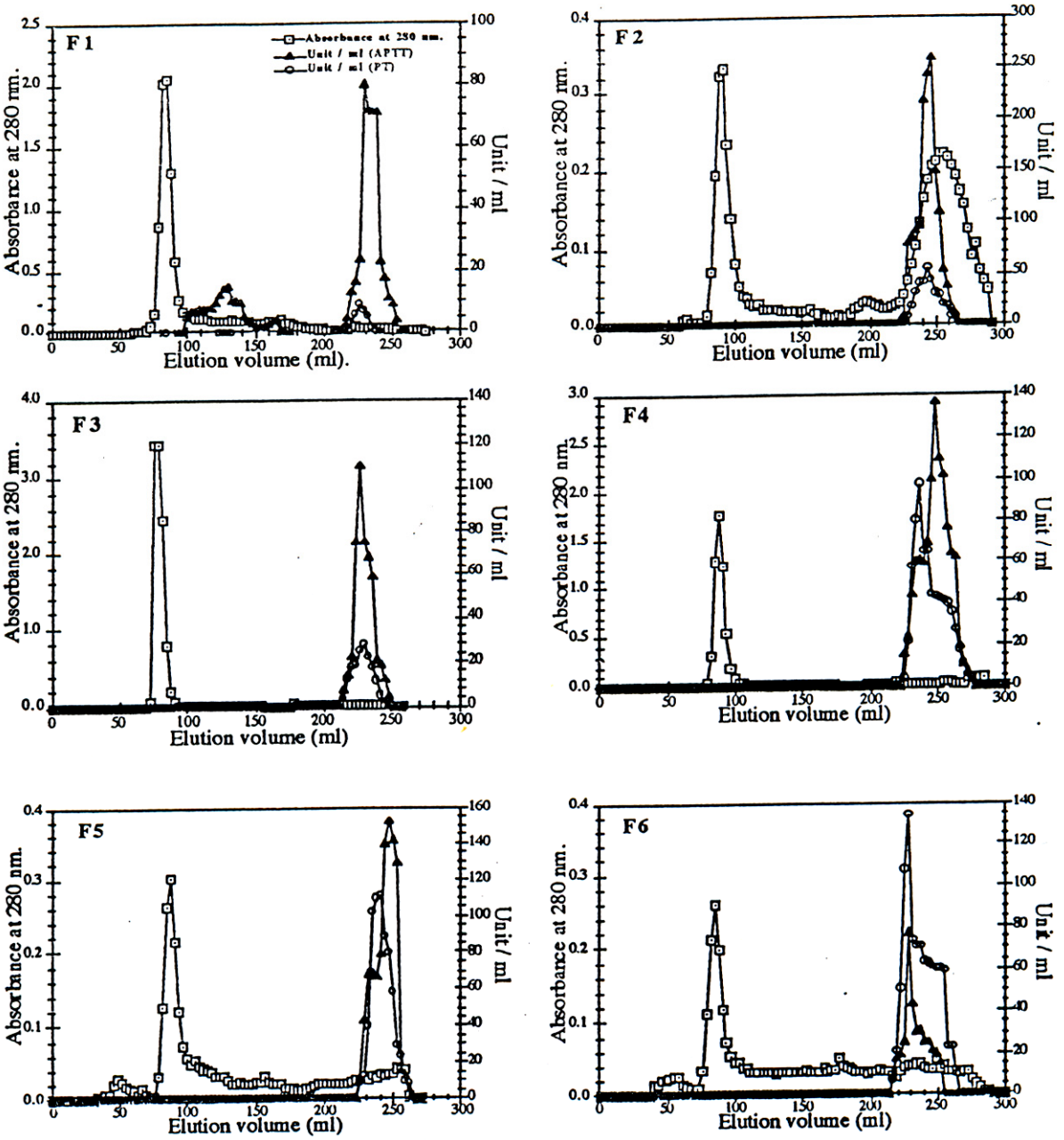

Figure 3. A typical elution profile for the chromatography of concentrated pooled DEAE-cellulose fractions of anticoagulants F1, F2, F3, F4, F5 and F6 on Sephadex G-50 column (92 x 1.6 cm i.d.) as described under the materials and methods section. The anticoagulant prolongs the prothrombin time (PT) and the activated partial thromboplastin time (APTT) of the camel plasma and expressed as unit / ml. The void volume is 85 ml as determined blue dextran (2000 kDa).

Table 1. Purification scheme of the anticoagulants from tick *H. dromedarii* embryo.

Fraction	Protein (mg)	Activated Partial Thromboplastin Time (APTT				Prothrombin Time (PT)			
		APTT (unit)*	Specific Activity	Recovery %	Fold Purification	PT (unit)*	Specific Activity	Recovery %	Fold Purification
Crude Extract	456.4	7998	17.5	100	1	79	0.173	100	1
DEAE-Cellulose Fractions:									
(1) –ve adsorbed	53.5	1561	29.2	19.5	1.67	54	1.0	68.3	5.78
(2) 0.05 M NaCl	9.5	1951	205.4	24.4	11.74	497	52.3	629.1	302.31
(3) 0.10 M NaCl	88.5	4117	46.4	51.5	2.65	957	10.8	1211.4	62.43
(4) 0.15 M NaCl	27.8	1530	55.0	19.1	3.14	503	18.1	636.7	104.62
(5) 0.20 M NaCl	7.2	1844	256.1	23.0	14.63	0	0	0	0
(6) 0. 50 M NaCl	8.7	4920	565.5	61.5	32.31	1473	169.3	1864.6	978.67
Sephadex G-50 Fractions:									
(1) –ve adsorbed	0.56	1442	2575.0	18.0	147.14	125	223.4	158.2	1255.06
(2) 0.05 M NaCl	0.42	2117	5040.5	26.5	288.03	1444	3438.1	1827.8	19873.41
(3) 0.10 M NaCl	0.98	2536	2587.8	31.7	147.87	1704	1738.8	2157.0	10050.87
(4) 0.15 M NaCl	0.31	1382	4458.1	17.3	254.75	2934	9464.5	3713.9	54708.10
(5) 0.20 M NaCl	0.30	2001	6670.0	25.0	381.14	2093	6976.7	2649.4	40327.75
(6) 0. 50 M NaCl	0.30	3051	10170.0	38.1	581.14	2188	7293.3	2769.6	42157.80

*One unit of anticoagulant activity (APTT or PT) was defined as the amount of the egg extract capable of prolonging the clotting time of 50 µl of citrated camel plasma by one fold above that of control samples.

Table 2. Activation of the crude anticoagulant which prolongs the prothrombin time (PT) of the camel plasma by NaCl increasing concentration.

NaCl Concentration (M)	Relative activity of the PT anticoagulant
0.00 M	100.0
0.05 M	103.9
0.10 M	133.0
0.15 M	192.2
0.20 M	234.0
0.50 M	1052.6

Table 3. The molecular weight of anticoagulant fractions which prolong both the APTT and PT of the camel plasma according to their elution volume from the sephadex G-50 column.

Anticoagulant fraction	Elution volume (ml)		Molecular weight (Dalton)	
	APTT	PT	APTT	PT
-ve Adsorbed (F1)	225	225	790	790
0.05 M NaCl (F2)	243	243	460	460
0.10 M NaCl (F3)	225	225	790	790
0.15 M NaCl (F4)	249	237	390	550
0.20 M NaCl (F5)	246	240	420	510
0.50 M NaCl (F6)	228	228	720	720

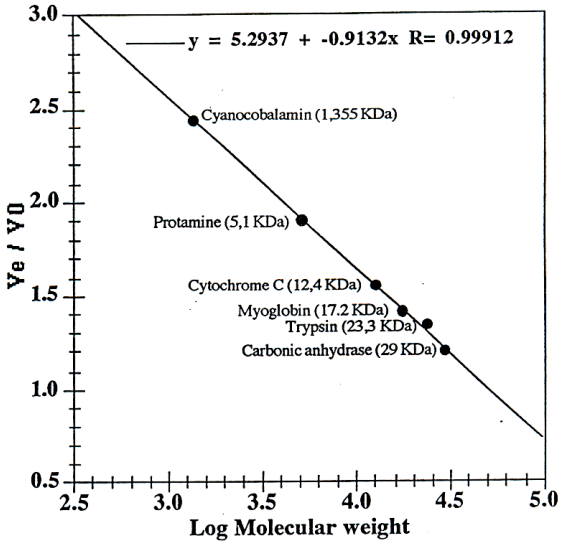

Figure 4. Calibration curve for determination of the molecular weights by gel filtration on Sephadex G-50 column (92 x 1.6 cm i.d.) as described in materials and methods section. The void volume was determined with blue dextran (2000 kDa) to be 85 ml.

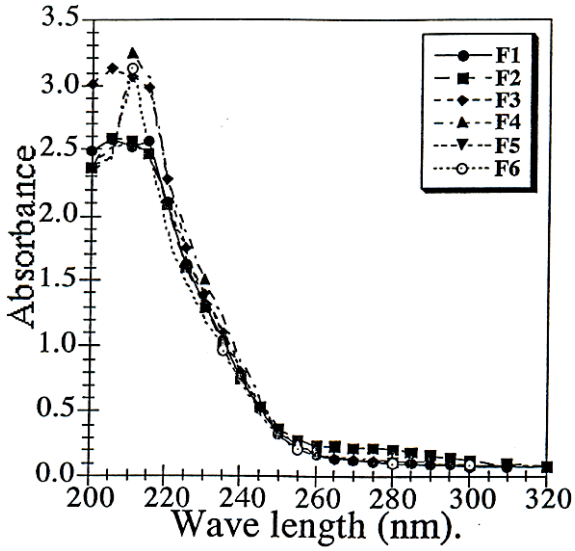

Figure 5. The ultraviolet absorption spectra of the purified anticoagulants F1, F2, F3, F4, F5, and F6 from tick; *H. dromedarii* embryos in 0.05 M sodium phosphate buffer pH 7.2 containing 0.02% NaN3.

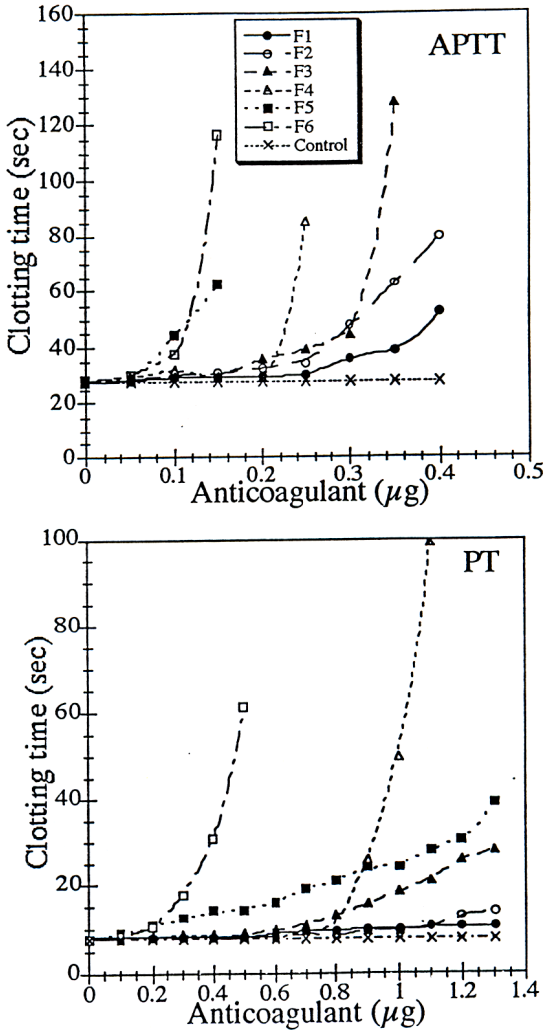

Figure 6. The activated partial thromboplastin time (APTT) and prothrombin time (PT) of the camel plasma in the presence of various concentrations of the purified six anticoagulant peptides from embryos of the camel tick *H. dromedarii*. The control is the clotting time of the camel plasma in absence of anticoagulant.

6- Effect of the anticoagulants concentration on the intrinsic and extrinsic coagulation pathways:

In order to determine the potency of the anticoagulants, various amounts of the six anticoagulants purified from the tick embryos were assayed for inhibition of the intrinsic and extrinsic coagulation pathways (Fig. 6) which prolong the APTT and PT of the camel plasma respectively. All of the six anticoagulants prolonged both the APTT and PT in a concentration dependent manner. The anticoagulant peptide F6 was found to be the most potent inhibitor of the intrinsic (APTT) and extrinsic

(PT) coagulation pathways. The maximum concentration of F6 per assay to prevent the coagulation completely was 0.2 µg protein for the intrinsic and 0.6 µg protein for the extrinsic pathway.

Discussion

It is well established that the total protein content of *H. dromedarii* eggs did not change significantly during embryogenesis (Kamel *et al.,* 1982). In the present study, the changes in the anticoagulant specific activity (Fig. 1) are presented which remained more or less constant during the embryonic development of the camel tick. This result suggests that the anticoagulants represent a fixed amount of the total embryonic proteins even in the just oviposited eggs coming from the maternal origin and their synthesis go on a parallel manner with the cell division.

Starting the purification procedure by chromatography of crude egg extract on the anion-exchanger DEAE-cellulose column (Fig. 2) was a successful step since the six peptide anticoagulants were resolved. The gel filtration on sephadex G-50 column (Fig. 3) was useful for the estimation of the very low molecular weights of the six peptide anticoagulants ranging from 390 to 790 dalton indicating that the purified peptides composed of either tripeptide or hexapeptide.

The obvious increase in the total units of the PT anticoagulant (Table 1) suggested the the NaCl used for elution of the protein fractions from DEAE-cellulose column may activate the PT anticoagulant. The activation effect of NaCl on the PT anticoagulant (Table 2) confirmed the previous postulation. However, the action of NaCl may be interpreted to result from the dissociation of the peptides anticoagulant from larger molecules.

Also, the chromatography on sephadex G-50 column separated the peptide anticoagulants from larger molecules which may bind to the anticoagulant and reduce its activity before their resolution on the sephadex G-50 column.

The lowest peptide anticoagulant (3530 dalton) was identified in the saliva of the tsetse fly (Cappello *et al.,* 1996) and tick anticoagulant peptide (TAP) (6 KDa) has been purified from the whole body extract of tick; *Ornithodoros moubata* (Waxman *et al.,* 1990). The tick embryos in the present study proved to have small peptide anticoagulants with molecular weights less than 1000 dalton.

The purity of the six anticoagulant fractions by polyacrylamide gel electrophoresis were not performed since their molecular weights are very low. As a task for the future, the purity and the identity of the anticoagulants could be verified by N-terminal amino acid sequence.

The multiplicity of the anticoagulants may be due to one of three possibilities; firstly these multiple forms of anticoagulants may be degradation products of a larger protein(s) but the presence of a large amount of protease inhibitors in the tick eggs (Hamed *et al.,* 1990) omits this suggestion. Secondly, these multiple forms of anticoagulants may be precursors synthesized during the embryonic stage for a larger one needed to play its role after hatching in other stages. This approach will be investigated by matching the sequence of the anticoagulants from the different developmental stages of the tick life cycle. Thirdly, these multiple forms of anticoagulants may be independent inhibitors of different blood coagulation factors and tissue specific such as the salivary gland and the gut. Whatever, further studies will define the target blood coagulation factor of each anticoagulant.

As a method of tick control, it is thus possible that antibodies directed to one or more of these anticoagulants will block the successful feeding of ticks. On the other hand, the potency and specificity of the tick anticoagulant peptide (TAP) in the inhibition of factor Xa, suggests that it may be effective in the treatment of thrombosis (Neeper *et al.,* 1990; Waxman *et al.,* 1990; Vlasuk *et al.,* 1991).

Maritz-Olivier *et al.,* (2007) reported that; tick anti-haemostatics could enhance our understanding of host coagulation as well as tick physiology. Even if tick derived anticoagulants are never approved for clinical use, any structural and functional information obtained from investigating these inhibitors will aid in the design of either synthetic peptides or peptidomimetics that might be developed further as novel therapeutics.

In this report, the anticoagulant peptide F6 was found to be the most potent inhibitor of the intrinsic (APTT) and extrinsic (PT) coagulation pathways. The minimum concentration of F6 per assay to prevent the coagulation completely was 0.2 µg for the intrinsic and 0.6 µg for the extrinsic pathway (Fig. 6), it may be a reasonable target for the development of small peptide, novel and potent anticoagulant for therapeutic applications.

In conclusion, this is the first study dealing with the anticoagulant activity in the tick eggs. The results of this study will be very helpful in two main

trends: (1) tick control and (2) therapeutic purposes of thrombosis. Firstly, as a method of tick control, it is thus possible that antibodies directed to one or more of these anticoagulants will block the successful feeding of ticks and consequently deprive the tick of the blood meal. Secondly, the study of the potency and specificity of the tick anticoagulants in the inhibition of blood coagulation particularly these small peptides and the definition of their primary structure will lead to the production of small anticoagulant effective in the treatment of thrombosis.

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REDUCING PASTURE INFESTATION BY *AMBLYOMMA VARIEGATUM* ADULTS THROUGH HERD MANAGEMENT DURING THE NYMPH INFESTATION PERIOD

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2. CIRDES, UR URBIO, Bobo-Dioulasso, Burkina Faso

Abstract

Amblyomma variegatum is the most harmful tick species of West African ruminants in particular because of the direct damage caused by the adult ticks, which attach during the rainy season. The distribution of adult ticks on pasture depends on the very heterogeneous drop off rhythm of engorged nymphs (80% detach between 14h30 and 17h00) and on suitability of the grazed pasture for tick survival. As nymphs infest their hosts during the dry season, the distribution of adult ticks may be influenced by herd management during this period. This possibility was assessed through a trial involving a herd grazing 4 different plots. During the early dry season, 2 of the plots were grazed in the morning and the 2 others in the afternoon. Six months later, Gudali zebus grazed these 4 plots alternately. The number of ticks picked up on each plot was highly variable, cattle capturing 3-fold more *A. variegatum* adults (1,040 vs 350) on the 2 pastures where the herd grazed in the afternoon during the dry season. The possibility of reducing pasture infestation using cost-free herd management is discussed.

Key-words

Amblyomma variegatum, tick control, drop off rhythm, cattle, Burkina Faso

Introduction

The life-cycle of *A. variegatum*, probably the most harmful tick species in West and Central Africa, has a very high seasonality in regions with tropical climate and one annual rainy season (Petney *et al.*, 1987). The adults infest their hosts mainly at the beginning of the rainy season, the larvae attach late in rainy season and the nymphs engorge during the dry season. Parasite loads of each stage peak 4 to 6 weeks after the onset of the infestation period.

Since engorged nymphs do not move over long distances to find a suitable place for their survival and development once they have detached, the distribution of unfed adults is determined by the spatial distribution of the hosts during the nymphal infestation period and by the drop off rhythm of the engorged nymphs (Minshull & Norval, 1982). Understanding the natural detachment rhythm of a tick species may thus lead to measures which could be included in an integrated tick control strategy. For example, Minshull (1982) considered that "advantages could be taken of the well defined drop off patterns exhibited by all three stages of *Rhipicephalus appendiculatus*" and proposed to delay the morning movement of cattle to the pastures since most engorged females detach early in the morning.

The drop off rhythm of *A. variegatum* nymphs has been studied in Guadeloupe by Barré (1989) who found that most of them detached in the morning.

On the other hand, Rechav (1978) observed that nymphs of *Amblyomma hebraeum*, whose biology is very close to that of *A. variegatum*, dropped off mainly in the late afternoon or shortly after dusk. Because these very divergent findings have very different implications, a study was carried out in Burkina Faso in order to identify the drop off rhythm of *A. variegatum* nymphs naturally attached to their cattle hosts. In light of these results, an experiment was then implemented to assess whether it was possible to reduce the number of *A. variegatum* adults in the grazed pastures.

Materials and methods

Experimental site and animals

The study to determine the natural detachment rhythm of engorged *A. variegatum* nymphs was carried out in two herds (A & C) belonging to Fulani cattle breeders settled for several decades in Tondogosso (11,10°N, 4,15°W) and Borodougou (11,15°N, 4,18°W), two villages located 13 and 15 km East from Bobo-Dioulasso. The monitored herds comprised respectively 50 and 65 head of cattle (mix of Baoulé, Zebus and cross-breeds). The animals involved in the study were chosen because they were quiet and easy to handle.

The experiment to assess whether herd management in the dry season could influence adult tick density on pasturelands was carried out on a 10 ha farm, belonging to Mr T. and located 8 km west of Bobo-Dioulasso (11,19°N, 4,39°W). The herd comprised 38 Gudali zebus which grazed on community pastures, in addition to the limited land of Mr T.

The tropical climate in these 2 areas is characterised by a mean annual rainfall of 1,050 mm. The rainy season generally occurs from mid-May to early-October (Diallo *et al.*, 1998). The natural vegetation is woody and bushy savannah.

Experimental designs

A. Nymph drop off rhythm. D During 4 weeks in November-December 1999, 4 animals (except during the last week in which only 3 animals were monitored because the infestation was too high and the time needed to record the ticks was therefore too long) chosen in herd A (weeks 1 and 3) and in herd C (weeks 2 and 4) were monitored over 4 successive days (from Monday morning to Friday morning) according to the following protocol. When the animals were examined for the first time, clusters of attached ticks were delimited by lines drawn on the skin with a marker pen (fig. 1) which allowed the establishment of a precise diagram of the distribution of the nymphs on the host. Nymph engorgement and detachment were then assessed on 6 occasions each day. Every morning, a 10 metre-long rope was tied around the horns of the selected animals which allowed their capture and immobilization in the pastures. The first tick count took place before the herd left the night paddock, between 9h30 and 10h45 (at 10h05 on average), and the last count was made just after the herd came back from pasture (between 18h00 and 19h00, at 18h20 on average), generally by lamplight. In the meantime, the animals were

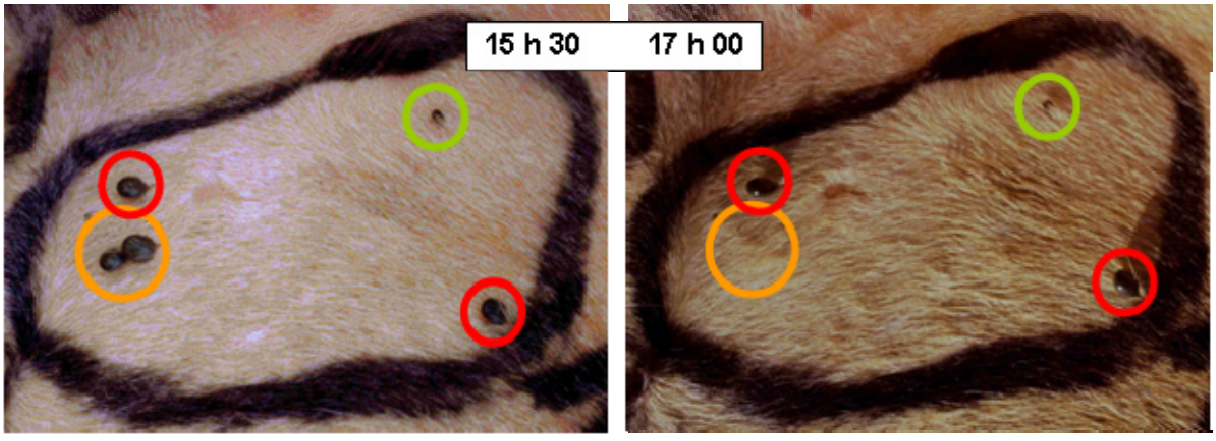

Figure 1: *Amblyomma variegatum* nymph engorgement and drop off survey. In this cluster attached to the chest and surrounded by a line drawn in marker pen, 2 nymphs (orange circle) were seen engorged at 15h30 and dropped off between 15h30 and 17h00; 2 others (red circles) dropped between 17h00 and 18h30, when the animal was checked again once back in the night pen; and the last nymph (green circle) engorged 2 days later.

caught and examined 4 times, at 11h00, 14h00, 15h30 and 17h00. Each change (observation of a new tick, a tick starting the final engorgement phase, detachment of an engorged nymph, disappearance of a nymph before engorgement) was immediately marked on the diagram. Nine cross-breeds animals in all were involved in this study: 3 in each herd were observed during 2 weeks whereas 3 others (2 in herd A and 1 in herd C) were monitored only during 1 week.

B. Alteration of adult tick density on pastureland. In the Bobo-Dioulasso region, almost all livestock owners use community pasture. It was very difficult to identify a farmer owning an area of land where fences could be built. Mr T.'s land was not very wide, half of it was covered by maize and sorghum fields, and the vegetation was very heterogeneous with regard to the available biomass and the presence of potential micro-habitats for ticks: small area of woody lush savannah and large areas of bushy and stony savannah or fallow land (fig. 2). Four plots were delimited on the land with wire fences erected in order to delimit 2 groups of plots of irregular shape, but with similar size and vegetation in each group. P1 and P2 plots (1.05 ha size), partly installed on lush pasture, were regarded as more suitable for tick survival than P3 and P4 (1.5 ha size) placed on poor fallow land and stony/ bushy savannah. P1 and P4 were randomly selected to be grazed by the herd in the morning, generally from 8h30 to 11h30, whereas P2 and P3 were selected to be grazed in the afternoon, between 15h00 and 18h00.

The plots were used alternately, the herd also foraging on the communal pastures, where cattle could drink from a river, in between times (from 11h30 to 15h00) and every other day. On day 1, P1 and P2 were used; on days 2 and 4, the herd grazed only community pasture; on day 3, cattle were brought on P3 and P4; on day 5, they grazed again on P1 and P2; etc. Each plot was consequently used once every 4 days between 01/12/2003 and 12/01/2004. This first part of the study was then stopped because tick infestation levels on the cattle had become too low, and because there was no more grass on the plots which remained ungrazed until the next rainy season. During the last 3 weeks of this first phase, it was necessary to give fodder to the cattle to offset the lack of grass. This was distributed in different parts of the plots to prompt the cattle to walk all over the enclosed area.

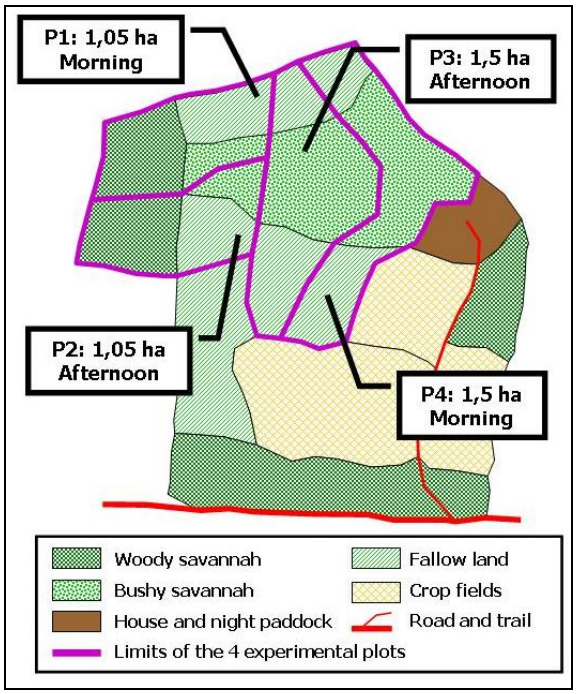

Figure 2: Map of the plots used during the study carried out in 2003/2004 to assess the influence of the grazing period on pasture tick density. "Morning" and "afternoon" refer to the period during which the herd grazed the plots during the dry season

Between 26/05/04 and 02/07/04, the 4 plots were grazed in turn, each one being used on 9 occasions. The Gudali zebus grazed plot P2 on day 1, plot P1 on day 2, plot P4 on day 3, plot P3 on day 4, and were brought again into plot P2 on day 5 (the grazing order was randomly determined). The 4 day period during which cattle successively grazed the 4 plots is designated as a "passage" in the results section. Cattle spent an average of 8.2 hours per day on the plots before being brought back into the night pen. During the first 28 days, the group consisted of the same 4 Gudali zebus; 2 other Gudali zebus were added for the 8 last grazing days. The study was stopped when it appeared that almost all ticks had been picked up from the pastures, and when the fodder had almost completely disappeared.

During the dry season, herd infestation was assessed once a week through tick counts on 5 randomly chosen animals. During the rainy season, the 4 (or 6) animals were examined twice daily, before and after each grazing session. All the attached ticks were removed in the morning, before the animals were brought onto the plots; and all ticks captured on the pasture were counted and removed in the evening when the cattle returned from the plots.

Results

A. Nymph drop off rhythm

During the 16-day study, 1,213 of the observed nymphs disappeared from the cattle. Sixty-four of them (5%) were not seen to be engorged or semi-engorged before they disappeared. Most of these ticks (53 of 64) disappeared during the night, when the animals were lying down in their night paddock. Although the detachment rhythm pattern was rather similar for all the monitored cattle, there were significant differences among hosts regarding the timing of tick detachment, particularly in herd C (fig. 3): Herd A - Chi^2 statistic = 16.5 ($p < 0.05$), Herd C - Chi^2 statistic = 43.6 ($p < 0.001$); tests calculated without the 10h00 to 11h00 time period in which no nymph dropped off.

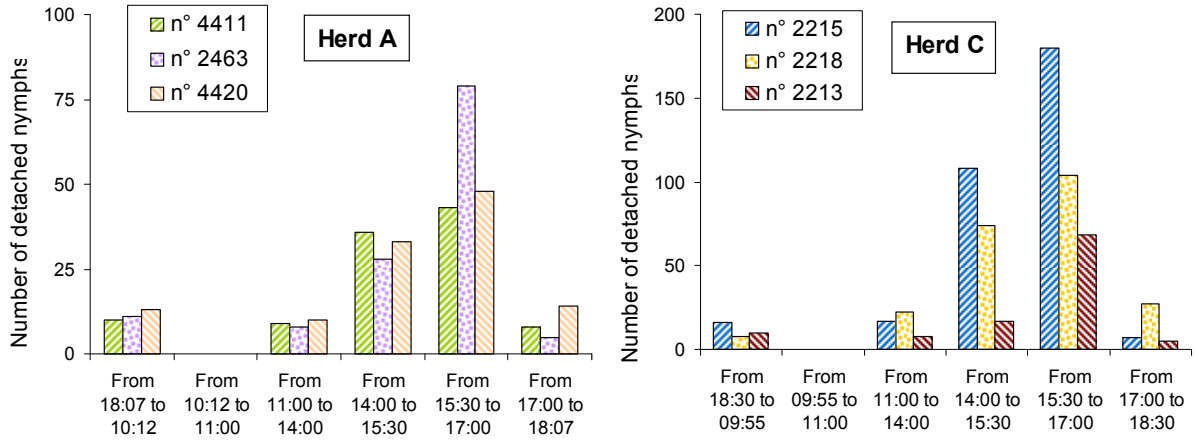

Figure 3: Patten of *Amblyomma variegatum* nymph drop off according to the host (only the 6 cattle monitored during 8 days are represented). The numbers represent the total over the 8-day period.

Detachment rhythm also varied over days (for example, week 4 - Chi^2 statistic = 50.0 ($p < 0.05$). On some days, more nymphs detached between 14h00 and 15h30 although most of them generally detached between 15h30 and 17h00 (fig. 4).

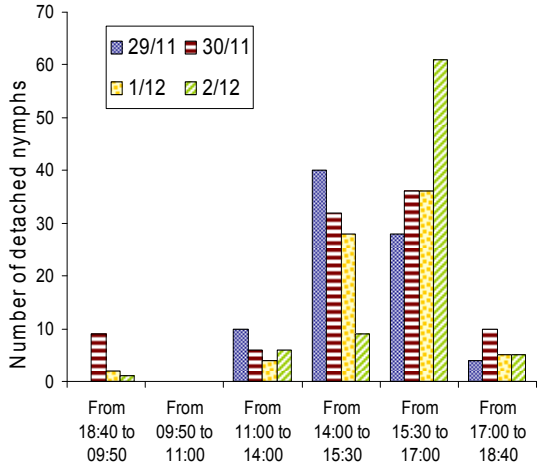

Figure 4: Pattern of *Amblyomma variegatum* nymph drop off according to the time of day during the 4th week of the study.

When the data from the two herds were merged into a single 24-hour period, the nymphal drop off pattern appeared very heterogeneous, 50% of the nymphs detaching between 15h30 and 17h00 and 29% between 14h00 and 15h30 (fig. 5). The respective percentages were 52% and 30% when only the ticks previously seen engorged or semi-engorged were taken into account. Only 6% of the nymphs dropped off in the night pens, where the cattle remained for 15,75 hours daily on average (i.e. 65% of the day, from 18h20 to 10h05), and this percentage was reduced to 2% if only the ticks previously seen engorged were considered.

B. Monitoring of herd T during the dry season

Due to the time needed to install the fences, the plots could not be used from the onset of the nymph infestation period, which starts mid-October. The first phase of the study therefore began during the infestation peak, which occurs between mid-November and mid-December. As the herd grazed also community pastures, the cattle were regularly infested by *A. variegatum* nymphs there.

Each plot was grazed on 11 occasions between 01/12/2003 and 12/01/2004. The mean infestation level of the Gudali zebus varied from 65 to 15 nymphs per animal during the course of the study, with a peak of 82 ticks on 09/12 (fig. 6). Mean

infestation for each day of the study period was assessed assuming that the change between two successive tick counts was linear. Because the herd comprised 38 animals and considering an average feeding period of 6.15 days for *A. variegatum* nymphs (Stachurski, 2000b), it was calculated that about 13,360 nymphs detached from the cattle during the study period, and 6,800 during the days when the herd grazed the experimental plots.

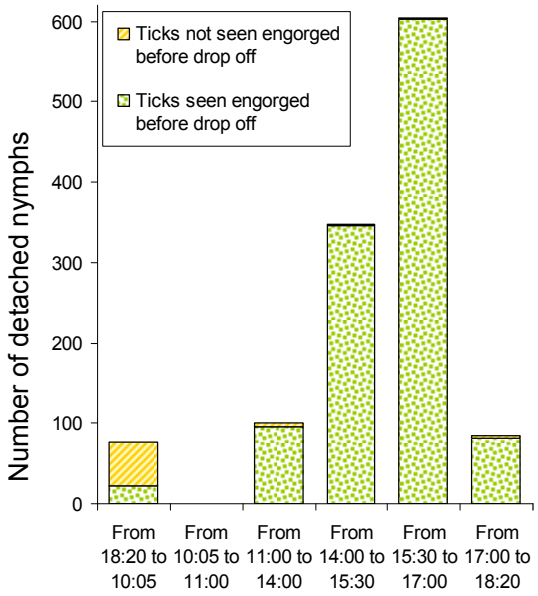

Figure 5: *Amblyomma variegatum* nymph drop off pattern for the 1,213 monitored ticks.

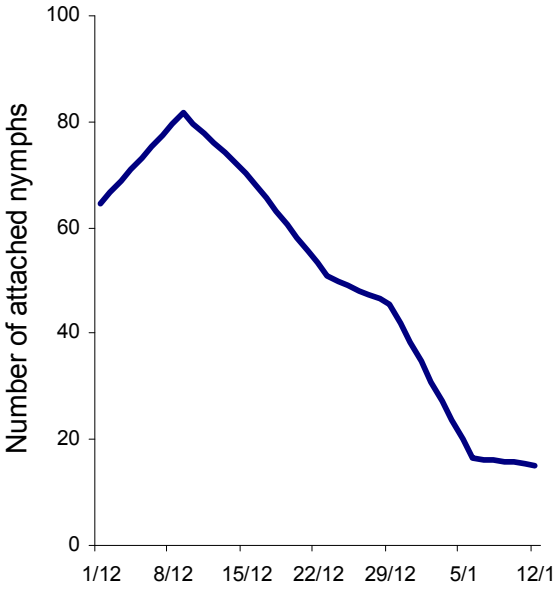

Figure 6: Mean number of *Amblyomma variegatum* nymphs attached to cattle in herd T during the dry season (first phase of the "infestation management" study).

C. Capture of A. variegatum adults during the rainy season

When the second phase of the study started, adult *A. variegatum* had already been active for a few weeks. The 4 Gudali zebus chosen to be monitored harboured in total 89 ticks of this species (69 males, 19 females and 1 nymph), as well as other ticks (108 *Hyalomma spp.*; 2 *Rhipicephalus spp.*; and 1 engorged female *Boophilus sp.*). All were manually removed on the morning of 26/05/04. On the next 35 mornings of this phase, a total of 45 male (of which 4 were found attached to the inter-digital areas) and 3 female *A. variegatum* were removed from the 4 monitored animals, as well as ticks of the other genera (*Hyalomma*, *Rhipicephalus* and *Boophilus*).

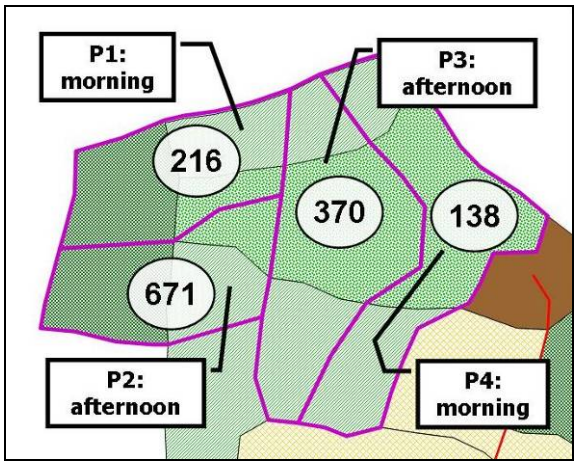

Figure 7: Number of *Amblyomma variegatum* adults captured in the 4 experimental plots by the 4 (or 6) monitored cattle during the 36 days of the second phase of the "infestation management" study. "Morning" and "afternoon" refer to the period during which the herd grazed the plots during the first phase of the study.

During the 36 days of the study (9 passages in each plot), the animals picked up 1,395 adult *A. variegatum* in the fenced plots: 216 in plot P1, 671 in P2, 370 in P3 and 138 in P4 (fig. 7). The total number of ticks captured by the 4 zebus during a passage dropped from 313 for the first passage to 75 for the 7th. It slightly increased to 96 and 94 ticks during the 2 final passages, when 2 more zebus were added to the 4 already involved. In each plot, the decrease in the number of captured ticks was roughly constant but sometimes irregular

(fig. 8). Some of the observed irregularities could be linked to climatic features. The decreases in plot P1 on passage 2, and in plot P3 on passage 4, occurred for example when it rained heavily for 2-3 hours in the morning. However, such an event was not recorded for the reduced captures noted in P4 on 4th passage and in P3 on the 3rd and 5th passages.

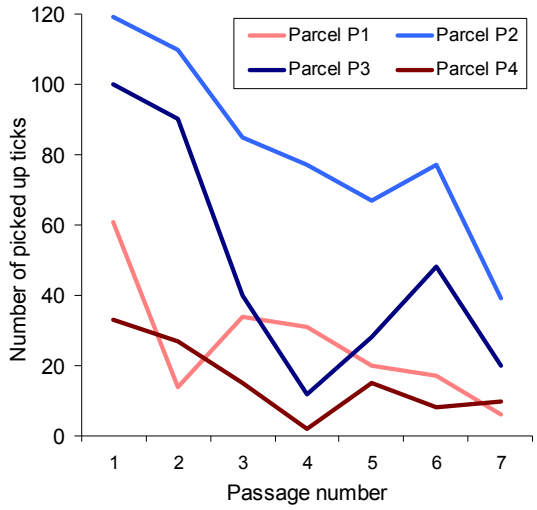

Figure 8: Total number of *Amblyomma variegatum* adults captured in the 4 experimental plots by the 4 monitored cattle during the 7 first passages of the second phase of the "infestation management" study.

More males (854) than females (541) were picked up, but the sex ratio changed dramatically during the course of the experiment: 71% of the captured ticks were males during the first 3 weeks, but only 40% during the last 3 weeks. Eighty-nine percent of these ticks (between 80% and 93% depending on the plot) were found attached to the inter-digital areas (fig. 9).The vegetation in the grazed plots influenced the number of adults picked up: although P3 and P4 were 50% larger than their counterparts used in the same half-day during the previous dry season, the zebus captured less ticks in these plots than in P1 (57% more than in P4) or in P2 (81% more than in P3). When the plots with the same size and surface were compared, it appeared that the density of the ticks in the plots used in the afternoon during the previous dry season was higher than that observed in the plots grazed in the morning, where the cattle picked up nearly 3-fold fewer adult *A. variegatum* (3.1-fold fewer for P1 compared to P2, $Chi^2 = 233$, $p < 0.001$; 2.7-fold fewer for P4 compared to P3, $Chi^2 = 105$, $p < 0.001$).

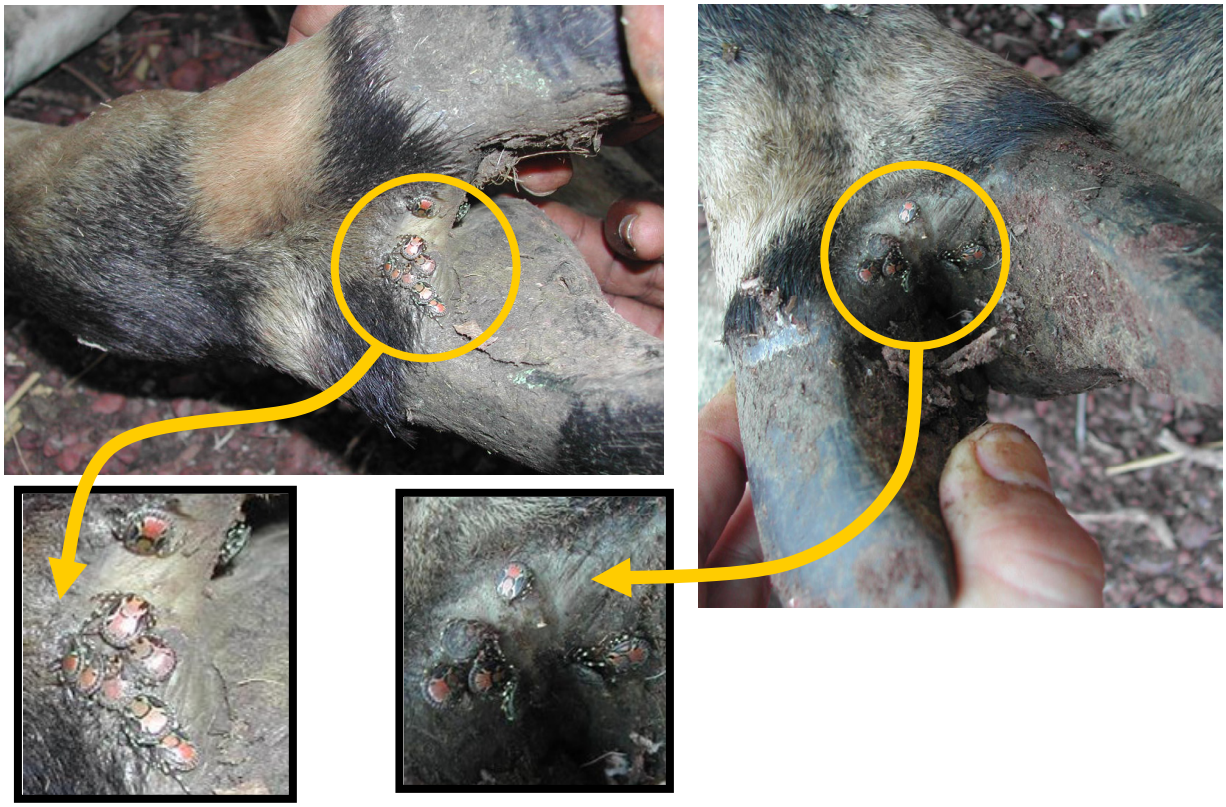

Figure 9: *Amblyomma variegatum* adults attached to the inter-digital areas, as found in the evening when cattle are brought back from the pasture: 9 males (left); 4 males and 3 females (right).

Discussion

The *A. variegatum* nymphal detachment pattern is clearly heterogeneous with about 80% of the ticks detaching between 14h30 and 17h00. Conversely, Barré (1989) observed in Guadeloupe that the detachment peak of *A. variegatum* nymphs occurred in the morning. That experiment was however carried out with goats and the ticks were put in cloth bags stuck on the skin: the conditions were therefore not those experienced during a natural infestation. Rechav (1978), studying the drop-off rhythm of *A. hebraeum* nymphs from experimentally infested rabbits, noticed a detachment peak (70% of the ticks) during the last 4 hours of the day and considered this "enables ticks to avoid desiccation".

It has been postulated that regulation of tick detachment results in the synchronization of the drop off rhythm with host behaviour, allowing the moult to occur in a suitable environment and/or facilitating the search for a host in the next life stage (Rechav, 1978; Belozerov, 1982). This seems also to be the case for *A. variegatum* nymphs. Detaching mainly in the afternoon, when the temperature is decreasing, enables engorged nymphs to have several hours of favourable climate to find a micro-habitat appropriate for survival and moulting. Dropping off the hosts earlier would expose the ticks to the hottest hours of the day which could lead to high mortality. In the same way, Wilkinson (1970) assumed that *B. microplus* was absent in some Australian regions because of the high temperatures at ground level. If engorged nymph detachment occurred later in the day, when the cattle were back in their night paddock, the ticks would be in a very unfavourable environment; no bush or clumps of grass are available to protect ticks from desiccation (favourite micro-habitats of engorged nymphs are bush and grass roots (or root networks) which retain humidity during the dry season: nymphs burrow along these roots up to depths of 10 cm) and there is a high risk of being crushed by the cattle or eaten by chickens which are efficient predators (Hassan *et al*., 1991; Dreyer *et al.*, 1997). Moreover, after moulting, unfed adults would not find hosts easily because the night pens are moved onto harvested fields during the dry season so that manure can fertilize the soil; during the next rainy season, after sowing the crops, cattle are no longer brought there.

The experiment described here was not designed to identify the factors affecting the drop-off rhythm or inducing the detachment of engorged nymphs. It however appears that this rhythm varied according to the day and the host. According to Rechav (1978), drop off is regulated primarily by an endogenous rhythm which could be influenced by some physiological rhythms of the host. Likewise, Bianchi & Barré (2003) considered that "the resumption of host activity after nocturnal resting of cattle may produce the increased early morning fall of engorged *B. microplus*". They also noticed that the drop off rhythm was different for ticks attached on parts of the host directly exposed to the sun compared to those attached on shaded areas, and therefore that sunlight may influence the tick detachment. Within a herd of cattle, animals have different behaviours. Some are always at the front of the herd, others are walking slowly behind, some are covering much greater distances than others, more indolent animals, etc. These differences might influence the nymph drop off rhythm since "movement of steers is one of the major factors that stimulate detachment of the ticks" (Bianchi & Barré, 2003). In November-December in Burkina Faso, there are days which are very sunny and others during which mist or clouds subdue the light; these variations might also act upon the drop off rhythm. However, as these factors were not recorded in the present study, the differences observed among days and hosts could not be explained. Nevertheless, the drop off rhythm was relatively stable. An experiment was therefore carried out to determine whether this detachment pattern could be used to modify tick density in the fields.

The invasion process by *A. variegatum* adults, previously described by Stachurski (2000a), was confirmed during the second phase of the study; the majority of the captured ticks attach to the inter-digital areas and move towards the predilection site (predilection sites are the anatomical areas where the ticks mainly attach and engorge. For *A. variegatum* adults, these sites include the udder or groin, chest and axilla, and perineal area) during the night, when the animals are lying down in the night pen. On this occasion, movements from one host (which has captured the tick in the pasture) to another (on which the tick will engorge) may occur (Stachurski, 2000a). This explains why, despite manual removal of the captured ticks in the evening, monitored cattle were infested by *A. variegatum* adults in the morning, and why these ticks were mainly attached to the predilection sites. The very low proportion of female ticks observed on cattle in the morning is likely the consequence of the biological characteristics of *A. variegatum*: no females attach to the predilection sites until males

have started to produce aggregation-attachment pheromone, 3 to 5 days after attachment (Norval & Rechav, 1979). Data collected during the present experiment suggested that cattle invasion by the *Hyalomma* species present in the area (mainly *H. marginatum rufipes* and *H. truncatum*) may also take place in this manner, since the majority of the *Hyalomma* found in the evening were attached to the inter-digital areas (75% of the 820 ticks captured) and because monitored cattle were often infested in the morning, despite evening tick removal. Of these ticks attached during the night (n = 258), only 30% were attached to the feet.

More ticks were picked up in the plots with denser vegetation than in those installed on stony savannah and fallow land, even though these latter plots were larger. This confirmed that the presence of favourable micro-habitats for nymphs in the pastures greatly influences adult distribution (Minshull & Norval, 1982). Cattle picked up nearly 3-fold more *A. variegatum* in the plots grazed in the afternoon during the dry season in comparison with the plots grazed in the morning. However, the difference between the plots was lower than expected, since about 10-fold more nymphs should have detached in the P2 and P3 plots, according to the drop off pattern previously determined (data not shown, calculated from fig. 5). Different reasons may be given for this observation. 1. Because the plots were not fenced from the beginning of the nymph period, cattle might have disseminated nymphs on the pasture before the start of the first phase of the study. 2. Wild animals (francolins, guinea-fowls, hedgehogs, etc.), infested by *A. variegatum* nymphs, may have disseminated nymphs in the plots after the installation of the fences (Ouédraogo, 1999; Stachurski, 2000b). 3. Density-dependent mortality of engorged nymphs might occur due to predators (like ants) or a lack of sufficient micro-habitats for large numbers of nymphs, thereby reducing the difference among plots with high and low infestation levels.

The difference observed between experimental plots confirmed however that herd management in the dry season can influence the number of *A. variegatum* adults present in the pasture six months later. How can livestock owners implement such a control method? Where could they bring their animals during the nymph drop off periods, i.e. in the afternoon? One solution could be to bring them back to the night pens earlier in the day, such that most ticks detach in this unsuitable environment. This could be considered if herdsmen had the possibility of leaving the night pens much earlier or if cattle could be left free to graze during the night. The latter is not possible when there are unharvested fields. From November onwards, cattle are allowed (and even encouraged) to graze crop residues (stems, leaves, tops... of maize, cotton, sorghum, groundnut...) in harvested fields. Herds can spend more than 3-4 hours daily in such fields (Stachurski, 2000b), which are unsuitable for nymph survival (no shade, no bush, no perennial grasses) and are not grazed in the next rainy season. The strategy during the dry season could therefore be to bring cattle into these harvested fields only after 14h00 or 15h00, and to let the animals graze community pastures in the first half of the day. At present, herds are brought indiscriminately morning and afternoon into the fields. This management strategy would not require an important change in current practices since herdsmen are already accustomed to alternating cattle between pasturelands and harvested fields. However, as most farmers use community pastures, such a herd management strategy would only be effective in reducing tick densities if all the herds sharing the same pastures (for example, all the herds of the same village) follow the same procedures. The implementation of this type of management strategy should therefore be preceded by a local awareness campaign.

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COMPARISON OF LIFE CYCLE PARAMETERS OF *PHYTOSEIUS GLEBA* (ACARI: MESOSTIGMATA) ON THREE PHYTOPHAGOUS HOST SPECIES

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Abstract

Mites of family Phytoseiidae are potential predators of various species of phytophagous mites throughout the world. The present studies were conducted in Acarology Research Laboratory, University of Agriculture Faisalabad. The aim of this work was to study the effect of different prey species on the biology of Phytoseiid mite, *Phytoseius gleba*. The results showed that *P. gleba* completed its development time from egg to adult in 9.00 days when fed on *Tetranychus urticae* as compared to 15.33 and 10.00 days when fed on *Panonychus citri* and *Eutetranychus orientalis* respectively. The maximum fecundity (40 eggs/female) was observed when feeding on *T. urticae* whereas when feeding on *P. citri* and *E. orientalis* it was 24.3 and 27.6 eggs respectively.

Key Words

Acari, life stages, prey species, Phytoseiidae

Introduction

The family Phytoseiidae includes potentially important predacious mite species found throughout the world on many crops including apple, citrus, grapes, tea, tomato, fig, cotton, coconuts, sweet potato, and potato (Nelson *et al*. 1973; Childers & Enns 1975; Muma 1975; Santos & Laing 1985; Sepasgosarian 1985; Thistlewood *et al*. 1996; Searle *etal.* 1998; Villanueva & Harmsen 1998; Naher & Haque 2007; Silva & Fernando, 2008). Some Phytoseiid species may play a significant role in controlling phytophagous mites and scale insects in North America, Europe, Africa, and Asia (Rasmy, 1975; Krantz, 1978; Abou-Setta and Childers 1989; Childers, 1994; Childers *et al.,* 2001). Few biological studies with *Euseius* species have been also conducted (Yue *et al*., 1994; Arturo *et al* 2004).

Phytoseius gleba Afzal and Bashir 2008 is a common species found in Faisalabad on different hosts like mango, citrus and bitter gourd where it preys on different life stages of the citrus red mite, *Panonychus citri* (McGregor) and two spotted spider mite *Tetranychus urticae* Koch (Chaudhri & Akbar 1985).

The objective of this research was to determine the comparative biological parameters of *P. gleba* (duration of life stages e.g., eggs, larvae, protonymph, deutonymph, adults, preoviposition period, total fecundity) when feeding on three different phytophagous mites under controlled laboratory conditions. The diet of *P. gleba* may provide one or more superior attributes for using in biological control of phytophagous mites on different crops in Pakistan.

Material and methods

Mite origin

Using sieve collection method, adult females of *P. gleba* were collected randomly from different citrus and mango orchards located in the vicinity of University of Agriculture, Faisalabad. Leaves carrying adult females were placed in a cooler, taken to the laboratory, and transferred to leaf arenas following the Chaudhri and Akbar (1985) method.

Rearing method for Phytoseius gleba

Adult females of *P. citri*, *T. urticae* and *E. orientalis* already collected from the mango orchard, University of Agriculture, Faisalabad were transferred individually into three citrus leaf arenas using a fine brush. The three leaf arenas with each of above mentioned prey species were kept at 25±2^{0}C and 70±2 % R.H. for whole life cycle of *A. gleba* (maximum 22 days). Adult females of *P. gleba* collected from citrus orchard were transferred using a fine brush to individual leaf arena containing phytophagous mites. Each arena with a prey species comprises one replication and repeated three times. Additional females including nymph and adults of phytophagous mites were added to each arena before all the preys were consumed. In this way following three treatments were compared.

T1= *Panonychus citri* as food.

T2= *Tetranychus urticae* as food.

T3= *Eutetranychus orientalis* as food.

Data regarding duration of development stages and fecundity were recorded twice daily at 0700 and 1900 hours. Development times and number of eggs deposited by *P. gleba* feeding on the three prey species were compared using (one way ANOVA) followed by LSD test for mean comparison.

Table 1. Mean duration in (days) and standard deviation of the different stages of *Phytoseius gleba* when feeding on three different phytophagous mites.

Stage	*Panonychus citri*		*Tetranychus urticae*		*Eutetranychus orientalis*	
	Mean	S.D	Mean	S.D	Mean	S.D
Egg	3.8b	0.29	2.5b	0.50	6.0a	1.32
Larvae	3.5a	0.05	1.5c	0.50	2.5b	0.50
Protonymph	2.1a	0.29	0.7c	0.30	1.5b	0.10
Deutonymph	2.8a	0.29	1.1b	0.50	1.5b	0.50
Adult female	20.5b	2.00	22.0a	1.06	20.8b	1.50

*Different letters represent different means.

Table 2. Life table parameters of *Phytoseius gleba* when feeding on three different phytophagous mites.

Parameters	*Panonychus citri*		*Tetranychus urticae*		*Eutetranychus orientalis*	
	Mean	S.D	Mean	S.D	Mean	S.D
Development time from egg to adult (days)	15.33a	1.0	9.0b	1.50	10.00a	1.00
Pre-oviposition Period(days)	1.60c	0.5	2.1c	0.01	3.30c	0.05
Oviposition Period(days)	11.00a	1.3	7.3b	0.70	8.23b	0.70
Total Average Fecundity (eggs/female)	24.30b	2.0	40.0a	2.50	27.60b	1.50

*Different letters represent different means.

Results and discussion

Effect of prey species on duration of different life stages of Phytoseius gleba.

The duration of the different life stages e.g., was lowest (2.5, 1.5, 0.7 and 1.1 days for eggs, larvae, protonymph, and deutonymph respectively) when fed on *T. urticae* as compared to the duration of life stages when feeding on *P. citri* and *E. orientalis* (Table-1). Duration of adults was highest (22 days)

when fed on *T. urticae* as compared to 20.5 and 20.8 days respectively when fed on *P. citri* and *E. orientalis.* So, it seems that *T. urticae* is a more favorable food source for the development of *P. gleba* than the other prey species tested. These results are similar to the findings on the life stages of Phytoseiid mite *Phytoseius gossipii* who tested the effect of two species of tetranychid mite species: Hafez *et al.* (1983) found that feeding on *T. urticae* favoured faster development as compared to feeding on *T. cucurbitaceaum*.

Prey preference of *P. gleba* are : *T. urticae> P. citri> E. orientalis.*

Life table parameters revealed that the maximum total fecundity (40 eggs/female) was observed when *P. gleba* fed on *T. urticae* as compared to 24.33 and 27.67 eggs/female respectively when fed on *P. citri* and *E. orientalis* (Table 2). There were significant differences in development time from egg to adult when fed on different prey species. The minimum development time (9.00 days) was observed feeding on *T. urticae* whereas after feeding on *P. citri* and *E. orientalis* females it was 15.33 and 10.00 days respectively.

The preoviposition of *P. gleba* was minimum (1.6 days) when fed on *P. citri* as compared to 2.1 and 3.3 days respectively after feeding on *T. urticae* and *E. orientalis.* These results are in accordance with the findings of Yousef *et al.* (1982) who studied the effect of prey species on biology and fecundity of a Phytoseiid mite *Phytoseius gossipi* and showed that fecundity was higher when mites were fed on *T. urticae* as compared to *T. granati.*

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THE EVALUATION OF THE ESTERASE AND GLUTATHION S-TRANSFERASE ENZYMES IN TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* KOCH. (ACARI: TETRANYCHIDAE) SELECTED WITH BIFENTHRIN

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Abstract

The bifenthrin resistance of the two-spotted spider mite was analyzed in relation with esterase and glutathione S-transferase activity. The resistance rate of the SAK strain selected twenty times with bifenthrin was increased from 1.81 to 21.84 fold. In addition, the interaction of some synergists with the bifenthrin was analyzed in the resistant BIF 20 strain. In this, while esterase enzyme activity was detected by gel electrophoresis and microplate reader method, glutathione S-transferase activity was detected only by microplate reader method. The esterase enzyme activity was raised from 19.08 mOD/min/mg proteins to 25.16 mOD/min/mg proteins. Additionally, it was observed that the band intensity of esterase enzyme in the electrophoresis method was increased. Besides, the GST enzyme activity increased from 11.91 mOD/min/mg proteins to 15.45 mOD/min/mg proteins.

Key-words

Tetranychus urticae, bifenthrin, resistance, esterase, glutathione S-transferase

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of key pests responsible for yield losses in greenhouse vegetables and other agricultural crops worldwide. Greenhouse is a suitable place for reproduction of this species. A major problem in the control of *T. urticae* is their ability to rapidly develop resistance to many important acaricides after only a few applications (Stumpf and Nauen 2002).

Bifenthrin is a member of the pyrethroid chemical class. It is an insecticide and acaricide, which affects the nervous system and causes paralysis in insects (Anonymous 1995). Several reports indicated the metabolism as an import mechanism responsible for bifenthrin resistance in *T. urticae* (Ay & Gurkan 2005; Leeuwen & Tirry 2007; Yang *et al*. 2002). The metabolic resistance has been inferred from the synergism of acaricidal activity of synthetic pyrethroids organophosphate compounds, suggesting that serine-dependent esterase may be involved in their detoxification (Leeuwen & Tirry 2007).

In the present study, the biochemical mechanisms of resistance-associated changes in susceptibility of the two-spotted spider mite populations selected with bifenthrin were evaluated.

Material and methods

Mite strains: The original population of *Tetranychus urticae* was collected from a commercial tomato (*Lycopersicum esculantum* L.)

greenhouse in Şarkikaraağaç district of Isparta province in August 6, 2003. This is designated as SAK. After collection, *T. urticae* was continuously maintained on pinto bean plants, *Phaseolus vulgaris* L. under laboratory conditions at 26 ± 2 °C, 60 ± 5 % RH and a 16 h photoperiod. A susceptible strain (GSS) had been obtained from Rothamsted Experimental Station, Harpenden (England), in 2001 and reared in laboratory conditions since 2001.

Toxicity tests

Chemicals: Bifenthrin was used as the formulated commercial product Talstar (EC 100g.l^{-1}, FMC). All other chemicals used were analytical grade.

These tests were conducted based on the method described by Ay (2005). The prepared suspension of bifenthrine was sprayed the internal surfaces of lids and bases of 60 mm diameter plastic Petri dishes and allowed to dry for 30 min. For each application, 1 ml suspension was sprayed on each base and lid pair by a Potter spray tower (Burckard Auto-Load, Rickmansworth, Herts, UK) at 14.504 psi. Adult female mites (≅30) were transferred to each dish using a hairbrush, and the dishes were closed and sealed with parafilm to prevent escape of spider mites. Thereafter, the mites in the dish were kept at 26 ± 2 °C, 60 ± 5 % RH and a 16 h photoperiod for 24 h after treatment. Survival of individual mite was determined by touching each mite with a fine brush. Mites, which were unable to walk at least a distance equivalent to their body length, were considered dead. The mortality tests were done before each experiment to determine a range of concentration that would produce approximately 10-95 % mortality. Each experiment was conducted using three replicates of a seven concentrations (plus distilled water control). Pooled data were subjected to probit analysis (POLO PC) (LeOra software1994) and $LC_{50,\ 60\ and\ 90}$ with respectively 95% CL were estimated. The LC_{50} values of selection population were compared to those of the susceptible population (GSS).

Selection for Resistance

Females of the original population (SAK) were selected for resistance to bifenthrine under laboratory conditions from March 2006 to October 2007. The used selection experiments were the modified methods of Yang *et al.* (2002). At least 400 adult female mites were transferred from SAK population into Petri dishes (40 mite/ petri dish) treated with bifenthrine concentrations equal to the LC_{60} for that cycle. After 24 h, surviving mites transferred back to plants and the populations were allowed to increase. The next selection cycle was conducted after the populations had increased, two or three generations later (approximately 15-20 days). A bioassay using the selection acaricide was conducted on mite population periodically when the number of surviving mites changed in the selection petri dishes. The new LC_{60} was applied as a selection pressure.

Synergism test

The effects of bifenthrin + synergist were tested using the methods of Kim *et al.* (2004). Mixed-function oxidase (MFO) and esterase inhibitor piperonyl butoxide (PBO) and others esterase inhibitor S-Benzyl-O, O-diisopropyl phosphorothioate (IBP) and triphenyl phosphate (TPP) were used to inhibit detoxification enzymes. Synergists were dissolved in acetone: distilled water (1:1) and 1 ml suspension sprayed on Petri dishes base and lid in the same manner as in toxicity test 30 min prior to acaricide application. Distilled water-acetone without a synergist was applied to the control. Synergists solutions were prepared in the following concentrations (mg.l^{-1}): PBO (500), IBP (400) and TPP (125). A synergistic ratio (SR) was calculated using the following formula:

SR= LC_{50} of bifenthrin without synergist / LC_{50} of bifenthrin with synergist

Biochemical assays

Electrophoresis: vertical non-denaturing polyacrylamide gel electrophoresis was performed following the procedures by Walker (1994), and Goka & Takafuji (1992). The gels were 1 mm thick and 80 mm x 80 mm in area. Acrylamide concentrations were 7.5 % in separating gels and 3.5 % in stacking gels. Adult female mites were homogenized individually in 10 µl of 32 % (w/v) sucrose with 0.1 % Triton X-100 in microtiter plates by a multiple-homogenizer (Moores *et al.* 1988). Electrophoresis was carried out at a constant current of 150 volts at 5-8 °C for about 1.5 h. Esterase was stained by placing the gels for 1 h. in 0.4 (w/v) fast blue BB salt after incubating them for 30 min in a 0.02 % (w/v) solution of α-naphthyl acetate in 0.2 M phosphate buffer (pH 6.5), containing 1 % acetone. Placing the gel in 7.5 % acetic acid stopped all staining reactions.

Table 1. Selection for resistance to bifenthrin in a strain of *Tetranychus urticae*; estimation of LC_{50} (µl of formulation/ 100ml of distilled water).

Strains	N[a]	Slope ± se	LC_{50} (µl/ 100ml) (95 % CI[b])	LC_{60} (µl/ 100ml) (95 % CI[b])	Resistance ratio LC_{50}[c]
SAK	633	1.332±0.13	98.45 76.24 - 122.73	152.57 122.37 - 189.67	1.81
BİF 1	734	1.132±0.11	117.32 70.10 - 176.29	196.44 127.82 - 297.17	2.15
BİF 2	630	1.048±0.10	165.61 123.34 - 216.30	288.97 221.33 -382.12	3.04
BİF 3	726	1.174±0.10	192.51 152.28-239.91	316.45 253.92-400.03	4.10
BİF 4	715	1.083±0.10	228.74 175.69-292.56	391.95 306.44-510.59	4.87
BİF 5	688	0.957±0.10	358.64 255.92-486.42	659.84 486.50-911.30	6.57
BİF 6	721	1.121±0.12	736.20 557.47-977.78	1238.80 934.60-1727.68	13.50
BİF 7	720	1.091+1.10	694.20 534.04-890.03	1184.91 924.01-1548.66	12.73
BİF 8	631	1.381±0.11	771.97 479.87-1200.75	1177.72 765.00-1956.45	14.15
BİF 9	726	1.285±0.11	532.67 411.91-670.63	838.61 665.97-1056.59	9.77
BİF10	727	1.030±0.10	827.06 631.84-1074.64	1456.92 1120.13-1961.02	15.16
BİF 11	730	1.082±0.10	846.32 654.46-1058.61	1453.39 1130.56-1917.65	15.52
BİF 12	724	1.002±0.09	816.97 629.24-1053.35	1462.07 1131.80-1956.40	14.98
BİF 13	722	1.110±0.10	925.12 716.92-1183.08	1564.87 1222.98-2055.25	16.96
BİF 14	721	1.076±0.105	1126.81 855.05-1472.93	1937.95 1482.35-2617.89	20.66
BİF15	723	1.100±0.10	1130.41 871.59-1455.61	1920.76 1490.87-2549.84	20.72
BİF16	723	1.092±0.10	1159.05 896.29-1490.32	1976.99 1536.41-2624.42	21.25
BİF 17	723	1.123±0.10	1129.86 867.39-1454.36	1899.04 1475.08-2504.11	20.71
BİF 18	722	1.223±0.10	914.42 730.41-1133.00	1473.21 1188.64-1855.38	16.76
BİF 19	720	1.367±0.11	985.84 794.24-1209.30	1510.66 1231.55-1871.76	18.07
BİF 20	723	1.213±0.11	1191.44 939.24-1497.75	1927.59 1532.96-2472.49	21.84
GSS	527	1.493±0.15	54.53 34.03 - 85.54	-	-

[a] Total number of mites used, [b] Confidence interval, [c] Resistance ratio = LC_{50} value of the BİF 20 strain / LC_{50} value of the GSS strain

Photometric Esterase assay: esterase assays were performed in compliance with Stumpf & Nauen (2002). The 10,000 g supernatant of mass homogenates of 100 adult females prepared in 500 µl ice-cold 0.1 M sodium phosphate buffer, pH 7.5, containing 0.1 % (w/v) Triton X-100, was diluted 10-fold and used as the enzyme source. Twenty five µl aliquots (0.5 mite equivalent) were added to the wells of a 96-well microplate, containing 25 µl of 0.2 M sodium phosphate buffer, pH 6.0. Wells with buffer-only served as control for the non-enzymatic reaction. The assay was started by adding 200 µl of substrate solution to each well, giving final volume of 250 µl. The substrate solution consisted of 15 mg of fast Blue RR salt dissolved in 25 ml of sodium phosphate buffer, pH 6.0, and 250 µl of 100 mM 1-naphthyl acetate in acetone. The esterase activity was measured continuously at 450 nm and 25 ^{o}C in Versamax kinetic microplate reader (Molecular Devices) for 10 min, utilizing Softmax software to fit kinetics plots by linear regression.

Photometric GST assay using 1-Chloro2,4-dinitrobenzene: glutathione S-transferase activities were evaluated according to Stumpf & Nauen (2002). GST activity was determined using 1-chloro-2,4-dinitrobenzene and reduced glutathione (GSH) as substrate. 100 adult females were homogenized in 1000 µl Tris-HCL (0.05 M, pH 7.5). The total reaction volume in each microtiter plate well was 300 µl, consisting of 100 µl each supernatant (10,000 g, 5 min), CDNB (Containing 0.1% (v/v) ethanol), and GSH in Tris-HCL (0.05 M, pH 7.5), giving final concentrations of 0.4 mM CDNB and 4 mM GSH.

Table 2. The synergistic effects of enzyme inhibitors (PBO, IBP and TPP) on bifenthrin resistance in *Tetranychus urticae* strain BİF 20; estimation of LC_{50} (µl of formulation/ 100ml of distilled water).

insecticide	N[a]	Slope ± se	LC_{50} (µl/ 100ml) (95 % CI[b])	LC_{90} (µl/ 100ml) (95 % CI[b])	SR[c]
Bifenthrin (only)	723	1.213±0.11	1191.44 939.24-1497.75	13583.14 9148.33-23136.41	-
Bifenthrin+PBO	721	1.377±0.12	888.35 695.58-1107.9	7571.38 5484.81-11557.67	1.34
Bifenthrin+IBP	721	1.392±0.11	680.75 428.35-994.66	5672.83 3389.16-13181.10	1.75
Bifenthrin+TPP	726	1.446±0.12	831.66 508.61-1244.84	6397.90 3735.84-15958.29	1.43

[a] Total number of mites used, [b] Confidence interval, [c] SR= LC_{50} of bifenthrin without synergist / LC_{50} of bifenthrin with synergist

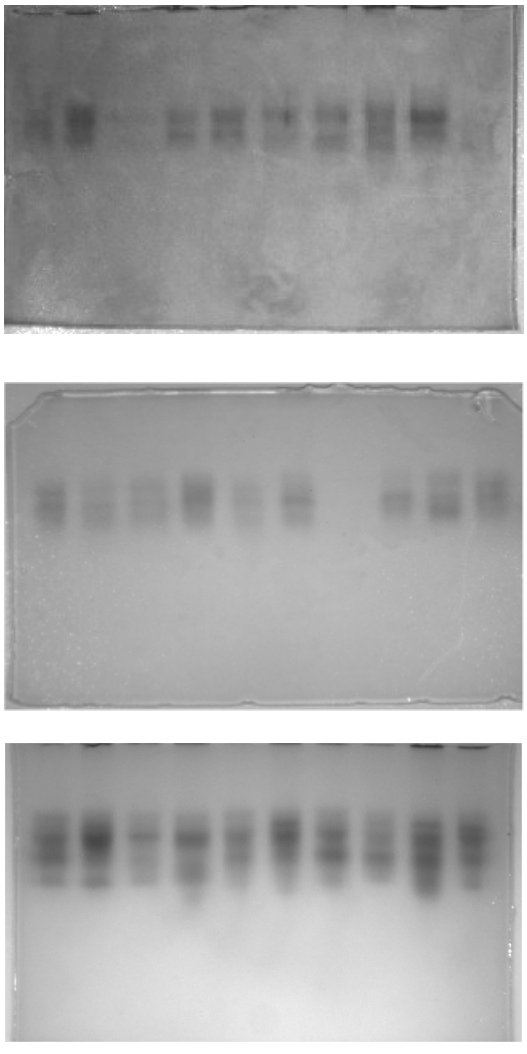
Table 3. Esterase and GST activities in susceptible strain GSS, parental strain SAK and bifenthrin resistant strain BİF 20 of *Tetranychus urticae* (P<0.05)[a].

Enzyme	Population	n[b]	Specific activity (±SE) mOD/min/mg proteins	R/S[c]
Esterase	GSS	3	10.11(±0.36) c	1.00
	SAK	4	19.08 (±0.31) b	1.89
	BİF 20	5	25.16 (±0.28) a	2.49
GST	GSS	4	13.67 (±0.50) b	1.00
	SAK	4	11.91 (±0.50) c	0,84
	BİF 20	6	15.45 (±0.41) a	1.13

[a]: Means with different letters in column for each enzyme are significantly different (P<0.05), [b:]number of replicates, [c]:enzyme activity SAK or BİF 20/ enzyme activity GSS strain.

The change in absorbance was measured continuously for 5 min. at 340 nm and 25 ^{o}C using the Versamax kinetic microplate reader (Molecular Devices). The non-enzymatic reaction of CDNB with GSH measured without homogenate served as control.

The activity of all enzymes was analysed by Softmax PRO software and presented as mOD/min/mg proteins. The data were analyzed using the General Linear Model (GLM) procedure of SAS (1999) by using strains in the model and



a

b

c

Figure 1. Acrylamide gels of esterase zones in individual of Susceptible strain GSS (a), parental strain SAK (b) and bifenthrin resistant strain BİF 20 (c) of *Tetranychus urticae*

PDIFF statement was used to compare strains' enzyme (Esterase and GST) activity means for dependent variables. Alpha level of 0.05 was accepted as significance level.

Results

Selection for Resistance

After twenty selections for resistance, the LC_{50} of bifenthrin for SAK strain increased from 98.45 µl (formulation)/100 ml water to 1191.44 µl/100 ml water (Table 1). Resistance ratio for SAK strain was raised from 1.81 to 21.84 fold at LC_{50} after selections.

Synergist test

The tree synergist PBO, IBO and TTP were used to investigate possible metabolic resistance to bifenthrin (Table 2). Synergistic effect on selected bifenthrin resistance was tested using BİF 20 population. IBP showed the highest synergistic effect on bifenthrin in selected BİF 20 population, PBO and TTP showed low effect.

Biochemical assays

In electrophoresis assay results, esterase bands were stained very dark in bifenthrin resistant strain BİF 20 than SAK and susceptible strains GSS (Figure 1). Also esterase activity was highest for BİF 20 in the microplate method. The esterase enzyme activity was raised from 19.08 mOD/min/mg proteins to 25.16 mOD/min/mg proteins after 20 selections with bifenthrin (Table 3). Additionally, it was observed that the band intensity of esterase enzyme in the electrophoresis method was increased.

Glutathione S-transferase activity was determined with the artificial substrate CDNB in mass homogenates and significantly higher ($P<0.05$) in the selected strain compared to the parental and susceptible strains. The GST enzyme activity increased from 11.91 mOD/min/mg proteins to 15.45 mOD/min/mg proteins (Table III).

Discussion and conclusion

Effective resistance management in agricultural insect pests depends on early detection of problem and assimilation of information on the resistant population including resistance mechanisms and accurate monitoring of resistance gene frequencies, so that rational pesticide choices can be made (Vontas *et al.* 2000). Esterase and GST play an important role in the detoxification of many agrochemicals including pyrethroids, organophosphates and carbamates (Wheelock *et al.* 2005; Konanz & Nauen 2004). In this study, the esterase and GST enzymes activity in the resistant

BIF 20 population was investigated. Both of the enzymes activities were increased when compared to the parental population. Ay & Gürkan (2005) have determined higher esterase activity in bifenthrin resistant two spotted spider mites that were collected form the cotton fields. Leeuwen & Tirry (2007) have found a high level resistance to bifenthrine in field-collected multi-resistant *T. urticae* population and defined high esterase activity in the same population. Yang *et al.* (2002) have reported an increase in esterase enzyme activity but a decrease in GST enzyme activity in bifenthrine selected *T. urticae* population. However, it was also reported that GSTs act as antioxidant–defence agents and confer pyretroid resistance in *Nilaparvata lugens* Stal and possibly in other insects (Vontas *et al.* 2001). In our study, a significant increase in esterase and GST enzymes activity in bifenthrin resistant population was observed compared to those in the sensitive and parental strains. In addition, IBF, a general esterase inhibitor, have shown high synergistic effect to bifenthrin. The other synergists, MFO and esterase inhibitor PBO and other esterase inhibitor TPP have also shown low synergistic effect with bifenthrin. These results have shown that both esterase and GST enzyme may play a role in the resistance to bifenthrin.

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COMPARAISON OF THE SUSCEPTIBILITY OF SEVERAL ALIMENTARY SUPPORTS TO THE OLD WORLD MITE *OLIGONYCHUS AFRASIATICUS* (ACARI: TETRANYCHIDAE)

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Abstract

The demographic parameters of *Oligonychus afrasiaticus* were compared at 32°C, on various plant-based food resources: date fruit of date palms cultivars 'Deglet Noor', 'Alig', 'Kentichi', 'Bessr', and 'Deglet Noor leaves' and 'sorghum leaves'.
Plant-based food, were categorized into three groups: 'Deglet Noor ' dates, sorghum leaves were susceptible, 'Bessr' dates and 'Deglet Noor ' leaves were resistant, 'Kentichi' and 'Alig' dates were of intermediate susceptibility.
At 32°C, *O.afrasiaticus* feeding on 'Bessr ', shows the lowest intrinsic rate of natural population increase (*r*m=0.136 day-1). Their virulence is low because of low fecundity and reduction in the fertility. Feeding on 'Deglet Noor dates', *O.afrasiaticus* presents the highest demographic performances (*r*m =0.213).This latter cultivar seems to be the most susceptible one.

Key words

Oligonychus afrasiaticus, *r*m, date palm, Southern Tunisia, Deglet Noor

Introduction

Tunisia produced 110,000 tons of dates annually, contributing around 13% to the agricultural exportations products. The Deglet Noor variety accounts for 62% of the total production in Tunisia (FAOSTA, 2005).The old world date mite, *Oligonychus afrasiaticus* (McGregor), is one of four major pests of date palms in Tunisia (Dhouibi 1991; Khoualdia et *al*., 1997). When present, it can cause very serious damage to fruits (Dhouibi, 1991).

Mite infestation usually starts in summer. Infested dates bunches strand, are covered with fine web. Immediately after fruit set, mite eggs are deposited to produce larvae which will feed on the fruits and later recover them with a web retaining sand particles. The cycle duration is of about ten to fifteen days depending on temperature (Hussain, 1974).

Mite development and fecundity depend on availability of appropriate nutrients, and thus to host plant resistance. Differences in varietals susceptibility to *O. afrasiaticus* have been reported from several areas. In Libya, the varieties 'Asabir', 'Aurig', 'Bestian', 'Apel' and 'Talise' were found to be the most susceptible while the cultivar 'Tafsirt' was found to be less susceptible (Edongali et *al*., 1988). The cultivars 'Hilali', 'Gibri' and 'Khanazani' in Oman were infested by *O.afrasiaticus* in April, whereas other cultivars were attacked later in the season (Elwan, 2000). In Israel, Palevsky (2005) reported that the cultivar 'Deglet Noor' was more attacked than the varieties 'Medjool' and 'Barhi'. The Iraqi variety 'Sayer' is relatively resistant to

mite attack (Hussain, 1974). The objective of this study is (1) to assess the suitability of fruits of four date palms cultivars as food source to *O. afrasiaticus* in order to identify 'susceptible' and 'resistant' cultivars, (2) to evaluate the role of 'Deglet Noor' leaves and sorghum leaves in the mite dispersal.

Material and methods

Oligonychus afrasiaticus was collected from date fruit 'Deglet Nour' variety in Segdoud near Tozeur southern Tunisia in July 2006 and maintained in the laboratory on sorghum plants (*Sorghum sp.*) at 25 ± 1 °C, 50-75% RH and 16:8 h (L:D) of photoperiod. *Oligonychus afrasiaticus* was reared in the laboratory on date fruit of the four date palms cultivars: 'Deglet Noor', 'Alig', 'Kentichi', 'Bessr', and on the leaves of the variety 'Deglet Noor' and of sorghum. The life tables were constructed to calculate the demographic parameters.

Dates and leaves were collected during the Kimri stage, characterised by the green colour of fruit from trees grown in a single plot of mixed cultivars at Segdoud near Tozeur southern Tunisia. Fruit dates and leaf disks (abaxial side up) were placed on water saturated foam mat in a plastic tray. The wet cotton wool prevented mite escape and maintained leaf freshness for two weeks. The cotton wool was maintained wet by adding water where necessary.

All the experiments were conducted into a climate-controlled chamber at 32 ± 1° C, 60±10 % RH and a photoperiod of 16:8 h. (L:D). The temperature 32°C corresponds to the monthly mean temperature of July and August in Tozeur. Mites rapidly multiply and population on dates reaches its peak at the end of July and in August.

Females were placed individually on each leaf disk or dates inside the plastic tray. The experiment was replicated 50 times for each support. They constituted the initial cohorts from which the life tables will be constructed to estimate demographic growth parameters. Three biological parameters on adult females of *O. afrasiaticus* were calculated: survival, fecundity and fertility. Leaf disks were checked daily and the number of eggs laid and immature mites born on each disk were recorded until all females died, and all the eggs either hatched or died, for evaluating the female fertility. When leaf disk started to deteriorate during the study, mites were removed to healthy leaf disks that were cut at the beginning of the trial but upon which no mites had been deposited.

The life table was constructed considering the females cohort studied. The net reproductive rate (R_0), the mean generation time (T), the intrinsic rate of natural increase(r_m), the doubling time (Dt), and the finite rate of increase (λ) were calculated using the method recommended by Birch (1948):

$$1 = \sum_{x=o}^{k} e^{-r_m} l_x m_x$$

With x pivotal age, l_x number surviving to age x, m_x age-specific fecundity

- $R_0 = \Sigma\, l_x m_x$
- $r_m = \ln(R_0)/ T$
- $Dt = \ln 2 / r_m$
- $T = \ln R_0 / r_m$
- $\lambda = e^{- r_m}$

Data on developmental time, duration of female reproductive periods and fecundity were analyzed using one-way ANOVA followed by the Sheffe test to compare data means.

Results

Development duration

At 32°C, duration of the whole immature phase (egg to adult emergence) was different between the supports tested (P= 0.01). Development duration was categorized as low on 'Deglet Noor' dates, 'Alig' dates and sorghum leaves (8.2, 8.3 and 8.3 days respectively), and high on 'Bessr' dates. Duration was intermediate on 'Kentichi' dates and leaves of 'Deglet Noor' cultivar (Figure 1).

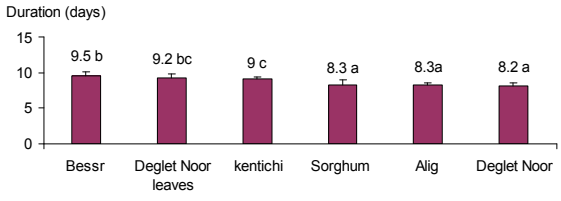

Figure 1. Mean (+Standard error) in days of the development duration of females of O.afrasiaticus at 32°C on dates of cultivars 'Deglet Noor', 'Alig', 'Bessr', 'Kentichi', leaves of sorghum and leaves of 'Deglet Noor' cultivar collected at Segdoud oasis, South of Tunisia. Means followed by the same letter are not significantly different (Sheffe test, P = 0.01).

Tetranychid development from egg to adult may vary from 6 to 10 days or more, depending on species, temperature, host plant, humidity and other factors (Helle & Sabelis, 1985).

Immature survival

The immature survival ranged from 92 to 75%. The lowest immature survival rate was observed on the 'Bessr' dates (75%) (Figure 2).

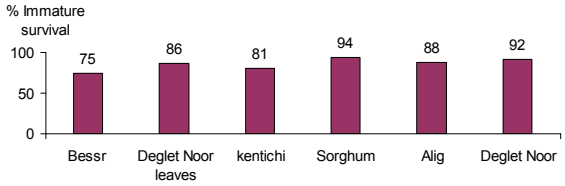

Figure 2. Immature survival of O.afrasiaticus at 32°C on dates of cultivars 'Deglet Noor', 'Alig', 'Bessr', 'Kentichi', leaves of sorghum and leaves of 'Deglet Noor' cultivar collected at Segdoud oasis, South of Tunisia.

Longevity of adult females

Food type seems to affect the longevity of adult females of *O. afrasiaticus.* Dates of 'Deglet Noor' cultivar appear to be the most favourable as mites had the highest longevity (10.4 days). The lowest longevity was recorded on sorghum leaves (5.9 days). No difference was observed between the dates of 'Bessr', 'Alig' and 'Kentichi' cultivars (Figure 3).

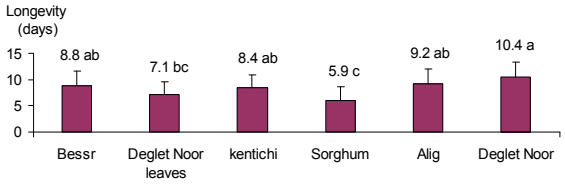

Figure 3. Mean (+Standard Error) longevity in days of adult females of *O.afrasiaticus* at 32°C on dates of cultivars 'Deglet Noor', 'Alig', 'Bessr', 'Kentichi', leaves of sorghum and leaves of 'Deglet Noor' cultivar collected at Segdoud oasis, South of Tunisia. Means followed by the same letter are not significantly different (Sheffe test, P = 0.01).

Fecundity

Results are reported on the figure 4. No statistical differences between the fecundity of females of *O. afrasiaticus* reared on 'Alig', 'Kentichi', 'Bessr' dates, sorghum leaves and leaves of 'Deglet Noor'. The values range from 8.1 to 11.6 eggs per female. Only on dates of 'Deglet Noor' cultivar, a significantly higher fecundity is observed (18.7 eggs / female).

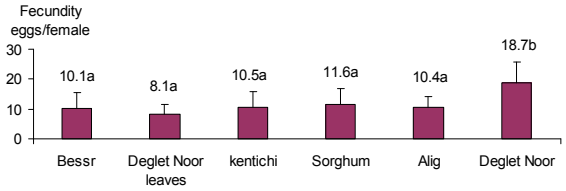

Figure 4. Mean (+Standard Error) fecundity in eggs/female of adult females of *O.afrasiaticus* at 32°C on different supports collected at Segdoud oasis, South of Tunisia. Means followed by the same letter are not significantly different (Sheffe test, P = 0.01).

Sex-ratio of the descendance

There were no significant effects of plant-based foods on sex-ratio of the descendant of *O. afrasiaticus* (Figure 5). The sex-ratio is always female biased. A ratio of 1 male to approximately 3 females is very often found, and is used to characterize tetranychid species (Helle & Sabelis, 1985).

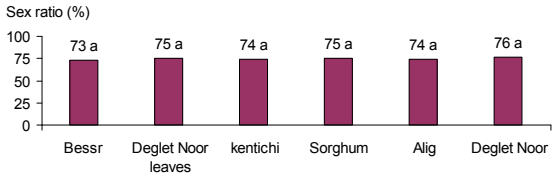

Figure 5. Sex-ratio female of the descendant of *O.afrasiaticus* at 32°C on different supports collected at Segdoud oasis, South of Tunisia. Means followed by the same letter are not significantly different. (Khi2 test).

Fertility

No significant difference in fertility was observed between the different modalities tested (Figure 6). The lowest fertility was observed on 'Bessr' dates (90.3%). Fertility is known to vary with the kind of host plant (Bengston, 1970).

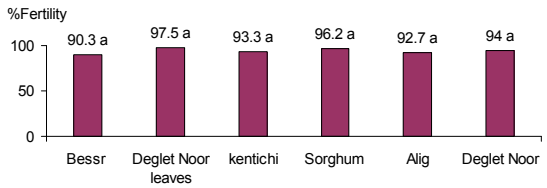

Figure 6. Fertility of *O.afrasiaticus* at 32°C on different supports collected at Segdoud oasis, South of Tunisia. Means followed by the same letter are not significantly different.

Table life

Calculated life table parameters are given in the

Table 1. Mean net reproductive rate (R_0) and intrinsic rate of natural increase (r_m) varied significantly among cultivars. Concurrently with the tendency observed for lowest duration of development and with the observed higher rates of oviposition, the mites reared on the 'Deglet Noor' fruits present the highest values of the intrinsic rate of natural increase (r_m =0.213 day^{-1}), Mean net reproductive rate (R_0=16.5 females) and finite rate of increase (λ=1.24 mite/day). Consequently, feeding on 'Deglet Noor' dates engenders the shortest mean generation time (T=13.16 day) and doubling time (Dt=3.25 days) of mites. Demographic performance of *O. afrasiaticus* was the lowest on the 'Besser' dates (r_m=0.136). The highest r_m was due to shortest development and generation time and a greatest rate of reproduction (Helle & Sabelis, 1985).

Population dynamics

The finite rate of increase (λ), ranged from 1.15 to 1.24 mite/day. It shows the population multiplication by time unit, represented by the equation: $N_t = \lambda N_0$ (N_t : population density at the time t, λ : the finite rate of increase, N_0: population density at initial time). The logarithmic transformation of this equation permits to illustrate the population variation in time (Figure 7).

Table 1. Demographic parameter of *O. afrasiaticus* at 32°C on different supports collected at Segdoud oasis, South of Tunisia: net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (r_m), doubling time (Dt) and finite rate of increase (λ).

Supports	Demographic parameters				
	R_0	T	λ	Dt	r_m
Deglet Noor	16.5	13.16	1.24	3.25	0.213
Alig	9.2	14.3	1.17	4.5	0.155
Kentichi	8.48	14.45	1.16	4.68	0.148
Bessr	7.43	14.74	1.15	5.09	0.136
Deglet Noor Leaves	6 .94	13.84	1.15	4.95	0.14
Sorghum leaves	9.49	11.76	1.22	3.41	0.203

An important difference between the six plant-based food resources is observed. After four generations (13, 12, 14, 15,14 and 14 days successively for 'Deglet Noor', Sorghum leaves, 'Kentichi', 'Bessr', 'Alig' and 'Deglet Noor' Leaves) , populations of *O. afrasiaticus* reared on 'Deglet Noor' and sorghum leaves would be in theory more abundant than on other food sources.

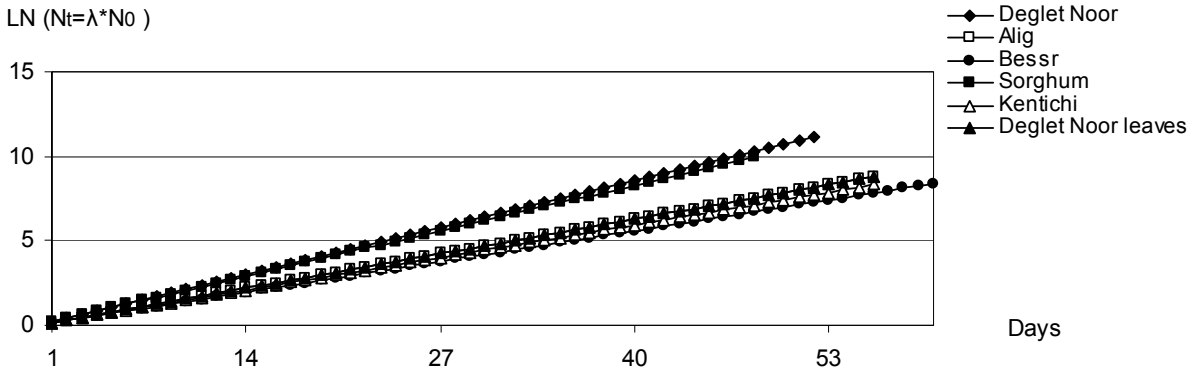

Figure 7. Population dynamics of *O. afrasiaticus* at 32°C on different supports collected at Segdoud oasis, South of Tunisia.

Discussion

Local population of *O. afrasiaticus* was able to feed and complete its development on the six tested kinds of food. The mite exhibited a high population increase when fed on 'Deglet Noor' dates with the highest intrinsic rate of increase (r_m=0.213). 'Kentichi' and 'Alig' dates were of intermediate value.

When *O. afrasiaticus* fed on 'Bessr' and 'Deglet Noor' leaves, we recorded a significant increase in development duration and a reduction in fecundity. The resultant low capacity of population growth suggests poor demographic performance of *O. afrasiaticus* on these cultivars.

Concordance between field observations and laboratory studies is observed. In Tunisian oasis, comparison of visible damage among selected date

palm cultivars: 'Deglet Noor', 'Alig', 'Bessr', 'Kentichi', showed significant and quantifiable differences in susceptibility to *O. afrasiaticus*. Dates of 'Deglet Noor' cultivar are very susceptible and most suffer and the 'Bessr' cultivar was more resistant (personal observation).

Differences in development, reproduction, longevity and population development of tetranychid mite on different host plants are common. These differences may be associated with impediments to feeding such as host plant texture, nutritional value of the host plant, host physiology (Helle & Sabelis, 1985; Kielkiewicz & Van de Vrie, 1990; Kerguelen & Hoddle, 2000). Tetranychids pierce the parenchyma tissue of leaves with their stylets and siphon out the cells' contents (Jeppson et *al.*, 1975; Van der Geest, 1985). Consequently, mite nutrition is directly affected by the chemical composition of ingested fluids. The present results suggest that the observed difference between 'Deglet Noor', 'Kentichi', 'Alig' and 'Bessr' cultivars susceptibility to *O. afrasiaticus* could be due to seasonal differences in nutritional quality of the cultivars.

In recent studies, fruit dates of cultivars: 'Barhi', 'Deglet Noor', and 'Mdjool' were analysed. It has been shown that the performance of *O. afrasiaticus* varied greatly depending on sugar levels in dates (Palevsky et *al.*, 2005).

Tetranychid mites have well-developed dispersal mechanisms, the initiation of dispersal phase appears to be a response to food shortage (Helle & Sabelis, 1985).

The results reported here show that the spider mite is able to survive and develop on 'Deglet Noor' and sorghum leaves, increasing the probability of a dispersing activity of great populations to the suitable hosts. In Tunisian oases, mite populations begin to decline with color change of fruit to yellow or red at the khalal stage. Mobile forms started leaving dates bunches and migrate to the palm crown and ground cover essentially, on *Sorghum* sp (personal observations). *Oligonychus afrasiaticus*, was rare in cold seasons (November-April), it was found over-wintering on pinnae and ground cover (Hussain, 1969; Palevsky, 2003).

Identifying differences in varietals susceptibility is crucial for developing efficient pest control programs. Cultivars that are less susceptible can be left non-sprayed, or sprayed at a low threshold. Identifying characteristics that enhance resistance to spider damage will enable plant breeders to produce resistant varieties. At last, increasing cultivar diversity in orchards should be considered as a strategy to reduce damage and associated yield reductions caused by *O. afrasiaticus*.

Further work is required to determine differences in chemical composition of resistant and susceptible cultivars, seasonal variation of these compounds, and their effect on the longevity and fecundity of *O. afrasiaticus*. A better understanding of the biochemical processes that may mediate cultivar resistance to *O. afrasiaticus* will also assist breeding efforts designed to select resistance cultivars.

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RESISTANCE MONITORING TO DELTAMETHRIN AND CHLORPYRIPHOS-ETHYL IN 13 POPULATIONS OF *TYPHLODROMUS PYRI* SCHEUTEN (ACARI: PHYTOSEIIDAE) FROM VINEYARDS IN THE SOUTHWEST OF FRANCE

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Abstract

The reduction in the susceptibility to deltamethrin and chlorpyriphos-ethyl of 13 populations of *Typhlodomus pyri* collected in vineyards in the Midi-Pyrénées region of France was demonstrated under laboratory conditions. The corrected mortality ranged from low to medium in all but one population, which was very susceptible to deltamethrin. This reduced toxicity of deltamethrin and chlorpyriphos-ethyl on the tested *T. pyri* populations would allow them to play an important role in IPM grapevines in the Midi-Pyrénées region, especially for the control of spider mites. There was no evidence of cross-resistance between deltamethrin and chlorpyriphos-ethyl.

Key words

Typhlodromus pyri, susceptibility, deltamethrin, chlorpyriphos-ethyl.

Introduction

Resistance of one *Typhlodromus pyri* Scheuten population to deltamethrin and of one *T. pyri* population to chlorpyriphos-ethyl, both from vineyards in the Midi-Pyrénées region of France, was previously demonstrated under laboratory bioassay end of sentence (Bonafos et al., 2007). This phenomenon has been known for *T. pyri* populations originating from Canada (Thistlewood 1991) and from New Zealand (Hoyt, 1972).

The susceptibility to deltamethrin and to chlorpyriphos-ethyl on 13 further populations of this species sampled in the Midi-Pyrénées region was investigated. The aim was to determine if resistance to these two active ingredients of *T. pyri* populations is a common phenomenon in vineyards in the Midi-Pyrénées region.

Material and methods

Predatory mite populations

The *T. pyri* tested populations came from the Midi-Pyrénées region of France. The vine plots were haphazardly selected from 10 different localities thoughout the region without prior knowledge of plant pesticides used in the past, despite the fact they all have been subjected to sprayings against the Hemiptera Cicadellidae *Scaphoideus titanus* Ball, the grape leafhoppers vector of the "Flavescence dorée" phytoplasma. The only requirement was to have at least one mobile form of the predatory mite per leaf of *T. pyri* per leaf during sampling. Twenty five leaves were taken from each vine plot, at a rate of one to two leaves per vine stock. This was done on both sides of the

rows and at the level of the fruit-bearing zone. Populations are listed in Table 1. Stock cultures of each population were kept in an environmental climatic chamber at 21±1,5°C, 55-85 % R.H. with a phtoperiod of 16: 8 L: D. They were maintained in artificial arenas as described by Overmeer and Van Zon (1982), with *Tetranychus urticae* Koch eggs as food source.

Insecticides

The synthetic pyrethroid deltamethrin (Décis Protech®, 15 g. l^{-1} SC, recommended field concentration 17.5 mgl^{-1}, Bayer Cropscience France) and the organophosphate chlorpyriphos-ethyl (228 gl^{-1} SC, recommended field concentration 855 mgl^{-1}, Dow Agrosciences France) were tested.

Bioassays

The susceptibility of females was observed in bioassays using only one diagnostic dose of deltamethrin (17.5 mgl^{-1}) and chlorpyriphos-ethyl (855 mgl^{-1}) which corresponded respectively to the highest concentrations recommended for French grapevines to control Tortricidea and Coccoidea. Both leaf disk (2.5 cm diameter, *Phaseolus vulgaris* L. Contender cv.) supports and phytoseiids were treated using a Potter Spray tower (Potter, 1952) (Burkard, Rickmansworth, Hertfordshire, UK). For each pesticide, young females were transferred from the stock culture using a fine camel hairbrush onto leaves placed into Petri dishes containing moistened cotton. After transferring females, Petri dishes were sprayed at 76 kPa with 1.5 ml of the solution or suspension for 2.30 s. This resulted in a wet deposit of 1.5 ± 0.2 $mg.cm^{-2}$. For each insecticide, four *P. vulgaris* leaf disks (four replicates) without females were sprayed under the same conditions. Four replicates of distilled water were used as control for each test. Following the treatment, twelve females were transferred onto each leaf disk, just after drying. Eggs of *T. urticae* were added at the beginning of the bioassays to be used as a food source. Mortality was assessed three days after spraying. Females unable of make some leg movements and unable to walk were scored as dead. Bioassays were conducted in an environmental climatic chamber at 21 ± 1°C, 60-80 R.H. and with 16: 8 L: D photoperiod. The tested populations were considered as susceptible or resistant to deltamehrin when mortality rates of 100 % and 30 %, respectively, were obtained. In the same way populations were considered as susceptible or resistant to chlorpyriphos-ethyl when mortality rates of 98 % and 91 %, respectively, were obtained. These classifications are based on the results obtained by Bonafos *et al.* (2007).

Table 1. *Typhlodromus pyri* tested populations and number of motile forms per leaf at the time of vine sampling.

Population number	Vine cultivar	Location	Number of mobile forms per leaf at the time of vine sampling
1	Negrette	Campsas	3.56
2	Negrette	Vacquiers	4.46
3	Negrette	Castelnau d'Estrefonds	3.84
4	Negrette	Campsas	3.04
5	Cabernet franc	Castelnau d'Estrefonds	2.20
6	Negrette	Villaudric	2.60
7	Negrette	Villematier	1.40
8	Negrette	Château Ferran	2.60
9	Negrette	Bouloc	3.12
10	Negrette	Fronton	5.12
11	Negrette	Campsas	5.56
12	Negrette	Villematier	2.68
13	Syrah	Castenau d'Estrefonds	2.48

Data analysis

The mortality data obtained for each population for deltamethrin and for chlorpyriphos-ethyl were corrected by Abbott's formula (Abbott, 1925).

Results

Number of Typhlodromus pyri motile forms per leaf in vine plot during sampling

Populations densities of T. pyri were higher than one motile form per leaf. Theoretically, this level allows the density regulation within populations of Tetranychidae. However, there were rather clear differences in density between the grapevine locations. The number of motile forms of T. pyri ranged from 1.40 to 5.56 per leaf. On the other hand, seven samples out of 13 had more than three motile forms of the predatory mite per leaf (Table 1).

Deltamethrin

Only one population of *T. pyri* was very susceptible (population 10). The concentration used in vine cultivation to control Tortricidea leds to 100 % female mortality in the laboratory bioassay. Deltamethrin resulted in 84 to 92 % mortality for populations 1, 2, 4 and 5. This group of predatory mites were susceptible to deltamethrin, but less than population 10. Populations 3, 9, 11 and 13 appeared to be rather tolerant of deltamethrin, with mortality values ranging from 61 to 75 %. Populations 6, 7, 8 and 12 appeared to be resistant to deltamethrin. Their corrected mortalities ranged from 5 to 31 % (Table 2).

Chlorpyriphos-ethyl

All populations of *T. pyri* were resistant. The concentration used in vine cultivation to control Coccoidea led to female mortality rates in populations 4, 9 and 10 of 55-74 % in the laboratory bioassay. Chlorpyriphos-ethyl results of 32-41 % mortality for populations 1, 2, 7 and 13. They were resistant to chlorpyriphos-ethyl, but less than populations 3, 5, 6, 8, 11 and 12, for which mortality values ranged from 7 to 25 % (Table 2).

Table 2. Corrected mortality of the 13 populations of *Typhlodromus pyri* submitted to a diagnostic concentration of deltamethrin (17.5 mg. l^{-1}) and chlorpyriphos-ethyl (855 mg. l^{-1})

Population number	Control mortality in %	Corrected mortality to deltamethrin in %	95% fiducial limit		Corrected mortality to chlorpyriphos-ethyl in %	95% fiducial limit	
			Lower	Upper		Lower	Upper
1	15.0	90.2	89.0	91.3	41.1	40.8	41.5
2	21.8	92.0	90.5	93.4	33.3	31.8	34.8
3	16.6	61.8	60.1	63.5	23.6	22.4	24.8
4	33.3	84.3	83.5	85.2	56.2	54.6	57.8
5	14.2	86.1	85.5	86.6	8.3	6.8	9.8
6	18.7	5.3	5.0	5.6	7.6	6.5	8.8
7	10.0	23.6	22.4	24.7	40.9	38.6	43.2
8	10.0	31.2	30.0	32.4	25.9	23.9	27.9
9	12.5	74.0	73.7	74.3	74.0	72.8	75.2
10	25.0	100.0	100	100	55.5	54.6	56.4
11	10.0	62.9	62.3	63.5	10.4	9.8	11.0
12	12.5	20.6	19.7	21.5	7.9	7.6	8.2
13	0.0	75.0	74.4	75.5	32.5	32.2	32.7

Discussion

Eight populations of *T. pyri* out of 13 were tolerant and four were resitant to deltamethrin, whereas all of the populations were resistant to chlorpyriphos-ethyl. An inventory of the spraying history carried out after this study revealed that a minimum of three treatments containing pyrethroid and organophosphate insecticides were usually applied in the vine plots in which the 13 populations of predatory mites originated. The main reason was that insecticide sprayings have been mandatory in the Midi-Pyrénées region for the control of *S. titanus* and this has been true for six years now.

It seems that the 13 populations tested were more resistant to chlorpyriphos-ethyl than to deltamethrin. It is possible that the populations of *T. pyri* tested are more exposed to treatments with organophosphates than to pyrethroid insecticides. The populations which had the highest number of motile forms per leaf were not more resistant to deltamethrin and chlorpyriphos-ethyl than the others. It would seem that there are factors other than the susceptibility to deltamethrin and to chlorpyriphos-ethyl, which could explain the levels of phytoseiid populations. The abundance of phytoseiid mites on *Vitis sp.* is influenced by the availability of prey, pollen and leaf surface characteristics (Karban *et al.,* 1995). In this way, it is very difficult to establish a correlation between the susceptibility of the populations studied with their type of vine origin and their geographical location. There was no evidence of cross-resistance. Populations tolerant or resistant to deltamethrin were not systematically resistant to chlorpyriphos-ethyl, and vice versa. For example, a grape vine population can acquire a resistance to deltamethrin over time if it is exposed to sprayings with this molecule for many years, but nevertheless remains susceptible to chlorpyriphos-ethyl because it is never or only rarely used.

The appearance of many strains of *T. pyri* tolerant or resistant to deltamethrin and resistant to chlorpyriphos-ethyl in the vineyards of the Midi-Pyrénées region in the southwest of France is of great interest. These insecticides which were considered to be intrinsically toxic to *T. pyri* (Sentenac et al., 2005), are now particulary compatible with the sprayings applied to control *S. Titanus* and Tortricidea in the Midi-Pyrénées because predatory mites are the main beneficial organisms present in this region.

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CHEMICAL COMPOSITION AND ACARICIDAL ACTIVITY OF 4 ESSENTIAL OILS AGAINST *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE)

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Abstract

Chemical composition and acaricidal activity of essential oils from the leaves of Mentha viridis, Laurus nobilis, Rosmarinus officinalis and Thymus palescens obtained by steam distillation and analysed by Gas Chromatography coupled to mass Spectrometry (GC/MS). Their toxicity against motile forms and eggs of T. urticae is estimated in laboratory conditions (25 ±2 °C; 75 ± 5% RH). The major components of essential oils are: carvone (24.28%), 1,8 cineole (eucalyptol) 17.42%, the α-pinene (15.25%) and thymol, (43.22%) respectively. Five doses in aqueous suspension (0, 1.25, 0.25, 0.5, 1%) are tested by dip methods disks of bean leaves. Toxicity of essential oils is estimated on 30 motile forms and 50 eggs. We estimate the mortalities and the LD50. The results showed a big efficiency of all essential oils (mortality 100 % with the strongest). According to the LC50 on the motile forms: the thyme is the most toxic (0.05 %) followed by the rosemary (0.1%), the laurel and mint (0.15%). On eggs the classification is the same but the LD50 are a little more raised.

Key-words

Chemical composition, essential Oils, steam distillation, GC/MS, toxicity. *Tetranychus urticae*

Introduction

Algerian agriculture knows each year considerable losses due to different destructive enemies. Among them, phytophagous mites and particularly the Tetranychidae became, the latter decade, major enemies of a big number of cultivated, spontaneous and ornamental plants, consecutively to the availability of the chemical products. Although effective, their repeated use results in the development of acaricidal resistance, undesirable effects on non-target organisms and have fostered environmental and human health concerns. These problems have highlighted the need for the development of new strategies for selective mite control. Plants may provide an alternative means because they constitute a rich source of bioactive chemicals. Much effort has been focused on plant extracts or phytochemicals as potential sources of commercial pest control agents (Isman 2000, Isman et al. 2001). The essential oils of herbs have been popularly applied commercially. Wink (2003) notes that secondary metabolites are present in all higher plants, usually in high structural diversity. Many of them have been found to protect plants against viruses, bacteria, fungi, and most importantly against herbivores. Therefore in the world, during the last 30 years many plant products have been tested as botanical pesticides to control spider mites. Several plant extracts have a good potential for acaricidal activity and are worth further investigation (Mansour et al. 1986; 2004). Labiatae is one of the large plant families used as a framework to evaluate the occurrence of some typical secondary metabolites. For example,

monoterpene limonene has shown deterrent and insecticide properties and carvone is used as sprouting inhibitors (Ibrahim et al. 2001, Aflatuni et al. 2003). Essential oils were extracted from many Labiatae and some of them caused mortality, induced repellency and reduced egg-laying in adult females of the Tetranychus cinnabarinus (Mansour et al. 1986). According to Miresmailli and Isman (2006), the essential oil of rosemary, entire or its major isolated components, are very effective acaricides and deteriorate in two days under the light effect. Algeria conceals important vegetable resources from various regions as coasts plains, mountains, steppe, Sahara and around water points. A very big number among them are used since last times in phytotherapy, protection of the stored foods, against the parasites of domestic animals… Currently our vegetable resources are very little exploited and one has recourse to the importation of the essential oils. Actually, studies of their distributions, chemical compositions and their effectiveness in different domains are more and more numerous. We propose in this paper a study of the chemical composition of essential oils of four aromatic plants and in order to evaluate their toxicity on the two-spotted spider mite, Tetranychus urticae Koch witch is an important and highly polyphagous pest in Algeria.

Material and methods

Plant material and essential oils extraction method

Leaves of Rosmarinus officinalis (Labiatae) were harvested in the horticultural station of the national agronomic institute El-Harrah (east of Algiers); Laurus nobilis (Lauraceae) from Tizi Ouzou 100 km south of Algiers); Mentha viridis (Labiatae) from Blida (50 km south west of Algiers) and Thymus palescens (Labiatae) from Tablat (150km south west of Algiers). Leaves were harvested and air-dried for about one week. Two hundred g of dried and pulverized leaves plants are submitted to hydro distillation for 4h, and the oils were collected by a modified Clevenger-type apparatus. The oils were separated from water, dried with Na2SO4, and stored in sealed vials at low Temperature 4°C before analysis. Yields were calculated and expressed by the percentage of oil obtained for dry vegetable.

GC/MS analysis

The chemical analysis of the essential oils was realized in the centre of development and researches laboratory of Moubydal Algiers. Gas chromatography/mass spectrometry: GC/MS was conducted using an Agilent 5973 GC/MS coupled to an Agilent 6890 gas chromatograph fitted with a split-splitless injector at 250°C (splitless mode). Analytical conditions have been fixed as follows: Agilent HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness), temperature program: from 50-250°C at 6°C/min, Helium was the carrier gas at 1.5 ml/ min. The injector and interface were operated at 230°C and 280°C, respectively. The identification of the components was performed on the basis of chromatographic retention indexes (RI) and retention times (RT), by comparison of the recorded mass spectra with computed data libraries (NIST98). For sesquiterpene hydrocarbons, further confirmations were obtained by comparing the mass spectra with data from the literature (Adam 2001).

Bioassays

Spider mite populations of T. urticae were collected from the experimental Station of INA greenhouses and reared on bean plants (Phaseolus vulgaris L.). Plants infested with mites were kept inside isolated cages within the greenhouse at 24± 6°C, 40-60% relative humidity (RH) and under natural daylight.

For direct contact toxicity, a leaf-dipping method was used with a control (water) at different concentrations (0.125, 0.25, 0.5 and 1%) of the tested essential oils. These concentrations were at X ½ intervals. Bean leaf disks 25 mm in diameter were dipped in each oil suspensions for 5 s. leaf disks were then drained and placed on moistened cotton in 14cm x 2cm Petri dishes. 30 motile forms were placed on each leaf disc in 10 replicates per treatment and the Petri dishes were covered with a ventilated lid and incubated (25 ± 2°C, 75 ±5 RH and 16 L: 8 D) for 24, 48 and 96h. At the end of these periods, the numbers of dead mites were counted under a dissecting microscope. Mites were considered dead if appendages did not move when prodded with a fine paintbrush.

To obtain eggs, 10 T. urticae females were taken from the stock colony and maintained on 2.5 cm diameter bean leaf disks on top of distilled water moistened cotton for oviposition and placed into 14 cm x 2 cm Petri dishes. After 24 h, the females were removed and 50 eggs were maintained per leaf disk and sprayed with same doses and in the same conditions as form mobiles tests. Non hatching eggs after six days are considered not living.

CONSTITUENTS	ABUNDANCE (%)			
	Laurel	**Rosemary**	**Mint**	**Thyme**
α-pinene	2.48	**15.25**	0.65	
Camphene	0.50	3.52		
Verbenene		0,51		
Sabinene			0.54	0.48
β- Pinene	0.84	0.60		
β-phellandrene			1.27	
Meta–mentha-1(7) ,8-diene			0.68	
α-Terpinene	0.40		0.16	
Limonene	1.16	3.08	**8.08**	
1,8cineole (Eucalyptol)	**17.42**	0.26		0.73
3-carene	0.85			
Menthol			1.37	
Iso-menthol			3.38	
Linalool	11.82			
Camphor	0.67	14.5		
Borneol	1.49	5.68		2.41
Para-cymene-8-ol	5.24	2.71		1.61
α-terpineol	6,56			
Trans dihydrocarvone			3.61	
Para cymene-9-ol	0.32	3.02		
Cis Sabinene hydrate acetate	0.53			
Z-ocimenone	1.64			
Thymol	**0.22**			**43.22**
Linalool acetate	2.32			
Carvenone			2.14	
Methyl ethyle carvacrol			0.24	
Carvone		1.41	**24.28**	8.35
Carvacrol	0.47			**24.40**
Iso-3-thujyl acetate	0.26		0.49	
Nonalole acetate	0.19			
α-Terpenyl acetate	0.74			
Limonene aldehyde	**10.53**			
Eugenol	2.40			
β-elemene	0.24			
δ-elemene			0,39	
Methyl eugenol	0.59		1,15	
Z-caryophyllene	**9.08**		1,17	
Caryophyllene	1.41		4,05	
β-humulene	0.34		0,45	
α-caryophyllene	0.38			
α-terpenyl iso butyrate	0.60			
γ-muurolene	0.33			
α-muurolene	0.30			
Trans-β- guaiene	0.66			
Bicyclogermacrene			2,42	
β-germacrene	0.78		0,28	
β-sesquiphellandrene	0.60			
Dihydroeugenol acetate	0.80		0,52	
Z-iso eugenol acetate	0.90			
Spathulenol	1.30		1.17	1.13
Caryophyllène oxide	0.78		1.24	0.64
14-hydroxy-9-epi-e-caryophyllène	1.71			
α-cymene		2.55		
Trans linalool oxide		0.76		
Cis-thujone		7.02		
Iso-3-thujanol		2.15		
Néo-3-thujanol		**11.89**		
3-thujanol		1.88		
α-terpineol		0.62		
Verbenone		**10.13**		
Isogeijerene		1.64		
Total	95.75	93.15	76.25	86.54

Table 1. Percentage composition of the essential oils of 4 plants from Algeria (detailed constituents –top, and chemical function –bottom).

Chemical function	**Essential oils constituents*(%)***			
	Laurel	R o s e m a r y	Mint	Thyme
Alcohols	25.73	28.87	8.56	5.6
Hydrocarbons	20.83	28.23	24.39	0.89
Ethers	17.4	0.26	8.30	0.73
Esters	16.36	2.32	1.39	0.53
Phenols	3.68	0	1.84	77.71
Cetones	2.7	32.17	30.53	0.97
Aldehyde	0.74	0	0	0
Oxides	0.95	0.76	1.24	0.64

Data analysis

Mortality observations were analyzed by analysis of variance (ANOVA). Newman and Keuls test was used to compare mean (Statitcf software, version 5). Probit analysis was used to determine LC_{50}; Abbott's formula was used for corrected mortalities.

Results and discussion

Chemical composition of the essential oils

The yields of the essential oils obtained by rosemary, laurel, thyme, and mint leaves extraction are respectively 1.4, 0.5, 2, and 1.6%v/w.

The chemical compounds found in these oils and their chemical functions are shown in Table1. The oil analysis by GC/MS permitted the identification of:

- 54 compounds representing 95.75% of the essential oil constituents from leaves of laurel. The main components are 1,8 cineole (17.42%), linalool (11.82%), α-terpenyl acetate (10.53%), Z-caryophyllene (9.80%), α-terpineol (6.56%), P-cymène-8-ol (5.24%) and α-terpinene (2.48%). Alcohols are dominant (25.73%) followed by hydrocarbons (20.83) and esters (16.36). The results of several authors confirm ours, at least for the major compound but with quantitative differences. Tajet (2002), the essential oil of the noble laurel harvested from forest of Yakourene (Algeria), Bouzouita et al.(2001) from Tunisia; Shatar & Altantseteg (2000) from Mongolia. For Macchioni et al. (2006), leaves of Laurus nobilis and L. novocanariensis include 91.84 % of mono terpene and only 1.4 % of sesquiterpenes.

- 25 compounds representing 95.76% of the essential oil constituents from leaves of rosemary with (α-pinene (15.25%), camphor (14.15%) neo-3-thujanol (11.89%), verbenone (10.13%), cis-thujone (7.02%) and borneol (5.68%). Cetones are dominant followed by alcohols and hydrocarbons 28.87, 28.23% respectively. Other reports have shown chemical composition percentages similar or higher than ours. For Boutekedjiret et al. (1999), the essential oil of the rosemary from Mountains of the Bibans situated 200 km east of Algiers, includes 35 compounds, of which eucalyptol (52.4%) is the major. From the Algerian Sahara Thirty compounds were characterized representing 98.2% with 1, 8-cineole (29.5%), 2-ethyl-4,5-dimethylphenol (12.0%) and camphor (11.5%) as the major components. (Touafek et al. 2004) From Tlemcen, west Algeria the major compounds to the spontaneous Rosemary are α- pinene (23.1%), camphor (15.3%) and ß-pinene (12.2%). To the cultivated Rosemary, the main compound is the camphor (13.8%), followed by α pinene (12.6%), 1,8-cineole (11.8%) and borneol (10.8%) (Atik Bekkara et al. 2007). In Egypt, we find two compositions, the one dominated classically by the camphor, α- pinene and the 1,8-cineole, and the other is verbenone and camphor. Finally, the rosemary of Corsica and Sardinia contains a rich essential oil there verbenone, acetate of bornyl and has α-pinene (Soliman et al. 1994). According to McCornick et al. 2006, the main cause of the variability of the major compounds of rosemary oil is due to the transformation its α-pinene to verbenone, while 1,8 cineole is constant.

- 50 compounds representing 95.25% of the essential oil constituents are found from leaves of mint. The main are carvone (24.28%), 1,8-cineole (8.30%), limonene (8.08%), caryophyllene (4.05%),

trans-dihydrocarvone (3.61%), iso-menthol (3.38%), bicyclogermacrene (2.42%), carvenone (2.14%) menthol (1.37%), β-phellandrene (1.27%); para-cymene and caryophyllene (1.24%), z-caryophyllene and spathulenol (1.17%)

Cetones are dominant (30.53 %) followed by some hydrocarbons (24.39 %). In Africa, mint essential oil showed a big difference in the number of identified constituents. But the carvone remains the major one (Ait-Ouazzou 2002; Younis & Bechir 2004). While for Kofidis *et al.* (2004), the essential oil of *Mentha spicata* leaves, from Greece, is characterized by a very high rate of linalool that is 85-93.9 % and only 2.1% of 1,8-cineole. They clarify that *Mentha spicata* L. [syn.: *M. viridis* L., *M.crispata* L., *M. microphylla* Koch, *M. tomentosa* Urv] plants are very polymorphic, both in their morphology and essential oils.

- 12 compounds representing 86.54 % of the essential oil constituents from leaves of thyme. The major compounds are thymol (43.22%), carvacrol (34.49%), carvone (8.35%), borneol (2.41%), para cymene8ol (1.61%) and spathulenol (1.13%). Phenols are dominant (77.71%). Our results are confirmed by authors: Bousbia (2004) from Algeria, Hudaib et al. (2002) from Jordan. For El-Guedoui (2003) the carvacrol is dominant (44.1%), followed by the thymol (26.4%).In Morocco Hmamouchi et al. (1997) note that essential oil of Thymus species. are also very rich in carvacrol or thymol. In Hungary, essential oil composition of three cultivated *Thymus* showed three different chemotypes: In the oil of *T. vulgaris* thymol (45.6%), in *T. x citriodorus* geraniol (39.2%) and *in T. x citriodorus*, "archer's gold," carvacrol (43.5%) was the main components. (Horváth et al. 2006)

This variability of the compounds is due to several factors. Indeed the quality and the return on extracted essential oil are influenced by: the method of extraction (Scalia et al. 1999; Chiasson et al. 2001); the fertilizer and the pH (the ideal, pH 4,5-5,4) grounds (Alvarez-Castellanos & Pascual-Villalobo 2003), the choice and the stage of the conditions of drying (Tateo & Riveted 1991), The geographical place (Maffei et al 1994), of the chemotype or the subspecies (Gören et al. 2001).

Bioassay Results

After 96h, all four essential were significantly lethal to *T. urticae* motile forms and eggs (Table 2). However, there were differences in the degree of toxicity of the oils to different stages of the two spotted spider mite.

The four oils caused 100 %mortality on the motile forms at 1%.If we consider the third dose (0.5%) to differentiate the toxicity of this oils, we notice that: The essential oil of thyme is more effective (99.60 %), followed by the rosemary (99.43%), the laurel (92.86%) and finally the mint with (84.13%.). Essential oil has acted on the motile forms of *T. urticae* dices the weakest dose (0,125%) and at the end of 24 hours. The mortality increased according to doses and time.

Table 2. Mortality (%) of eggs and mobiles forms (MF) of de *T. urticae* (±SD) after 96h of exposure.

Doses	%		D_0 control	D_1	D_2	D_3	D_4
essential oils	rosemary	MF	4.8 ± 0.76	48.02±3.33	68.65±3.19	99.43±4.7	100
		eggs	9 ± 2.94	45.9±4.42	55.82± 0.85	84.84±2.53	96.92±1.87
	thyme	MF	4.8 ± 0.76	58.09± 3.08	68.65± 3.57	99.60 ± 0.66	100
		eggs	9±2.94	46.6±4.17	60.88± 5.5	81.76±3.58	98.46±1.31
	laurel	MF	4.8±0.76	47.22±2.6	51.19±3.70	92.86±3.35	100
		eggs	9±2.94	24.6±3.43	47.91± 7.7	78.90± 4.08	96.70± 1.45
	mint	MF	4.8±0.76	49.60±4.4	52.38±4.7	84.13±4.01	100
		eggs	9± 2.94	17.1±4.11	38.7±6.67	62.0±6.44	85.9±5.66

MF=motile forms

On eggs the oils are less toxic. At the 1%, rosemary oil is more effective provoking 96.92 %, of mortality, the thyme 98.48 %, followed by the laurel (96.70 %) and finally the mint (85.9 %). The

LD_{50}, calculated from the various rights of regression (table 3) confirms the classification according to the mortalities.

Table 3. Functions and LC50 of essential oils (%).

	ESSENTIAL OILS			
	thyme	rosemary	laurel	mint
motiles	y=1.2103x + 3.0321 R^2=0.8317	y = 1.2782x + 1.8354 R^2=0.8797	y=1.3656x + 1.2177 R^2=0.913	y=1.2613x + 1.5653 R^2=0.8484
LD_{50}	0.050%	0.12%	0.16%	0.15%
eggs	y=1.0632x + 2.0249 R^2=0.9285	y=0.9801x + 2.2408 R^2=0.9557	y=1.2147x + 1.1532 R^2=0.9894	y=1.038x + 1.2046 R^2=0.9293
LD_{50}	0.16%	0.16%	0.22%	0.38%

The analysis of the variance in two classification criteria revealed a highly significant differences for product factors (F=76.74 and P=0,000) and stages (F 863.09 and P=0.000) as well as for their interaction (F=41.77 and P=0.000). The test of Newman and Keuls shows that the thyme and the rosemary act in a similar way (group A), the laurel and the mint are in 2 different groups (B, C).

As regards the results of our biological tests on the motile forms and the eggs of *T. urticae,* four essential oils was lightning in 1% provoking 100% of mortality of the mobile forms and showed a good effect ovicide engendering mortalities of eggs highly superior to 50% from 0.25%. Several works were realized on essential oils and their major compounds to estimate their acaricidal effects. Amer and Momen (2001) studied the repulsive and toxic effect of two essential oil, *Majorana hortensis* Moench and *Rosmarinus officinalis* L. against *Tetranychus urticae* Koch and *Eutetranychus orientalis* Klein in conditions of laboratory. Both oil showed more toxicity for *E. orientalis* than *T. urticae* with a significant increase of their efficiency with the rise of doses. They explain this toxicity by the ascendancy of the oxygenated compounds. On *T. cinnabarinus*, essential oils of *Cuminum cyminum*, *Pimpiinella ansium*, *Origanum syriacum*) and *Eucalyptus camaldulensis* are toxic by fumigation (Tuni and Sahikaya 1998 in Isman and Muray 2000). Won-il Cho *et al*. (2003) estimated fifty three essential oils of plants for their toxicity against eggs and the adults of *Tetranychus urticae* as well as on the adults of *Phytoseiulus persimilis* Athias-Henriot, by vapors and direct contact of the essential oil, the results showed a very important action by inhalation of various oil but more particularly that of peppermint and mint. The acaricidal activity of *Laurus nobilis* oil against *Psoroptes cuniculi*, at a concentration of 10%, led to a mortality rate of 73%; at 5% the average activity was significantly reduced to 51%, while dilutions of 2.5%, 1.25% and 0.625% were ineffective (Macchioni et al. 2006).

Saad El-Zemity et al. (2006) showed the effectiveness of fourteen essential oils and their monoterpenoidal constituents against house dust mites *Dermatophagoides pteronyssinus* Trouessart. These studies suggest that essential oils and their major constituents are potentially effective, environmentally acceptable, inexpensive, simple and alternative approach for the control of house dust mites.

Conclusion

Essential oil of four plants containing major compound very studied and very used in control management of various phytophagous enemies of plants. So thymol eucalyptol, eugenol, carvacrol, α-pinene, limonene, limonene aldehyde, Z-caryophyllène, the carvone, the linalool are widely used as insecticidal, acaricidal, bactericidal. The results show that herb essential oils, in particular those of thyme, rosemary, laurel and mint was proved to have potent acaricidal activity. Essential oil of thyme is significantly the most toxic against motile forms and eggs respectively. Acaricidal activity of these essential oils is a promising way to control mites in closed environments. Further studies should be made to confirm that and to evaluate their toxicity against natural enemies.

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DIRECT EFFECTS OF SOME PESTICIDES ON TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* KOCH AND ITS PREDATORY MITES (*PHYTOSEIULUS PERSIMILIS* ATHIAS-HENRIOT, *NEOSEIULUS CALIFORNICUS* [MCGREGOR]) ON CUCUMBER PLANTS UNDER GREENHOUSE CONDITIONS

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Abstract

Sub lethal dose of three commonly used pesticides in Turkey were evaluated on the two-spotted spider mite (TSSM) *Tetranychus urticae* and its predatory mites, (*Phytoseiulus persimilis*, *Neoseiulus californicus*) under greenhouse conditions during the season in 2007. The three pesticides (hexythiazox: a selective acaricide; bifenthrin: a pyrethroid insecticide-acaricide; and dimethoate: an organophosphate insecticide-acaricide) at of one-third the recommended field concentration as a low dosage were applied directly after releasing the predators to compare with pesticides without predators or with predators alone for controlling TSSM population. Additionally, IOBC toxicity criteria was used to evaluate the toxicity of tested pesticides against predators. Greenhouse results indicated that bifenthrin has harmful effects on *P. persimilis* while it was moderately harmful on *N. californicus*. While the toxicity of dimethoate and hexythiazox against tested predators was moderately harmful and harmless, respectively.

The efficacy of the combination of hexythiazox, bifenthrin combined with predators (*P. persimilis* or *N. californicus*) and dimethoate with *N. californicus* on TSSM population can effectively controlled the TSSM on cucumber plants when compared to the predatory mites alone. But on other hand, hexythiazox and dimethoate could not control the TSSM population properly. While the functions of *P. persimilis* seemed to be influnced negatively with dimethoate effect therefore, *P. Persimilis* combined with dimethoate can not provided rapidly and satisfactory control. Therefore, the sub lethal effects of pesticides may be used for controlling the TSSM population combined with predators in IPM programs.

Key–words

Sub lethal effects, IPM, IOBC toxicity criteria, *Tetranychus urticae,* predatory mites

Introduction

Pesticides may destroy or harm natural enemies following exposure by contact, ingestion or less commonly by respiration. They may also affect natural enemies indirectly by killing or contaminating their hosts or prey (Picone & Tassel 2002). Natural enemies are usually more susceptible to the effects of pesticides than their plant-feeding hosts or prey owing to their generally smaller size, searching habits, usually less-developed enzyme-based detoxification systems. For a given population of natural enemies exposed to a pesticide over a range of doses, some natural enemies will generally die within a relatively short period (e.g., 48 hours) while others may survive beyond that period (Charlet 1995). There are many different ways to test the effect of a pesticide or other compound on predatory mites, beginning with a slide-dip study and progressing in complexity to a field-scale study (Hassan & Oomen 1985). Sublethal effects are those effects that

occur in the pesticide-exposed survivors. The behavioural or physiological nature of sublethal effects on natural enemies tends to be fundamentally similar, although less severe, compared with that of lethal effects (Verkerk 2001). Farmers need to control a whole range of pests, not just one, and crop diseases as well. Often the most effective control programs are based on a combination of biological and chemical control. For this to be possible, scientists must select strains and species of natural enemies which are resistant to chemicals. They must also identify those chemicals which are compatible with natural enemies (Anonymous 2001).

The two-spotted spider mite (TSSM) is a common cause of crop damages in greenhouses in Turkey. This species is difficult to control with chemicals. Most populations have become resistant, and the mite hides underneath leaves where sprays and powders cannot reach them (Ay 2005). A species of predatory mite, *Phytoseiulus persimilis* Athias-Henriot, is being mass reared in Turkey as a biological control agent of two-spotted spider mite in vegetables. This predatory mite was recorded in natural colonies along the Turkish Mediterranean coast (Şekeroğlu & Kazak 1993). *Neoseiulus californicus* McGregor is also found naturally in Turkey and was first recorded in 2001 on strawberry, peach, bean and pepper plants (Çakmak & Çobanoğlu 2006).

The aim of this study is to evaluate the effect of three pesticides (sublethal dose) on *T. urticae*, *P. persimilis* and *N. californicus* populations as part of a mite management program and to define the toxicity of used pesticides against predators according to IOBC toxicity criteria under greenhouse conditions.

Material and Methods

Chemicals

Two insecticides-acaricides, bifenthrin and dimethoate, and a selective acaricide and mite growth regulator hexythiazox, were used in this experiment. These pesticides are commonly used to control spider mites and insects on vegetable crops in Turkey. The recommended field concentration of bifenthrin is 0.06 g a.i. l^{-1} (60 ppm) (Talstar® 10 EC, 100 g a.i. l^{-1}, Bayer): Pyrethroid insecticide-acaricide. The recommended field concentration of dimethoate is 0.45 g a.i. l^{-1} (450 ppm) (Poligor® EC, 400 g a.i. l^{-1}, Hektaş): Organophosphate insecticide-acaricide. The recommended field concentration of hexythiazox is 0.05 g a.i. l^{-1} (50 ppm) (Twister® 5 EC, 50 g a.i. l^{-1}, Hektaş). The experimental concentration of the pesticides was one-third of the recommended field concentration i.e. bifenthrin 0.02 g a.i. l^{-1} (20 ppm), dimethoate 0.15 g a.i. l^{-1} (150 ppm) and hexythiazox 0.017 g a.i. l^{-1} (17 ppm).

Source of mites

Tetranychus urticae was reared on bean plants (*Phaseolus vulgaris* L. cv. Barbunia) at 25 ± 1 °C and 65 ± 10 % RH under a 16-h light regime. Clean plants were grown in a climate room (same regime) until they were 2-weeks old and were subsequently added bi-weekly to the spider mite culture. The predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* were reared at 25 °C on detached bean leaves infested with two-spotted spider mites. These leaves were put on an inverted flower pot in a water-containing tray covered with a Plexiglas container. Some 2–3 leaves from the spider mite culture were added to the cultures bi-weekly. *Phytoseiulus persimilis*, Turkish strain, was obtained from fields in Hatay, Turkey. *Neoseiulus californicus,* Spical®, was obtained from Koppert BV and reared on bean leaves with spider mites.

Greenhouse trials

Cucumber plants (*Cucumis sativus* L.) were used in the greenhouse as host plant. Cucumber seedlings were prepared for planting and then transferred to the greenhouse. Blocks of plants were separated from each other to prevent plants touching and mites moving between blocks and were surrounded with cloth barricades. Plants were infested in 01 April 2007 with TSSM (30 females/plant) when they became mature to the 4 real leaves phase. Predatory mites (*P. persimilis* or *N. californicus*) were released i.e. 4 predatory females per plant, after 17 days from infestation with TSSM. Thereafter, pesticides were applied with a hand sprayer seven days after predators release. Leaf samples were taken from all blocks by the intervel of 7, 14 and 21 days from application of pesticides and also before releasing and application. The number of predatory mites and also the egg, immature and adult stages of TSSM were counted on an area of 8 cm^2 of cucumber leaf in laboratory. Four leaves one from each plant (middle part), were sampled from each block. The corrected efficacy percent was calculated according to Sun-Shepard formula (Püntener 1981):

Table 1. Mean density of predatory mites for treatments of pesticides on an area of 8 cm^2 of cucumber leaf.

Treatment	Before spraying	3 days	7 days	14 days	21 days
Dimethoate + *P. persimilis*	3	0.75	0.5	0.75	1.5
Dimethoate + *N. californicus*	3	1	0.75	1	0.5
Bifenthrin + *P. persimilis*	3.75	0.5	0	0	0
Bifenthrin + *N. californicus*	4.25	2	3	1	0
Hexythiazox + *P. persimilis*	3.25	2.5	4	0.5	0
Hexythiazox + *N. californicus*	3.5	2.75	3	1.25	0

Table 2. Mean mortality ratio (%) of predatory mites for treatments of pesticides and classification of pesticides toxicity according to IOBC category

Treatment	Mortality % (for third day)	IOBC category (for third day)
Dimethoate + *P. persimilis*	75	M
Dimethoate + *N. californicus*	66.66	M
Bifenthrin + *P. persimilis*	90.90	T
Bifenthrin+ *N. californicus*	52.94	M
Hexythiazox + *P. persimilis*	23	N
Hexythiazox + *N. californicus*	21.4	N

Table 3. Mean of corrected efficacy (± s.e) for greenhouse treatments against TSSM populations

Treatment	Corrected efficacy± s.e		
	7 days	14 days	21 days
P. persimilis + dimethoate	75.34 ± 1.04 d	85.30 ± 0.79 c	85.32 ±1.17 d
N. californicus + dimethoate	80.46 ± 1.00 c	90.49 ± 0.61 b	94.50 ± 1.00 b
Dimethoate	56 ± 1.51 e	37.96 ± 3.46 d	31.55 ± 2.49 f
P. persimilis + bifenthrin	95.41 ± 1.06 a	99.62 ± 0.21 a	99.82 ± 0.17 a
N. californicus + bifenthrin	92.89 ± 0.27 a	99.48 ± 0.17 a	99.83 ± 0.16 a
Bifenthrin	86.89 ± 0.94 b	98.70 ± 0.27 a	98.75 ± 0.26 a
P. persimilis + hexythiazox	94.28 ± 0.67 a	99.62 ± 0.21 a	99.81 ± 0.18 a
N. californicus + hexythiazox	88.77 ± 0.85 b	99.31 ± 0 a	99.83 ± 0.16 a
Hexythiazox	35.62 ± 2.04 g	42.05 ± 2.15 d	49.43 ± 2.06 e
P. persimilis	65.58 ± 1.65 e	84.99 ± 0.84 c	89.13 ± 1.14 c
N. californicus	66.66 ± 1.51 e	87.34 ± 0.62 c	88.27 ± 0.79 c
Control (water)	0 ± 0 h	0 ±0 e	0 ± 0 g
Statistic (Type: split plot;	LSD 0.05 = 2.88	LSD 0.05 = 4.17	LSD 0.05 = 3.45
Duncan's Test at *P*=0.05)	R^2 = 0.99; df = 47	R^2 = 0.99; df = 47	R^2 = 0.99; df= 47

$$\textbf{Corrected efficacy \%} = \frac{Change\%\ in\ related\ plot \pm Change\%\ in\ control\ plot\ population}{100 \pm Change\%\ in\ control\ plot\ population} * 100$$

Where:

$$\text{Change \% in control} = \frac{Population\ in\ control\ plot\ after\ treatment - Population\ in\ control\ before\ treatment}{Population\ in\ control\ plot\ before\ treatment} * 100$$

$$\text{Change \% in treated} = \frac{Population\ in\ treated\ plot\ before\ treatment - Population\ in\ treated\ after\ treatment}{Population\ in\ treated\ plot\ before\ treatment} * 100$$

The classification of the side-effect of a pesticides was evaluated according to IOBC category (International Organization for Biological Control) for field and semi-field tests against natural enemies where; 0-50 % mortality is harmless or slightly harmful (N), 51-75 % mortality is moderately harmful (M) and >75 % mortality is harmful (T) (Boller *et al.* 2006).

The greenhouse experiments were designed in twelve blocks with 4 plants in each block. Six blocks containing pesticides and predatory mites (*P. persimilis* or *N. californicus*); three blocks containing TSSM and pesticides without predatory mites and two blocks containing TSSM and predatory mites without pesticides and the last block was designed as a control block (TSSM + water). The average temperature and relative humidity were 23.05 °C and 51 % during overall trail period, respectively. Data were analyzed with ANOVA by using the computer program Cohort Software and means were separated by the aid of Duncan's Test.

Results

Effect of dimethoate

Results in Table 1 and 2 show that, the population of predators decreased and influenced by dimethoate effect which caused 75 % and 66.66 % mortality for *P. persimilis* and *N. californicus,* respectively. The efficacy of *N. californicus* with dimethoate gradually had increased over time; therefore, density of TSSM decreased and also caused decreasing the population of *N. californicus* (Figure 1). While the efficacy of *P. persimilis* with dimethoate against TSSM population increased from 75.34 % to 85.30 % and then no significant increase was recorded. The efficacy of predators with dimethoate had showed significant difference when compared with dimethoate alone over time. Also, there was significant difference within the treatments which involved dimethoate with predators (Table 3). *Effect of bifenthrin*

The effect of bifenthrin was very toxic to *P. persimilis* and caused 90.90 % mortality and then was not observed during the test time. While the toxicity of bifenthrin against *N. californicus* caused 52.94 % mortality and then population of *N. californicus* was observed (Table 1 & 2) despite the low density of TSSM population, which remained under the influence of bifenthrin effectiveness (Figure 2). The efficacy of bifenthrin agansit TSSM population showed no significant difference when compared with the treatments which involved predatory mites with bifenthrin for 14 or 21 days after application (Table 3).

Effect of hexythiazox

Density of predators populations (*P. persimilis* and *N. californicus*) was not significantly affected for 3 days after the application of hexythiazox which caused 23 % and 21.4 % mortality sequentially (Table 1 & 2). Obviously, there was a complete and an energetic (synergistic) effect between the predators and hexythiazox against TSSM population and significant difference was found between the efficacy of predators with hexythiazox and the efficacy of hexythiazox alone or the efficacy of predators alone (Table 3).

The efficacy of predators with hexythiazox was effective to TSSM population over time. The decrease in density of TSSM population was accompanied by gradual decrease in the density of predators population (Figure 3). Comparison of treatments against TSSM

The efficacy of hexythiazox and bifenthrin combined with predators (*P. persimilis* or *N. californicus*) on TSSM population showed significant difference ($P<0.05$) with the treatments which involved predatory mites or with dimethoate and hexythiazox alone. Additionally, there was a significant difference between hexythiazox and dimethoate efficacy when compared with the predators efficacy during the test time. On the other hand, there was significant

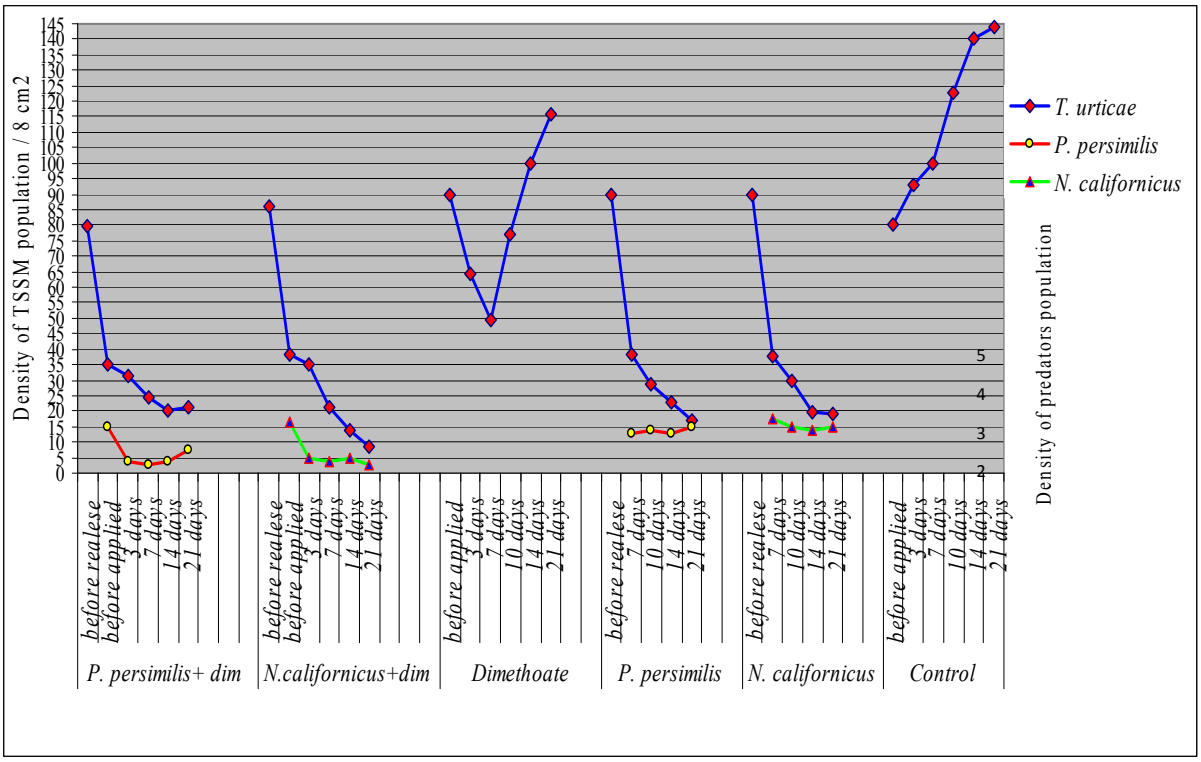

Figure 1. Mean density of TSSM and predatory mites population for treatments of dimethoate on an area of 8 cm^2 of cucumber leaf

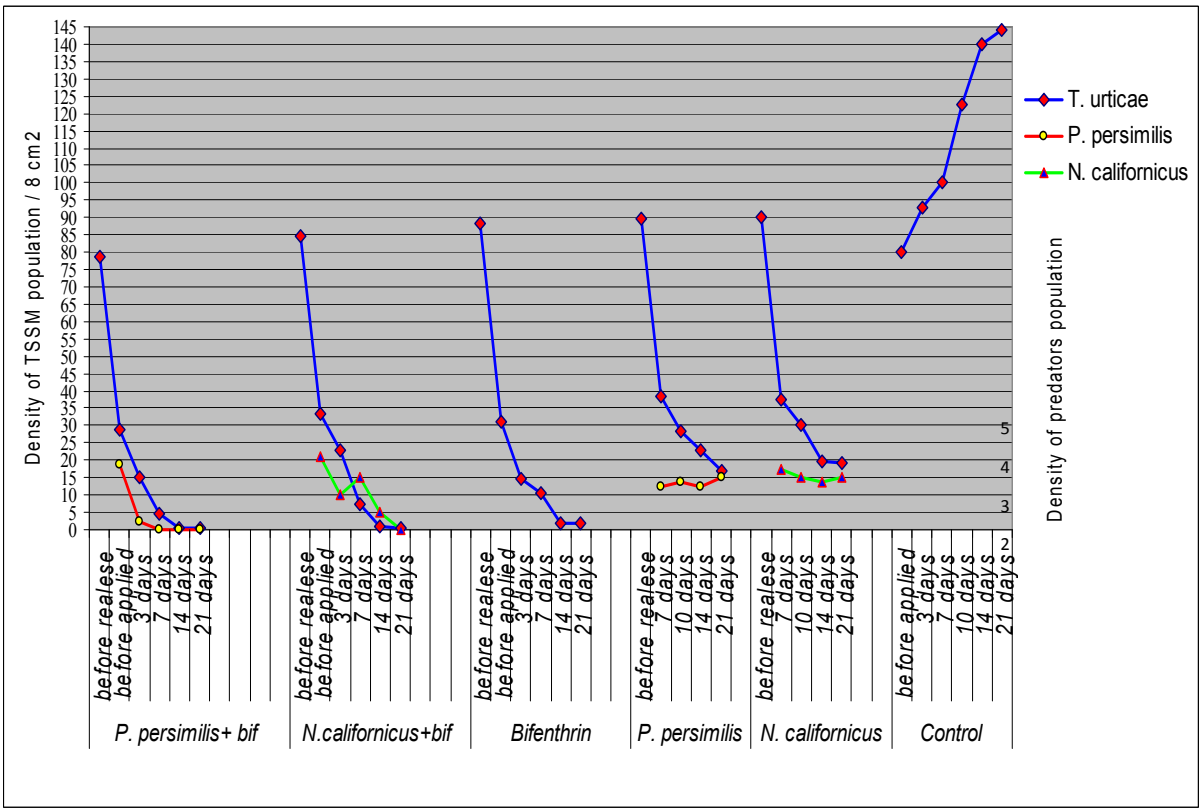

Figure 2. Mean density of TSSM and predator mites populations for treatments of bifenthrin on an area of 8 cm^2 of cucumber leaf

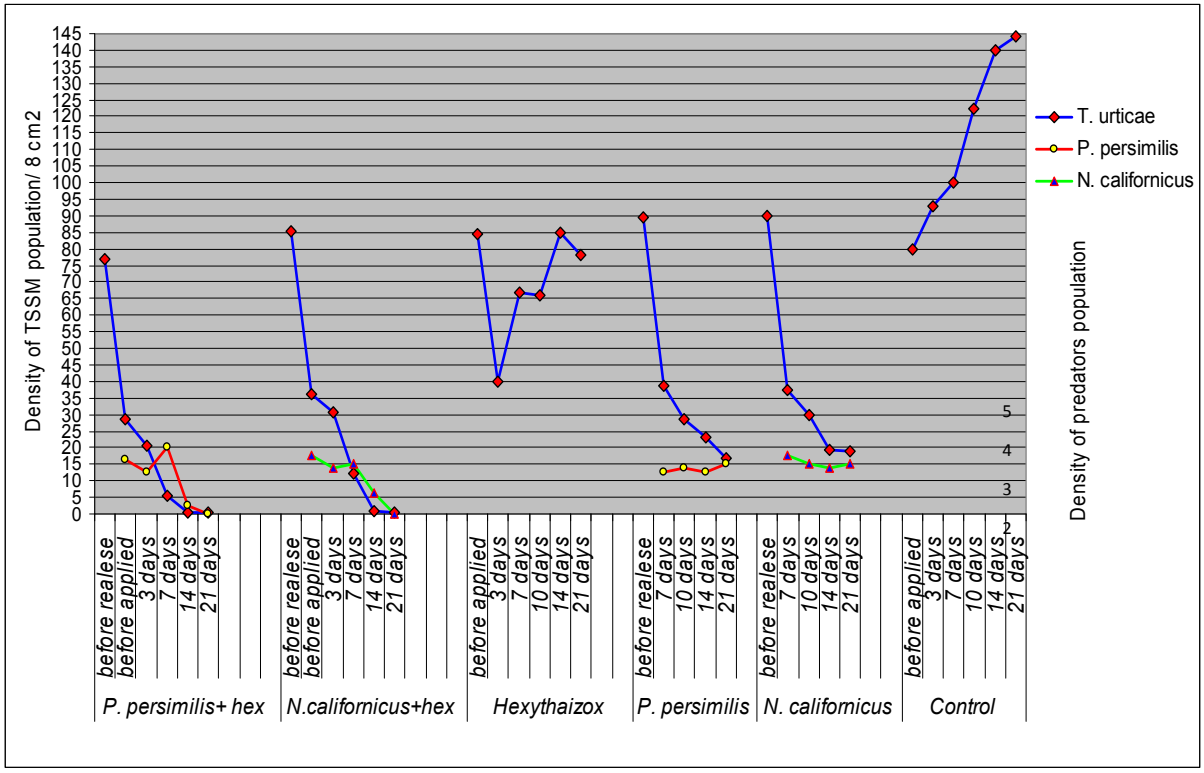

Figure 3. Mean density of TSSM and predator mites populations for treatments of hexythiazox on an area of 8 cm^2 of cucumber leaf

difference within the treatments which involved dimethoate with predators and also significant difference was found with hexythiazox and bifenthrin combined with predators (Table 3).

Discussion

Toxicological studies on predaceous mites of economic importance are mainly concerned with measuring the possible adverse effects of pesticides on these tiny animals. Data on initial toxicity of pesticides are indispensable to the development of safe spray programs for integrated control in orchards, fields, or greenhouses. Several test methods have been designed for the evaluation of pesticides in this respect. Although such methods do not always allow an exact prediction of what will happen in the field, they will give a fair clue as to what risks can be expected. Those pesticides that prove absolutely harmless in laboratory tests are in all probability also harmless in the field. In case of doubt semi-field tests or field tests should provide the answers (Hele & Overmeer 1985).

Our study was designed as a 'semi-field' trial primarily to assess compatibility of some pesticides with *P. persimilis* and *N. californicus* to control spider mites under a realistic greenhouse conditions with cucumber as the host plant. In this paper, we used low concentrations of various pesticides as direct effect after the release of predatory mites to control TSSM. From our results, we concluded that after the application of hexythiazox the predators, *P. persimilis* or *N. californicus* can effectively control the two-spotted spider mite (TSSM) on cucumber as compared to the predatory mites alone or to hexythiazox alone which could not control the mite properly. While after the application of dimethoate, *P. persimilis* can effectively control the TSSM only first 7 days when compared to the predatory mites alone or to dimethoate alone. Thereafter, significant difference was found with the efficacy of predators alone. Verkerk (2001) reported that the sub lethal dose of pesticides on natural enemies may have behavioural and physiological effects such as attack ratio, handling time, rate of discovery, development time and reproduction (Verkerk 2001). Therefore, *P. persimilis* efficiency seems to influence with direct effect of dimethoate and caused negative effects to predator functions.

However, *N. californicus* or *P. persimilis* had supported the hexythiazox effects but only *N.*

californicus had supported the dimethoate effects and provided effective rapid control of TSSM populations when compared to the predatory mites alone or to dimethoate and hexythiazox alone. Part of these results seem to agree with Richard and Campbell (1999) who reported that the integrated control of *T. urticae* using clofentezine in conjunction with *P. persimilis* was likely to be more effective than an approach based on chemical or biological measures alone (Richard & Campbell 1999). In addition, Verkerk reported that the effects of pesticides on natural enemies are often negative; pesticides can sometimes enhance natural enemy function particularly if they are selective against the pests or are used at low dosages (Verkerk 2001). Although, the effectiveness of phytoseiid predators for the biological control of spider mites on their host plants, the predators alone may not be able to maintain spider mite populations below the economic injury level for an extended period of time (Field & Hoy 1986, Kim Paik 1996a, Ibrahim & Yee 2000). Therefore, biological control of spider mites can be accomplished by the selective use of pesticides that are more toxic to the pest species than to its natural enemies (Hoy & Ouyang 1986, Zhang & Sanderson 1990, Kim Paik 1996).

Bifenthrin was effective to TSSM population. Therefore, no significant difference was found within bifenthrin treatments particularly, after 14 and 21 days, and there was significant difference with predators alone.

Bifenthrin was extremely toxic to *P. persimilis* but not to *N. californicus*. According to IOBC category, bifenthrin has harmful effects on *P. persimilis* but has moderately harmful effects on *N. californicus*. While the toxicity of dimethoate against tested predators is moderate, the toxicity of hexythiazox against tested predators is nil.

Kenneth *et al.* (2002) found that abamectin, Gowan 1725, hexythiazox, horticultural oil, neem oil, pyridaben, and spionosyn residues caused no mortality to *P. persimilis* 1, 3, 7, or 14 days after application, and that *T. urticae* mortality from hexythiazox and spinosad residues was not significantly greater than the control; and bifenthrin and chlorfenapyr residues were toxic to *P. persimilis* (Kenneth *et al.* 2002). Sato *et al.* (2002) reported that fenpyroximate, fenpropathrin, dimethoate, propargite, sulfur, and benomyl were innocuous to *N. californicus* which used against TSSM on strawberry under field conditions (Sato *et al.* 2002).

Therefore, TSSM control can be successfully achieved by combining biological and chemical measures using chemicals with low dosage.

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INFLUENCE OF DIFFERENT CONTROL PRACTICES ON THE TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* (KOCH) ON STRAWBERRY UNDER LOW TUNNELS IN EGYPT

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Abstract

The efficacy of the predatory mites, *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus californicus* McGregor, *Euseius scutalis* (Athias-Henriot) and *N. barkeri* (Hughes) and of Vertimec®, Plant guard, micronised Sulfur and Sumite against *Tetranychus urticae* Koch, the main pest of Sweet Charlie and Camarosa strawberry cultivars, was tested from 2000 to 2002 at Qalubia Governorate. Results revealed that the reduction percentage of the active stages of *T. urticae* achieved by releasing of *P. persimilis* and *N. californicus* was higher than the one observed with *E. scutalis* and *N. barkeri* when the release rates was about 5-10 individuals /plant. Also, acaricidal activity, termed as reduction percentage, ranged between 50-80 % for vertimec® while that of Plant guard, Sumite and micronised Sulfur ranged only between 5-35%. It was further observed that the populations of *T. urticae* as well as of the released predatory mites were more abundant on Camarosa than on Sweet Charlie plots.

Key words

Strawberry, *Tetranychus urticae*, Phytoseiidae mites, chemical control

Introduction

Strawberry (*Fragaria* x *ananassa* Duch) is a highly valuable crop, spread worldwide. In Egypt, at Nile Delta, it was produced in Qalubia Governorate since the 1930s. The indigenous cultivar Balady, a juicy cultivar, characterized by strong scent, high sugar content, and small fruit size, was the dominant variety. However, during the 1990s strawberry farmers replaced this cultivar by several exotic ones, because of their high yield, their extended fruiting period (from November till June), and their larger fruit size and lower sugar content. Several arthropod and disease pests attack strawberry plantation (Johnson 1998). The most serious pests are the two-spotted spider mite *Tetranychus urticae* Koch and the sweet peach aphid *Myzus persicae* (Sulzer) (Oatman & McMurtry, 1966; Wysoki, 1985; Price, 2002). Spider mite population may build up rapidly during the growth season, and leaf damages reduce the quantity and quality of harvestable fruits (Mckinlay & Thomson, 1987; Kunimoto, 2000; Walsh et al., 2002). In Egypt, *T. urticae* is considered as the key pest for strawberry; infestation starts early during transplantation in September and continues until the end of season in June. Biological control of *T. urticae* on strawberry has been widely studied either through mass release of phytoseiid predatory mites or through testing naturally-occurring predatory mites or insects (Waite, 1988; Garcia-Mari & Gonzalez-Zamora, 1999). A pilot experiment has been carried out by EL-Laithy in 1998 on the biocontrol of *T. urticae* using

Neoseiulus californicus McGregor on strawberry planted in plastic tunnels. The results of the experiment (see EL-Laithy *et al*., 2004) has encouraged further field studies. The present study aims at (1) evaluating the impact of some exotic and indigenous predatory mite species, namely *Phytoseiulus persimilis* Athias-Henriot, N. *californicus*, *Euseius scutalis* (Athias-Henriot) and N. *barkeri* (Hughes) and (2) examining the acaricidal activity of Vertimec®, Plant guard, micronised Sulfur and Sumite in the light of the average strawberry yield obtained.

Material and Methods

Two newly Californian cultivars of strawberry were chosen for this study. The first cultivar is Camarosa, characterized by an abundant vegetative growth and fruits with bright shape and sweet taste. The second cultivar is Sweet-Charlie, which has similar characteristics, yet by a lesser degree.

General experimental design: experiment was conducted at Qalubia Governorate over two successive seasons 2000/2001 and 2001/2002 in a complete randomized block design with the objective of studying the effect of different biological and chemical control methods on the spider mite infesting the two strawberry cultivars. Treatments included releasing of four phytoseiid predatory mites (*P. persimilis*, N. *californicus*, *E. scutalis* and *N. barkeri)* and four pesticide compounds (Vertimec®, Plant guard, micronised Sulfur and Sumite). Each treatment was replicated three times. The replicate consisted of two raised beds 4m x 1.2m, each having four lines of strawberry plants. The total number of strawberry plants in each replicate was 128 (16 x 4 x 2). A vertical plastic sheet of 70 cm height (to fit with the tunnel covering sheet) isolated the replicates from each other.

Sampling procedure: samples were taken each 2 weeks. Ten leaves were randomly collected from each replicate (i.e. 30 leaves for each treatment). Collected samples were kept in polyethylene bags, tightly closed with rubber bands and preserved in an ice box. The leaves sampled were transferred for examination in the laboratory using a stereomicroscope. Counts were made for active stages of *T. urticae* and predatory mites. The first spraying has been done the 17/02/2001 (for both chemical and biological applications) and a respraying has been carried out on the 24/03/2001. In 2002, the first spraying was performed on the 13/01/2002 and a respraying has been carried out the 17/02/2002.

A. *Chemical control*:

Micronised Sulfur (70% wettable powder), Sumite (10 % Etoxazole), the biopesticide Vertimec® (1.8 % Abamectin) and Plant guard (10 million cells of *Trichoderma harizianum* / L) were used. The rate of application was 2g / L, 0.5ml / L, 0.4 ml / L and 3ml / L, respectively. Treatments started when the population density approached an average of 2-5 *T. urticae* individuals / strawberry leaflet. Repetition of application occurred according to the *T*. urtica*e* population rebuild up density, fruit picking date and after brunning of strawberry plants for removing of old and highly infested leaves. Samples were taken randomly before and after spraying. Reduction percentages of *T. urticae* active stages were estimated following Henderson and Tilton (Fleming & Retnakaran, 1985).

Reduction %= 1-(**treatment after** x control before **/ treatment before** x control after)

B- Biological control:

Four predatory mite species were used as agents for biological control against *T. urticae*. The strain of *P. persimilis* used is a cross breading colony between one population from Italy (Prof. S. Ragusa, 1998) and one population from Morocco (Prof. M. El-osmani, 2007). The population of *N. californicus* is a French strain (Dr. G. Fauvel, 1996). The strain of *N. barkeri* used was freshly collected from *Bermuda grass* Giza city Egypt summer 1999. The population of *E. scutalis* was collected from mulberry leaves*,* Giza city summer 1999*/* Egypt.

The Phytoseiidae species were reared using methods modified by McMurtry and Scriven (1965). Large plastic boxes (26 x 15 x 10 cm) were used. Cotton pads were put in the middle of each box, leaving a space containing water to act as a barrier preventing predatory mites from escaping. Tangle foot strips were further placed at the edges of the box. Cotton pads were kept saturated with water. Excised bean leaves highly infested with *T. urticae* were provided every day as a food source. Plastic boxes were kept in an incubator at 28 °C ± 2 and 70 ± 10% relative humidity, but without adjustment of photoperiod (the used incubators are not provided with unit for photoperiod adjustment). The colony of *T. urticae* was maintained on potted beans, namely *Phaseolus vulgaris* L., in a rearing experimental glasshouse (1.5 x 2 x 3 m).

Phytoseiid mite release was initiated when the population density of *T. urticae* on strawberry samples averaged 2-5 individuals / leaflet. The ratio between predator and prey ranged between 1:10 to 1:7. As a strawberry plant in early growing stage has 6-10 leaves/plant, hence we release one predator /plant which equal 1: 6-10 (predator – prey ratio) The required population size of predatory mite individuals / replicate was calculated according to the following formula:

Released number = Total number of *Tetranychus urticae* per replicates per plant / /proposed prey - predator ratio

Bean leaves with predatory mites (formerly counted, mix of mobile stages) were transferred in an ice box (10- 3 °C) to strawberry fields. Distribution was performed on infested strawberry plants (i.e. on patches of *T. urticae*). Repetition of releasing depended on the population size of *T. urticae.* After releasing, samples were taken weekly. Active stages of each of *T. urticae* and predatory mites were counted. The reduction percentage of spider mite densities was determined following Henderson and Tilton (Fleming & Retnakaran, 1985).

***Statistical analysis*:** the obtained data (biweekly mean of *T. urticae*) were analysed witha variance analysis (ANOVA) with mean separation at 5% level of significance (Snedecor & Cochran, 1967), followed by a LSD mean comparison test.

Results and Discussion

Chemical control trial

2000/2001 season

Sprayings of the selected biocides started on the two strawberry cultivars in mid February (Table 1), but the population of *T. urticae* was not significantly reduced except in the plots treated with Vertimec® on Sweet Charlie cv.. On this variery, for the treatment Sumite, the density increased after spraying. Therefore, a second spray was applied 5 weeks later (24 /3 /2001). The resurgent population of *T. urticae* peaked in mid April and then decreased continuously until the end of the season (Table 1). Maximal acaricidal activity (mean population density) was obtained with Vertimec®, while Sumite, micronized Sulfur, Plant guard showed weak activity.

Similar results were obtained for the cultivar Camarosa, even if the population increase was more relevant during the season, particularly in April (Table 1). The highest reduction of population density on Sweet Charlie was 71.7% for Vertimec compared to 20.6, 28.1 and 16.1 % for Plant guard, micronised Sulfur and Sumite, respectively. Percentages for Camarosa were 57.5, 24.4, 9.9 and 6.4%. Population fluctuation of *T. urticae* within treatments are due to not only the applied treatments but in addition winded days (in particular that strawberries are planted in sandy soil and winds cause an extra infestation with mites), the physiological state of strawberry plants in particular fruiting cycle which affect suitability of plants for spider mite. As shown in the Table 1, the population starts to consistently decline from early April in all treatments including control even there is fruiting until June.

2001/2002 season

Results obtained in 2001/2002 indicated an early infestation of *T. urticae* in mid January (Table 1). Acaricidal activity was not detectable on Sweet Charlie for Sumite, micronised Sulfur and Plant guard while population reduction calculated for vertimec® reached 83.9%, the second spraying being unnecessary. Population of *T. urticae* peaked during March except in Vertimec® plots (Table 1). Acaricidal activity on Camarosa reflected also a lower efficiency of Sumite, micronised Sulfur, Plant guard, as the percentage of reduction of these products were 14.6, 37.5, 30.4 % compared with 72.2 % for Vertimec®. Along the season on Camarosa cv, mean population of *T. urticae* did not exceed 38.4 individuals / leaf in Vertimec® plots but it peaked to 317.7, 239.5, 298.4 and 317.7 individuals / leaf in Sumite, micronised Sulfur, Plant guard and control plots, respectively. We can not see difference between sumite, sulfur and control, a quite better result for plant guarded but surely not sufficient and a very good reduction with vertimec;

The lower acaricidal activity of micronised Sulfur found herein could be due to the unsuitable climatic conditions prevailing during the experimental seasons i.e. temperature and relative humidity, as mentioned by Auger *et. al*. (2003). Temperature varied between 17-25˚C and relative humidity between 55-65% during the 1st season and the same parameters were between 18-23˚C and 55-68 %, respectively, during the 2nd season. In the present study, *T. urticae* reached its highest density during the period from the end of February till April. Thereafter, population started a consistent decrease. As interpreted by Rodriguez *et a*l. (1960) and Hamilton- Kemp *et al*. (1988) the physiological changes of the plant could no longer be suitable for mite reproduction.

Table 1. Mean number of Tetranychus urticae active stages / leaf of two cultivars of strawberry affected by different pesticide compounds at Qaliobia Governorate during 2000 /2001 and 2001/2002 seasons.

2000/2001							2001/2002					
Cultivar	Sampling date	**Vertimec**	**Plant-Guard**	**Micronised sulfur**	**Sumite**	**Control**	Sampling date	**Vertimec**	**Plant-Guard**	**Micronised sulfur**	**Sumite**	**Control**
Sweet Charlie	**17/2 +**	**7.3**	**6.4**	**6.8**	**6.7**	**9.0**	**13/1 +**	**5.9**	**4.0**	**5.7**	**5.1**	**9.6**
	24/2	0.7	1.4	3.8	9.7	12.4	**20/1**	0.8	15	11.5	8.6	33.5
	10/3	3.2	12.5	9.3	16.5	32.4	**3/2**	0.8	13.1	15.9	17.9	34
	24/3 ++	3.7	10.6	20.3	18.2	21.2	**17/2 + +**	0	14.9	16.4	21	38.5
	7/4	43.1	75.1	54	48.6	99.5	**3/3**	10.1	68	73.5	83.2	118.2
	21/4	28.7	141.2	129.7	143	148.4	**17/3**	13.3	76.2	90.7	107.1	96.4
	5/5	6.5	46.1	41.4	31.3	41.1	**31/3**	2	40.6	49.1	41.5	49.3
	19/5	0.4	1.8	1.8	1.9	4.7	**14/4**	0.5	3.8	40.9	3.6	5.6
	2/6						**28/4**	0	0.4	0.2	0.3	1.1
	16/6						**21/5**	0.9	1.1	0.6	1.7	1.3
	Treatments Mean	**11.7b**	**37.0a**	**33.38a**	**34.49a**	**45.97a**	**TreatmentMean**	**3.43c**	**23.7 b**	**26.77 ab**	**29.2 ab**	**38.75 a**
	Mean reduction%	**47.8**	**7.18**	**6.36**	**0.0**		**Mean reduction%**	**87.1**	**31.7**	**8.3**	**8.3**	
Camarosa	**17/2 +**	**16.3**	**16.9**	**8.2**	**8.2**	**23.3**	**13/1 +**	**38.5**	**32.5**	**46.7**	**21.9**	**27.5**
	24/2	9.5	30	25	29.2	64.4	**20/1**	1.6	34.9	39.5	32.5	60.8
	10/3	30.3	61.6	42.9	76.8	98.6	**3/2**	5.5	73.9	75.9	78.3	141.7
	24/3 ++	61.5	105.9	92.8	127	159.5	**17/2 + +**	30.1	247.8	226.5	209.2	225
	7/4	60.4	239.1	192.8	208.7	237.1	**3/3**	7.0	232.2	207.6	317.7	339.8
	21/4	29.3	81.2	73.3	63.4	120.3	**17/3**	19.4	298.4	239.5	295.3	265.1
	5/5	0.3	42.5	33.6	33.1	45.8	**31/3**	4.9	55.9	52.7	47	57
	19/5	0.7	2.2	1.9	2.2	2.7	**14/4**	0	4.2	3.6	4.9	7.3
	2/6						**28/4**	0	1.3	1.1	1.5	2.4
	16/6						**212/5**	1.3	3.8	4.2	3.8	4.4
	Treatments Mean	**26.04 c**	**72.42 ab**	**58.82 b**	**68.57ab**	**93.95 a**	**Mean**	**10.82 c**	**96.40 ab**	**89.73 ab**	**101.21 ab**	**113.10 a**
	Mean reduction%	**58.2**	**13.60**	**20.4**	**14.1**		**Mean reduction%**	**76.2**	**16.8**	**27.5**	**15.1**	

+Pretreatment sample and spraying date

+= respray

N.P. Vertimec was not resprayed on Sweet Charlie during 2nd season

Table 2. Mean number of Tetranychus urticae stages/leaf of two cultivars of strawberry affected by releasing four predacious mite species at Kalubia Governorate during 2000 /2001 and 2001/2002 seasons.

2000/2001							2001/2002					
Cultivar	Sampling date	***P persimilis***	***A. californicus***	***E. scutalus***	***A .barkeri***	**Control**	Sampling date	***P persimilis***	***A. californicus***	***E. scutalus***	***A .barkeri***	**Control**
SweetCharlie	**17/2+**	**10.0**	**9.7**	**15.6**	**19.5**	**9.0**	**13/1 +**	**13.4**	**13.1**	**7.9**	**9.6**	**9.6**
	24/2	1.1	2.7	1.5	3.1	7.4	**20/1**	2.6	2.7	12.6	14.7	33.5
	10/3	0	0	2.9	2.2	32.4	**3/2**	0.2	0.4	5.3	9.6	34
	24/3 ++	1.9	1.2	6.5	11.0	21.2	**17/2 + +**	0.2	0	4.8	18.5	38.5
	7/4	0	0	9	18.8	99.5	**3/3**	0	0	19.3	67.1	118.3
	21/4	2.5	0	10.7	30.6	148.4	**17/3**	09	1.3	32.3	15.8	96.4
	5/5	0	0	0	4.0	41.1	**31/3**	0	0	14	10.1	49.3
	19/5	0	0	0.2	0.1	4.7	**14/4**	0	0	1.4	1.0	5.6
	2/6						**28/4**	0	0	0	0	1.1
	16/6						**12/5**	0.8	0.5	1.3	0.6	1.3
	Treatments Mean	1.93 c	1.7 c	5.18 b	11.16 b	45.47	TreatmentsMean	1.8 c	1.78 c	9.86 b	8.8 b	38.75 a
	Mean reduction %	95.8	93.7	78.6	79.4		Mean reduction %	94.0	98.9	31.5	46.8	
Camarosa	**17/2+**	**11.2**	**21.2**	**15.4**	**17.4**	**23.3**	**13/1 +**	**50.5**	**39.9**	**42.9**	**32.4**	**27.5**
	24/2	7.7	14.6	19.9	19.2	64.4	**20/1**	3.3	8.3	20.8	25.8	60.8
	10/3	12.2	9.0	23.6	51.0	98.6	**3/2**	16.0	17.6	42.2	46.2	141.7
	24/3 ++	26.2	23.4	45.9	61.4	159.5	**17/2 + +**	32.6	23.4	121	107.1	225.0
	7/4	38.7	16.9	78.3	147.7	237.1	**3/3**	7.9	3.4	81.9	55.6	339.8
	21/4	2.2	0	13.0	26.8	120.3	**17/3 ++**	2.4	1.7	59.6 ++	53.9 ++	265.1
	5/5	0	0	0	3.1	45.8	**31/3**	5.4	1.5	18.2	18.8	57.0
	19/5	0	0	0.2	1.2	2.7	**14/4**	0	0	0.7	0.8	7.3
	2/6						**28/4**	0	0	0	0	2.4
	16/6						**12/5**	1.0	0.1	1.3	1.1	4.4
	Treatments Mean	**12.3 c**	**10.6 c**	**24.53 c**	**39.97 b**	**93.95 a**	**Treatments Mean**	**11.92 c**	**9.59 c**	**38.9 b**	**34.2 b**	**113.1a**
	Mean reduction %	**60.8**	**77.7**	**49.7**	**43.04**		**Mean reduction %**	**82.3**	**96.2**	**95.6**	**62.6**	

Pretreatment sample and releasing date, ++ re-releasing date N.P. Both of *P. persimilis* and *A. californicus* were released one time on Sweet Charlie during 2nd. Season and *E.scutalis* and *A. barkeri* were released on Camarosa three times.

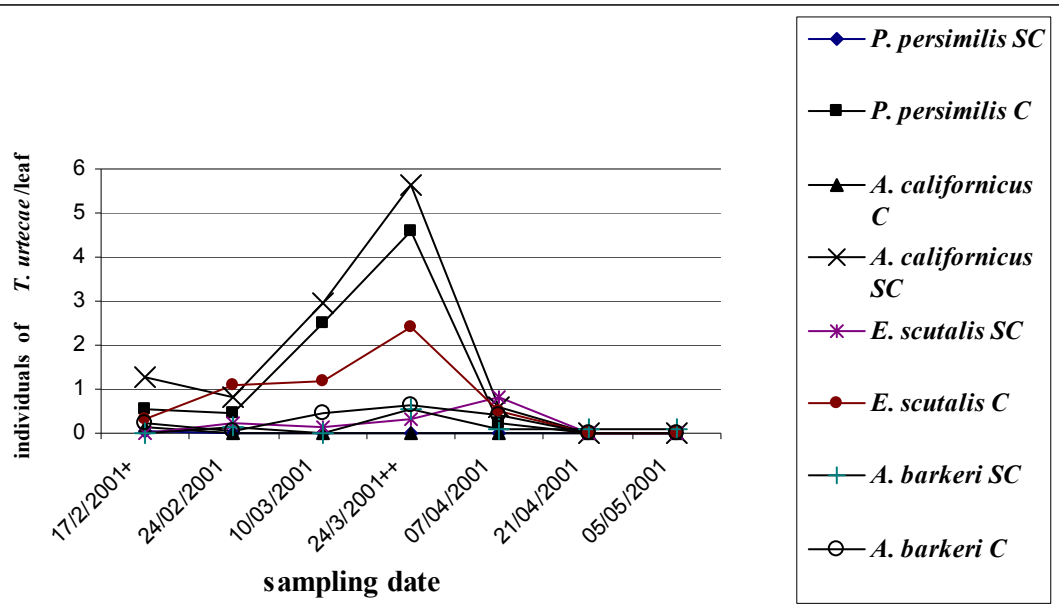

Fig.1 : Mean numbers of the predatory mites on Sweet Charlie(SC) and Camarosa(C) strawberry cultivars after releasing at Kalubia Governorate during 2000-2001 season.

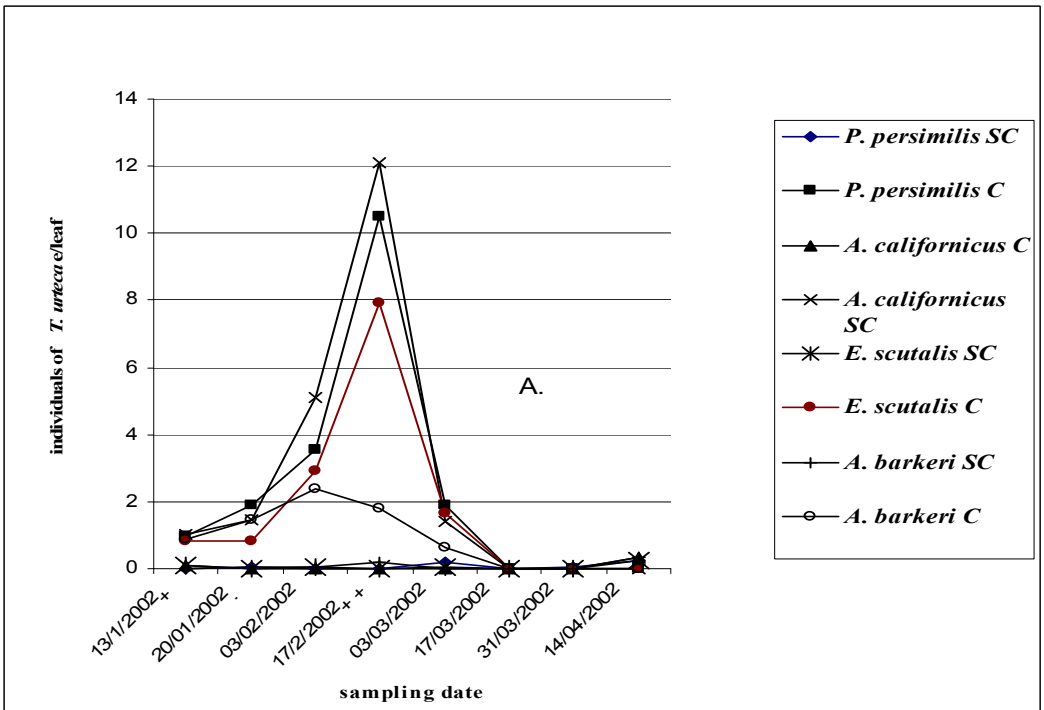

Fig. 2: Mean numbers of the predatory mites on Sweet Charlie(SC) and Camarosa (C) strawberry cultivars after releasing at Kalubia Governorate during 2001-2002 season.

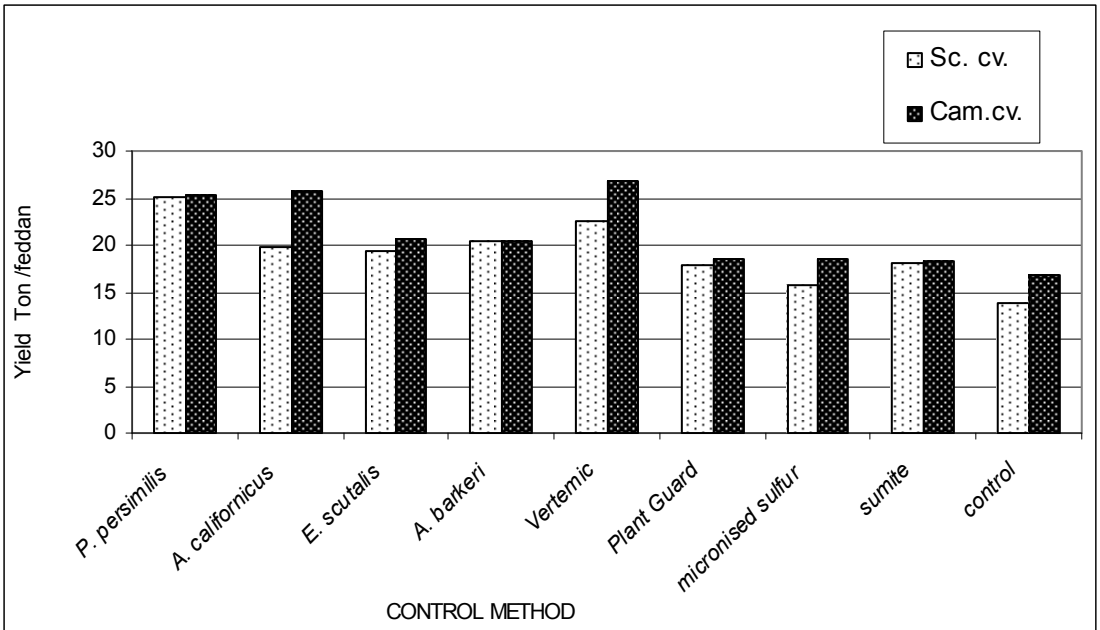

Fig.3 Strawberry yield of two cultivars under different control methods for *Tetranychus urticae* during 2000/2001 season

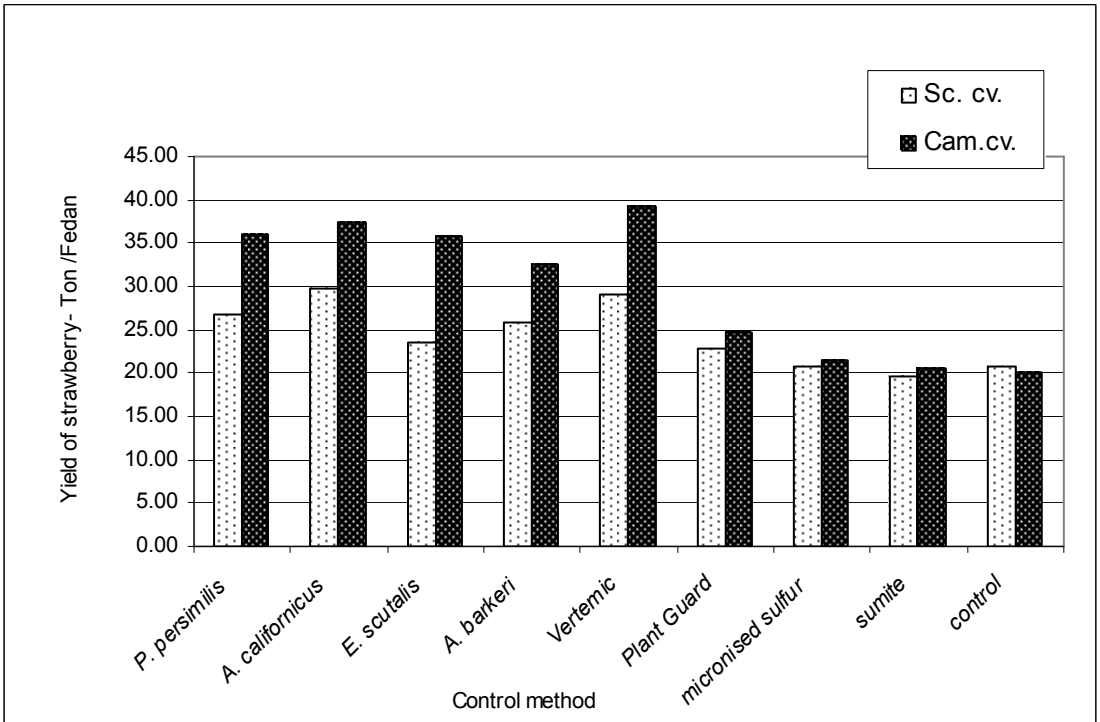

Fig. 4 Strawberry yield of two cultivars under different control practices for *Tetranychus urticae* during 2001/2002

Biocontrol trials

2000/2001 season

As shown in the table 2, the densities of *T. urticae* approached 15 individuals / leaf in mid February. The release of predatory mites was made on the 17 th of February at the ratios of 1;7 : 1:10 predator /prey . The second release was made at the end of March (five weeks later). Population reduction of *T. urticae* on Sweet Charlie was 93.7 and 95.6% in *P. persimilis* and *N. califonicus* plots respectively, and 84.4 and 77.8% for *E. scutalis* and *N. barkeri* respectively.

Population density reached a maximum of 2.5, 2.7, 10.7 and 30.6 individuals /leaf following the two releases of the above-mentioned predatory mites compared with 148.4 individuals / leaf for control on Sweet Charlie. Results recorded on Camarosa cv. followed a growing trend (Table 2) different from that of Sweet Charlie cv. The two releases of predatory mites were carried out, but the population of *T. urticae* increased to 38.7,16.9, 78.3, 147.7 and 237.1 individuals /leaf for *P. persimilis*, N. *californicus E. scutalis*, *N. barkeri* and control, respectively, . Thereafter, *T. urticae* populations were adequately controled. The highest reduction was 84.4% for *N*. *californicus* followed by 44% for *P. persimilis*, 60.4% for *E. scutalis* and 39.3% for *A. barkeri*.

Season 2001/2002

During the second season, the infestation with *T. urticae* started in mid January with a fast build up in particular on Camarosa (Table 2). The four predatory mite species were released two times; on the 13th of January and the 17th of February. However, concerning Sweet Charlie, *P. persimilis*, and *N*. *californicus* plots did not need a repeated release. On the other hand, *E*. *scutalis* and *N. barkeri* plots required a third release on Camarosa cv (Table 2). The highest reduction was 96.2% with *N. californicus* and the lowest was 35.28 for *N. barkeri* on Sweet Charlie cv. On Camarosa cv, reduction reached 90.4% for *N. californicus* and 62.43% for *E. scutalis*.

Population density of the released predatory mites *P. persimilis*, *N*. *californicus*, *E. scutalis* and *N. Barkeri* (Figures 1, 2) was higher on Camarosa cv than on Sweet Charlie cv . Such results may reflect a negative interaction between both *T. urticae* and predatory mites and the physical and chemical properties of the Sweet Charlie, the case which is opposite for Camarosa cv. (Dicke 1996).

The releasing rate (5-10 individuals /plant) and the prerelease mean number of *T. urticae* are a topic of discussion. Several factors are relevant in this context such as the prevailing climatic factors, percentage of infested leaves, times of pruning, mulching time which influence dispersing of predatory mites and wind velocity and direction as mentioned by Coop and Croft (1995) using *Neoseiulus fallacis* (Garman). Waite (1988) in Queensland concluded that *P. persimilis* could be released at the rate of 2 individuals / strawberry plant when *T. urticae* numbers averaged 6 individuals /leaf to get satisfactory biocontrol practices. The studies of Oatman et al. (1968), (1976), (1977) in California are very interested because of similarity with the Egyptian climatic condition. Two releasing rates of 5 and 10 individuals /plant of *P. persimilis* did not lead to a significant difference in *T. urticae* population or fruit yield of strawberry Tufts cultivar. They compared between *P. persimilis*, *N. californicus* and *Galendromus occidentalis* Nesbitt released at 10 individuals / plant of strawberry when the density of *T. urticae* was 1 active stage / leaflet. The highest fruit yield was recorded with *P. persimilis* and the lowest with *G*. *occidentalis*. The higher sensitivity of Camarosa to *T. urticae* infestation is obviously in the present study in both control and treatments plots (Tables 1and 2) which are in agreement with results of Walsh et al. (2003).

Different control tactics of *T. urticae* performed herein influenced fruit yield during the two seasons as shown (figures 3, 4). Data revealed that the highest strawberry yield and increase percentage was found in the Vertimec® treatment. This is particularly true for the Camarosa cv. which yielded 39.3 Ton comparing to 20.18 Ton / Feddan for control. (1 Feddan is equal to 0.42 hectare), which in turn, means an increase of 94.5% during 2001/2002 season. Following Vertemic®, are the plots treated with *N. californicus* and *P. Persimilis* with a yield rise of 85% and 80.6%, respectively during 2000/2001 (for the Sweet Charlie cv). The Camarosa cv. showed a high increase of yield rate with the two indigenous predatory mites *E. scutalis* and *N*. *Barkeri* during 2001/2002. However, in 2000/2001, this yield increase was only of 21-22%. The increase in the yield of Sweet Charlie on the other hand, amounted only 39.6 and 46.8% respectively in the second season. The increase in the percentage of Sweet Charlie yield produced by Sumite, micronized Sulfur and Plant guard did not exceed 28.1 and 10.1% in the two seasons, respectively. Camarosa also showed a slight increase recording 10.1% and 22.3% in the two seasons respectively. The over all high fruit yield found with Vertimec

herein is in conformance with the findings of Trumble (1996). The high yield increase 76.7% observed in *E. scutalis* plot is expected to enhance more trials to replace exotic predatory mites by the indigenous species.

Conclusion

Results obtained on applicable biocontrol of *T. urticae* in strawberry fields in Nile delta enhance more experiments on a large scale in particular with private sector. There are also other successful experiments carried out in new reclaimed areas in Egypt (under publication). However several questions need to be studied:

- On the use of efficient exotic predatory mites as *N californicus* which may influence native species because of its high tolerance to many environmental stresses (such as drought, prey shortage, high temperature). Further studies should be performed to compare native predatory mites with exotic ones, besides cost benefit ratio analysis. The majority of the native predatory mite species can furthermore be mass reared on pollen and other alternative food substances;

- On the widespread tendency for organic farming and the using of biocides such as Vertimec® which are not allowed in the organic system. In the present study, we show the higher yield retained due to Vertimec spraying as an acaricide (and insecticide) which are preferred for growers.

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ABILITY OF *PHYTOSEIULUS LONGIPES* TO CONTROL SPIDER MITE PESTS ON TOMATO IN EUROPEAN GREENHOUSES

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Abstract

Even if many studies dealt with the biological control of spider mites on tomato in greenhouses, no efficient solution has still been found beyond chemicals to get rid of those phytophagous mites. Among them, two species, *Tetranychus evansi* and *T. urticae*, can be considered as very serious pests, leading to great damages in tomato crops in Southern Europe. Preliminary experiments showed that *Phytoseiulus longipes* is a very promising predator of these two species. In the present study, life tables of a Chilean strain of this predator have been calculated, at 25 °C, 80 ± 10 % RH and 16/ 8 (L/ D), in several prey/ plant conditions: *P. longipes* feeding on *T. evansi* on tomato, *P. longipes* feeding on *T. urticae* on tomato and *P. longipes* feeding on *T. urticae* on bean. 88.9 % of the predators did not complete their immature phase while feeding on *T. evansi*, and life tables could not be calculated. However, while feeding on *T. urticae*, immature survival was 99.8 % and 90.0 % on bean and tomato, respectively. Immature durations of *P. longipes* fed with *T. urticae* were not different, being 4.35 and 4.21 days, on bean and tomato, respectively. The intrinsic rate of increase (r_m) was 0.368 and 0.116 female/ female/ day, on bean and tomato, respectively. Those results suggest that the Chilean strain of *P. longipes* would not be able to control neither *T. evansi* nor *T. urticae* in tomato crops. However, it seems able to eat and develop on *T. urticae* in other crops. Another strain of *P. longipes*, originating from Brazil, is currently being studied and lead to very enthusiastic perspectives to control spider mites on tomato greenhouses, both *T. urticae* and *T. evansi*. Furthermore, experiments are being conducted to try to explain the surprising differences in feeding habits and host plants between the two strains of this predaceous mite.

Key-words

Tetranychus evansi, *Tetranychus urticae*, life history, biological control, Solanaceae

Introduction

Although many predatory mite species have been studied to control tetranychids, those pests still cause serious damages to several crops, and especially to tomato in greenhouses. In Southern Europe, the main problem has been *Tetranychus urticae* Koch for many years (Zhang 2003). But since the early nineties, an invasive pest, *Tetranychus evansi* Baker & Pritchard, which seems to be originated from South America (Gutierrez & Etienne 1986) spreads through Southern Europe (Ferragut & Escudero 1999; Bolland & Vala 2000, Migeon 2005, Castagnoli 2006, Tsagkarakou 2007) and causes severe injuries, especially to Spanish tomato crops (Ferragut pers. comm.).

In order to get rid of *T. evansi*, many methods have been experimented, like pesticides (Blair 1989, Mabeya *et al.* 2003), plant resistance development (Maluf *et al.* 2001, Resende *et al.* 2002, Gonçalves *et al.* 2006, Resende *et al.* 2008), entomopathogenic fungi (Humber & Moraes 1981, Wekesa *et al.* 2005, 2006, 2007) and several predators (Sarmento *et al.* 2004, Ho *et al.* 2005, Oliveira *et al.* 2005) including predatory mites

(Moraes & Lima 1983; Moraes & McMurtry 1985a; Moraes & McMurtry 1985b; Moraes et McMurtry 1986; Escudero et Ferragut 2005; Rosa *et al.* 2005, Ferrero *et al.* 2007, Furtado *et al.* 2007, Koller *et al.* 2007). The only enthusiastic perspective up to now has been pointed out by Furtado *et al.* (2006) and Ferrero *et al.* (2007), with the discover and the study of a Brazilian strain of *Phytoseiulus longipes* Evans (named *P. longipes* B thereafter). Following those studies, this type I predator (McMurtry & Croft 1997), feeding preferentially on mites of the subfamily *Tetranychinae*, seems actually to be specific of *T. evansi* (Furtado *et al.* 2007). More recently, a Chilean strain of this species (written *P. longipes* C thereafter) has been found (Ragusa pers. comm.). In order to use these strains in biological control for controlling *T. evansi*, further biological studies are required. This paper aims to determine the life history of *P. longipes* C on several plant substrates/ prey conditions.

Material and Methods

Species and strains studied

The strain of *P. longipes* studied was obtained from a colony initiated with specimens collected in Chile, fed with *T. urticae*, in Nogal, Los Andes, Valparaiso Region (Ragusa, pers. comm.). Mites were fed with all stages of *T. urticae*, offered on leaves of *Phaseolus vulgaris* L. cv. Contender, placed underside up in rearing units constituted of plastic trays (10 × 15 cm) bordered with water-saturated cotton to avoid mite escapes and to maintain the turgescence of the leaves. Rearing units were placed in climatic units at 25 ± 2 °C, 75 ± 10 % RH and 16/ 8 [L/ D]. The *T. evansi* stock colony was initiated with specimens collected from a tomato screenhouse at Saint-Jeannet (Alpes-Maritimes, 06, France) in October 2007 (Migeon pers. comm.), and reared on *Lycopersicon esculentum* Miller in rearing units similar to those described above.

The *T. urticae* stock colony initiated with specimens collected in Montpellier, France, was reared on *P. vulgaris* cv. Contender in a greenhouse.

Immature development

Experimentations were performed at 25 ± 2 °C, 75 ± 10 % RH and 16/ 8 [L/ D]. Three items were tested, each one being characterised by a prey (*T. evansi* or *T. urticae*) and a plant substrate (tomato or bean): *T. evansi* / tomato, *T. urticae* / bean and *T. urticae* / tomato. The combination *T. evansi* / bean has not been tested because this plant was unsuitable for rearing our strain of *T. evansi*. Groups of five prey females were placed using a thin paintbrush in experimental units, consisting of a leaf disk of a plant substrate (2 cm in diameter) placed underside up onto a moist disk of filter paper, inside a Petri dish (2 cm in diameter, 1 cm high). One day later, eggs of *P. longipes* (between 0 and 6 hours old) obtained from the intermediate stock colony (consisting in 30 to 50 females reared on a leaf of *P. vulgaris* cv. Contender placed in a plastic tray as described above) were transferred to each experimental unit (one egg/ unit); then closed with a transparent plastic film. To maintain humidity, distilled water was added on the filter paper every day. Periodically (once a week on tomato, twice on bean) *P. longipes* individuals were transferred to new leaves infested with preys as previously reported.

Observations were carried out every 12 hours to determine the duration and the survivorship for each stage.

Reproduction

Recently emerged adult *P. longipes* females obtained were transferred to new experimental units. A male taken from the stock colony was then added to each unit containing one female, and a new one was added for every male that died or escaped. At least 30 couples were observed. Daily observations were conducted to determine female fecundity and survivorship. The eggs laid were placed daily in a single unit and reared to adulthood to determine the secondary sex ratio (female percentage of the studied female cohort offspring).

Life Table

The life table was constructed considering the females of the cohort studied. The net reproductive rate (Ro), the mean generation time (T), the intrinsic rate of increase (r_m), the doubling generation time (Dt), and the finite rate of increase (λ) were calculated using the method recommended by Birch (1948):

$Ro = \Sigma (l_x \times m_x)$

$T = \Sigma (x \times l_x \times m_x) / \Sigma (l_x \times m_x)$

$r_m = Ln (Ro) / T$

$Dt = Ln (2) / r_m$

$\lambda = \exp (r_m)$

In those equations, *x* is the age (with 0.5 for the

day when eggs had been laid), l_x, the cumulative female survivorship, and m_x, the number of female descendants per female at *x*.

Calculation of a corrected r_m value was performed by iteration. The method, aiming to find r_m for which $(1 - \Sigma \exp(-r_m \times x) \times l_x \times m_x)$ is minimal, was given by Maia *et al.* (2000).

Analysis of variance (ANOVA) and related Tukey HSD mean comparison tests were performed to determine differences between duration of the immature phases and adult stages between the different items tested.

The r_m iteration and statistical analysis were computed with R (R project 2008).

Results

Immature development

Egg to adult duration ranged from 4.10 to 4.21 days, for the T. evansi/ tomato and T. urticae/ tomato conditions, respectively (Table 1). The egg stage was the longest, varying from 1.80 to 1.96 days. The larval stage was the shortest, varying from 0.35 to 0.55 days. No statistical analysis could be performed for the item T. evansi/ tomato for the protonymphal, deutonymphal and egg to adult stages because too many individuals had died prematurely (11.1 % of the immatures tested

Table 1. Mean duration (± Standard Error) in days of the immature instars of a Chilean strain of *Phytoseiulus longipes* for several items prey/ plant substrate, number of replicates (*N* and immature survival rate).

Stage	Item			
	T. evansi/ tomato	*T. urticae*/ bean	*T. urticae*/ tomato	$F_{(df1, df2)}$ ($\alpha = 0.05$)
Egg	1.80 (0.21) a	1.96 (0.20) b	1.83 (0.00) a	$Ft < F_{(2, 109)} = 10.99$; $P = 4.48 \times 10^{-5}$
Larva	0.44 (0.17) ab	0.55 (0.22) a	0.35 (0.23) b	$Ft < F_{(2, 105)} = 8.91$; $P = 2.66 \times 10^{-4}$
Protonymph	1.05 (0.35)	0.85 (0.26) a	1.01 (0.26) b	$Ft < F_{(2, 88)} = 4.39$; $P = 1.51 \times 10^{-2}$
Deutonymph	1.00 (0.00)	0.99 (0.18) a	1.01 (0.29) a	$Ft > F_{(2, 80)} = 0.11$; $P = 8.9 \times 10^{-1}$
Egg to adult	4.10 (0.00)	4.36 (0.27) a	4.21 (0.35) a	$Ft > F_{(2, 80)} = 2.91$; $P = 6.03 \times 10^{-2}$
N	27	41	44	
Immature survival (%)	11.1	99.8	90.9	

Ft (F from tables) < $F_{(df1, df2)}$ (F calculated) mean that there are differences between the mean durations for a stage (ANOVA, $\alpha = 0.5$). Durations followed by a different letter for a stage are significantly different (Tukey HSD test, $\alpha = 0.5$). No statistical analysis could be performed for some stages of the *T. evansi* / tomato condition because of high mortality at the larval stage.

Table 2. Mean durations (± Standard Errors) in days of adult phases, longevity and ovipositional rates of *Phytoseiulus longipes* C feeding on *Tetranychus urticae* on bean or tomato, number of replicates (*N*).

Stage	Plant substrate		
	Bean	Tomato	$F_{(df1, df2)}$ ($\alpha = 0.05$)
Pre-oviposition	1.22 (0.93) a	0.95 (0.69) a	$Ft > F_{(1, 40)} = 1.05$; $P = 3.11 \times 10^{-1}$
Oviposition	3.74 (1.85) a	2.63 (2.41) a	$Ft > F_{(1, 40)} = 2.70$; $P = 1.08 \times 10^{-1}$
Post-oviposition	0.30 (0.46) a	1.21 (1.64) b	$Ft < F_{(1, 40)} = 6.10$; $P = 1.79 \times 10^{-2}$
Longevity	8.95 (2.18) a	8.98 (2.62) a	$Ft > F_{(1, 51)} = 6.00 \times 10^{-4}$; $P = 9.81 \times 10^{-1}$
Eggs / female / day	1.34 (0.89)	0.57 (0.48)	
Total eggs / female	6.77 (5.79)	3.29 (2.78)	
N	30	23	

Ft (F from tables) < $F_{(df1, df2)}$ (F calculated) mean that there are differences between the mean durations for a stage (ANOVA, $\alpha = 0.5$). Durations followed by a different letter for a stage are significantly different (Tukey HSD test, $\alpha = 0.5$).

Table 3. *Phytoseiulus longipes* C demographic parameters on two substrates fed with *Tetranychus urticae* at 25 °C, 80 ± 10 % RH and 16/ 8 (L/ D). Net reproductive rate (Ro), mean generation time (T), finite rate of increase (λ), doubling generation time (D_t), estimated and iterated intrinsic rate of increase (r_m).

Substrate	Demographic parameter					
	Ro	*T*	λ	D_t	r_m (Birch)	r_m (iterated)
Bean	3.92	4.04	1.40	2.05	0.338	0.368
Tomato	1.39	2.91	1.12	6.12	0.113	0.116

survived). No significant differences were found between the two other conditions tested for the egg to adult and deutonymph durations. For the three other immature stages (i.e. egg, larva and protonymph), significant differences were always found between the items T. urticae/ bean and T. urticae/ tomato.

Reproduction

Adult phase durations and oviposition rates were observed only when *P. longipes* was feeding on *T. urticae*, on bean or tomato (Table 2), because of the high mortality of *P. longipes* when fed with *T. evansi*. The longevity was not significantly different between the two conditions, varying from 8.95 and 8.98 days, on bean and tomato, respectively. The pre-oviposition period ranged from 1.22 to 0.95 days, oviposition from 3.74 to 2.63 days and post-oviposition from 0.30 to 1.21 days, on bean and tomato, respectively. The only significant difference found between these two conditions was for the post-oviposition period. The mean oviposition rate and total oviposition were highest when *P. longipes* was fed with *T. urticae* on bean than on tomato, being 1.34 eggs/ female/ day and 6.77 eggs/ female, respectively.

Life tables

Calculated life table parameters are given in Table 3. Congruently with the previous observations, the best features were obtained for the item *T. urticae*/ bean. The intrinsic rate of increase (iterated) was more than three times higher on bean than on tomato, while the doubling time and the net reproductive rate were almost three times lower on bean than on tomato. The calculated mean generation time was 4.04 and 2.91 days, and the finite rate of increase 1.40 and 1.12, on bean and tomato, respectively.

Discussion, conclusions and perspectives

This study has been performed in order to determine first the ability of the predaceous mite *P. longipes* C to develop and reproduce fed with tetranychids on tomato, through the analysis of its life parameters The second goal was to compare the biological characteristics of the studied strains of this species. Up to 2007, experiments had been performed on two other strains of this predator: a South-African strain (reported as *P. longipes* SA thereafter) and a Brazilian strain (Badii & McMurtry 1983, 1984; Moraes & McMurtry 1985b; Takahashi & Chant 1992, Badii *et al.* 1999). It has been reported that the South-African strain is unable to control *T. evansi* in the conditions tested, even if promising results had been found to control *Tetranychus pacificus* (McGregor) (Badii *et al.*, 1999). On the opposite, the results obtained for the Brazilian strain, discovered in 2005 (Furtado et al. 2006), are enthusiastic for the control of *T. evansi* but also of *T. urticae* on tomatoes (Ferrero *et al.* 2007; Furtado *et al.* 2007.

Concerning the developmental phase of *P. longipes* C, several points could be pointed out. At first, for the item *T. evansi*/ tomato, only 3 individuals out of 27 reached the adult stage, and none of these 3 mites survived enough to mate. These conditions thus seem to be unsuitable for the Chilean strain of *P. longipes*, which is a key difference to notice with *P. longipes* B, which seems specific to *T. evansi* (Furtado *et al.* 2007). For the other conditions tested in the present paper (see table IV), egg to adult durations were lower to what has been observed previously with 4.7 and 4.8 days while *P. longipes* B was feeding on *T. evansi* and *T. urticae*, respectively, on *Canavalia ensiformis* (L.) (Furtado *et al.* 2007), 4.9 days while P. longipes B was feeding on T. evansi on Solanum americanum Miller (Ferrero et al. 2007) and 5.23 days for P. longipes SA feeding on T. pacificus on bean (Badii & McMurtry 1984). The Chilean strain of P. longipes thus seems to develop faster than the other strains feeding on T. urticae.

The whole immature phase durations are of the same order of magnitude or longer for the other *Phytoseiulus* species, with 5.5 days for *Phytoseiulus*

Table 4. Comparison of immature development durations (in days), longevity (in days) and intrinsic rates of increase (female/ female/ days) for several *Phytoseiulus* species, depending on the prey provided, the plant substrate and climatic conditions.

Phytoseiulus species	Prey species	Plant substrate	Climatic conditions	Immature development duration in days	Longevity in days	Intrinsic rate of increase r_m (female/ female/ day)	Reference
P. longipes B	*Tetranychus evansi*	*Solanum americanum*	25 ± 2 °C, 80 ± 10 % RH, 12/ 12 L/ D	4.9	20.3	0.293	Ferrero *et al.* 2007
	T. urticae	*Canavalia ensiformis*	25 ± 1 °C, 83 ± 12 % RH, 12/ 12 L/ D	4.8	29.7	0.32	Furtado *et al.* 2007
	T. evansi			4.7	31.1	0.363	
P. longipes SA	*T. pacificus*	*P. lunatus*	25 ± 1 °C, 80 ± 7 % RH, 14/ 10 L/ D	5.23	34.12	0.366	Badii & McMurtry 1984
P. macropilis	*T. urticae*	*C. ensiformis*	26 °C, 60 ± 5 % RH, 12/ 12 L/ D	4.8	44	0.193	Silva *et al.* 2005
P. persimilis	*T. evansi*	*P. vulgaris*	25 ± 1 °C, 70-80 % RH, 12/ 12 L/ D	6.91	NA	0.116	Escudero & Ferragut 2005
	T. urticae			4.16	NA	0.373	
P. fragariae	*T. urticae*	*S.americanum*	25 ± 0.8, 12/ 12 L/ D	5.5	23.3	0.273	Vasconcelos *et al.* 2008

fragariae Denmark & Schicha feeding on *T. urticae* on *S. americanum* (Vasconcelos *et al.* 2008), 4.8 days for *Phytoseiulus macropilis* (Banks) feeding on *T. urticae* on *C. ensiformis* (Silva *et al.* 2005) and 6.91 and 4.16 days for *Phytoseiulus persimilis* Athias-Henriot feeding on *T. evansi* and *T. urticae* on bean (Escudero & Ferragut 2005). Results obtained here on the developmental phase of *P. longipes* C showed that this strain is not able to develop, thus to control, *T. evansi* on tomato, but can develop in the same way on tomato and bean while feeding on *T. urticae*. At last, as defined by McMurtry & Croft (1997), type I phytoseiids, belonging to the genus *Phytoseiulus*, do not need food until they reach the protonymphal stage. This has also been observed during the present experiment for the Chilean strain of *P. longipes*

Adult phase durations of the Chilean strain of *P. longipes* were calculated only with *T. urticae* as prey. These durations were very short compared to all the data previously compiled (table IV). Longevity was only 8.95 and 8.98 days in our data for items *T. urticae*/ bean and *T. urticae*/ tomato, respectively, while Ferrero *et al.* (2007) showed a 20.3 days longevity for *P. longipes* B and Badii (1984) a 34.12 days longevity for *P. longipes* SA. For the other *Phytoseiulus* species studied feeding on *T. urticae* at the same temperatures than in the present study, longevity is also variable, from 44.0 and 23.3 days, for *P. macropilis* and *P. fragariae*, respectively (Silva *et al.* 2005; Vasconcelos 2008). The only difference that could explain those variations beyond the species might be the photoperiod, which was 16/ 8 (L/ D) in our experiment, and 12/ 12 or 14/ 10 (L/ D) on the others (Overmeer 1985). Values of r_m reported by Furtado (2007) for *P. longipes* B, and by Badii (1984) for *P. longipes* SA, both reared on bean, are very similar to what was obtained in the present study for *P. longipes* C feeding on *T. urticae* on bean, with 0.320, 0.366 and 0.368 female/ female/ day, respectively. Similar or lower figures were found for other species feeding on *T. urticae* on bean, ranging from 0.373 to 0.193 female/ female/ day, for *P. persimilis* and *P. macropilis*, respectively (Escudero & Ferragut 2005; Silva *et al.* 2005). However, the low r_m found in the present experiment for *P. longipes* C feeding on *T. urticae* on tomato can suggest that the item *T. urticae*/ tomato is not suitable for this strain, thus that no effective control of *T. urticae* could be considered by releasing this predaceous mite on tomato greenhouses.

This is the first report of a study on the biology of a Chilean strain of *P. longipes*. This work showed results different than those already obtained for other strains of this species. *Phytoseiulus longipes* C was, as the SA strain, unable to complete its development feeding on *T. evansi* on tomato, while the B strain could. Those observations lead to the first major conclusion of this work which is that *P. longipes* C would be unable to control *T. evansi* populations in tomato greenhouses. Great differences in longevity had been also pointed out between the Chilean strain and the two others. These differences could be due to differences in the setup (photoperiod), or to the strain itself. Values of r_m showed that *P. longipes* C would be able to develop and reproduce well feeding on *T. urticae* on bean, but apparently not enough feeding on *T. urticae* on tomato to settle an enthusiastic prognosis for its use in biological control.

In this paper, particular attention was paid to the differences between the three strains of *P. longipes* already studied: differences in the suitability of plant substrate and in prey items, in differences in longevity and oviposition at the same conditions. All these observations lead the authors to settle a whole study to compare those strains in combined conditions. Along with more biological studies, other tools like genetic markers or morphological analysis are presently being used to determine and explain some of those differences. Many conclusions on the usefulness and the efficiency of those strains to control tetranychids on tomato greenhouses should be given later.

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EFFECT OF FIVE COTTON CULTIVARS ON LIFE TABLE PARAMETERS OF *TETRANYCHUS URTICAE* (ACARI: TETRANYCHIDAE)

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Abstract

The influences of five cotton cultivars (Varamin, Mehr, Bakhtegan, Sahel, Saiokra) on *T. urticae* were studied in laboratory conditions at 25±1ºC, 70±10% RH and 16L: 8D photoperiod. Leaf disc method was used in all the experiments. Reproduction rate, fertility and other life table parameters including intrinsic rate of natural increase (r_m), net reproductive rate (R_0), mean generation time (T), finite rate of increase (λ) and doubling time (DT) of the two-spotted spider mite on different cotton cultivars were calculated. Life table parameters were estimated using the Birch and Jacknife methods. The results indicate that the Varamin cultivar is the best suitable cultivar for the two-spotted spider mite displaying a higher intrinsic rate (0.181), whereas Bakhtegan was the least suitable cultivar because with a low intrinsic rate (0.152). The lowest r_m value suggested an antibiosis resistance in this cultivar in comparison with the other ones.

Key words

Cotton cultivars, Fertility life table parameters, Two-spotted spider mite, Jacknife method, Antibiosis resistance.

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is an extremely polyphagous pest that has been reported from economically important agricultural and ornamental plants (Mondal & Ara 2006). It feeds by sucking contents of plant cells (Helle & Sabelis 1985). *Tetranychus urticae* is an important pest of cotton in many parts of the word. As spider mites can cause significant reduction, in yield, fiber quality and seed viability of cotton (Wilson 1993). Many different cultivars of cotton are planted in the word and each of them may influence the development of mite populations. In this study, the effects of five important cotton cultivars in Iran (Bakhtegan, Mehr, Sahel, Saiokra and Varamin) were studied on life table parameters of *T. urticae*.

Material and methods

Plants

25 plants from each of the investigated cotton cultivars (Bakhtegan, Mehr, Sahel, Saiokra and Varamin) were planted and kept at 16L: 8D photoperiod in a greenhouse.

Mite colony

Cotton cultivar leaves that were infested with *T. urticae* were collected from cotton field of Varamin, Iran. The mites were transferred to cotton cultivars which kept separated from non-infested plants. Two generation of mites were reared on each cultivar (on plant) and the third generation was used in experiments.

Rearing unit

For experiments, leaf discs of 28 mm diameters,

placed upside down on a 5 mm layer of agar (2%) inside a 60 mm diameter Petri dish. All dishes were kept in a growth chamber maintained at 25±1ºC, 70±10% RH and 16L: 8D photoperiod. During the experiments, the leaf discs and agar were replaced weekly.

Bioassay

One mite female per rearing unit was placed on the leaf discs and allowed to lay eggs over a period of 8 h. Thereafter the female mite and all of the eggs except one egg were removed. The remaining eggs were kept individually in growth chamber until the mites were matured. After emergence of a female, one male was introduced to the leaf disc. Eggs laid per female were recorded daily and Observations continued until all mites dead. To determine the sex ratio, three random samples (at least 60 eggs in each sample) were taken from the eggs that were laid in the beginning, middle and near the end of females' lifespan. Collected eggs were allowed to develop to adulthood and then the sex ratio was determined.

Statistical analysis

Data of life tables were used to calculate the intrinsic rate of natural increase (r_m), net reproductive rate (R_0), mean generation time (T), doubling time (DT) and finite rate of increase (λ) of the two-spotted spider mite on different cotton cultivars. Life table parameters were estimated using the Birch's and Jacknife methods by use of PersianRm software (Naveh *et al*. 2004).

Results and discussion

Life table parameters were studied including intrinsic rate of natural increase (r_m), net reproductive rate (R_0), mean generation time (T), doubling time (DT) and finite rate of increase (λ) of the two-spotted spider mite on different cotton cultivars.

The lowest λ (1.164), r_m (0.152) and R_0 (15.552) were observed for *T. urticae* on the Bakhtegan cultivar, where the population doubles in 4.542 days (Table 1).

Table 1. Life table parameters of *T.urticae* on five cotton cultivars (mean±SE)[a].

Cotton cultivars	R_0	r_m	T	DT	λ
Bakhtegan	15.552±1.952[b]	0.152±0.006[c]	18.066±0.361[a]	4.542±0.198[a]	1.164±0.007[c]
Mehr	27.743±1.889[a]	0.182±0.003[a]	18.214±0.211[a]	3.795±0.074[b]	1.200±0.004[a]
Sahel	21.943±1.373[a]	0.165±0.004[b]	18.655±0.183[a]	4.182±0.104[a]	1.180±0.004[b]
Saiokra	26.816±2.251[a]	0.182±0.004[a]	18.016±0.231[a]	3.790±0.090[b]	1.200±0.005[a]
Varamin	26.203±2.176[a]	0.181±0.004[a]	17.981±0.196[a]	3.810±0.089[b]	1.199±0.005[a]

[a] Mean ± SE followed by the same letter within columns were not significantly different based on Duncan multiple range tests at α = 0.05.

The intrinsic rate of natural increase (r_m) is an important parameter for describing the growth potential of a population under specific climatic and food conditions, as it reflects the overall effects of temperature and food on development, reproduction and survival of the pest (Southwood & Handerson 2000). Statistical analysis indicated that there are significant differences between r_m values on different cotton cultivars (F = 8.35, df = 4, P < 0.0001). The lowest r_m value in Bakhtegan variety suggests an antibiosis resistance in this cultivar in comparison to the other cultivars.

Studies indicate that okra-leaf cotton cultivars are more resistant to the two-spotted spider mites than normal cotton leaves (Bailey et al. 1978, Bailey & Meredith 1983, Wilson 1994b). Wilson (1994b) compared fertility life table parameters of *T.urticae* on okra-leaf cotton (Saiokra) and normal-leaf cotton (Deltapine 90). Results showed that there were no significant differences in any parameters measured (r_m, R_0, T) between spider mites reared on the okra- and normal-leaf. in present study, which all tested cultivars had normal leaf except Saiokra, also there were no significant differences in measured parameters between saiokra cultivar with Varamin and Mehr cultivars, which have less resistant to *T.urticae*. This result demonstrates that spider mites don't discriminate between leaf tissue from either okra- or normal-leaf for feeding or oviposition. Wilson in the same study researched the effect of leaf shape on development of mite population on okra- and normal-leaf. He observed that mite colonies began near the junction of leaf blade and petiole and developed along the major veins of the leaf and

into the leaf folds, which were found on normal-shaped leaves and were absent from okra-shaped leaves. Therefore, mite colonies on okra-leaf developed in close proximity to the major veins. In contrast, colonies on normal-leaf appear to be less restricted and developing more widely over the leaf surface, by this reason okra leaves have more resistant to *T.urticae* but this result don't obtain in the present study because we used leaf discs for experiments and all leaf discs had equal area for mite distributions. Another factor which can attributes in cotton resistance to spider mites is presence of glandular or non-glandular hairs on leaf surface (Kamel & Elkassaby 1965). Hairy types of cotton cultivars confer a higher resistance to spider mites than the smooth leaf varieties (Abul-Nasr 1960), because of reducing physical activity of spider mites (Steinite & Levinsh 2003). Also in this study, Bakhtegan cultivar which had more trichome on leaves than the other ones was the most resistant variety. Likewise, chemical compounds of host plant such as gossypol and tannin can also play important roles in determining the increase rate of pest population (Wilson 1994, Zummo *et al*. 1984).

This study demonstrates that cotton cultivars could have different effects on increase of mite population. One of the suitable methods to decrease pest damage is the use of varieties which have more resistance against pests, thus, use of cotton cultivars which have more resistance to *T.urticae*, such as Bakhtegan variety, can be useful in areas where damage of this pest is abundant.

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LOCAL AND SYSTEMIC RESPONSES INDUCED BY *TETRANYCHUS URTICAE* (ACARI: PROSTIGMATA: TETRANYCHIDAE) FEEDING IN CUCUMBER PLANTS TRANSFORMED WITH THAUMATIN II GENE

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Abstract

Results of our studies seem to show that defense protein - thaumatin II is not involved neither in local nor in systemic resistance of cucumber against *T. urticae.* Thaumatin II gene transformation of cucumber plants leads to the elevation of the abundance of some leaf polypeptides, but their level do not further increase upon mite infestation. In the soluble fraction of leaves of non-transformed cucumber plants, the pattern of peroxidase isoforms changes, suggesting the involvement of this oxidative enzyme in local but not in systemic response to *T. urticae* feeding. However, in the presence of thaumatin II in leaf tissues the local increase in the activity of peroxidase caused by *T. urticae* feeding is much weaker than in mite-infested leaves of untransformed control plants of line B.

Key-words

Two-spotted spider mite, *Cucumis sativus* L., SDS-PAGE protein, peroxidase

Introduction

A wide variety of induced defense mechanisms to one herbivore allow crop-plants to cope with other herbivores, omnivores or natural enemies (Karban & Baldwin 1997, Smith 2005). Among them the accumulation of proteins known as defense-related (proteinase inhibitors, phenylalanine ammonia lyase, chalcone synthase, polyphenol oxidase, lipoxygenase) and pathogenesis-related (PR) proteins (β-1,3 glucanase (BGL2), PR-3 – basic chitinase, PR-9 family with peroxidase properties) is the most frequently observed event upon insect-pest infestation (Duffey & Felton 1989, Felton et al. 1989, Bowles 1990, Ryan 1990, Duffey & Felton 1991, Duffey & Stout 1996, Mayer et al. 1996, Stout et al. 1996, Bi et al. 1997, Inbar et al. 1998, Forslund et al. 2000, Walling 2000, Moran et al. 2001, Thaler et al. 2001, Thaler 2002, Thaler et al. 2002, Spence et al. 2007). They are also known to be correlated with induced defense responses to mite attack (Bronner et al. 1991, Kielkiewicz 1996, Kielkiewicz 1998, Stout et al. 1996, Arimura et al. 2000, Takabayashi et al. 2000, Tomczyk 2001, Kielkiewicz 2002, Kielkiewicz 2003, Kant et al.2004, Grinberg et al. 2005, Spence et al. 2007).

Thaumatin, the sweet tasting protein found in the Western African shrub (*Thaumatococcus danielli*) share a significant amino acid homology to PR-5 proteins, known as thaumatin-like (TL) proteins (Van der Wel & Loeve 1972, Van der Wel & Bel 1980, Vigers et al. 1992, Witty 1992, Thompson et al. 2006). TL-proteins are often induced in plants in

response to a range of abiotic and biotic factors (Koiwa et al. 1997, Van Loon 1997, Schweizer et al. 1998, Forslund et al. 2000, Voeckel et al. 2004, Zhu-Salzman et al. 2004, Hernandez et al. 2005, Sanz-Alferez et al. 2007).

Cucumber plants carrying the thaumatin II gene have been modified to accumulate sweet protein to improve the taste of fruits (Szwacka et al. 2002). In the open-field experiment, transgenic cucumber plants of T5 generation expressing the thaumatin II gene affected the abundance of some common cucumber pests including spider mites (Kielkiewicz et al. 2006). However, the role of thaumatin II in cucumber-spider mite interaction is still unclear and broader significance of these compound is under current investigations.

In this study, Western blot, SDS-PAGE and peroxidase (POX) activity staining were used to study local and systemic protein changes caused by two-spotted spider mite (TSSM) (*Tetranychus urticae* Koch, Acari: Tetranychidae) in T5 generation of the genetically modified (GM) cucumber plants of line T212 01 with relatively high level of thaumatin II, both in leaves and fruits (Szwacka et al. 2002, Kielkiewicz et al. 2006).

Material and Methods

Plants of T5 generation of line T212 01 and non-GM inbred line of *Cucumis sativus* L. Borszczagowski (line B) were grown under greenhouse conditions. Four-week-old plants were used in the experiment. Plants were infested with TSSM by placing 10 young females on the 8th leaf from the bottom, for 7days. Non-infested plants were used as a control. Each group consisted of 5 plants.To assess involvement of thaumatin II, polypetides and POX in defense response of transgenic cucumber expressing thaumatin II, the Western blot, SDS-PAGE and POX analyses were performed, respectively. Samples for all analyses were collected from the 8th mite-injured leaves and from the 10th uninjured leaves from above the feeding site of both B (untransformed, control) and T 212 01 lines, 7 days after the infestation.

Western blot analysis

Total soluble proteins from leaf samples were extracted with a mixture of 50mM Tris buffer pH 8.0 containing 1mM ethylenediaminetetraacetic acid (EDTA) pH 8.0, 15 mM β-mercaptoethanol, 1% phenylmethylsulphonyl fluoride (PMSF). The homogenates were centrifuged 3 times (15 000 g x 10 min.) at 4^{o}C. Total protein was determined according to Bradford (1976) using bovine albumin as a standard and 25 µg of protein was loaded onto each lane of 12% polyacrylamide gel. After electrophoresis the proteins were blotted to a Immun-Blot® PVDF membrane (Bio-Rad, USA). Thaumatin II from *T. danielli* (0.25 µg, Sigma, St. Louis, MO, USA) was used as a positive control and Protein Marker (Sigma, St. Louis, MO, USA) was used as molecular mass marker.

The membranes were blocked overnight (at 4^{o}C) in 5% fet-free milk in TBS, and washed with TBS. Rabbit anti-thaumatin polyclonal antibody (diluted 1:2500, v/v) was used as a primary antibody. An anti-rabbit IgG-alkaline phosphatase (Sigma, St. Louis, MO, USA) (diluted 1:15000, v/v) was used as a secondary antibody. To develop blots Western Blotting detection, reagents (5-bromo-4-chloro-3-indolyl/nitro blue tetrazolium, BCIP/NBT) were used according to manufacture's manual.

Protein assay and SDS-PAGE

Leaf samples were grounded in liquid nitrogen. Total protein and enzyme were extracted from liquid nitrogen powder in 5 ml of extraction buffer (50mM TRIS-HCl pH 7.5) containing 1% (w/v) insoluble polyvinyl-pyrrolidone (PVP) and 0.1mM EDTA. The extraction was carried out at 4^{o}C for 1 h. The extracts were centrifuged (20 000 g for 20 min. at 4^{o}C) and the supernatants were used directly for SDS and native polyacrylamide gel electrophoresis. The protein concentration in supernatants was measured according to Bradford (1976) using bovine albumin as a standard. Protein samples and molecular weight Protein Marker (Sigma, St. Louis, MO, USA) were loaded onto 12% polyacrylamide gel with 4% stacking gel. SDS-PAGE was carried out according to Laemmli (1970). Thirty micrograms of protein per lane was used. After electrophoresis gel was incubated overnight in staining solutions (0.1% Coomassie Brilliant Blue R-250, 50% methanol and 10% glacial acetic acid) and afterwards gel was rinsed several times in destaining solution (50% methanol and 10% glacial acetic acid) until background of the gel was fully destained.

Native PAGE and POX active staining

Electrophoresis under non-denaturing conditions was performed on 12 % polyacrylamide gel by the method of Laemmli (1970). Sixty micrograms of protein per lane was used. Staining of POX isoforms was achieved by incubating gels 20 min. after electrophoresis in 50mM sodium acetate buffer, pH 5.0, containing 2mM benzidine dissolved in dimethyl sulfoxide (DMSO). The reaction was initiated by adding 3mM H_2O_2 and was allowed to continue for the next 20 min. (Polle

at al. 1994).

Results

Effect of transgenesis

Immunoblotting results (Figure 1) demonstrate that thaumatin II gene expression was stronger in mature than in younger leaves for T5 generation of cucumber line T212 01.

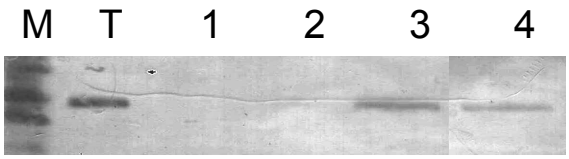

Figure 1. Western blot analysis of total protein samples of leaves of non-transformed control line B (lane 1 - mature leaves; lane 2 – young leaves) and transgenic line T212 01 (lane 3 – mature leaves; lane 4 - young leaves). 25 micrograms of total soluble protein were loaded in each lane, separated by 12% PAGE and blotted. The membrane was probed with anti-thaumatin II antibody. M – protein molecular mass marker. T-positive control, thaumatin (22 kDa) from *T. daniellii*.

The results obtained by SDS-PAGE of the soluble leaf proteins of cucumber line T212 01 show the changes in the protein profiles after transformation (Figure 2). Compared to the untransformed control (cucumber line B), in young and fully expanded (mature) leaves of transformed plant the abundance of 3 polypeptide bands (31, 45 and 66.2 kDa) increased with the strongest in the 31kDa band (Figure 2).

Thaumatin II gene insertion into cucumber resulted in decreased POX abundance in both mature and young leaves of line T212 01 (Figure 3), compared to the line B (non-infested, control). POX in mature leaves of non-infested plants of line B appears as four well-visible isoforms, while very weak band of the only one isoform of POX was visible after electrophoresis of extracts of mature leaves of line T 212 01 (Figure 3, lane 1 *vs* 3).

Local and systemic effect of mite-feeding

Thaumatin II abundance did not change neither in leaves injured by TSSM feeding nor in leaves distant (non-infested) from the feeding site of mites (Figure 4). This seems thus to exclude the thaumatin II is involved in the constitutive and induced defense response to TSSM.

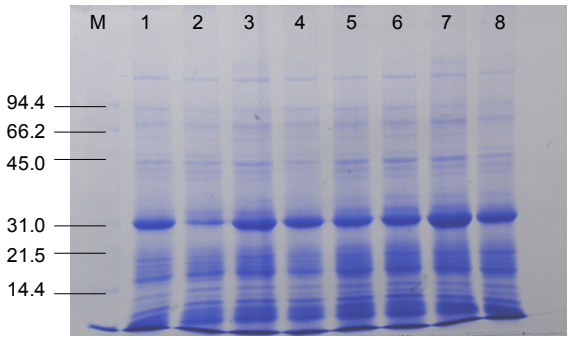

Figure 2. SDS_PAGE analysis of the soluble protein samples of leaves of non-transformed control line B and transgenic line T212 01 in response to localized TSSM feeding on mature leaves. M - molecular mass marker (Sigma, USA); lane 1: line B- control mature leaves; 2 – line B - mite-infested mature leaves; 3 – line T212 01 – control mature leaves; lane 4 – line T212 01 - mite-infested mature leaves; 5 – line B, young leaves from non-infested (control) plant; 6 – line B, non-infested young leaves from mite-infested plant; 7 – line T212 0, young leaves from non-infested (control) plant; 6 – line T212 01, non-infested young leaves from mite-infested plant. 30 micrograms of proteins were loaded in each lane.

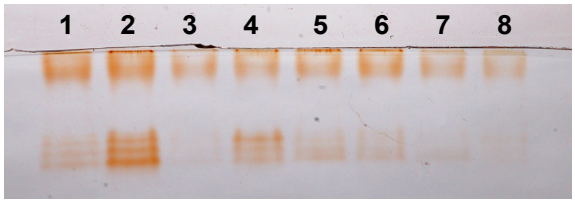

Figure 3. Native PAGE of POX isoforms detected in the soluble protein samples of leaves of non-transformed control line B and transgenic line T212 01 in response to localized TSSM feeding on mature leaves. Lane 1: line B – control mature leaves; 2 – line B - mite-infested mature leaves; 3 – line T212 01 – control mature leaves; lane 4 – line T212 01 - mite-infested mature leaves; 5 – line B, young leaves from non-infested (control) plant; 6 – line B, non-infested young leaves from mite-infested plant; 7 – line T212 0, young leaves from non-infested (control) plant; 6 – line T212 01, non-infested young leaves from mite-infested plant. 60 micrograms of proteins were loaded in each lane.

Locally, TSSM feeding resulted in a slight reduction of the abundance of 3 polypeptide bands (31, 66.2 and 97.4 kDa) in mite-injured site of the leaf of line B, and 5 bands (14.4 - 21.5, 31, 45, 66.2 and 97.4 kDa) in mite-injured site of the leaf of transgenic T212 01 line (Figure 2). No systemic induced changes in leaf protein profile have been recorded for the line B plants (Figure 2). However, in uninjured leaves of transgenic line T212 01 that were distant from TSSM infested ones the abundance of 4 protein bands (31, 45, 66.2, and 97.4 kDa) decreased as compared to the control (line B) (Figure 2).

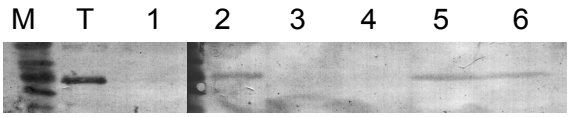

Figure 4. Western blot analysis of total protein samples of leaves of non-transformed control line B (lane 1 – non-infested mature leaves; lane 2 – mite-infested mature leaves) and transgenic line T212 01 (lane 3 – non-infested mature leaves; lane 4 – mite-infested mature leaves; lane 5 – young leaves, from non-infested plant; lane 6 – young leaves from mite-infested plant). 25 micrograms of total soluble protein were loaded in each lane, separated by 12% PAGE and blotted. The membrane was probed with anti-thaumatin II antibody. M – protein molecular mass marker. T-positive control, thaumatin (22 kDa) from *T. daniellii*.

Native PAGE analysis indicated that much stronger increase in abundance of POX isoforms has been found in TSSM-infested mature leaves of control line B than in infested leaves of transgenic line T212 01 (Figure 3). In non-infested young leaves of both control line B and line T212 01, distant from the leaves infested by TSSM the activity of POX isoforms was extremely low related to the activity of POX isoforms in mature leaves and declined to undetectable level in non-infested young leaves of mite-infested plants (Figure 3).

Discussion

Our results suggesting stronger expression of thaumatin II gene in mature than in young leaves of T5 generation of transgenic cucumber plants of line T212 01 are in agreement with previous findings (Kielkiewicz et al. 2006) that showed higher level of thaumatin II in mature than in young leaves of T4 generation of two transgenic cucumber lines T224 09 and T212 01. Therefore, thaumatin II accumulation in leaves of transgenic cucumbers expressing thaumatin II seems to progress with leaf age. Similarly, higher level of thaumatin II was observed in matured cucumber fruits (10-14 cm length) than in young ones (6-9 cm length) (Gajc-Wolska, data unpublished).

Although, results of other studies reported strong and rapid accumulation of PR-5 (TL-like) proteins in other plant species in response to mechanical wounding (Schweizer et al.1998), bacterial, fungal, phytoplasmal infection (Koiwa et al. 1997, Van Loon 1997, Zhong & Shen 2004, Hernandez et al. 2005), aphid infestation (Forslund et al. 2000,Voeckel et al. 2004) or nematode feeding (Sanz-Alferez et al. 2007),thaumatin II gene in transformed cucumber plants does not seem to be induced by TSSM feeding neither locally nor systemically.

In this study, the transformation of cucumber with thaumatin II gene led to the induction of abundance of some polypeptides both in fully developed and young leaves, however, their abundance did not further increase neither at local feeding site nor in the distance. Thus, these polypeptides seem unlikely to be involved in the activation of defense responses against TSSM.

The induction of defensive proteins is the most frequently observed biochemical response upon mite infestation (Bronner et al. 1991, Kielkiewicz 1996, Kielkiewicz 1998, Stout et al. 1996, Arimura et al. 2000, Takabayashi et al. 2000, Tomczyk 2001, Kielkiewicz 2002, Kielkiewicz 2003, Kant et al.2004, Grinberg et al. 2005, Spence et al. 2007). Recent studies by Grinberg et al. (2005) documented that the broad mite (*Polyphagotarsonemus latus* (Banks), Acari:Tarsonemidae) feeding caused in infested leaves of cucumber the activation of genes involved in defense: lipoxygenase (*LOX*), β-1,3-glucanase (*BGL2*), and *POX*. In cotton plants infested by *Tetranychus turkestani* (Ugarov and Nikolski), strong increase in the activity of peroxidase resulted in the production of the condensed tannin proanthocyanidin, which has anti-nutritive properties (Spence et al. 2007). POXs (hydrogene peroxide (H_2O_2) oxido-reductase) have shown to be involved in regulation of reactive oxygen species removal (Kawano 2003, Li-Juan Quan et al. 2008), oxidation of polyphenols and/or flavonoids (Gaspar et al. 1982), oxidative cross-linking of cell wall polymers (Fry 1986, Polle et al. 1994) and/or anti-nutritive compounds biosynthesis (Duffey & Felton 1989, Felton et al. 1989, Duffey & Felton 1991, Duffey & Stout 1996). Thus, an increase in POX activities is believed to be involved in plant defense responses against broad range of herbivores. Our 'in-gel' evaluation of POX reveals that transformation changed cucumber plant making it unable to keep POX activity at the level found in untransformed plant. In response to TSSM, POX activity was elevated locally, but not systemically in both non-transgenic and transgenic plants. However, in response to TSSM feeding local POX isoforms increase to a lower abundance in mite-injured leaves of cucumber encoding thaumatin II gene than in control plants (line B). It seems existing a suppressed involvement of POX in defense response of transgenic cucumber plant against TSSM. The link between anti-oxidative mechanisms and mite-stress tolerance should be further investigated to elucidate the mechanisms underlying the observed changes in transformed

cucumber leaf proteins.

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PREDATORY EFFICENCY OF *AGISTEMUS YUNUSI* CHUADHRI (STIGMAEIDAE: ACARINA) AND *AMBLYSEIUS DEDUCTUS* CHUADHRI (PHYTOSEIIDAE: ACARINA) FEEDING ON THREE PHYTOPHAGOUS MITE SPECIES.

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Abstract

Mites of the family Stigmaeidae and Phytoseiidae are potential predators of various species of phytophagous mites throughout the world. The aim of this work was to study the predatory efficency of different stages (larva, nymph and adult) of one stigmaeid (*Agistemus yunusi*) and one Phytoseiid (*Amblyseius deductus*) against three phytophagous mites species (*Panonychus ulmi*, *Panonychus citri* and *Tetranychus urticae*). Results showed that *A. deductus* has better predation rates than *A. yunusi* when both species fed on three different phytophagous mites. The maximum predation of all stages of the two predatory mites was achieved when fed on the nymph and adults of *T. urticae* whereas the predation rate was the lowest on *P. ulmi.*

Key words

Feeding preference, *Amblyseius*, Predation , *Agistemus, Tetranychidae*

Introduction

The study of the feeding range of predaceous mites is essential for evaluating their role in reducing the associated plant pests. Some of the predaceous mites, particularly Stigmaeid and Phytoseiid species, have been studied in this regard by numerous authors e.g., Collyer (1964), Herbert (1959), McMurty (1964), El-Badry & Afify (1968), Santos (1976), Clements & Harmsen (1990) and Fan & Zhang (2005). The Stigmaeidae and Phytoseiidae includes potential important predaceous species found throughout the world on many crops including apple, citrus, mango, grapes, tea, tomato, fig, cotton, sweet potato, potato and also on weed plants (Nelson *et al*. 1973; Childers and Enns 1975; Muma 1975; Santos and Laing 1985; Sepasgosarian 1985; Thistlewood *et al*. 1996; Searle and Smith Meyer 1998; Villanueva and Harmsen 1998). Some species of these two families may play a significant role in controlling phytophagous mites and scale insects in North America, Europe, Africa, and Asia (Rasmy 1975; Krantz 1978; Childers 1994; Childers *et al*., 2001). Whereas several phytoseiid species have been studied for their predatory performances, until now few biological studies on *Agistemus* species have been conducted (Yue *et al.,* 1994).

Agistemus yunusi and A. deductus are common species found in Faisalabad on different hosts like mango, citrus and bitter gourd where. They prey on different life stages of the citrus red mite, *Panonychus citri*, *Panonychus ulmi* and the two spotted spider mite *Tetranychus urticae* (Chaudhri and Akbar 1985). The objective of this work was to determine the comparative predatory efficiency of different life stages (larvae, nymph and adult) of *A. yunusi and A. deductus* using three different phytophagous mites as food source under

controlled laboratory conditions. The aim was to detect relevant attributes to use these predatory mites for biological control of phytophagous mites on different crops in Punjab, Pakistan.

Material and methods

Culture of Mite

Using sieve collection methods, adult females of *A. yunusi* and *A. deductus* were collected from mango and citrus orchards located in the vicinity of the University of Agriculture, Faisalabad. Leaves with adult females were placed in an ice box, taken to the laboratory, and transferred to leaf arenas following the Chaudhri and Akbar (1985) methods. Nymph and adults of three different phytophagous mite species already collected from the field were transferred separately into leaf arenas (figure 1) using a fine sable brush. Each leaf arena measured 1.2 cm in depth and 1 cm in width. Host species were kept at 25±2^{0}C and 70±2%R.H. Each arena with a predatory mite species comprises one replication. Nymph and adults of phytophagous mites (*P. ulmi, P. citri* and *T. urticae*) were added to each arena at one per day basis prey consumption, before all were consumed.

Figure 1. Rearing cell for predatory and phytophagous mites.

The experiment was repeated twice and data regarding predatory efficacy of predatory mites were recorded twice a day at 0700 and 1900 hours.

Table 1. Feeding *efficiency* of several stages of *Agistemus yunusi and Amblyseius deductus* on three Phytophagous mite species Mean (±standard deviation) of the number of preys consumed.

Predator	Predator Stages (Days)	*Panonychus ulmi*		*Panonychus citri*		*Tetranychus urticae*	
		Nymph	Adult	Nymph	Adult	Nymph	Adult
Agistemus yunusi	Larvae (1-2)	2.5±0.3	1.5±0.2	3.9±1.1	2.6±0.5	6.2±1.3	2.1±0.2
	Nymph (6-7)	6.5±0.9	2.5±0.8	34.4±1.9	21.7±1.4	49.5±1.8	12.5±1.1
	Adult (14-16)	64.5±4.5	44.5±3.4	67.7±3.2	81.7±4.1	98.2±4.4	104.6±6.6
Amblyseius deductus	Larvae 2-3)	5.1±102	2.6±1.3	6.9±1.4	4.9±1.1	10.6±2.2	2.7±0.6
	Nymph (7-9)	18.9±1.4	8.3±0.8	51.7±2.0	33.4±2.3	71.3±2.7	41.3±1.9
	Adult (17-19)	77.7±2.6	88.8±3.4	105.1±4.3	116.6±4.3	132.2±6.0	152.0±6.9

Table 2. Consumption by *Agistemus yunusi and Amblyseius deductus* of nymphs and adults of three phytophagous mite species.

Predator	*Panonychus ulmi*		*Panonychus citri*		*Tetranychu urticae*	
	Nymph	Adult	Nymph	Adult	Nymph	Adult
Agistemus yunusi	73.5±5.7	48.5±4.4	106.0±6.3	106.0±6.2	153.9±7.56	119.2±8.0
Amblyseius deductus	101.7±5.2	99.7± 5.0	163.7±7.7	154.9±7.8	214.1±10.9	196.0±9.4

Table 3. Total consumption by *Agistemus yunusi and Amblyseius deductus* of three phytophagous mite species.

Predator	*Panonychus ulmi*	*Panonychus citri*	*Tetranychus urticae*
Agistemus yunusi	122.0±10.1	212.0±12.5	273.1±15.6
Amblyseius deductus	201.4±10.2	318.6±15.5	410.1±20.3

Results and discussion

The experiment of predatory efficiency of *A. yunusi* and *A.deductus* show that, as expected, the

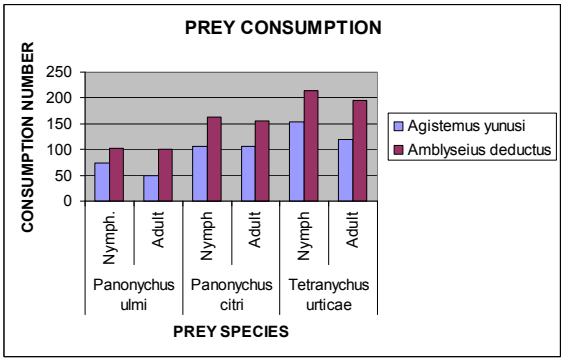

Figure 2. Consumption by predatory mites of nymphs and adults of three phytophagous species

maximum feeding (measured in a per day basis) was recorded during the adult stage of both species (table-1). Larvae, nymph and adults of both predatory mites maximally fed on *T. urticae and P. ulmi* was fed. The larvae of *A. deductus* lived for 2-3 days and fed maximally on nymphs of *T. urticae* than its adults, whereas its minimum feeding was on the adults of *P. ulmi. The* nymph of *A. deductus* lived for 7-9 days and fed maximally on nymphs of *T. urticae* than on other mites. Adults of *A. deductus* lived for 17-19 days, and the maximum feeding was observed on the adults of *T. urticae* (table-1).These results are similar to results obtained by Hafez *et al.,* (1983) who determined the effect of tetranychid mites on the life stages of genus *Amblyseius.* The larvae, nymph and adults of *A. yunusi* lived for 1-2, 6-7 and 14-16 days respectively. Adults of *A. yunusi* fed more on adult of *T. urticae* than on nymphs of either *T. urticae* or the other tetranychid. These results are concordant with the findings of Yousaf *et al.,* (1982) who studied the maximum predation on *T. urticae*, and the effect on the biology and fecundity of stigmaeid mites. In conclusion, both *A. yunusi* and *A. deductus* fed more on *T. urticae* nymphs

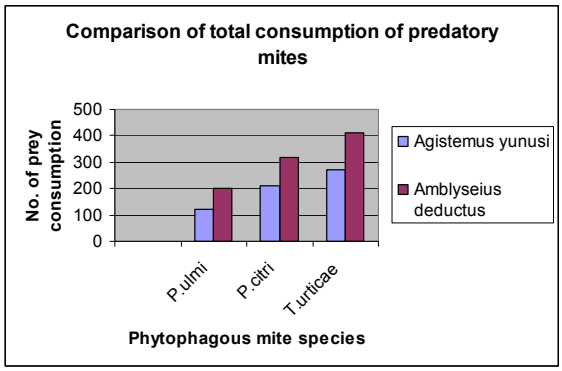

Figure3. Total consumption by predatory mites of three phytophagous mite species.

than on all other phytophagous mites during their life stages (table 1 and 2), the same pray choice appears in the results of the total consumption (table 3 and figure 3). In addition, the two studied predatory mites prefer to feed on *P. citri* rather than on *P. ulmi.* The Feeding preference of *A. deductus* and *A. yunusi* can be resumed as follows: *T. urticae > P .citri > P .ulmi.*

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DETERMINATION OF THE RESISTANCE CHARACTERISTIC OF THE *ETETRANYCHUS URTICAE* KOCH' S (ACARI: TETRANYCHIDAE) STRAIN SELECTED WITH PROPARGITE

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Abstract

The biochemical mechanism of propargite resistance in *Tetranychus urticae* strain collected from a tomato greenhouse (CUM) and maintains in the laboratory was determined in this study. LC_{50} level of the CUM strain selected seventeen times with propargite was increased from 13.16 water to 417.70 µl / 100 ml water. Selected strain showing 32.75 fold resistance was named PROP 17 strain. Application of propargite with synergists piperonyl butoxide (PBO), S- Benzyl-O, O-diidopropyl phosphorothioate (IBP) and triphenyl phosphate (TPP) resulted in 1.53, 1.46 and 1.35 fold synergistic ratio, respectively, in the PROP 17 strain. The esterase activity was evaluated using 1-naphtyl acetate, but 1-chloro-2, 4-dinitrobenzene (CDNB) was used as a substrate for investigation of GST activity. Esterase enzyme activity was increased in propargite resistant the PROP 17 strain from 7.69 to 13.97 mOD/min/mg proteins. In addition, the band intensity of esterase enzyme increased in the electrophoresis method. Besides, the GST enzyme activity increased from 9.75 to 13.71 mOD/min/mg proteins.

Key-word

Tetranychus urticae, propargite, synergist, esterase, gluthathione S-transferase

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important agricultural pest with a global distribution (Gorman *et al.* 2001). It causes important losses in ornament plants, horticultural crops and greenhouse production. *Tetranychus urticae* can increase strain density very rapidly in the suitable host plant and climate condition. Two spotted spider mite causes most important economic losses and reach high density in short time because of the suitable environment along year in the greenhouse condition (Tsagkarakou *et al.* 1999). It affects crops by direct feeding; thereby reducing the area available for photosynthetic activity and in severe infestations may cause leaf abscission (Leeuwen *et al.* 2005). For a successful control it is necessary to know the spread, biology, ecological necessity, type of damage, and recognition of this pest.

Many insecticides and acaricides have been registered to control *T. urticae* in Turkey (Ay *et al.* 2005). One of the major problems in the control of *T. urticae* is their ability to rapidly develop resistance to many acaricides after a few applications (Stumpf *et al.* 2001). In addition intense and continuous chemical use may cause more problems such as environmental pollution and disruption natural balance.

Propargite is a non- systemic acaricide used for controlling a variety of phytophagous mites on many crops (Kumar *et al.* 2005). It is a selective

acaricide has been used to control *T. urticae* in many crops in Turkey (Ay 2005). Propargite is contact and stomach poisons acting on all stages *T. urticae* (Ay *et al.* 2005).

In this study were to confirm resistance strain, the PROP 17, in the laboratory in comparison with a susceptible strain, GSS, and to calculate resistance ratios. Synergistic effects and detoxifying enzyme activities in the susceptible and resistant strain of *T. urticae* were also examined to understand possible mechanisms of propargite resistance.

Materials and methods

Mite strains: The original strain of *Tetranychus urticae* was collected from a commercial tomato (*Lycopersicum esculantum* L.) greenhouse in Antalya province in Kumluca district in May 21, 2003. This strain was named as the CUM. After collection, *T. urticae* was reared continuously on pinto bean plants (*Phaseolus vulgaris* L.) under laboratory conditions at 26 $\pm$ 2 ^{o}C, 60 $\pm$ 5 % RH and a 16 h photoperiod. A susceptible strain (GSS) were obtained from Rothamsted Experimental Station, Harpenden (England), in 2001 and reared in laboratory conditions since 2001. The propargite resistant (the PROP 17) strain had been produced by selecting a field colony (the CUM) with propargite for seventeen selections.

Toxicity test

These tests were conducted based on the method described by Ay (2005). The prepared suspension of propargite was sprayed the internal surfaces of lids and bases of 60 mm diameter plastic Petri dishes and allowed to dry for 30 min. For each application, 1 ml suspension was sprayed on each base and lid pair by a Potter spray tower (Burckard Auto-Load, Rickmansworth, Herts, UK) at 14.504 psi. Adult female mites (≅30) were transferred to each dish using a hairbrush, and the dishes were closed and sealed with parafilm to prevent escape of spider mites. Thereafter, the mites in the dish were kept at 26 $\pm$ 2 ^{o}C, 60 $\pm$ 5 % RH and a 16 h photoperiod for 24 h after treatment. Survival of individual mite was determined by touching each mite with a fine brush. Mites, which were unable to walk at least a distance equivalent to their body length, were considered dead. Mortality tests were done before each experiment to determine a range of concentration that causing approximately 10-95 % mortality. Each experiment was conducted using three replicates of a seven concentrations (plus distilled water-only control). Pooled data were subjected to probit analysis (POLO PC) (LeOra software1994) and $LC_{50,\ 60\ and\ 90}$ with respectively 95 % CL were estimated. The LC_{50} values of selection strain were compared to those of the susceptible strain (GSS). A resistance ratio (RR) was calculated according to the following formula:

RR= LC_{50} value of the PROP 17 strain / LC_{50} value of the GSS strain

Selection for Resistance

Females of the original strain, the CUM were selected for resistance to propargite under laboratory conditions from September 2006 to June 2007. Selection experiments were conducted by modified method of Yang *et al.* (2002). At least 400 adult female mites were transferred from the CUM strain into petri dishes (40 mite/ Petri dish) treated with propargite concentrations equal to the LC_{60} for that cycle. After 24 h, surviving mites transferred back to plants and the populations were allowed to increase. The next selection cycle was conducted two or three generations later (approximately 15-20 days) when the populations had increased. A bioassay using the selection acaricide was conducted on mite strain periodically when the number of surviving mites changed in the selection petri dishes. The new LC_{60} was applied as a selection pressure.

Synergism test

The effects of propargite and synergists were tested using the methods of Kim *et al.* (2004). Mixed-function oxidase (MFO) and esterase inhibitor piperonyl butoxide (PBO) and others esterase inhibitor S-benzyl-O, O-diisopropyl phosphorothioate (IBP) and triphenyl phosphate (TPP) were used to inhibit detoxification enzymes. Synergists were dissolved in aceton: distilled water (1:1) and 1 ml suspension sprayed on base and lid of petri dishes in the same manner as in toxicity test 30 min prior to acaricide application. Only aceton + distilled water was applied to the control. Synergist solutions were prepared at following concentration ($mg.l^{-1}$): PBO (500), IBP (400) and TPP (125). A synergistic ratio (SR) was calculated using following formula:

SR= LC_{50} of propargite without synergist / LC_{50} of propargite with synergist

Biochemical assays

Electrophoresis: vertical slab polyacrylamide gel electrophoresis was performed following previously reported procedures by Walker (1994), Goka & Takafuji (1992). The gels were 1 mm thick and 80 mm x 80 mm. in area. Acrylamide concentrations were 7.5 % in separating gels and 3.5 % in stacking gels. Adult female mites were homogenized individually in 10 µl of 32 % (w/v) sucrose with 0.1% Triton X-100 in microtiter plates

by a multiple-homogenizer. Electrophoresis was carried out at a constant current of 150 volt at 5-8 ^{0}C for about 1.5 h. For esterase activity gel was stained by placing the gels for 1 h. in 0.4 (w/v) fast blue BB salt after incubating them for 30 min in a 0.02 % (w/v) solution of α-naphthyl acetate in 0.2 M phosphate buffer (pH 6.5), which contained 1 % acetone. All staining reactions were stopped by placing the gel in 7.5 % acetic acid.

Photometric Esterase assay: esterase assays were performed according to Stumpf & Nauen (2002). The 10,000 g supernatant of mass homogenates of 100 adult females prepared in 500 µl ice-cold 0.1 M sodium phosphate buffer, pH 7.5, containing 0.1 % (w/v) Triton X-100, was diluted 10-fold and used as the enzyme source. Twenty five µl aliquots (0.5 mite equivalent) were added to the wells of a 96-well microplate, containing 25 µl of 0.2 M sodium phosphate buffer, pH 6.0. Wells with buffer-only served as control for the non-enzymatic reaction. The assay was started by adding 200 µl of substrate solution to each well, giving final volume of 250 µl. Substrate solution consisted of 15 mg of fast Blue RR salt dissolved in 25 ml of sodium phosphate buffer, pH 6.0, and 250 µl of 100 mM 1-naphthyl acetate in acetone. The esterase activity was measured continuously at 450 nm and 25 ^{o}C in Versamax kinetic microplate reader (Molecular Devices) for 10 min, and analyzed utilizing Softmax software to fit kinetics plots by linear regression.

Photometric GST assay Using 1-Chloro2,4-dnitrobenzene: glutathione S-transferase activities were performed according to Stumpf & Nauen (2002). GST activity was determined using 1-chloro-2,4-dnitrobenzene and reduced glutathione (GSH) as substrate. One hundred adult females were homogenized in 1000 µl Tris-HCL (0.05 M, pH 7.5). The total reaction volume per well microtiter plate was 300 µl, consist of 100 µl each supernatant (10,000 g, 5 min), CDNB (Containing 0.1 % (v/v) ethanol), and GSH in Tris-HCL (0.05 M, pH 7.5), giving final concentrations of 0.4 mM CDNB and 4 mM GSH. The change in absorbance was measured continuously for 5 min at 340 nm and 25 ^{o}C using the Versamax kinetic microplate reader (Molecular Devices). The nonenzimatic reaction of CDNB with GSH measured without homogenate served as control.

The activity of all enzymes was analyzed by Softmax PRO software and presented as mOD/min/mg proteins. The data were analyzed using the General Linear Model (GLM) procedure of SAS (1999) by using strains in the model and PDIFF statement was used to compare strains' enzyme activity means for dependent variables. Alpha level of 0.05 was accepted as significance level.

Results

Selection for resistance

The CUM strain of Tetranychus urticae were analyzed propargite for resistance character. LC50,60 levels of the CUM strain of T. urticae were determined by dry film method. Then, the selection was completed by applying the LC60 level that was determined for each new population. When the CUM strain was exposed to seventeen selection with propargite, 32.75 fold resistance was developed. After seventeen selections with propargite the LC50 of the CUM strain increased from 1.03 to 32.75 µl (formulation)/ 100 ml water (Table 1). The strain 32.75 fold resistant to propargite was named PROP 17.

Synergistic effects

In order to gain information on the propargite resistance mechanisms synergists such as PBO (MFO and EST inhibitor) (Young *et al.* 2005, Kim *et al.* 2006), IBP and TPP (EST inhibitors) (Kim *et al.* 2004, Kang *et al.* 2006, Wang & Wu 2007) were used for bioassay (Table II). Treatment with PBO exhibited remarkably higher level of synergism to propargite in the PROP 17 strain (SR=1.53). Treatment with IBP and TPP resulted in 1.46 and 1.35 fold synergism ratio respectively, in the PROP 17 strain. Additionally, 1.18, 1.30 and 1.40 fold synergism ratio by PBO, IBP and TPP was determined in the GSS strain.

Detoxifying enzyme activity

In the propargite selected resistant strain the PROP 17 activities of esterase and glutathione S-transferase (GST) enzymes were analyzed. While esterase enzyme activity was detected by gel electrophoresis and microplate reader methods, glutathione S-transferase activity was detected only by microplate reader method. The esterase activity was visualized using 1-naphtyl acetate, but CDNB was used as a substrate for GST enzyme activity. The PROP 17 strain which was resistance against propargite the esterase enzyme activity was increased from 7.69 to 13.97 mOD/min/mg proteins. The band intensity of esterase enzyme increased in the electrophoresis method (Figure I). Besides, the GST enzyme activity increased from

Table 1. Resistance ratio and LC_{50} levels determined after selection with propargite from the CUM and GSS populations (µl of formulation/ 100ml of distilled water).

Strain	N[a]	Slope ± SE	LC_{50} (µl/ 100ml) (0.95 CI[b])	LC_{60} (µl/ 100ml) (0.95 CI[b])	Resistance ratio LC_{50}[c]
CUM	718	1.064 ± 0.097	13.16 10.28-16.79	22.77 17.81-30.11	1.03
select 1	721	1.284 ± 0.108	14.94 11.93-18.58	23.53 18.92-29.80	1.17
select 2	720	1.079 ± 0.102	23.39 18.04-30.27	40.16 31.00-53.98	1.83
select 3	720	1.163 ± 0.097	35.00 27.18-44.29	57.80 45.69-73.86	2.74
select 4	722	1.188 ± 0.111	39.25 30.24-50.38	64.13 49.97-84.25	3.07
select 5	722	1.047 ± 0.097	51.56 40.25-66.22	90.03 69.93-120.74	4.04
select 6	727	1.082 ± 0.100	60.03 46.37-77.12	102.91 80.04-136.25	4.70
select 7	725	1.083 ± 0.101	86.20 66.70-110.97	147.71 114.6-196.73	6.75
select 8	726	1.042 ± 0.097	104.35 81.28-133.92	182.67 142.02-244.13	8.18
select 9	725	1.079±0.098	113.35 88.73-144.07	194.63 152.90-255.36	8.88
select 10	722	1.119 ± 0.099	131.06 103.15-165.35	220.77 174.81-285.94	10.27
select 11	722	1.014 ± 0.096	188.70 145.81-243.86	335.50 259.05-452.22	14.79
select 12	723	1.036 ± 0.097	228.77 177.56-294.16	401.83 311.88-537.45	17.93
select 13	720	1.085 ± 0.098	261.42 204.88-332.48	447.55 351.35-588.52	20.49
select 14	725	1.129 ± 0.104	330.18 256.81-422.17	553.54 432.67-729.09	25.88
select 15	722	1.057 ± 0.102	376.64 288.16-489.62	653.97 502.71-881.32	29.52
select 16	721	1.095 ± 0.099	398.92 313.65-506.86	679.58 533.95-894.24	31.27
PROP 17	725	1.210 ± 0.106	417.70 329.09-525.50	676.47 537.55-868.86	32.75
GSS	724	1.214 ± 0.101	12.75 10.17-15.82		

[a] Total number of mites used, [b] Confidence interval, [c] Resistance ratio = LC_{50} value of the PROP17 strain / LC_{50} value of the GSS strain

Table 2. Toxicity of propargite with and without synergist to susceptible, GSS and propargite-resistant, the PROP 17 strains of *Tetranychus urticae* (µl of formulation/ 100ml of distilled water).

Treatment	N[a]	Slope ± SE	LC_{50} (µl/ 100ml) (0.95 CI[b])	LC_{90} (µl/ 100ml) (0.95 CI[b])	SR[c]
PROP 17 strain					
Propargite only	725	1.210 ± 0.106	417.70	4786.42	-
			329.09-525.50	3215.04-8190.72	
+PBO	723	1.355 ± 0.112	272.29	2402.97	1.53
			192.64-369.13	1541.02-4637.97	
+IBP	723	1.260 ± 0.107	285.11	2968.07	1.46
			224.42-355.58	2082.26-4748.96	
+TPP	719	1.322 ± 0.107	308.76	2875.23	1.35
			249.20-378.43	2056.67-4457.64	
GSS strain					
Propargite only	724	1.214 ± 0.101	12.75	144.89	-
			10.17-15.82	99.71-238.14	
+ PBO	725	1.200 ± 0.103	10.73	125.45	1.18
			5.60-18.82	56.30-687.46	
+ IBP	722	1.433 ± 0.122	9.74	76.40	1.30
			7.65-12.10	56.21-113.98	
+ TPP	723	1.423 ± 0.117	9.06	72.13	1.40
			6.44-12.22	47.343-133.720	

[a] Total number of mites used, [b] Confidence interval, [c]Synergistic ratio: LC_{50} for propargite alone / LC_{50} for propargite with synergist

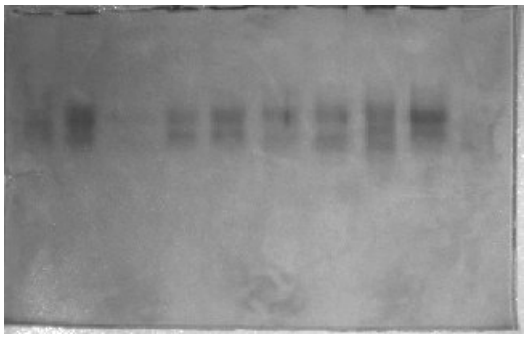
Table 3. Detoxifying esterase and GST enzymes activities in susceptible (GSS), the CUM and the propargite-resistant the PROP 17 strains of *Tetranychus urticae* (P<0.05)[a]

Enzyme	Strain	n[b]	Specific activity (± SE) mOD/min/mg proteins	R/S[c]
Esterase	GSS	4	10.35 (± 0.72) b	1.00
	CUM	4	7.69 (± 0.72) c	0.74
	PROP 17	5	13.97 (± 0.64) a	1.34
GST	GSS	5	13.73 (± 0.65) a	1.00
	CUM	4	9.75 (± 0.73) b	0.70
	PROP 17	5	13.71 (± 0.65) a	0.99

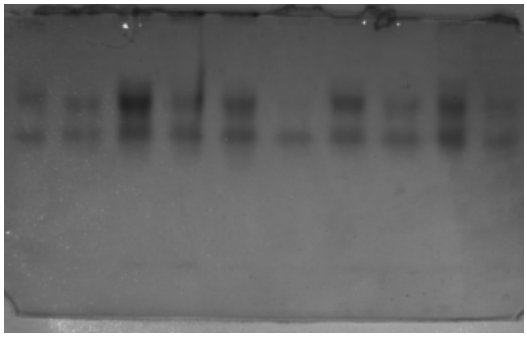
[a]: Means with different letters in column for each enzyme are significantly different ($P < 0.05$), [b]:number of replicates, [c] :enzyme activity CUM or PROP 17/ enzyme activity GSS strain.

9.75 to 13.71 mOD/min/mg proteins (Table 3). The levels of esterase and GST enzyme activity were found statistically significantly higher in selected strain (PROP 17) when compared to parental strain (P<0.05).

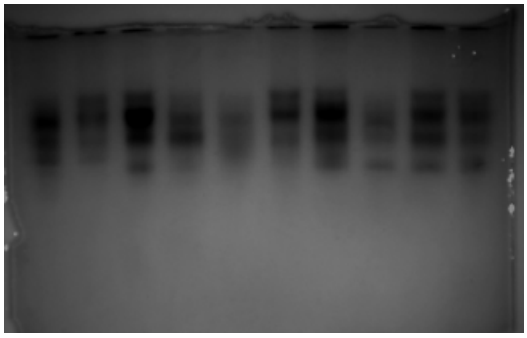
Moreover, the esterase enzyme activity of PROP 17 was found statistically significantly higher than susceptible GSS strain ($P < 0.05$).



a



b



c

Figure 1. Esterase zones in the different populations of the *Tetranychus urticae* (a: esterase zones of the GSS strain, b: esterase zones of the CUM strain and c: esterase zones of the PROP 17 strain)

Discussion and conclusion

Acaricide resistance continues to be a major problem in the control of the two-spotted spider mite, *Tetranychus urticae*, one of the most important pest species in many cropping systems worldwide (Leeuwen & Tirry 2007).

The CUM populations of *T. urticae* were analyzed to propargite for resistance character. The CUM strain has increased development while becoming seventeen selection propargite. The CUM strain exposed to seventeen selection with propargite 32.75 fold resistance were developed (Table I). Also synergists activity of piperonyl butoxide (PBO), triphenyl phosphate (TTP) and S-benzyl-O,O-diisopropyl phosporothioate (IBP) with propargite in resistant the PROP 17 strain was studied. PBO, TTP and IBP synergists significantly restored propargite toxicity in the PROP 17 population. But three synergists were affected toxicity in the susceptibility population. The activity spectrum of the three synergists suggests that metabolic detoxification via EST enzyme activity likely plays a major role in propargite resistance in the PROP 17 strain.

Resistant the PROP 17 strain was investigated the enzyme activities of esterase and glutathione S-transferase. Esterase enzyme plays a role in the detoxification of many agrochemicals including pyrethroids, organophosphates and carbamates (Wheelock *et al.* 2005). Esterase enzyme was determined polyacrylamide gel electrophoresis and microplate reader methods. Additionally GST enzyme activity was determined kinetically at the microplate reader method. The PROP 17 strain that was resistance against propargite the esterase enzyme activity was increased from 7.69 to 13.97 mOD/min/mg proteins. The band of esterase enzyme increased in the electrophoresis method. Elevated levels of GST play a major role as a mechanism of resistance to insecticides and acaricides in resistant pest insects and mites (Nauen & Stumpf 2002). Besides, the GST enzyme activity increased from 9.75 to 13.71 mOD/min/mg proteins.

The resistance to propargite developed by *T. urticae* was reported (Kabir *et al.* 1993: Ay *et al.* 2005). Rauch & Nauen (2003) found 1.2 –fold and 1.2-fold increase in both esterase and GST enzyme activity in spirodiclofen selected *T. urticae* population. Results of this study revealed that there is a positive relationship between esterase and GST enzyme activity and resistance to propargite. In addition, IBF and TPP, general esterase inhibitors and MFO inhibitor PBO have shown synergistic effect to propargite. In conclusion, based on these findings, general esterase and GST enzymes may play a role in propargite resistance in two-spotted spider mite.

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