Parthenogenesis and Sexuality
in Oribatid Mites

Phylogeny, Mitochondrial Genome Structure and Resource Dependence

vom Fachbereich Biologie der Technischen Universität Darmstadt

zur Erlangung des akademischen Grades

eines Doctor rerum naturalium

genehmigte Dissertation von

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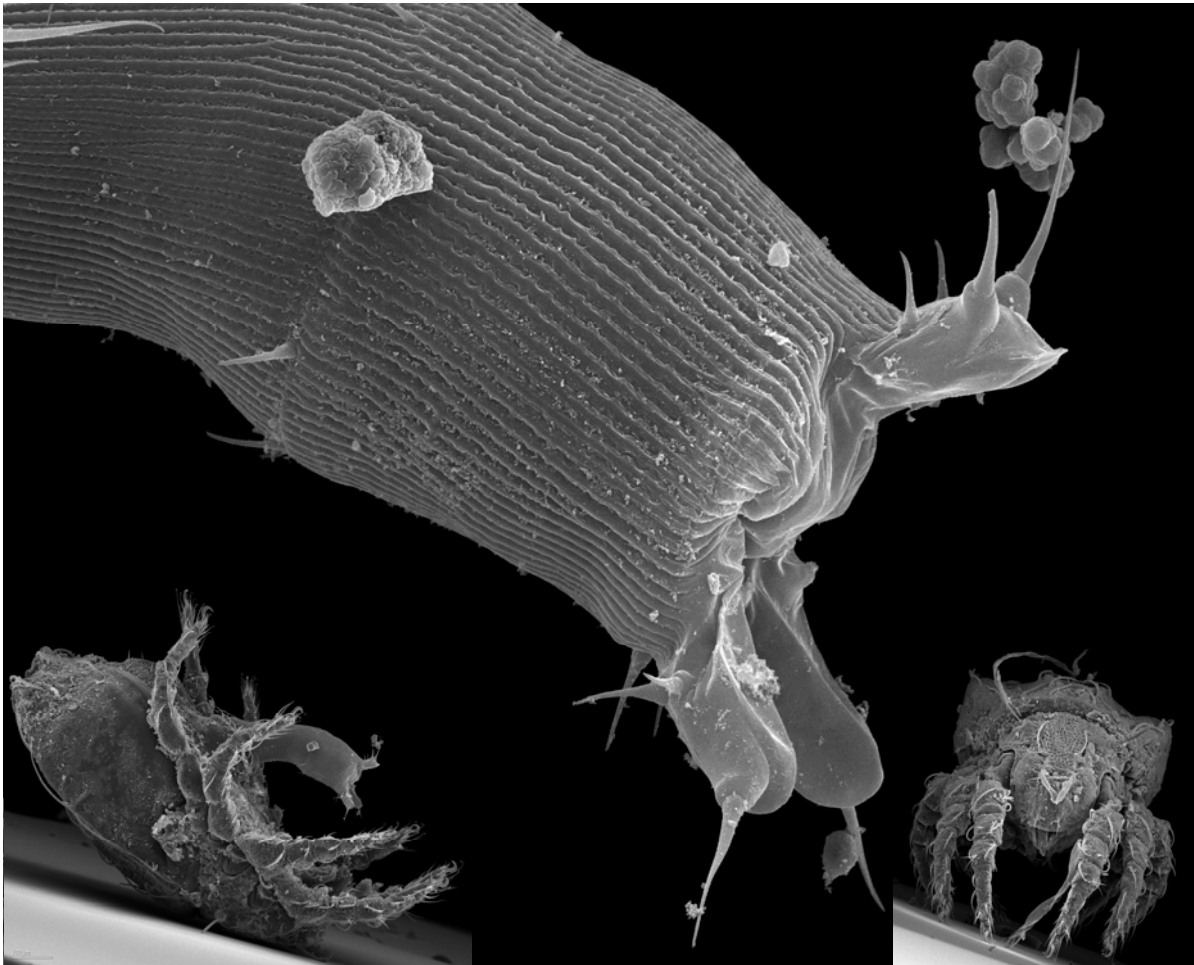
aus Frankfurt a. M.

For my family...

... and in remembrance of

Walter Maier

(*1933-†2004)

"When you have eliminated the impossible,

whatever remains, however improbable, must be the truth."

Sherlock Holmes

"Daring ideas are like chessmen moved forward;

they may be beaten,

but they may start a winning game."

Johann Wolfgang von Goethe

Curriculum Vitae

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1999-2001	Basic study period of biology at the University of Technology, Darmstadt
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<u>Further education and stay abroads</u>

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February 2005	Sequencing Workshop, Scientific Research and Development GmbH, Oberursel
May 2005	Arlequin Workshop, meeting of the "Junge Systematiker", Kassel
August 2005	Workshop on Molecular Evolution, Woodshole (USA)
December 2005	SuperTree Workshop, Iffelsdorf
Sept.-Nov. 2007	Stay abroad in Canberra, Australia, for sequencing the mitochondrial genome of two oribatid mite species

<u>Symposia and meetings</u>

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April 2005	Mesofauna meeting Göttingen; talk
August 2005	X. Congress of the ESEB, Krakow, Poland; talk
February 2006	DZG meeting on "New markers and new theory", Potsdam; poster

March 2006	"Orthologous Gene" meeting Göttingen; poster
March 2006	Mesofauna meeting Aachen; talk
August 2006	XII. International Congress of Acarology, Amsterdam, Netherlands; poster
February 2007	DZG meeting on "Life-history evolution", Bayreuth
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August 2007	XI. Congress of the ESEB, Uppsala, Sweden; poster

Other experiences

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2002-2004	Student assistant at the TU Darmstadt, Department of Biology: supervision of practical courses in Zoology and Botany
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May 2006/07/08	Supervision of the alternative practical course for "Spezielle Zoologie"
July-August 2006	Supervision of student excursions

Further supervisions

Martin Rosenberger	"Biogeographische Untersuchung der parthenogenetischen Hornmilbenart *Platynothrus peltifer* und der sexuellen Hornmilbenarten *Carabodes coriaceus*, *Carabodes femoralis* und *Carabodes marginatus* anhand des Elongation Factors 1α", research internship 2006
Max Althammer	„Molekular-phylogenetische Untersuchungen zur Stellung der Astigmata innerhalb der Acarina", state examination 2006
Martin Rosenberger	„Postglaziale Rekolonization von Zentraleuropa durch die parthenogenetische Oribatide *Platynothrus peltifer* und die sexuelle Oribatide *Steganacarus magnus*", Diploma thesis 2007
Patrick Pachl	„Phylogenie basaler Oribatiden: Konvergente Entwicklung des ptychoiden Verteidigungsmechanismus zur Feindabwehr", research internship 2008
Alexander Weigand	„Populationsgenetische Untersuchungen an der Hornmilbe *Platynothrus peltifer* (Acari, Oribatida): Intraspezifische Cytochrom C Oxidase I-Variabilität & mitochondriale Heteroplasmie", research internship 2008

Publications Resulting From This Dissertation

CHAPTER 2

Domes K, Althammer M, Norton RA, Scheu S, Maraun M (2007) The phylogenetic
relationship between Astigmata and Oribatida (Acari) as indicated by molecular markers.
Experimental and Applied Acarology 42, 159-171.

CHAPTER 3

Domes K, Norton RA, Maraun M, Scheu S (2007) Re-evolution of sexuality breaks Dollo's
law. Proceedings of the National Academy of Science USA 104, 7139-7144.

CHAPTER 4

Domes K, Maraun M, Scheu S, Cameron SL (2008) The complete mitochondrial genome of
the sexual oribatid mite *Steganacarus magnus*: genome rearrangement and loss of tRNAs.
BMC Genomics 9, 532.

CHAPTER 5

Domes K, Rosenberger M, Weigand A, Maraun M, Scheu S, Cameron SL (in preparation) Is
the complete mitochondrial genome of the parthenogenetic oribatid mite *Platynothrus peltifer*
heteroplasmic?

CHAPTER 6

Domes K, Scheu S, Maraun M (2007) Resources and sex: soil re-colonization by sexual and
parthenogenetic oribatid mite species. Pedobiologia 51, 1-11.

CHAPTER 7

Domes K, Scheu S, Maraun M (in preparation) Does resource quality foster the oribatid mite
community? A laboratory experiment.

Further Publications

Schaefer I, Domes K, Heethoff H, Schneider K, Schön I, Norton RA, Scheu S, Maraun M (2006) No evidence for the 'Meselson effect' in parthenogenetic oribatid mites (Oribatida, Acari). Journal of Evolutionary Biology 19, 184-193.

Heethoff H, Domes K, Laumann M, Maraun M, Norton RA, Scheu S (2007) High genetic divergences indicate ancient separation of parthenogenetic lineages of the oribatid mite Platynothrus peltifer (Acari, Oribatida). Journal of Evolutionary Biology 20, 392-402.

Maraun M, Domes K, Schaefer I, Scheu S (2008) Molekulare Phylogenie von Oribatiden (Hornmilben). In: Theorie in der Ökologie Band 12. Bodenzoologie und Ökologie: 30 Jahre Umweltforschung an der Freien Universität Berlin. Jopp F, Pieper S (Hrsg.). Peter Lang Internationaler Verlag der Wissenschaften, Frankfurt/Main.

Chahartaghi M, Maraun M, Scheu S, Domes K (2009) Resource depletion and colonization: A comparison between parthenogenetic and sexual Collembola species. Pedobiolgia 52, 181-189.

Maraun M, Erdmann G, Scheu S, Domes K (submitted) Multiple convergent evolution of aboreal life in oribatid mites indicates the primacy of ecology.

Norton RA, Pachl P, Scheu S, Maraun M, Domes K (in preparation) A molecular test of body-form convergence in oribatid mites.

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Zusammenfassung

Die vorliegende Arbeit untersucht molekulare und ökologische Konsequenzen verschiedener Reproduktionsformen auf Hornmilben (Acari, Oribatida) als Modellorganismen. Die Erforschung der Evolution und Aufrechterhaltung von Sex und Parthenogenese ist eins der interessantesten Gebiete der Evolutionsbiologie. Bis heute ist nicht geklärt, warum Sex trotz der damit verbundenen Kosten für den Organismus die vorherrschende Vermehrungsweise im Tierreich ist: Weibchen müssen Zeit und Ressourcen in Partnersuche und Paarung investieren, sie müssen ihr Genom reduzieren und erzeugen im Vergleich zu parthenogenetischen Weibchen nur die Hälfte an reproduktiven Nachkommen (Kosten der Meiose + Kosten der Männchen = "twofold costs of sex"). Obwohl diese Kosten erdrückend erscheinen, vermehren sich nur 1% aller Metazoen parthenogenetisch. Drei so genannte „ancient asexual scandals" - bdelloide Rotatorien, darwinulide Ostracoden und einige Linien der Oribatiden - widersprechen Theorien über die Funktionalität und Aufrechterhaltung von Sex, indem sie über lange Zeit ohne Mixis und Rekombination existieren.

Oribatiden sind ideale Modellorganismen für die Erforschung molekularer und ökologischer Auswirkungen von Sex und Parthenogenese. Von den ca. 10.000 hauptsächlich boden-lebenden Arten vermehren sich etwa 9% parthenogenetisch; ein Prozentsatz, der ungewöhnlich hoch ist. In Waldböden sind außerdem bis zu 80% der Individuen parthenogenetisch. Dass sexuelle und parthenogenetische Oribatiden-Arten im selben Habitat koexistieren, vereinfacht es, den Einfluss ökologischer Faktoren (z.B. Ressourcen-Verfügbarkeit) auf die Verbreitung der beiden Reproduktionsweisen zu untersuchen.

Die erste Studie der vorliegenden Arbeit untersuchte das bisher kontrovers diskutierte phylogenetische Verhältnis von Astigmata und Oribatida. Wir untersuchten die Hypothese, dass Astigmata aus den Desmonomata hervorgegangen sind anhand von DNA-Sequenzen für die Gene 18S und *ef1α*. Phylogenetische Analysen mit drei verschiedenen Methoden unterstützen den Ursprung der Astigmata innerhalb der Oribatida nicht, sondern gruppieren Astigmata außerhalb der Oribatida als Schwestergruppe der Endeostigmata.

Die zweite Studie untersuchte die phylogenetischen Verwandtschaftsverhältnisse von sexuellen und parthenogenetischen Vertretern der Desmonomata. Dafür wurden drei Gene (*ef1α*, *hsp82* und 18S) sequenziert und der kombinierte Datensatz phylogenetisch analysiert. Die Analyse der Merkmalsentwicklung und -verteilung von Sex und Parthenogenese ergab, dass Crotoniidae Sexualität von dem parthenogenetischen Cluster der Camisiidae zurück evolviert haben. Diese Umkehrung der Reproduktionsform ist einmalig im Tierreich und widerspricht „Dollo`s law", das besagt, dass komplexe Merkmalszustände, nachdem sie einmal verloren gegangen sind, nicht mehr zurück erlangt werden können.

In der dritten und vierten Studie wurden die mitochondriellen Genome der sexuellen Art *Steganacarus magnus* und der parthenogenetischen Art *Platynothrus peltifer* sequenziert. Dem Genom von *S. magnus* fehlen 16 tRNAs und die Gen-Reihenfolge unterscheidet sich von dem hypothetischen Grundplan. Die Sekundärstruktur der vorhandenen tRNAs unterscheidet sich stark von der typischen Kleeblatt-Struktur, was auch für *P. peltifer* gezeigt wurde. Die Gen-Reihenfolge bei *P. peltifer* unterscheidet sich ebenfalls vom Grundplan und von der bei *S. magnus*, wobei hauptsächlich die Gene *nad1* und *nad2* betroffen sind. Im Gegensatz zu unserer Hypothese ist keine Duplikation des Gens *cox1* in *P. peltifer* vorhanden, stattdessen verursachen einzelne Nukleotid-Polymorphismen ein geringes Maß an intraindividueller Variabilität, die die hohe intraspezifische Diversität aus anderen Studien allerdings nicht erklären kann.

Die fünfte Studie untersuchte die Wiederbesiedlung von Boden und Streu durch sexuelle und parthenogenetische Oribatiden und ihre Reaktion auf Ressourcen-Limitierung in Mikrokosmen über 10 Monate. Wie erwartet waren parthenogenetische Taxa von einer Ressourcen-Limitierung stärker betroffen als sexuelle. Dennoch lassen die Ergebnisse vermuten, dass die Wiederbesiedlung freier Habitate stärker von Faktoren wie Körpergröße und Generationszeit abhängt als von der Reproduktionsform. Generell hatten Weibchen sexueller Arten mehr Eier als parthenogenetische Weibchen. Die Ergebnisse deuten allerdings darauf hin, dass parthenogenetische Arten ihre Investition in Reproduktion anpassen können, z.B. durch die Produktion weniger Eier bei abnehmender Ressourcen-Verfügbarkeit.

Die sechste Studie untersuchte die Effekte der Verfügbarkeit von Ressourcen erhöhter Qualität auf die Abundanz sexueller und parthenogenetischer Taxa bei unterschiedlichen Temperaturen (10, 15, 20°C). Entgegen unserer Erwartungen sanken die Abundanzen aller Arten in allen Versuchsansätzen bei allen Temperaturen sowohl bei sexuellen als auch bei parthenogenetischen Taxa. Diese Ergebnisse deuten darauf hin, dass sich Oribatiden im Freiland von anderen Ressourcen als leicht verfügbarem Kohlenstoff ernähren, z.B. von Mykorrhiza-Pilzen.

Die Ergebnisse der vorliegenden Arbeit erweitern und festigen die Stellung von Oribatiden als Modellorganismen in der Evolutionsbiologie. Die Umkehrung der Reproduktionsform bei Crotoniidae ist einmalig für „ancient asexuals" und erlaubt die Untersuchung und den direkten Vergleich der genetischen Konsequenzen von Sex und Parthenogenese. Das Vorkommen spanandrischer Männchen in parthenogenetischen Taxa ermöglicht die Untersuchung von Genen, deren Genprodukte an Meiose, Spermatophoren-Produktion und Geschlechtsbestimmung beteiligt sind. Themen für zukünftige Studien beinhalten die Untersuchung von Populationsstrukturen anhand von Mikrosatelliten, die Suche nach transposablen Elementen und die Verifizierung, welche Rolle Qualität und Quantität von Ressourcen für das starke Auftreten von Parthenogenese bei Bodenmikroarthropoden spielen.

Summary

The present thesis investigates molecular and ecological effects of different reproductive modes on oribatid mites as model organisms (Acari, Oribatida). The investigation of the evolution and maintenance of sex vs. parthenogenesis is one of the most exciting fields in evolutionary biology. It is still puzzling why sexual reproduction dominates in the animal kingdom beside its high costs: females need to invest time and resources in searching mating partners and copulation, they dilute their genomes and halve the amount of reproductive progeny by producing males (cost of meiosis + cost of males = twofold costs of sex). Although these costs seem overwhelming, only 1% of all Metazoa reproduce by parthenogenesis. Three so-called "ancient asexual scandals", namely bdelloid rotifers, darwinulid ostracods and several lineages of oribatid mites, challenge theories on the maintenance of sex as they persist over evolutionary timescales.

Oribatid mites are ideal model organisms to investigate molecular and ecological consequences of sex and parthenogenesis. Among the roughly 10,000 mainly soil-dwelling oribatid mite species about 9% are parthenogenetic, a percentage which is unusually high; further, up to 80% of oribatid mite individuals in forest soil reproduce by parthenogenesis. Sexual and parthenogenetic oribatid mites coexist in the same habitat allowing to investigate the role of ecological factors, e.g., the availability of resources.

The first study of this thesis aimed at clarifying the relationship of Astigmata and Oribatida which is controversially discussed. We tested the hypothesis that Astigmata evolved from within Desmonomata which renders Oribatida paraphyletic by sequencing two genes (18S, *ef1α*) for a representative sample of oribatid and astigmatid mite species. Phylogenetic analyses using three different analytical approaches did not support the hypothesis on the origin of Astigmata within Oribatida. Astigmata always clustered outside Oribatida with or close to Endeostigmata, usually in a sister-group relationship.

The second study of this thesis investigated phylogenetic positions of sexually vs. parthenogenetically reproducing Desmonomata. Three genes *(ef1α, hsp82* and 18S) were sequenced and phylogenetic analyses were performed on a concatenated dataset. Character history and ancestral state evolution analyses revealed that Crotoniidae re-evolved sexuality from the parthenogenetic cluster of Camisiidae. This reversal in reproductive mode is unique in the animal kingdom and violates Dollo's law that complex ancestral states can never be reacquired.

In the third and fourth study of this thesis the complete mitochondrial genomes of the sexual species *Steganacarus magnus* and the parthenogenetic species *Platynothrus peltifer* were sequenced. The genome of *S. magnus* lacks 16 tRNAs and the gene order differs from the hypothetical ancestral chelicerate arrangement as conserved in *Limulus polyphemus*. The structure of tRNAs differs remarkably from the typical cloverleaf structure which also was the case in *P. peltifer*. The mitochondrial gene arrangement of *P. peltifer* differs from the hypothetical ground plan and to the arrangement in *S. magnus* mainly in the positions of *nad1* and *nad2*. In contrast to our hypothesis, no gene duplication of *cox1* was found but single nucleotide polymorphisms causes a low level of intraindividual heteroplasmy that cannot explain the high intraspecific diversity found in other studies.

The fifth study of this thesis investigated the re-colonization of soil and litter by sexual and parthenogenetic oribatid mites and their response to resource depletion in laboratory microcosms over ten months. In agreement with our expectations, parthenogenetic taxa suffered more from resource shortage than sexual species. However, the results suggest that the recovery from disturbances and the recolonization of soil and litter is more influenced by body size and generation time than by reproductive mode. In general, egg numbers were higher in sexual than in parthenogenetic species. The results further suggest that parthenogenetic taxa adjusted the investment in reproduction by reducing egg production with declining availability of resources.

The sixth experiment of this thesis explored effects of increased availability of high quality resources on the abundance of sexual and parthenogenetic oribatid mite species at different temperatures (10, 15 and 20°C). In contrast to our expectations, species abundances declined in each of the treatments at each temperature and sexuals and parthenogens were equally affected. The results indicate that oribatid mites in the field feed on other resources than easily available carbon, potentially mycorrhizal fungi.

Overall, the results of the present thesis expand and strengthen the use of oribatid mites as model organisms in evolutionary biology. The reversal of the reproductive mode in Crotoniidae is unique for "ancient asexuals" and allows the investigation of genetical consequences of sex and parthenogenesis. The presence of spanandric males in parthenogenetic taxa allows investigating factors responsible for the persistence of genes involved in meiosis, sperm production and sex determination. Further topics for future studies comprise the investigation of population structures using microsatellites as neutral markers, the existence transposable elements, and the role of resource quality and quantity for the high frequency of parthenogenesis in soil microarthopods.

Sterkste dier ter wereld is blinde mijt

"…Reden voor die stelling is dat de kleine geleedpotigen -minder dan een millimeter groot- in staat zijn ongeveer 1.200 keer hun eigen gewicht te heffen. De mijt is daarmee vijf keer sterker dan in theorie van een dergelijk organisme te verwachten is, zo schrijven de wetenschappers.

De in de tropen vaak voorkomende hoornmijt (*Archegozetes longisetosus*) weegt een tienduizendste van een gram en leeft op de bodem van rottende organismen. Ze heeft sterke klauwen van ongeveer een twintigste van een millimeter groot…"

(De Tijd)

Mites are the primary source of poison arrow frog toxins

"…Mites -- not ants as long believed -- appear to be the primary source of toxins used by poison arrow frogs to defend against predators, reports new research published in the early online edition of *Proceedings of the National Academy of Sciences* (PNAS).

Screening ants, mites, and other arthropods collected from the native habitat of the Central American poison frog *Oophaga pumilio* for various alkaloids, researchers led by Ralph A. Saporito, a biologist at Florida International University, found more than 80 alkaloids present in free-living, soil-dwelling oribatid mites. Of these toxic compounds, 42 were present in the skin glands of *O. pumilio*, suggesting that mites are the dominant source for frog poison…"

(mongabay.com)

Клещи вновь открыли радость секса

"… Исследования показали, что представители группы Crotoniidae, которые размножаются обычным половым способом, произошли от представителей группы Camisiidae, у которых оно полностью отсутствует. Это первый пример возвращения в процессе эволюции такого сложного механизма, как половое размножение.

Необходимость полового размножения у Crotoniidae ученые объясняют тем, что эти клещи обитают на деревьях, где условия более суровые, чем в почве, где живут Camisiidae. Половое размножение обеспечивает вариабельность особей, что служит лучшему приспособлению в условиях постоянных изменений. В почве, где условия более стабильные, партеногенез обеспечивает высокую скорость размножения вида.…"

(http://www.polit.ru/science/2007/04/17/mites.html)

CHAPTER 1

General Introduction

Sex is widespread in the animal kingdom. But although the majority of Metazoa reproduce by sexuality (Bell 1982), this reproductive mode is characterized by a plethora of peculiarities. As numerous as the peculiarities of sex are the consequences for the organism. Experts just agree in one point: sex is essential for long-term survival and non-sexual species (parthenogens and asexuals) are doomed to extinction. However, even more interesting than the functioning of sex is the question if all non-sexuals indeed are doomed to extinction. The surprising answer is no, there are at least three non-related animal groups that challenge almost all theories on the evolutionary role of sex. These groups, namely bdelloid rotifers, darwinulid ostracods and some lineages of oribatid mites, have been termed "ancient asexual scandals" since they persist since millions of years while being parthenogenetic (Maynard Smith 1978, Judson and Normark 1996). Oribatid mites probably form the oldest, the most numerous, most beautiful and most fascinating of these groups. Therefore, combining the investigation of the maintenance of parthenogenesis over evolutionary timescales with oribatid mites as model organisms is one of the most exciting fields in biology and turns the question from "why are some parthenogens able to establish themselves?" to "why do parthenogens not regularly replace sexuals?" as previously asked by John Maynard Smith (1978).

1.1 Sex and Parthenogenesis

Sexual reproduction (or amphimixis) is characterized by the reduction of the genetic material by meiosis (segregation) and the following restoration of the original chromosome number by fusion of gametes from two different parents at fertilization (syngamy; Hughes 1989). Thereby, intra- and inter-chromosomal recombination during meiosis causes genetic diversity in the offspring. Since more than 95% of all Metazoa reproduce by sexuality it is the predominant reproductive mode in the animal kingdom (Bell 1982).

In contrast, defining the terms "parthenogenesis" and "asexuality" is more controversial. While several authors use parthenogenesis and asexuality synonymously (Gerritsen 1980, Van Doninck et al. 2002, Barraclough et al. 2003, Normark et al. 2003, see review of de Meeus et al. 2007), others refer to asexuality as "clonal reproduction" (de Meeus et al. 2007) which includes some forms of parthenogenesis, i.e. apomictic thelytoky (see below). The latter authors argue for avoiding the term "parthenogenesis" since it is collective for different reproductive modes, figuratively a "paraphyletic term".

Generally, parthenogenesis is defined as the development of offspring from unfertilized eggs (Hughes 1989) and was first described in 1745 (Bonnet 1745). In some cases sperm presence is still necessary to trigger egg development (e.g., in gynogenesis or hybridogenesis) whereas "true" parthenogenesis does not require males at all (Hughes 1989). In contrast to asexuality (defined as reproduction from somatic cells), the production of gametes during parthenogenesis depends on the germline; while syngamy is always absent, recombination and segragation can be involved depending on the cytogenetic mechanisms (Hughes 1989; Fig. 1.1).

Parthenogenesis is usually further divided in arrhenotoky, pseudo-arrhenotoky, deuterotoky and thelytoky (Bell 1982, Suomalainen et al. 1987, Hughes 1989; Fig. 1.1). Following the definition above, only thelytoky can be named "true" parthenogenesis since all others include progeny from fertilized eggs. While arrhenotokous species produce diploid females from fertilized and haploid males from unfertilized eggs (e.g., widespread in Hymenoptera and cyclical parthenogens), in pseudo-arrhenotokous species both males and females develop from fertilized eggs but males secondarily become haploid.

```
                                    Reproduction
                  somatic cells    |              germline
          ┌───────────────────────────────┬───────────────────────────────┐
          |                                |                                |
      Asexuality                    Parthenogenesis                     Sexuality
                              ┌──────────────┴──────────────┐
                              |                         „true" parthenogenesis
                         Arrhenotoky                    Thelytoky
                      Pseudo-arrhenotoky          meiosis  |  mitosis
                         Deuterotoky         ┌─────────────┴─────────────┐
                                             |                           |
                                         Automixis                   Apomixis
                              pre- or post-meiotic chromosome duplication
                              suppression of first or second meiotic division
                                 central or terminal fusion of gametes
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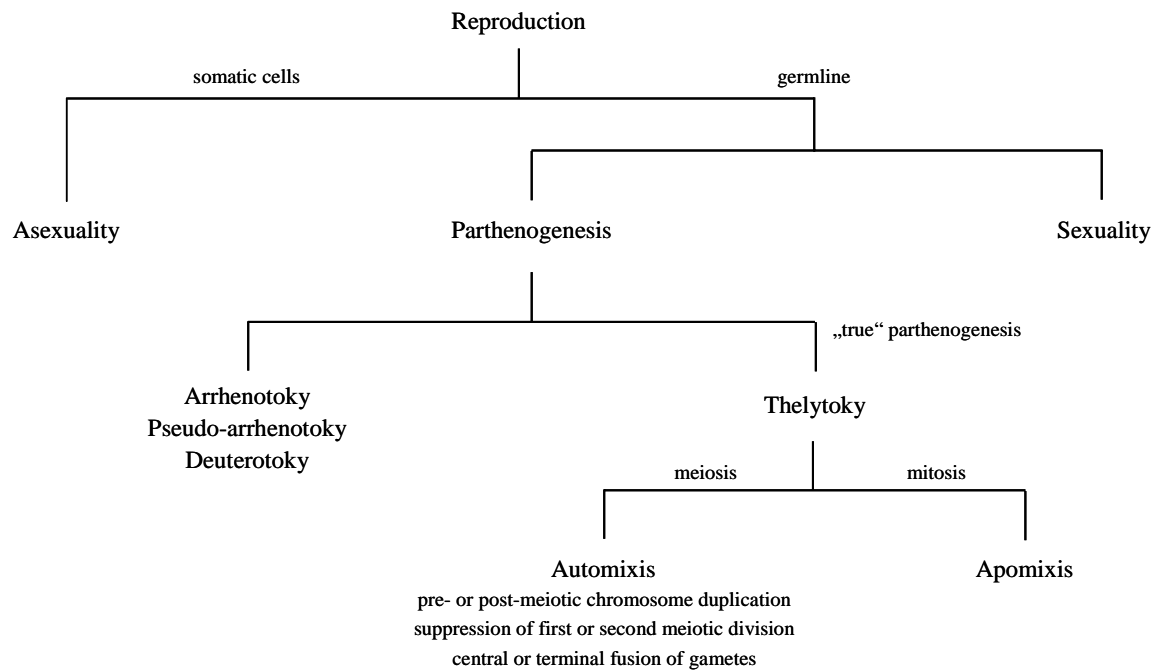
Figure 1.1 Classification of reproductive modes.

In deuterotokous species both males and females develop from reduced eggs and diploidy is restored postmeiotically by the fusion of the first two cleavage nuclei. Therefore, arrhenotoky, pseudo-arrenotoky and deuterotoky are a kind of mixed reproductive mode since two sexes are involved. In contrast, thelytokous species comprise exclusively of females which develop from unfertilized eggs (e.g., common in bdelloid rotifers and oribatid mites). Thelytoky can further be divided in apomixis and automixis. While apomictic females produce their gametes via mitosis without a reductional division and genetic mixing (which usually ensures the identical replication of the maternal genome), automixis includes a meiotic process (Figs. 1.1, 1.2). The original number of chromosomes can be restored by pre- or post-meiotic chromosome duplication, by intrameiotic supression of either the first or second meiotic division or by central or terminal fusion of the meiotic products, each having different consequences for the genetic variability of the offspring (Figs. 1.1, 1.2). While central fusion restores the maternal genome except at recombination sites and therefore largely keeps the maternal heterozygosity, terminal fusion results in a homozygous genome different from the mother (Suomalainen et al. 1987, Hughes 1989).
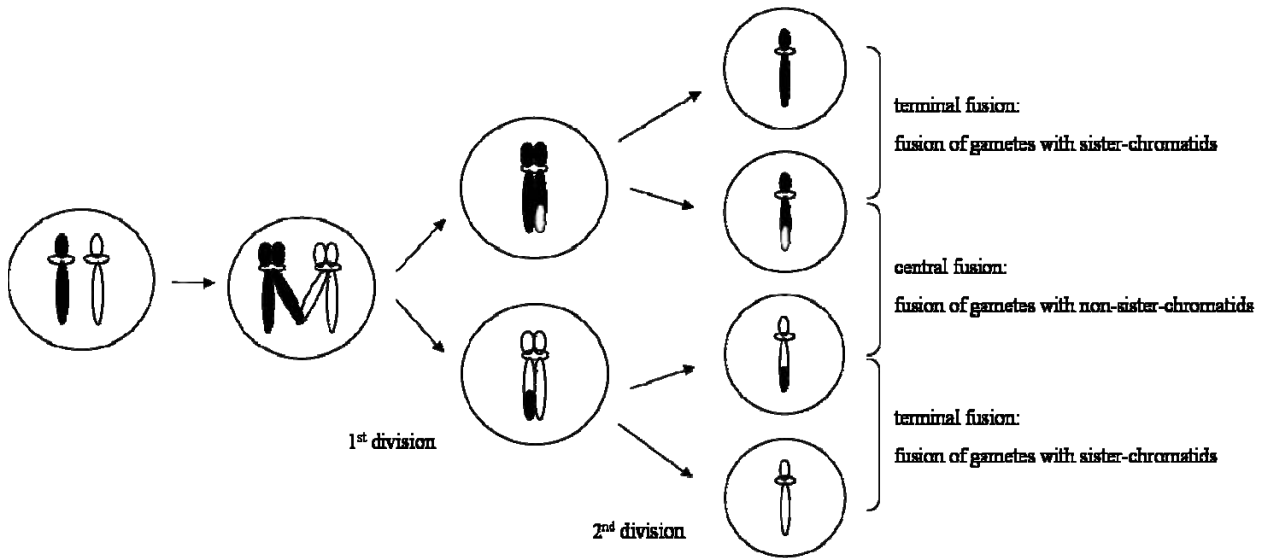
Figure 1.2 Automictic thelytoky with central or terminal fusion of the meiotic products and genetic recombination before the first meiotic division (modified from Hughes 1989).

1.1.1 Theories on the Evolutionary Consequences of Sex and Parthenogenesis

Although sexual species suffer from the so-called two-fold costs of sex by the production of males (and therefore the reduction of the number of reproductive offspring) and the dilution of their genome (Williams 1975, Maynard Smith 1978), only 1% of all Metazoa reproduce parthenogenetically showing an unbalanced taxonomic distribution (Bell 1982). The ubiquitous existence and maintenance of sex is an enigma in evolutionary biology and one of the most exciting unanswered questions. In theory, sexually reproducing species should be outcompeted by parthenogens in most environments since the latter have a faster population growth rate as compared to sexual populations due to the avoidance of searching mating partners and copulation; further, parthenogens produce all-female progeny resulting in twice the number of daughters that can reproduce in the next generation. But although parthenogens should regularly outcompete their sexual relatives, sexual reproduction still dominates.

A bewildering array of theories have been proposed to explain why sex prevails in most Metazoa (e.g., Muller 1964, Williams 1975, Maynard Smith 1978, Bell 1982, Stearns 1987, Birky 1996, Barton and Charlesworth 1998, Vrijenhoek 1998, West et al. 1999, Birky et al. 2005). Roughly, these theories can be categorized by the long- or short-term benefit for the organism. Theories on the short-term advantages of sex support the accelerated rate of adaptive evolution to changing environments or host-genotypes by the production of

genetically diverse offspring in one generation (e.g., Best-Man-hypothesis: Williams 1975, Tangled-Bank-Hypothesis: Ghiselin 1974, Red Queen hypothesis: Van Valen 1973, Hamilton 1980, biotic uncertainty hypothesis: Glesener and Tilman 1978). On the other hand, theories on the long-term advantages of sex focus on the prevention of the accumulation of deleterious mutations by gene recombination (e.g., Mullers ratchet: Muller 1964, Mutational Load Theory: Kondrashov 1988). However, none of the theories can satisfactorily solve the "queen of problems in evolutionary biology" (Bell 1982) and even pluralistic approaches as proposed by West et al. (1999) are not able to solve the paradox of sex (Schön et al. 2008).

In general, all theories uniformly predict the extinction of parthenogenetic species in the long-term. While persisting over evolutionary timescales so-called "ancient asexuals" such as bdelloid rotifers (360 species; Mark Welch and Meselson 2000), darwinulid ostracods (26 species; Martens et al. 2003) and some groups within oribatid mites (> 400 species; Maraun et al. 2003, Heethoff et al. 2007a) challenge this view and are considered "evolutionary scandals" (Maynard Smith 1978, Judson and Normark 1996).

1.2 Oribatida

1.2.1 Systematics

Mites (Acari) are among the oldest, most abundant and most diverse arthropod groups (Walter and Proctor 1999). More than 42,000 species are clustered in three major clades: Parasitiformes, Opilioacaridae and Acariformes (Krantz 1978, Evans 1992, Walter and Proctor 1999). With about 30,000 species Acariformes are the largest subgroup. Members of the Acariformes have been grouped into Prostigmata, Astigmata and Oribatida. A fourth set of species is grouped as the paraphyletic Endeostigmata (OConnor 1984, Walter 2001). A number of phylogenetic relationships of acariform mite groups have been proposed (Berlese 1897, Krantz 1960, Zachvatkin 1953, OConnor 1984, Grandjean 1937, 1954) - especially concerning the position of Astigmata - and there is an ongoing controversy over higher classification of Acariformes (Norton 1998, Domes et al. 2007c).

Oribatid mites (Oribatida, Acari) are the largest subgroup of Acariformes. Traditionally, based on morphological data they consist of six major groups (Fig. 1.2): (1) the basal Palaeosomata, (2) the specious Enarthronota including the Brachychthonoidea and Hypochthonioidea, (3) the small group Parhyposomata, (4) the paraphyletic "Mixonomata" including for instance the box mites, (5) the species-rich group of "Desmonomata" that includes mainly parthenogenetic taxa such as Nothridae, Camisiidae, Trhypochthoniidae and

Malaconothridae and (6) the higher and very species rich Circumdehiscentiae (=Brachypylina) (Grandjean 1953, 1965, 1969). Circumdehiscentiae are further separated into Opsiopheredermata, Eupheredermata, dorsodeficient Apheredermata, pycnonotic Apheredermata and Poronota (Travé et al. 1996). This classification has been modified by several authors according to morphological and molecular data (Haumann 1991, Weigmann 1996, Maraun et al. 2004, Domes et al. 2007c, R.A. Norton, unpublished data, P. Pachl, unpublished data); while some genera were moved in different groups (e.g., Lohmanniidae seem to cluster inside Enarthronota and not in "Mixonomata") the main classification remained unchanged.
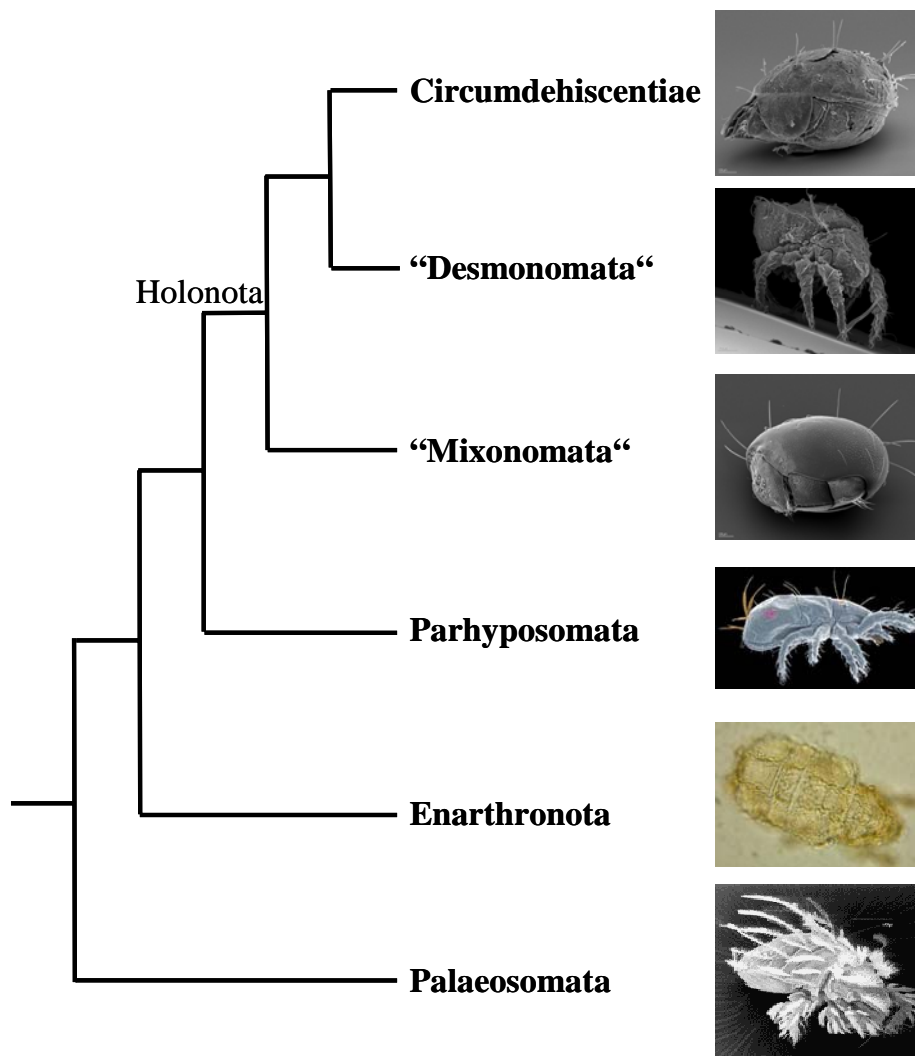
Figure 1.2 Oribatid mite phylogeny based on morphological data (after Grandjean 1953, 1965, 1969, Haumann 1991, Weigmann 1996).

1.2.2 Reproduction and Development

About 9% of all known oribatid mite species reproduce by parthenogenesis which is one to two orders of magnitude higher than in other animal groups (Norton and Palmer 1991). Offspring presumably are produced by thelytokous automixis with terminal fusion of the meiotic products (Taberly 1987, Wrensch et al. 1994, Heethoff et al. 2006). Although this mechanism should lead to complete homozygosity (except at recombination sites), fixed heterozygosity was shown for nine parthenogenetic oribatid mite species using isozyme techniques which may indicate the occurrence of central fusion (Palmer and Norton 1992). However, heterozygosity and the so-called Meselson effect (stating that an allele within a species is more similar to an allele of another species than to its homologous allele; see Butlin 2002 for description) could not be confirmed using molecular data of the elongation factor 1 alpha (*ef1α*) and the heat-shock protein 82 (*hsp82*) (Schaefer et al. 2006). This discrepancy might be due to holokinetic chromosomes in combination with inverted meiosis, in which the reductional and equational divisions are inverted as compared to normal meiosis leading to the reversal of effects of terminal and central fusion (Wrensch et al. 1994, Heethoff et al. 2006).

Unfortunately, the mechanism of sex determination is unknown in oribatid mites; they lack sex chromosomes despite they are diploids (2n=18) (Taberly 1987, Wrensch et al. 1994, Heethoff et al. 2006). Sexual oribatid mite species have a balanced sex ratio of 1:1 while most parthenogenetic species consist of 98-100% females (Norton and Palmer 1991, Palmer and Norton 1991, Cianciolo and Norton 2006, Domes et al. 2007a). As is the case in many parthenogenetic species, in some parthenogens non-functional spanandric males occur; their spermatophores have been proven to be sterile and ignored by females (Grandjean 1941, Taberly 1988, Palmer and Norton 1992). Why they persist over evolutionary timescales is unknown so far.

In sexual species insemination occurs indirectly via species-specific spermatophores. All oribatids have six postembryonic instars: inside the egg the prelarva develops into the larva which hatches from the egg and develops from a pro-, deuto- and trionymphe into the adult. Most oribatid mites are slowly reproducing (Travé et al. 1996), but the generation time varies from two to three weeks in Oppiidae (under lab conditions with high temperature; reviewed in Norton and Palmer 1991) up to 950 days in *Steganacarus magnus* (Travé et al. 1996) and up to five years in *Ameronothrus lineatus* (Søvik and Leinaas 2003). Generally, eggs develop slowly in Enarthronota (Forsslund 1942, 1957), Phthiracaridae and Desmonomata (Luxton

1981, Travé et al. 1996), whereas in Oppiidae and Suctobelbidae they develop quickly (Luxton 1981, Travé et al. 1996). The time of reproduction also differs between species; some taxa produce eggs during the whole year, in others egg production depends on season. Further, the numbers as well as the size of eggs vary among taxa (Travé et al. 1996, Domes et al. 2007a).

1.2.3 Density and Diversity

About 10,000 oribatid mite species are described so far (Subias 2004) but a total number of 100,000 may exist (Schatz 2002). Oribatid mites are predominantly soil-dwelling detritivorous or fungivorous microarthropods playing an important role in decomposition processes in particular forest ecosystems (Maraun and Scheu 2000, Schneider et al. 2004a, b). They reach densities up to 400,000 ind/m² in acidic boreal forests whereas densities in mountain rainforests or calcareous forests are rather low (e.g., tropical montane rainforest about 15,000 ind/m²; Maraun et al. 2008). Densities on the bark of trees are also low as compared to soil (Erdmann et al. 2006).

Oribatid mite densities in soil are relatively constant during seasons reflecting the stability of the soil habitat. Nevertheless, species co-exist in small soil patches where mircohabitat conditions can vary strongly even at small distances (Mitchell 1979, Maraun et al. 1999). Densities are lowest at sites with high disturbances, caused by e.g. earthworms or other macrofauna (Maraun et al. 2001) and at sites with low quality resources (Maraun et al. 2008). Top-down control of oribatid mite densities through predators is of minor importance since adult individuals are usually strongly seclerotized (Peschel et al. 2006, but see Schneider and Maraun 2005).

Their high diversity in soil is probably due to trophic niche differentation (Schneider et al. 2004a, b) although laboratory feeding choice experiments suggest that they are generalistic feeders behaving as "choosy generalists" (Maraun et al. 2003, Schneider et al. 2004a, b, Schneider and Maraun 2005). In general, oribatid mite species feed on numerous resources such as living and dead plant material, organic matter, fungi and lichens and leaving or dead animals such as nematodes.

1.3 Molecular Markers

The field of molecular biology developed rapidly during the last century; techniques improved, the analyses became cheaper and their speed increased dramatically. Today molecular markers are routinely used in numerous fields such as molecular taxonomy and barcoding, phylogeny, population genetics, medicine and forensics. While researchers can choose between numerous methods for investigating a specific problem (e.g., restriction fragment length polymorphisms (RFLP), denaturing gradient gel electrophoresis (DGGE), hybridization, southern plot, investigation of gene expression patterns), the most accurate results and the ones most straightforward to explain are obtained from the comparison of DNA sequences.

DNA sequences of specific genes are used to answer evolutionary and taxonomic questions; depending on the question differently conserved genes, either nuclear or mitochondrial (or chloroplasts in plants), are used. The nuclear genome of animals includes coding and non-coding DNA regions and varies between $9.8x10^7$ basepairs (bp) in *Caenorhabditis elegans* (Riddle et al. 1997) and $1.3x10^{11}$ bp in *Protopterus aethiopicus* (Animal Genome Size Database, http://www.genomesize.com/statistics.php?stats=fish). It is arranged in a species-specific number of linear chromosomes and is more than the simple sum of the organism's genes due to interactions of genes (epistasis), the involvement of genes in mutliple pathways (pleiotropy) and control mechanisms. While some genes are present as a single copy (e.g., *hsp82*, *ef1α*), in others multiple copies of the same sequence exist (e.g., ribosomal DNA genes such as 5,8S, 18S or 28S); of course, in diploid organisms at least two alleles of each gene are present.

DNA barcoding and molecular taxonomy are extensively discussed since their first announcement by Scherer and Sontag (1986) and Hebert et al. (2003a, b). While some authors distinguish between these two molecular approaches (Vogler and Monaghan 2007), others describe the first as an instance of the latter (Will and Rubinoff 2004). However, the basic principle of the molecular identification and description of a species is the uniqueness of the specific gene sequence obtained from a certain sample (Hebert et al. 2003a). The most frequently used molecular marker for barcoding in animals is the 5'-end partial sequence of the mitochondrial cytochrome c oxidase I gene (*cox1*) (Hajibabaei et al. 2007). While numerous studies report sufficient power of *cox1* for using as a species marker (Hebert et al. 2003b, Ball and Armstrong 2006, Hajibabaei et al. 2007), others stress the inadequateness of *cox1* in delineating species relationships caused by maternal inheritance of mitochondria,

different mutation rates across taxa and heteroplasmy (Will and Rubinoff 2004). As alternatives to *cox1* the mitochondrial genes of the large ribosomal DNA (16S rDNA) und cytochrome b (*cob*) (Chu et al. 2006, Lemer et al. 2007) as well as the nuclear internal transcribed spacer regions (ITS1 and ITS2) and the 18S ribosomal DNA (18S rDNA) are commonly used as DNA barcoding markers (Noge et al. 2005, Ben-David et al. 2007, Hajibabaei et al. 2007).

Nuclear non-coding DNA regions further contain neutrally evolving markers since there is no selective pressure on their function as it is the case for coding genes. The most famous non-coding markers are microsatellites (also known as variable number of tandem repeats (VNTRs), simple sequence repeats (SSR) or short tandem repeats (STR)); they consist of short DNA sequences (10-1000 bp) with a locus-specific number of tandemly repeated DNA motives of 2-6 bp (e.g., $(CA)_{13}$ or $(TAT)_7$). Units of these repetitive motives can easily be added or removed during DNA replication which causes high variability of the repetitions at a given locus. Since microsatellites are highly polymorphic, neutrally evolving, co-dominant markers they can be used in forensics, the diagnosis of diseases, conservation biology and population genetics. Especially for the last, analyses of microsatellites can help to understand the impact of forces such as mutations, recombination, migration, bottlenecks and genetic drift (Goldstein and Schlötterer 1999).

Molecular markers can record the pattern of evolutionary history and therefore reveal the evolutionary past (Hartl and Clark 1997). The first molecular research on oribatid mites focused on allozymes for elucidating the genetic diversity of populations of parthenogenetic species (Palmer and Norton 1992) and to differentiate between steganacarid mites from the Canary-Islands (Avanzati et al. 1994). While still focusing on Canary-Island specimens, Salomone et al. (1996, 2002) later used the mitochondrial *coxI* gene. After studies focusing on the genetic variability within and among parthenogenetic and sexual taxa using the ribosomal ITS1 region (Heethoff et al. 2000) as well as a partial sequence of the ribosomal 28S DNA (D3) (Maraun et al. 2003), the first molecular phylogeny of oribatid mites was published in 2004 including 64 sequences of the D3 region (Maraun et al. 2004). Molecular phylogeny (not always explicitly named as that) was than expanded to the elongation factor 1 alpha (*ef1α*), the heat-shock protein 82 (*hsp82*) and the ribosomal 18S region (18S) (Schaefer et al. 2006, Domes et al. 2007b, c, Laumann et al. 2007, M. Maraun et al., submitted, R.A. Norton et al., in preparation, P. Pachl, unpublished data).

1.3.1 Mitochondria

Mitochondria, commonly kown as the cell "powerhouses", are maternally inherited cell organelles that contain a circular double-stranded genome of about 14-19 kb in length which is an indication of their descendence from free-living bacteria. In general, each mitochondrion contains 2-10 copies of the genome and several hundred mitochondria may be present in a single cell (Scheffler 2001). The mitochondrial DNA (mtDNA) typically codes for 13 proteins, 22 transport RNAs (tRNA) and two ribosomal RNAs (rRNA). Mitochondrial (mt) genes are of bacterial origin but why they persisted over time is still puzzling since they can easily be transfered to the nucleus; however, all species with mitochondria have more or less exactly the same gene content (Boore 1999, Scheffler 2001, Lane 2005).

The two strands of mtDNA are distinguished by their unbalanced guanine content: the heavy (H or +) strand is guanine rich while the light (L or -) strand is guanine poor (Anderson et al. 1981) and the mitochondrial genes are asymmetrically distributed between these two strands (Fonseca et al. 2006; Fig. 1.3). The replication process of mtDNA takes about 2 h (Clayton 1982) and starts from the origin of the H-strand. After two-thirds of the H-strand replication of the L-strand starts in the opposite direction when the origin of the L-strand is reached (Fonseca et al. 2006). Therefore, the parental H-strand remains single-stranded until the new L-strand is synthesized and during that time the H-strand may be subject to mutational damage (Fonseca et al. 2006).

MtDNA differs from nuclear DNA by an almost twenty times faster mutation rate (of course varying depending on the respective gene). Mutation rates are accelerated by free radicals generated during the respiratory chain located in the mitochondrial membrane, but rates stay relatively constant over time. This enables the clock-like analysis of events over several thousands of years. Further, due to the "asexual" transfer of mtDNA from mother to daughter mitochondrial genes allow the tracking of the fate of individuals and their descendents; while nuclear genes are shuffeld around in every generation by recombination, mtDNA stays the same over time (except for mutational changes) since only the maternal mitochondria are propagated (Scheffler 2001, Lane 2005).

Although rearrangements of mitochondrial genes are assumed to be rare and therefore be useful phylogenetic markers (Boore 1999), the arrangement, especially of the small genes for tRNAs, varies remarkebly among arthropods (Black and Roehrdanz 1998). While the arrangement of the hypothetical ancestor of arthropods is present in the horseshoe crab *Limulus polyphemus* (Lavrov et al. 2000; Fig. 1.3) and in three soft and two hard ticks (Shao

et al. 2005), the arrangement varies in species such as *Varroa destructor* (Parasitiformes, Navajas et al. 2002), the genus *Leptotrombidium* (Acariformes; Shao et al. 2006), the sea spider *Nymphon gracile* (Pycnogonida, Podsiadlowski and Braband 2006) and in lice (Insecta, Covacin et al. 2006).
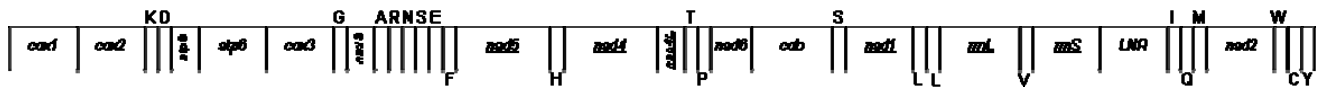
Figure 1.3 Arrangement of the mitochondrial genes of the horseshoe crab *Limulus polyphemus* (Lavrov et al. 2000). Underlined genes and genes written beneath the box are located on the opposite strand. Gene abbreviations: *nad1-6,4L*=NADH dehydrogenase subunit 1-6,4L; *cox1-3*=cytochrome oxidase subunit I-III; *cob*=cytochrome b; *atp6,8*=ATP synthase subunit 6,8; *rrnL*=large ribosomal subunit RNA; *rrnS*=small ribosomal subunit RNA; LNR=large non-coding control region. Genes for tRNAs are symbolised by an one-letter code of their amino acid: I=Isoleucine; Q=Glutamine; M=Methione; W=Tryptophan; C=Cysteine; Y=Tyrosine; K=Lysine; D=Aspartate; G=Glycine; A=Alanine; R=Arginine; N=Asparagine; S=Serine; E=Glutamate; F=Phenylalanine; H=Histidine; T=Threonine; P=Proline; L=Leucine; V=Valine.

Mitochondrial genes, especially those for *coxI* and *cob* are commonly used as barcoding markers and for biogeographical studies across a wide range of taxa (Evans et al. 2007, Hajibabaei et al. 2007). However, since mitochondrial genes are known to evolve more rapidly than nuclear genes they are more useful for resolving recent splits (Boore 1999). Whole genome approaches have been used to investigate species relationships (Smith et al. 1993, Boore et al. 1995) and this may provide a powerful tool to improve doubtful phylogenies. As indicated above, variations of gene arrangements between taxa provide unique apomorphic characters for delineating species relationships since gene rearrangements are unlikely to have evolved by convergent evolution (Boore 1999).

1.4. Ecological Factors: Resources

Population dynamics and community structures are driven by ecological factors. Traditionally, ecological factors are divided in abiotic and biotic factors with abiotic referring to the non-living world (e.g., weather, temperature) and biotic referring to interactions between individuals (e.g., predation, co-operation). Biotic and abiotic factors (either predictable or unpredictable) can both function as disturbances and therefore prevent exponential population groth (Pickett and White 1985).

An important ecological factor for the evolution and maintenance of sexuality presumably is the availability and quality of resources (Scheu and Drossel 2007). The term "resources" encompasses food sources and nutrients entering the food web as well as available habitats.

Belowground systems essentially rely on plant resources entering the system as litter materials. On a worldwide basis, nearly 95% of the annual net primary production enters the decomposer subsystem where it serves as food and habitat resource for the decomposer community (Fioretto et al. 2003).

Models explaining the maintenance of sexual reproduction such as the Tangled Bank (Ghiselin 1974; see 1.1) imply that different genotypes within or between populations are able to exploit different ranges of resources. Usually, sexual species are thought to exploit a wider range of resources since there genotypes are more diverse. Therefore, theory predicts the dominance of sexuality in unstable habitats since higher genetic diversity allows a faster reaction to changing environmental conditions (Williams 1975, Hamilton 1980). Consequently, parthenogenesis should be favoured in stable habitats (such as litter layer or deeper soil of forests) since there is no need for fast adaptation (Scheu and Drossel 2007). Changes in resource availability can therefore influence the predominance of sexual or parthenogenetic taxa (Korpelainen 1990); while parthenogenesis correlates with resource availability, sexuality is often associated with resource shortage, as shown in cyclical parthenogens such as aphids or cladocerans (Redfield 1999).

1.5 Objectives

The present work investigates evolutionary aspects of sex and parthenogenesis using oribatid mites as model organisms. First, phylogenetic studies based on molecular techniques were conducted to solve long-standing questions on relationships of different taxonomic groups (Chapter 2 and 3). A second approach extended the available data on genetic diversity to complete genome approaches and therefore provides a promising tool for resolving deep-splits in acarine mites in future projects: the complete mitochondrial genomes for the sexual mite *Steganacarus magnus* (Chapter 4) and the parthenogenetic mite *Platynothrus peltifer* (Chapter 5) were sequenced to investigate gene rearrangements, heteroplasmy and phylogenetic patterns. Both genomes are the first mitochondrial genomes available for the large mite subgroup of Sarcoptiformes. Third, for linking the mode of reproduction and fitness, i.e. reproductive success, to ecological factors such as resource availability and resource quality two microcosm experiments with varying amounts of resources were performed (Chapter 6 and 7).

Chapter 2 investigates the phylogenetic relationship of Astigmata and Oribatida, which is controversially discussed on the basis of morphological and molecular data. A fragment of the

ribosomal 18S region and the *ef1α* of selected species of Astigmata and Oribatida were sequenced and phylogenetic analyses using e.g. neighbour-joining and Bayesian algorithms were performed. We tested the hypothesis that Astigmata evolved from within Desmonomata which renders Oribatida paraphyletic.

In Chapter 3, based on molecular phylogenies evolutionary patterns of sexually vs. parthenogenetically reproducing Desmonomata are analysed, especially the re-evolution of sex in Crotoniidae. For this purpose, specimens of Desmonomata and related groups were sampled and the genes *ef1α*, *hsp82* and 18S were sequenced. Again, phylogenetic analyses using established methodologies were performed and the history and ancestral state of character evolution (sex vs. parthenogenesis) were investigated using parsimony and likelihood algorithms. We hypothesized that the sexually reproducing genus *Crotonia* clusters within parthenogenetic Desmonomata, especially to Camisiidae, and has therefore re-evolved sex.

Chapter 4 presents the first complete mitochondrial genome for an oribatid mite, the sexually reproducing *S. magnus*. The genome was obtained for individuals collected in the Kranichstein forest using long-PCR techniques including newly designed primers. Since gene arrangements in chelicerates, especially arachnids, are highly divers, we expected the mitochondrial genome of *S. magnus* to contribute to the general diversity. We further hypothesized that the gene arrangement differs from the hypothectical ground plan as conserved in *Limulus polyphemus* (Lavrov et al. 2000) due to the deep-split speciation event of the species.

Chapter 5 further investigates the high genetic variability of the *cox1* gene in *P. peltifer* which was previously described by Heethoff et al. (2007a). For this purpose, the complete mitochondrial genome was sequenced to check for the presence of gene duplictations. Further, we searched for heteroplasmy within clones of a single individual and investigated the local genetic variability of *cox1* at the Kranichstein forest; the sequences were compared to those published in Heethoff et al. (2007a). We expected that the genome of *P. peltifer* is heteroplasmic due to a gene duplication of *cox1*.

In Chapter 6 the re-colonization of soil and litter by sexual and parthenogenetic oribatid mites and their reaction to resource depletion are investigated. Soil and litter devoid of soil fauna was allowed to be re-colonized by oribatid mites, i.e. we followed the re-colonization of habitats where resources were in ample supply. Oribatid mites were determined to species level, sexed and in females the number of eggs was counted. Particular attention was paid to the development of sexual vs. parthenogenetic species. We hypothesized that parthenogenetic

species will suffer more from resource depletion than sexual taxa, and that they will colonize habitable space faster due to their faster mode of reproduction.

Continuing and extending the experiment presented in Chapter 6 and inspired by the work of Scheu and Drossel (2007), Chapter 7 explores the consequences of the availability of high quality resources on the abundances of sexual and parthenogenetic oribatid mite species in laboratory microcosms. The treatments comprised those with litter of reduced quality, untreated litter as well as glucose enriched litter material. Again, oribatid mites were determined to species level, adults were sexed and the eggs were counted in adult females. We expected the number of specimens to decline in the reduced litter quality treatment but to increase in the glucose enriched treatment which we expected to be more pronounced at higher temperatures. We further expected sexual species to be less affected than parthenogens in the reduced litter quality treatment due to their higher genetic diversity, but to be outcompeted by parthenogens in the glucose enriched treatment due to faster reproduction.

The Phylogenetic Relationship between Astigmata and Oribatida (Acari) as Indicated by Molecular Markers

Astigmata comprise a diverse group of acariform mite species with a remarkable range of life histories, most of which involve parasitic or commensal relationships with other organisms. Several authors have suggested that Astigmata evolved as a paedomorphic clade from within Oribatida, and both morphology and gland-chemistry strongly suggest that their sister-clade is within the oribatid subgroup Desmonomata. The biologies of these groups contrast greatly, since oribatid mites are mostly soil-living detritivores and fungivores, and have life cycles that are much longer than those in Astigmata. We tested the hypothesis that Astigmata evolved from within Desmonomata using two molecular markers, the ribosomal 18S region (18S) and the nuclear elongation factor 1 alpha (*ef1α*) gene. Representative acariform mites included 28 species of Oribatida, eight of Astigmata, two of Prostigmata and two of Endeostigmata; outgroups included members of Ricinulei and Myriapoda. To minimize the possibility of long-branch attraction artifacts, we limited highly variable sites by removing gaps (18S) and third codon positions (*ef1α*) from the sequences. Maximum parsimony, neighbour-joining and Bayesian algorithms formed trees that consistently placed Astigmata outside a monophyletic Oribatida, usually as sister-group of the endeostigmatid mite *Alicorhagia* sp. Analyses with and without outgroups resulted in similar topologies, showing no evidence for long-branch artifacts and leaving the conflict with morphological and biochemical data unexplained.

2.1 Introduction

Mites (Acari) are among the oldest, most abundant, and most diverse arthropod groups, including plant and animal parasites, free-living predators and soil-dwelling decomposers (Walter and Proctor 1999). The more than 42,000 species have been grouped in either two or three major clades depending on whether the small group Opilioacariformes is included within Parasitiformes or is considered its sister group (Krantz 1978, Evans 1992, Walter and Proctor 1999). With about 30,000 species, Acariformes comprise about 70% of the described mite species.

Most members of the diverse Acariformes have been included in one of three groups: Prostigmata, Astigmata and Oribatida (roughly 15,000, 5,000 and 10,000 species, respectively). Species in the first two show a great diversity of life histories, while oribatid mites are predominantly free-living detritivores and fungivores in soil. A small fourth set of species, grouped as the paraphyletic Endeostigmata, comprises a basal cluster of lineages, with some uncertainty over membership and relationships; some seem more closely related to Prostigmata, and others to Oribatida (OConnor 1984, Walter 2001). However, the principal source of controversy over higher classification of Acariformes relates to Astigmata.

As reviewed by Norton (1998), many acarologists have envisioned a close relationship between Astigmata and Oribatida, with the two forming Sarcoptiformes. There are essentially three different hypotheses of relationships: (1) Oribatida are derived from within Astigmata (Berlese 1897), (2) Oribatida and Astigmata are sister-groups (Krantz 1960) or (3) Astigmata are derived from within Oribatida (Zachvatkin 1953, OConnor 1984, Norton 1998). Others, particularly Grandjean (1937, 1954), rejected Sarcoptiformes and placed Oribatida close to Prostigmata, with Astigmata being an isolated group. Van der Hammen (1972) had speculated about a sister relationship of Astigmata and Tarsonemina, but Lindquist (1976) rejected this conclusively on morphological grounds. OConnor (1984) and Norton (1998) used morphological characters and cladistic principles to support the hypothesis that Astigmata evolved as a paedomorphic clade within the traditional Oribatida. More than two dozen progressive and regressive synapomorphies supported the hypothesis that they group within Desmonomata (Norton 1998). Biochemical studies of the opisthonotal glands, exocrine glands known only from Astigmata and non-basal clades of oribatid mites, added a second source of information that supported the origin of Astigmata within middle-derivative oribatid mites (Sakata and Norton 2001, Raspotnig 2006).

DNA studies on this question have been unconvincing so far. They were based on few taxa and single genes, and had varying results. Maraun et al. (2004) and Murrell et al. (2005), who studied two different rRNA regions (28S and 18S, respectively) both found the single astigmatid mite they studied to cluster within Oribatida, but with only poor to moderate statistical support and with positions external to Desmonomata. By contrast, maximum parsimony analysis of 18S sequences of 14 oribatid, three astigmatid, one endostigmatid and two prostigmatid mites showed 100% bootstrap support for a clade of Oribatida from which Astigmata were separated (Norton 2007).

The resolution of the origin of Astigmata will strongly influence the perception of the evolution of their unique and diverse life histories; this includes biological relationships with other organisms – as parasites and commensals – which rarely occur in oribatid mites, as well as attendant traits such as the use of high-quality resources and having much faster life cycles than do oribatid mites (OConnor 1994, Norton 1994, 2007). Since current DNA evidence conflicts with that from morphology and gland biochemistry, our objective is to test if Astigmata originated within Oribatida by using an improved sampling of both mite taxa and genes. We analyse these data with multiple algorithms and take into account that the vastly different generation times of these mites may result in evolutionary rate differences and lead to artifacts in the analyses (Bergsten 2005).

2.2 Materials and Methods

2.2.1 Gene Selection

We combined data from two molecular markers, one coding gene – elongation factor 1 alpha (*ef1α*) – and one non-coding region – small subunit ribosomal RNA (18S). These markers are commonly used for phylogenetic studies (Danforth et al. 1999, Robillard and Desutter-Grandcolas 2006) and have already been used for investigating phylogenetic and evolutionary relationships of mites (Dobson and Barker 1999, Klompen 2000, Lekveishvili and Klompen 2004, Schaefer et al. 2006, Klompen et al. 2007). Since they are differently conserved we expected these two markers to resolve both recent and deep splits and the relationship of Astigmata and Oribatida, especially Desmonomata.

2.2.2 Species Selection

In order to sample a sufficient range of acariform mite taxa, we investigated both genes from 28 species of Oribatida, eight species of Astigmata, two species of Prostigmata and two species of Endeostigmata (Table 2.3). For Oribatida we used members of five out of six commonly recognized groups: Palaeosomata, Enarthronota, Mixonomata, Desmonomata and Circumdehiscentiae (Grandjean 1969, Weigmann 2006); the small group of Parhyposomata, was not sampled, but has never been related to the origin of Astigmata. The middle-derivative Desmonomata were most heavily sampled, since they were inferred to have a close relationship with Astigmata. Astigmata were represented by five members of Acaridae and one member each of Aeroglyphidae, Carpoglyphidae and Glycyphagidae, respectively. Outgroup taxa, with sequences obtained from GenBank, included two members of Ricinulei – an arachnid lineage often linked to Acari – and a centipede as a more distant outgroup.

2.2.3 Sample Preparation, PCR and Sequencing

DNA was extracted from single individuals. Each mite was placed in an Eppendorf tube, frozen in liquid nitrogen and crushed with a plastic rod. Total DNA was extracted using Qiagen DNeasy® Kit for animal tissues according to the manufacturer's protocol (elution was performed in 30 µl instead of 400 µl; Qiagen, Germany).

Amplifications were performed in 25 µl volumes containing 0.5-0.7 µl of each primer (100 pmol/µl), 5-8 µl DNA and 12.5 µl HotStarTaq Mastermix (1.25 units HotStarTaq® polymerase, 100 µM of each dNTP and 7.5 mM $MgCl_2$ buffer solution; Qiagen, Germany). Primer sequences and PCR programs are given in Table 2.1 and Table 2.2, respectively.

PCR products were visualized on 1% agarose gels and purified using QIAquick® PCR Purification Kit (Qiagen, Germany); PCR products were directly sequenced by Macrogen Inc. (Seoul, South Korea). All sequences are available at GenBank (see Table 2.3 for accession numbers).

Table 2.1 Primer sequences for PCR of the elongation factor 1 alpha (*ef1α*) gene and the ribosomal 18S region (18S).

gene	primer name	sequence (5'-3')	references
ef1α	40.71F	TCN TTY AAR TAY GCN TGG GT	Klompen (2000)
	52.RC	CCD ATY TTR TAN ACR TCY TG	
18S (PCR)	18Sforward	TAC CTG GTT GAT CCT GCC AG	
	18Sreverse	TAA TGA TCC TTC CGC AGG TTC AC	
18S (sequencing)	18S554f	AAG TCT GGT GCC AGC AGC CGC	modified after R.H.
	18S1282r	TCA CTC CAC CAA CTA AGA ACG GC	Thomas, pers. comm.
	18S1150f	ATT GAC GGA AGG GCA CCA CCA G	
	18S614r	TCC AAC TAC GAG CTT TTT AAC C	

Table 2.2 PCR conditions of the elongation factor 1 alpha (*ef1α*) gene and the ribosomal 18S region (18S).

	ef1α		18S	
	temperature (°C)	time	temperature (°C)	time
initial denaturation step	95	15 min	95	15 min
denaturation	95	50 sec	95	45 sec
annealing	46	70 sec	57	1 min
elongation	72	2 min	72	1 min
number of cycles	9		34	
denaturation	95	50 sec	-	-
annealing	50	70 sec	-	-
elongation	72	2 min	-	-
number of cycles	34		-	
final elongation	72	10min	72	10 min

2.2.4 Alignment and Phylogenetic Analysis

DNA sequences of the ribosomal 18S region and the *ef1α* gene of all taxa were combined in a super-matrix and aligned using the default settings in ClustalX (Thompson et al. 1994, 1997); the alignment was modified by eye. The alignment of *ef1α* was unambiguous, but the alignment of 18S included gaps. To minimize saturation in the molecular dataset and long-branch attraction artifacts in the trees, sites with the highest rates of substitutions were removed by excluding all alignment gaps from the 18S sequences and the third codon positions from the *ef1α* sequences. To detect long-branch attraction artifacts all analyses were done on the whole dataset and on a dataset in which the outgroups were excluded. For comparison, we also performed analyses with gaps and third codon positions included, and with only one astigmatid mite species included (data not shown).

Table 2.3 Name of sequenced individuals and GenBank accession numbers for all specimens analysed in this study.

Taxa		Accession Numbers	
		18S	ef1α
outgroup			
Myriapoda	*Zelanion antipodus* (Pocock, 1891)	AY859620	AY310183
Ricinulei	*Pseudocellus pearsei* (Chamberlin & Ivie, 1938)	PPU91489	-
	Cryptocellus centralis Fage, 1921	-	AF240839
Astigmata			
Acaridae	*Acarus gracilis* (Hughes, 1957)	EF203769	EF203783
	Acarus siro (Linné, 1758)	AY022023	EF203784
	Aleuroglyphus ovatus (Troupeau, 1879)	EF203770	EF203782
	Tyroborus lini (Oudemans, 1924)	EF203768	EF203780
	Tyrophagus putrescentiae (Schrank, 1781)	DQ025510	EF203782
Aeroglyphidae	*Austryglycyphagus (=Glycycometus) geniculatus*	EF203773	EF203787
Carpoglyphidae	*Carpoglyphus lactis* (Linné, 1758)	EF203772	EF203785
Glycyphagidae	*Lepidoglyphus destructor* (Schrank, 1781)	EF203771	EF203786
Prostigmata			
Erythraeidae	*Balaustium* sp. Von Heyden, 1826	EF203775	EF203795
Labidostommatidae	*Labidostomma mammillata* (Say)	EF203774	EF203796
Endeostigmata			
Alicorhagiidae	*Alicorhagia* sp. Berlese, 1910	AF022024	EF203788
Terpnacaridae	*Terpnacarus gibbosus* (Womersley, 1944)	AY620904	AF256521
Oribatida			
Paleosomata			
Acaronychidae	*Zachvatkinella* sp. Lange, 1954	EF203776	EF203792
Palaeacaridae	*Palaeacarus hystricinus* Trägardh, 1932	EF204472	EF203793
Enarthronota			
Eniochthoniidae	*Eniochthonius minutissimus* (Berlese, 1903)	EF091428	EF081329
Hypochthoniidae	*Hypochthonius rufulus* C.L. Koch, 1835	EF091427	AY632861
	Eohypochthonius gracilis (Jacot, 1936)	EF203777	EF203794
Lohmanniidae	*Lohmannia banksi* Norton et al., 1978	AF022036	EF081330
Mixonomata			
Euphthiracaridae	*Rhysotritia duplicata* (Grandjean, 1953)	EF091417	EF081310
Nehypochthoniidae	*Nehypochthonius porosus* Norton & Metz, 1980	EF081308	EF081328
Phthiracaridae	*Steganacarus magnus* (Nicolet, 1855)	AF022040	AY632837
	Atropacarus striculus (C.L. Koch, 1835)	EF091416	EF081309
Desmonomata			
Camisiidae	*Camisia segnis* (Hermann, 1804)	EF203778	EF203790
	Heminothrus paolianus (Berlese, 1913)	EF091423	EF081316
	Platynothrus peltifer (C.L.Koch, 1839)	EF091422	AY632851
Hermanniidae	*Hermannia gibba* (C.L. Koch, 1839)	EF091426	EF081327
Nanhermanniidae	*Nanhermannia coronata* Berlese, 1913	EF091421	AY632825
Malaconothridae	*Malaconothrus gracilis* v.d. Hammen, 1952	EF091424	EF081311
	Trimalaconothrus sp. Berlese, 1916	EF204474	EF203789
Nothridae	*Nothrus silvestris* Nicolet, 1855	EF091425	AY573591
Trhypochthoniidae	*Archegozetes longisetosus* Aoki, 1965	AF022027	EF081321
	Mainothrus badius (Berlese, 1905)	EF081301	EF081318
	Mucronothrus nasalis (Willmann, 1929)	EF081299	EF081319
	Trhypochthonius americanus (Ewing, 1908)	EF081298	EF081317
Circumdehiscentiae (=Brachypylina)			
Achipteriidae	*Achipteria coleoptrata* (Linnaeus, 1758)	EF091418	AY632776
Carabodidae	*Carabodes femoralis* (Nicolet, 1855)	EF091429	EF081325
Eutegaeidae	*Eutegaeus curviseta* Hammer, 1966	EF081297	EF081326
Neoliodidae	*Poroliodes farinosus* (Koch, 1839)	EF203779	EF203791
Phenopelopsidae	*Eupelops plicatus* (C.L. Koch, 1836)	EF091418	AY632797
Tectocepheidae	*Tectocepheus velatus* (Michael, 1880)	EF093781	EF093763

The evolutionary model parameters were determined with Modeltest 3.7 (Posada and Crandall 1998) using a hierarchical likelihood ratio test (hLRT). The model of evolution was TrN+I+G (Tamura and Nei 1993) with base frequencies A=0.2995, C=0.2180, G=0.2381, Gamma distribution shape parameter α=0.5874 for four categories of among-site variation, and fraction of invariant sites I=0.5117. Substitution rates were estimated as A-C, A-T, C-G and G-T=1.0, A-G=2.2648 and C-T=4.1463. The evolutionary model for the dataset without the outgroups was also TrN+I+G with base frequencies A=0.2984, C=0.2192, G=0.2373, Gamma distribution shape of α=0.5744 and a fraction of invariant sites I=0.5237. The substitution rates were A-C, A-T, C-G and G-T=1.0, A-G=2.2501 and C-T=4.1077. Model parameters for the separate datasets were very similar so their combination seemed valid.

Phylogenetic trees were constructed using neighbour-joining (NJ) and maximum parsimony (MP) as implemented in PAUP* 4b10 (Swofford 1999). Maximum parsimony (MP) trees were constructed with heuristic search of 100 random additions, and the tree bisection-reconnection (TBR) branch-swapping algorithm with the option to collapse zero branch length. A strict consensus tree was constructed. Reliability of the branches was ascertained by bootstrap analyses for NJ (100,000 replicates) and MP (10,000 replicates) in PAUP*. Bayesian phylogenetic analysis was performed with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) using the settings of GTR+I+G with three independent runs of 3,000,000 generations and four chains each; rate matrix and base frequencies were estimated and trees were sampled every 300 generations. A majority consensus tree was generated using a burn-in of 2,000.

2.3 Results

Phylogenetic analyses with neighbour-joining, maximum parsimony and Bayesian algorithms were based on a super-matrix of the 18S region and the *ef1α* gene (gaps and third codon positions excluded) with 1,776 base pairs and 39 taxa in total (37 taxa for analyses without outgroups). Of the 1,776 positions 1,209 (1,238; analysis without outgroups) were conserved and 567 (538) were variable with 372 (344) positions being parsimony-informative. There were no differences in nucleotide composition among all taxa and all four nucleotides were uniformly distributed. The maximum likelihood distance of the whole dataset averaged 10% and had a maximum value of 24%. The average likelihood distance (5% and 8%) as well as the maximum (both 13%) and the minimum (0.4% and 0.7%)

distances within Oribatida and Astigmata were very similar; values after exclusion of the outgroups were identical to those calculated with outgroups.

All phylogenetic algorithms gave nearly identical tree topologies. Astigmata were always monophyletic, supported by high bootstrap support values and posterior probabilities (Figs. 2.1-2.3). Acaridae were always monophyletic and supported by high bootstrap support values and posterior probabilities; within it, *Tyroborus lini* and *Tyrophagus putrescentiae* were sister species, as were the two *Acarus* species; the position of *Aleuroglyphus ovatus* varied. Relationships among the other astigmatid lineages varied, but collectively they were always the sister taxon of Acaridae.

Topologies for the two species of Endeostigmata and two of Prostigmata varied with algorithm, and none was supported by high bootstrap support values or posterior probabilities (Figs. 2.1-2.3). The two species of Endeostigmata, *Alicorhagia* sp. and *Terpnacarus gibbosus,* never were sister taxa, consistent with the common assumption that Endeostigmata are paraphyletic. By contrast, the two prostigmatids, *Balaustium* sp. and *Labidostomma mammillata,* were expected to be sister taxa, but this only happened in the MP tree. In the NJ and MP trees *Alicorhagia* was the sister-taxon of Astigmata; in the Bayesian tree the sister-taxon of Astigmata was represented by *Alicorhagia* sp. and *Balaustium* sp. In all analyses without outgroups, *Balaustium* sp. was the most basal taxon.

Oribatida were always monophyletic, supported by moderate bootstrap values and posterior probabilities (70-85 and 0.54-0.83, respectively, Figs. 2.1-2.3). Within them, Enarthronota including *Hypochthonius rufulus, Eohypochthonius gracilis, Lohmannia banksi* and *Eniochthonius minutissimus,* always formed the most basal taxon, which was well supported (81-98; 1.00). Palaeosomata with the genera *Zachvatkinella* and *Palaeacarus* always formed the next monophyletic branch (100; 1.00); its sister-taxon consistently comprised those oribatid taxa having the opisthonotal gland (Mixonomata, Desmonomata and Circumdehiscentiae). Phthiracaridae and Euphthiracaridae (in the subgroup Ptyctima of Mixonomata) consistently formed the next branch with moderate to strong support (68-100; 1.00). Since *Nehypochthonius porosus* clustered on a separate branch, Mixonomata appeared to be paraphyletic.

Figure 2.1 Neighbour-joining tree (TrN+I+G) based on combined sequences of the ribosomal 18S region and the nuclear elongation 1 alpha gene. Gaps and third codon positions are excluded. *Zelanion antipodus* was used as an outgroup. Upper numbers at nodes indicate bootstrap support values of 10,000 neighbour-joining bootstrap replicates, lower numbers result from the same analysis with the outgroups excluded. Only values over 50 are indicated. The arrow labels the most basal taxon if the outgroups are excluded.

Figure 2.2 Maximum Parsimony tree based on combined sequences of the ribosomal 18S region and the nuclear elongation 1 alpha gene. Gaps and third codon positions are excluded. *Zelanion antipodus* was used as an outgroup. Upper numbers at nodes indicate bootstrap support values of 10,000 neighbour-joining bootstrap replicates, lower numbers result from the same analysis with the outgroups excluded. Only values over 50 are indicated. The arrow labels the most basal taxon if the outgroups are excluded.

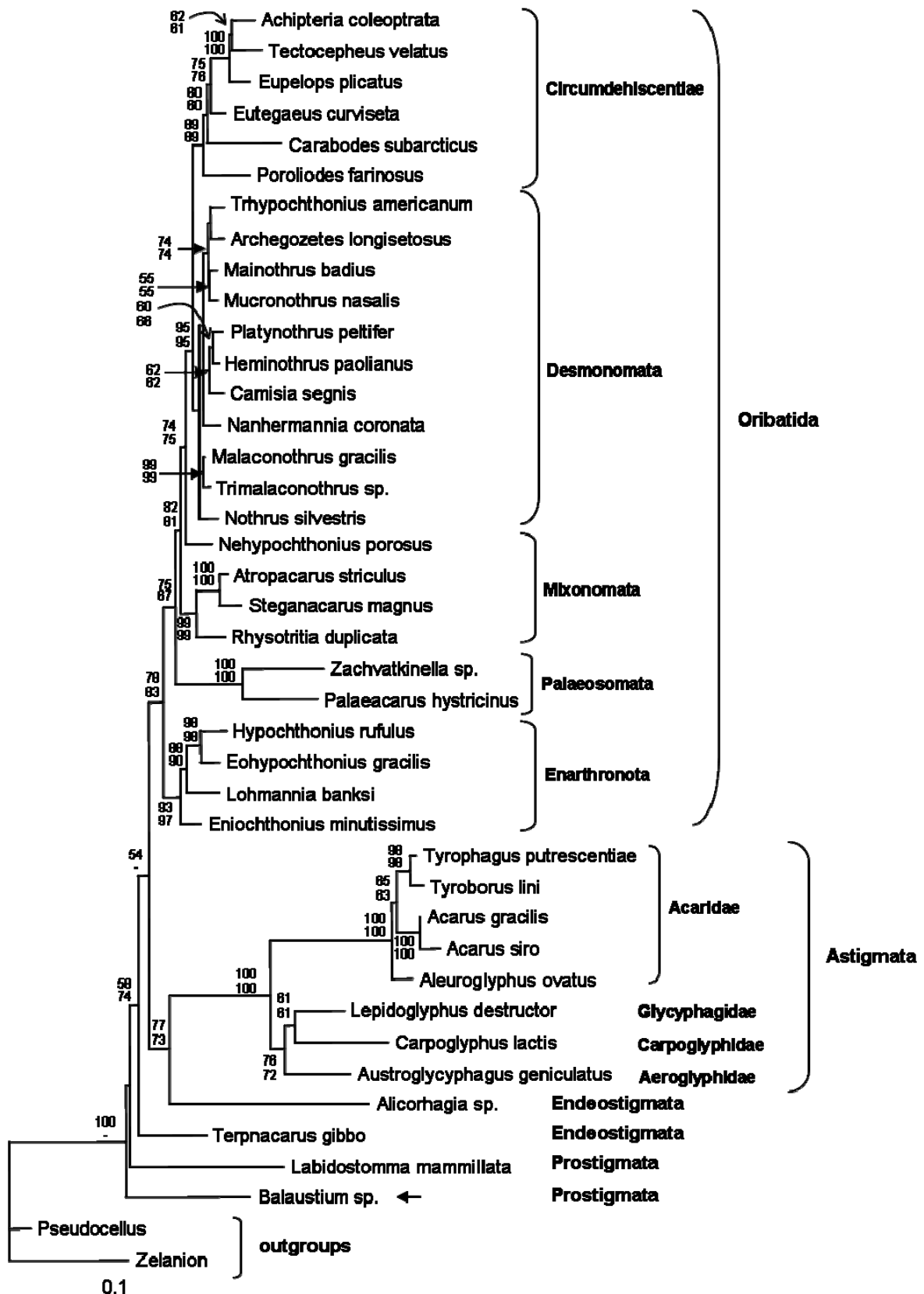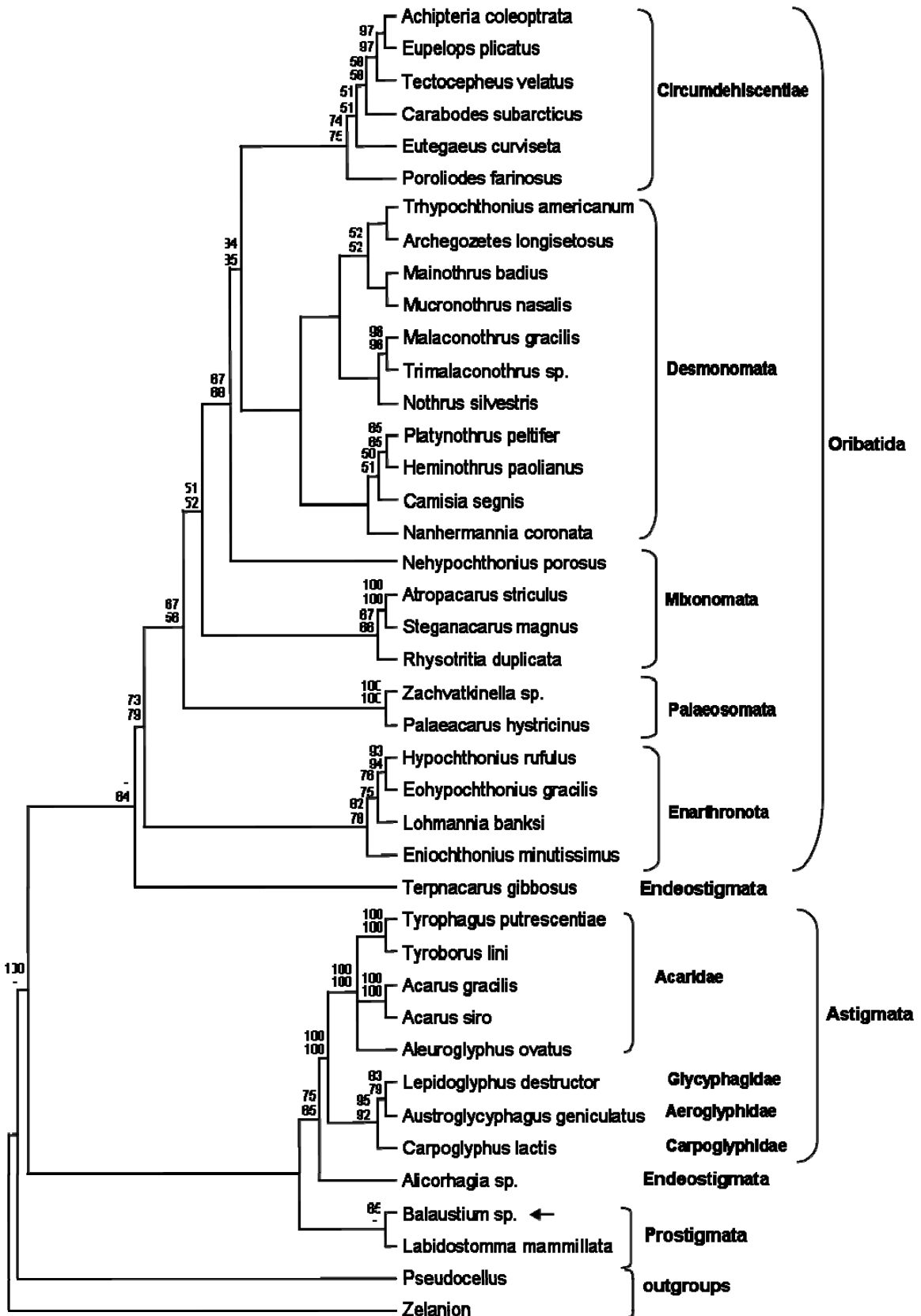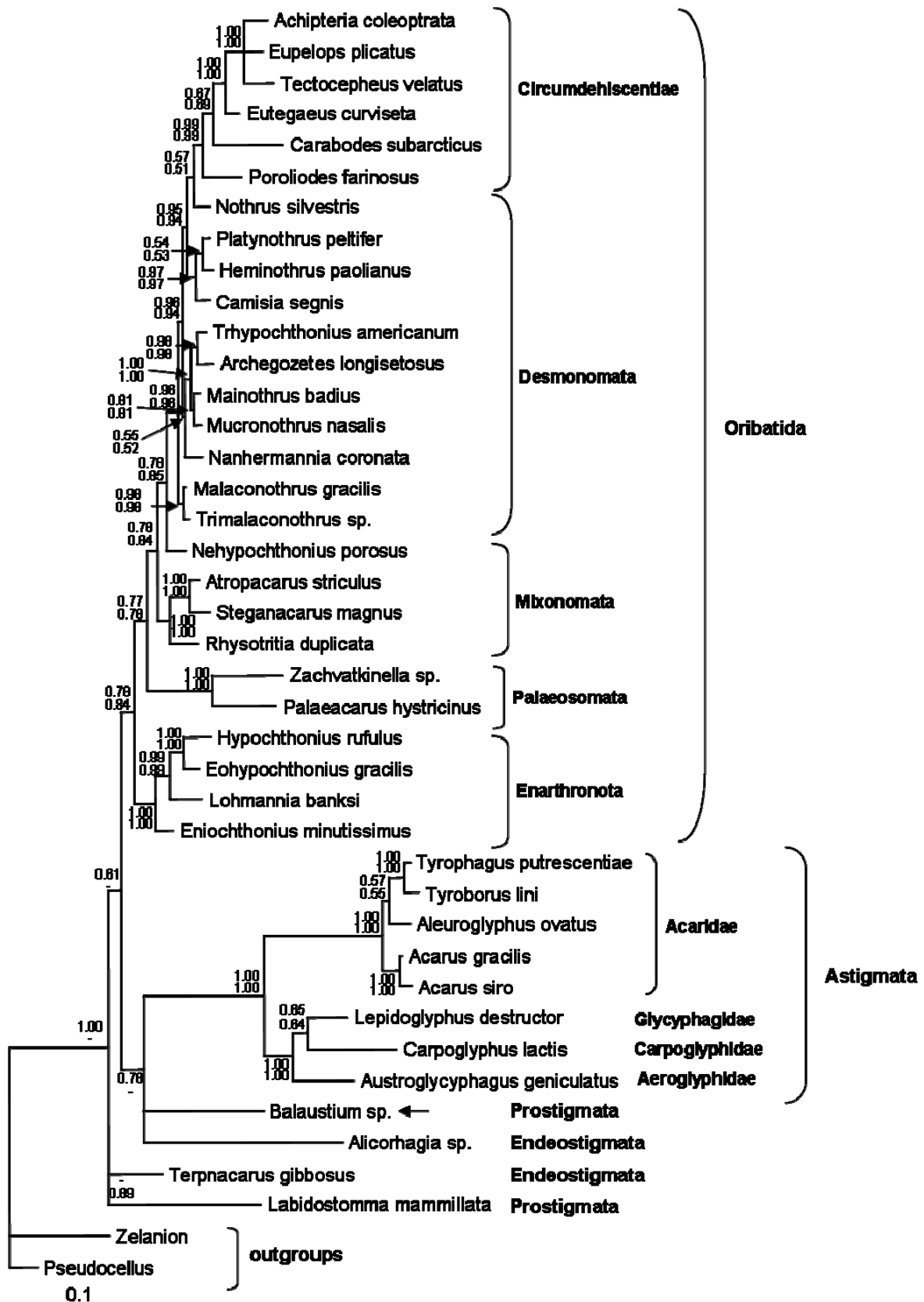**Figure 2.3** Bayesian tree based on combined sequences of the ribosomal 18S region and the nuclear elongation 1 alpha gene. Gaps and third codon positions are excluded. *Zelanion antipodus* was used as an outgroup. Upper number at nodes indicate Bayesian support values, lower numbers result from the same analysis with the outgroups excluded. The arrow labels the most basal taxon if the outgroups are excluded.

Desmonomata were monophyletic in the NJ and MP trees (unsupported by bootstrap values; Figs. 2.1, 2.2), but paraphyletic in the Bayesian tree (Fig. 2.3). However, families within Desmonomata were always monophyletic, e.g. Camisiidae (*Platynothrus peltifer, Heminothrus paolianus* and *Camisia segnis*), Malaconothridae (*Malaconothrus gracilis* and *Trimalaconothrus* sp.) and Trhypochthoniidae (*Trhypochthonius americanus, Archegozetes longisetosus, Mainothrus badius* and *Mucronothrus nasalis*). Circumdehiscentiae were always monophyletic, but with only weak support (75-89; 0.51-0.59); they formed the sister-group of Desmonomata in the NJ and MP trees (Figs. 2.1, 2.2) but in the Bayesian tree the desmonomatan *Nothrus silvestris* was their sister-taxon (Fig. 2.3). In all trees the most basal taxon of Circumdehiscentiae was *Poroliodes farinosus*.

In summary, no analysis supported the origin of Astigmata within desmonomatan Oribatida. This is true regardless of whether outgroups were included or not, and whether gaps and third codon positions were excluded or included (data not shown). Astigmata always clustered with or close to *Alicorhagia* sp., usually in a sister-group relationship.

2.4 Discussion

2.4.1 Phylogenetic Relationship of Astigmata and Oribatida

In contrast to evidence from morphology and gland chemistry, sequence analyses of the ribosomal 18S region and the nuclear elongation factor 1 alpha gene do not support any hypothesis on the origin of Astigmata within Oribatida, i.e. Zachvatkin (1953), OConnor (1984) or Norton (1998). In addition, our data do not support an origin of Oribatida within Astigmata (Berlese 1897), or a sister-group relationship between these two groups (Krantz 1960). Therefore, there is no molecular support for Sarcoptiformes in its early and most-used sense (Reuter 1909, Vitzthum 1925). Of studied taxa, Astigmata appear to be more closely related to the endeostigmatan *Alicorhagia* sp., although the latter is also within Sarcoptiformes in the broader sense of OConnor (1984).

Our molecular analyses correspond partly to the view of Grandjean (1937, 1954) on relationships of Astigmata. Grandjean's hypothesis, summarized by Norton (1998), did not use cladograms or cladistic terminology, but his idea can be presented as Astigmata being the sister-clade of the combined Prostigmata+Oribatida+Endeostigmata; he recognized that these four groups together comprise a monophyletic unit (Acariformes = his Actinochitinosi). Consequently, the molecular trees that link Astigmata to *Alicorhagia* sp. support his rejection

of a close connection between Astigmata and Oribatida, but not the premise of his argument, which was that there is no close connection between Endeostigmata and Astigmata.

While the morphological evidence for a close relationship of Astigmata and Desmonomata is strong, there were also several conflicting characters (Norton 1998). Only one of those was incongruent with the more general hypothesis of OConnor (1984) that Astigmata evolved within some non-basal clade of oribatid mites: Alberti (1991) noted that Astigmata sperms were unlike those of oribatid mites and similar in some ways to those of an endeostigmatid mite. However, Liana and Witalinski (2005) recently considered Astigmata spermatozoa to be so highly modified that their ultrastructure could not help in determining the external relationships of the group.

No morphological trait of Astigmata seems synapomorphic with those of *Alicorhagia* sp., *Terpnacarus gibbosus*, or any other member of Endeostigmata or basal Prostigmata. Also, no member of the latter groups has glands that are similar to the opisthonotal glands shared by most Astigmata and Oribatida. Therefore, in our molecular trees each of the many morphological and biochemical similarities between the two groups must be attributed to convergence.

2.4.2 Long-branch Attraction

Astigmata and Oribatida differ strongly in life history traits (Norton 1994, OConnor 1994). While oribatid mites are characterized by slow development and low fecundity, i.e. have K-style traits, Astigmata have fast development and a high fecundity, i.e. are "r-selected" (Crossley 1977, Behan-Pelletier 1999). In addition, while a majority of Desmonomata reproduce parthenogenetically, most Astigmata are sexual (Norton and Palmer 1991, Norton et al. 1993). While such differences do not themselves argue against relationships, they can influence molecular evolution and may introduce distortion in molecular trees. One way is by long-branch attraction (LBA), describing that species or clades with long branches are attracted by each other or by the long branch of the outgroup (Felsenstein 1978). LBA is commonly recognized or suspected in molecular phylogenetic studies, which are more susceptible to this artifact than morphological studies (reviewed by Bergsten 2005).

We used several methods to detect and correct for LBA. We excluded highly variable nucleotide positions in 18S and *ef1α* (gaps and third codon positions, respectively) from the analysis and we compared methods that are less sensitive to LBA (e.g., Bayesian) with those that are more sensitive to it (e.g., maximum parsimony). To see if outgroups "attracted"

Astigmata to the base of the trees, we compared tree topologies having outgroups included and excluded. All analyses yielded the same result; although members of Astigmata have longer branches in the tree (probably due to a higher substitution rate) they were always monophyletic and never clustered within Oribatida, nor did relationships within Oribatida change regardless of which method was used. Therefore, none of the tests showed evidence of LBA as a distorting factor.

2.4.3 Conclusions

Our comprehensive study – based on the combination of two different genes that probably have different substitution rates (Klompen 2000, Schaefer et al. 2006) and on a relatively wide taxon sampling – does not support the origin of Astigmata within Oribatida. Unlike the simple parsimony approach used in morphological studies, three different analytical approaches were applied to the molecular data, and all gave topologies that were consistent and had moderate to good support values. No indication of long-branch attraction artifacts was found, and the molecular trees are mostly consistent with morphology-based classifications within Oribatida.

Molecular studies have changed our interpretation of many aspects of metazoan phylogeny (Halanych 2004). DNA data are often considered more objective; their analysis can be both parsimony- and model-based, and important artifacts (i.e. LBA) can be tested for. However, care is necessary especially if a contradicting morphological phylogeny is strongly supported, which is true for Astigmata. Further research will be necessary to resolve the phylogenetic relationships of the major sarcoptiform lineages and the position of Astigmata therein. These may involve a wider sampling of Endeostigmata and Astigmata, including basal groups like Histiostomatoidea, but also other approaches may be useful. Mitochondrial gene arrangements might be conserved enough in Astigmata to provide a useful test of competing hypotheses (e.g., Boore and Brown 2000, Roehrdanz et al. 2002). Also, a combined analysis of morphological and DNA characters, as in studies of parasitiform mites (Klompen et al. 2000, Lekveishvili and Klompen 2004), might prove synergistic (Jenner 2004, Bergsten 2005).

CHAPTER 3

Re-evolution of Sexuality Breaks Dollo's Law

The dominance of sexual reproduction is still an unresolved enigma in evolutionary biology. Strong advantages of sex have to exist since only a few parthenogenetic taxa persist over evolutionary timescales. Oribatid mites (Acari) include outstanding exceptions to the rule that parthenogenetically reproducing taxa are of recent origin and doomed to extinction. In addition to the existence of large parthenogenetic clusters in oribatid mites, phylogenetic analyses of this study and model-based reconstruction of ancestral states of reproduction imply that Crotoniidae have re-evolved sexuality from parthenogenetic ancestors within one of those clusters. This reversal in reproductive mode is unique in animal kingdom and violates Dollo's Law that complex ancestral states can never be reacquired. The re-evolution of sexuality requires that ancestral genes for male production are maintained over evolutionary time. This likely is true for oribatid mites since spanandric males exist in various species, although mechanisms that enable the storage of genetically ancestral traits are unclear. Our findings present oribatid mites as an unique model system to explore the evolutionary significance of parthenogenetic and sexual reproduction.

3.1 Introduction

The enigma of the evolution of sex comprises two processes, the origin and the maintenance of sex. Theories on the advantages of sex mainly refer to the improvement of the progeny's fitness in sexual populations despite reducing the overall number of offspring (Maynard Smith 1978, Bell 1982). Nevertheless, one of the enduring mysteries of biology is the prevalence of sexual reproduction in eukaryotes. Since parthenogenetic species do not waste resources in producing males (the now-classic "two-fold" advantage) and do not break up favourable gene combinations they should rapidly outcompete sexual species in most environments (Maynard Smith 1978, Bell 1982). Why this is not true has been debated for decades, with so many answers having been proposed (Birky 1996, Vrijenhoek 1998, Barraclough et al. 2003) that a second enigma has emerged: How could a few animal lineages have maintained parthenogenetic reproduction over considerable evolutionary time, avoiding extinction long enough to radiate and form monophyletic clades? The most studied examples of such "ancient asexual scandals" (Maynard Smith 1978) are darwinulid ostracods (Martens et al. 1998), bdelloid rotifers (Mark Welch and Meselson 2000) and several large clusters within oribatid mites (Norton and Palmer 1991, Maraun et al. 2004, Schaefer et al. 2006, Hetthoff et al. 2007a).

Mites exhibit a bewildering array of genetic systems and reproductive modes (Cruickshank and Thomas 1999, Weeks et al. 2001) and parthenogenetic reproduction has evolved numerous times. Parthenogenesis is most common in Oribatida, a widespread and abundant group of soil invertebrates. An estimated 9% of species are parthenogenetic, which is one to two orders of magnitude higher than in other animal groups (Norton and Palmer 1991). Most parthenogenetic oribatid mites are clustered in species-rich clades with no known sexual species, making each such clade an independent "asexual scandal" (Norton and Palmer 1991, Maraun et al. 2004, Heethoff et al. 2007a, Norton et al. 1993).

The pattern of reproductive modes is most varied in Desmonomata, a speciose group with an age of at least 100 million years (Heethoff et al. 2007a, Norton et al. 1988, 1989), probably predating the break-up of Pangea (Hammer and Wallwork 1979). While most families in this group are either entirely parthenogenetic or sexual, there is also one with mixed reproductive modes (Table 3.1) (Palmer and Norton 1991). All parthenogenetic species have a highly female-biased sex-ratio with most populations having over 99% females whereas sexual species comprise at least 30% males (Norton et al. 1993, Palmer and Norton 1991). Evidence of these patterns comes from culturing and population studies of a wide range of species

throughout the world, representing most known genera (Norton et al. 1993, Palmer and Norton 1991). However, phylogenetic relationships among the sexual and parthenogenetic taxa have been addressed only superficially.

Of the sexual taxa, Crotoniidae are most puzzling (Palmer and Norton 1991). The sexuality of these soil and tree-dwelling mites may simply reflect the ancestral reproductive mode of Desmonomata, but unlike the other taxa, they are not globally distributed; their range is essentially Gondwanan (Table 3.1). Also, they are morphologically similar to *Camisia*, a widespread and rather derived genus of the parthenogenetic Camisiidae. The Gondwana distribution and the morphological similarity suggest that Crotoniidae may have evolved from within Camisiidae and thereby re-evolved sexuality. The regain of sex would contrast Dollo's Law stating that complex characters never re-evolve once they are lost (Gould 1970). If true the re-evolution of sexuality in oribatid mites would be the first such reversal known in the animal kingdom and add to the mystique of sex as "the queen of problems in evolutionary biology" (Bell 1982).

We tested the hypothesis that sexuality re-evolved in Crotoniidae by investigating its phylogenetic position among a wide range of sexual and parthenogenetic oribatid mites, using a combined dataset of partial sequences of the ribosomal 18S region (18S), the heat shock protein 82 gene (*hsp82*) and the elongation factor 1 alpha gene (*ef1α*).

3.2 Materials and Methods

3.2.1 Taxon Sampling

In total, 30 oribatid mite species were sampled. Oribatid mites are commonly ascribed to six major groups, Palaeosomata, Enarthronota, Parhyposomata, Mixonomata, Desmonomata, and Brachypylina (Grandjean 1969, Taberly 1988). Parthenogenetic clusters are most common in Enarthronota and Desmonomata, which are early- and middle-derivative groups, respectively. We focused on Desmonomata, comprising seven families with 36 genera and about 500 described species (Palmer and Norton 1991, Grandjean 1969, Subias 2004). In addition to having the large parthenogenetic families Trhypochthoniidae (68 spp.), Malaconothridae (104 spp.), Camisiidae (92 spp.) and Nanhermanniidae (56 spp.), Desmonomata include two families, Crotoniidae (45 spp.) and Hermanniidae (80 spp.), that reproduce only sexually and one family, Nothridae (54 spp.), that has both sexual and parthenogenetic genera. Representatives of all seven families of Desmonomata were included to ascertain whether sexuality in these families appeared to be ancestral or derived, with respect to other

Desmonomata (Table 3.1). Camisiidae were most heavily sampled since a close relationship to Crotoniidae was hypothesized. Other desmonomatan families were represented by a single genus since their reproductive modes is internally constant.

Several species of Brachypylina, the "higher" oribatid mites, were sampled to ascertain monophyly or paraphyly of Desmonomata. Members of Enarthronota and Mixonomata were sequenced for use as respective outgroups, and were selected based on earlier phylogenetic studies (Maraun et al. 2004, Grandjean 1969, Haumann 1991). Parhyposomata and Palaeosomata were not included since they are small taxa having no apparent bearing on our objectives.

Oribatid mites were collected from litter and soil at different localities in Germany, Poland, USA, New Zealand and Russia. We complemented the dataset with sequences available at GenBank (Table 3.1).

Table 3.1 Species list, gene length, GenBank accession numbers, geographical distribution and mode of reproduction for specimens analysed in this study.

Taxa	18S	hsp82	ef1α	ef1α (acc)	hsp82 (acc)	18S (acc)	distribution[§]	reproductive mode	references
Enarthronota									
Hypochthoniidae									
Hypochthonius rufulus C.L. Koch, 1835	1782	531	543	AY632861	DQ090776	EF091427	Holarctic, Seychelles	parthenogenetic	*, $, pers. observ.
Eniochthoniidae									
Eniochthonius minutissimus (Berlese, 1903)	1759	535	543	EF081329	DQ090773	EF091428	Cosmopolitan	parthenogenetic	*, pers. observ.
Lohmanniidae									
Lohmannia banksi Norton et al., 1978	1794	513	543	EF081330	DQ090777	AF022036	USA	parthenogenetic	pers. observ.
Mixonomata									
Nehypochthoniidae									
Nehypochthonius porosus Norton & Metz, 1980	1741	535	543	EF081328	DQ090779	EF081308	USA, Hawaii	parthenogenetic	∈
Phthiracaridae									
Steganacarus magnus (Nicolet, 1855)	1733	513	543	AY632837	DQ090781	AF022040	Holarctic, USA	sexual	*
Atropacarus striculus (C.L. Koch, 1835)	1742	522	543	EF081309	DQ090782	EF091416	Holarctic, Oriental, Australian	parthenogenetic	¥
Euphthiracaroidea									
Rhysotritia duplicata (Grandjean, 1953)	1741	513	543	EF081310	DQ090780	EF091417	Palaearctic	parthenogenetic	£, pers. observ.
Desmonomata									
Camisiidae[p]									
Heminothrus paolianus (Berlese, 1913)	1741	528	543	EF081316	DQ090794	EF091423	Holarctic	parthenogenetic	#, *, +
Platynothrus peltifer (C.L. Koch, 1839)	1741	525	543	AY632851	DQ090793	EF091422	Holarctic, Oriental, New Zealand, Neotropic	parthenogenetic	#, ¤, *, ¢
Camisia horrida (Koch, 1839)	1741	522	543	EF081312	EF081331	EF081302	Holarctic	parthenogenetic	#
Camisia spinifer (C.L. Koch, 1835)	1741	522	543	EF081313	EF081332	EF091420	Holarctic, Oriental, South America	parthenogenetic	#, *
Crotoniidae[s]									
Crotonia brachyrostrum (Hammer, 1966)	1741	522	543	EF081314	DQ090796	EF081303	Gondwanan	sexual	#
Crotonia cf *caudalis* (Hammer, 1966)	1741	519	543	EF081315	DQ090795	EF081304	Gondwanan	sexual	#
Hermanniidae[s]									
Hermannia gibba (C.L. Koch, 1839)	1739	510	543	EF081327	DQ090800	EF091426	Holarctic, Seychelles	sexual	#, *
Nanhermanniidae[p]									
Nanhermannia coronate Berlese, 1913	1741	535	543	AY632825	DQ090799	EF091421	Holarctic, Neotropic	parthenogenetic	#, *, pers. observ.
Malaconothridae[p]									
Malaconothrus gracilis v.d. Hammen, 1952	1741	528	543	EF081311	EF081339	EF091424	Holarctic, Neotropic	parthenogenetic	#, *
Nothridae[s,p]									
Nothrus silvestris Nicolet, 1855	1741	535	543	AY573591	DQ090802	EF091425	Holarctic, Australian	parthenogenetic	#, *, pers. observ.
Nothrus silvestris histilus Jacot, 1937	1741	535	543	EF081323	EF081333	EF081305	East of USA	parthenogenetic	#
Nothrus truncatus Banks, 1895	1742	535	543	EF081322	EF081334	EF081306	USA	parthenogenetic	#
Novonothrus flagellatus Hammer 1966	1741	531	543	EF081324	DQ090801	EF081307	New Zealand, Australia, Gondwanan	sexual	#
Trhypochthoniidae[p]									
Archegozetes longisetosus Aoki, 1965	1748	510	543	EF081321	DQ090798	AF022027	Pantropical	parthenogenetic	#, *, +, pers. observ.
Mainothrus badius (Berlese, 1905)	1741	519	543	EF081318	EF081338	EF081301	Holarctic, Neotropic	parthenogenetic	#
Mucronothrus nasalis (Willmann, 1929)	1741	528	543	EF081319	DQ090797	EF081299	Boreal, Australian, Neotropic	parthenogenetic	#, +, Ψ
Trhypochthonius americanus (Ewing, 1908)	1741	528	543	EF081317	EF081337	EF081298	USA	parthenogenetic	#, *, +
Trhypochthoniellus crassus (Berlese, 1904)	1741	525	543	EF081320	EF081336	EF081300	Holarctic, Australian, Neotropic, Ethiopic	parthenogenetic	#, +
Brachypylina									
Achipteriidae									
Achipteria coleoptrata (Linnaeus, 1758)	1741	510	543	AY632776	EF081335	EF091418	Holarctic, Neotropic, Oriental	sexual	pers. observ.
Carabodidae									
Carabodes femoralis (Nicolet, 1855)	1740	510	543	EF081325	DQ090786	EF091429	Holarctic, Pantropic	sexual	pers. observ.
Eulaegaeidae									
Lintegaeus curviseta Hammer, 1966	1741	510	543	EF081326	DQ090789	EF081297	Gondwanan	sexual	pers. observ.
Phenopelopsidae									
Eupelops plicatus (Koch, 1835)	1740	522	543	AY632797	DQ090783	EF091419	Holarctic, Nearctic	sexual	*, pers. observ.
Tectocepheidae									
Tectocepheus velatus (Michael, 1880)	1746	516	543	EF093763	EF093770	EF093781	Cosmopolitan	parthenogenetic	*, pers. observ.

[T] *Caraboides subarcticus* for 18S
[§] inferred from Hammer and Wallwork (1979) and Subias (2004)
[s] sexual
[p] parthenogenetic

* Grandjean (1941)
§ Luxton (1981)
# Palmer and Norton (1991)
+ Harding (1969)
∈ Travé (1973)

¥ Cianciolo and Norton (2006)
£ Norton and Metz (1980)
¤ Taberly (1988)
¢ Palmer and Norton (1990)
Ψ Taberly (1951, 1958)

3.2.2 Sample Preparation, PCR and Sequencing

Total DNA was extracted from one to ten individuals using Qiagen DNeasy® kit for animal tissues following the manufacturer's protocol (but elution in 30 µl instead of 400 µl; Qiagen, Germany). Amplifications for the18S region, *hsp82* and *ef1α* were performed either in 50 µl volumes containing 1 µl of each primer (100pmol/µl), 4-8 µl DNA and 25 µl of HotStarTaq Mastermix (2.5 units HotStarTaq® Polymerase, 200 µM of each dNTP and 15 mM $MgCl_2$ buffer solution; Qiagen, Germany) or in 25 µl volumes using half the amount of reagents. The primers used and the PCR programs are given in Appendices 1 and 2. PCR products were visualised on 1% agarose gels and purified using QIAquick® PCR Purification Kit (Qiagen, Germany). PCR products were either prepared for direct sequencing or cloned using Qiagen PCR Cloning Kit (Qiagen, Germany) and transformed into *E. coli* Nova Blue Singles[TM] competent cells (Novagen, Germany) by heat shock using the manufacturer's protocol. The plasmids were purified using FastPlasmid Mini Kit (Eppendorf, Germany). DNA was sequenced by Scientific Research and Development GmbH, Oberursel (Germany), Qiagen Genomic Services, Hilden (Germany), or Macrogen Inc., Seoul (Korea). All sequences are available at GenBank (for accession numbers see Table 3.1).

3.2.3 Alignment and Phylogenetic Analysis

Since the parameters of the evolutionary models of the three datasets were very similar, DNA sequences of 18S, *hsp82* and *ef1α* of 30 oribatid mite taxa were combined in a super-matrix and aligned using the default settings in ClustalX (Thompson et al. 1997); the alignment was modified by eye. The evolutionary model parameters were determined with Modeltest 3.7 (Posada and Crandall 1998) using hLRT. The model of evolution was TrN+I+G (Tamura and Nei 1993) with base frequencies A=0.3082, C=0.2238, G=0.2484, Gamma distribution shape parameter α=0.5819 for 4 categories of among-site variation, and fraction of invariant sites I=0.5915. The substitution rates were estimated as A-C, A-T, C-G and G-T=1.0, A-G=2.7550 and C-T=4.8958. Phylogenetic trees were constructed using neighbour-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) algorithms as implemented in PAUP* 4b10 (Swofford 1999). Maximum parsimony (MP) and maximum likelihood (ML) trees were constructed with heuristic search of 100 random additions, and the tree bisection-reconnection (TBR) branch-swapping algorithm with the option to collapse zero branch length. A strict consensus tree was constructed for both. Reliability of the

branches was ascertained by bootstrap analyses for NJ (100,000 replicates), ML (100 replicates, heuristic search), and MP (10,000 replicates, heuristic search) in PAUP*. Bayesian phylogenetic analysis was performed with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) using the settings for GTR+I+G with three independent runs of 3,000,000 generations and four chains each; rate matrix and base frequencies were estimated and trees were sampled every 300 generations. A majority consensus tree was generated using a burn-in of 2,000.

Ancestral states and the history of character evolution were investigated with parsimony and likelihood algorithms, using the StochChar package in Mesquite (Maddison and Maddison 2003, 2005). Likelihood analyses were calculated under a symmetrical model with equal rates for the loss and regaining of sex and an asymmetrical model with independent rates estimated by maximum likelihood algorithm. Asymmetrical models with higher rates for the loss of sex (5:1, 10:1) were also tested. Probabilities were calculated assuming equal length for all branches based on the topology of the ML and Bayesian tree.

Separate analyses (NJ, MP, ML, Bayesian) of the three datasets gave slightly different topologies among desmonomatan families, but internal topologies were identical (data not shown). The Camisiidae/Crotoniidae group was always supported by high support values and *Novonothrus* occupied a basal position within Nothridae.

3.3 Results

Phylogenetic analyses with neighbour-joining, maximum likelihood, maximum parsimony and Bayesian algorithms were based on a super-matrix with 2,897 base pairs and 30 taxa. All algorithms gave nearly identical tree topologies which largely agree with those based on morphological data and earlier molecular studies (Fig. 3.1) (Maraun et al. 2004, Grandjean 1969, Haumann 1991). While Desmonomata as a whole were paraphyletic, all internal taxa except Camisiidae were monophyletic. The sexual genus *Novonothrus* was basal in Nothridae, supported by high bootstrap and posterior probability values. Maximum likelihood and maximum parsimony analyses of character evolution consistently assigned sexuality as the ancestral state of Nothridae (Fig. 3.2a, b).
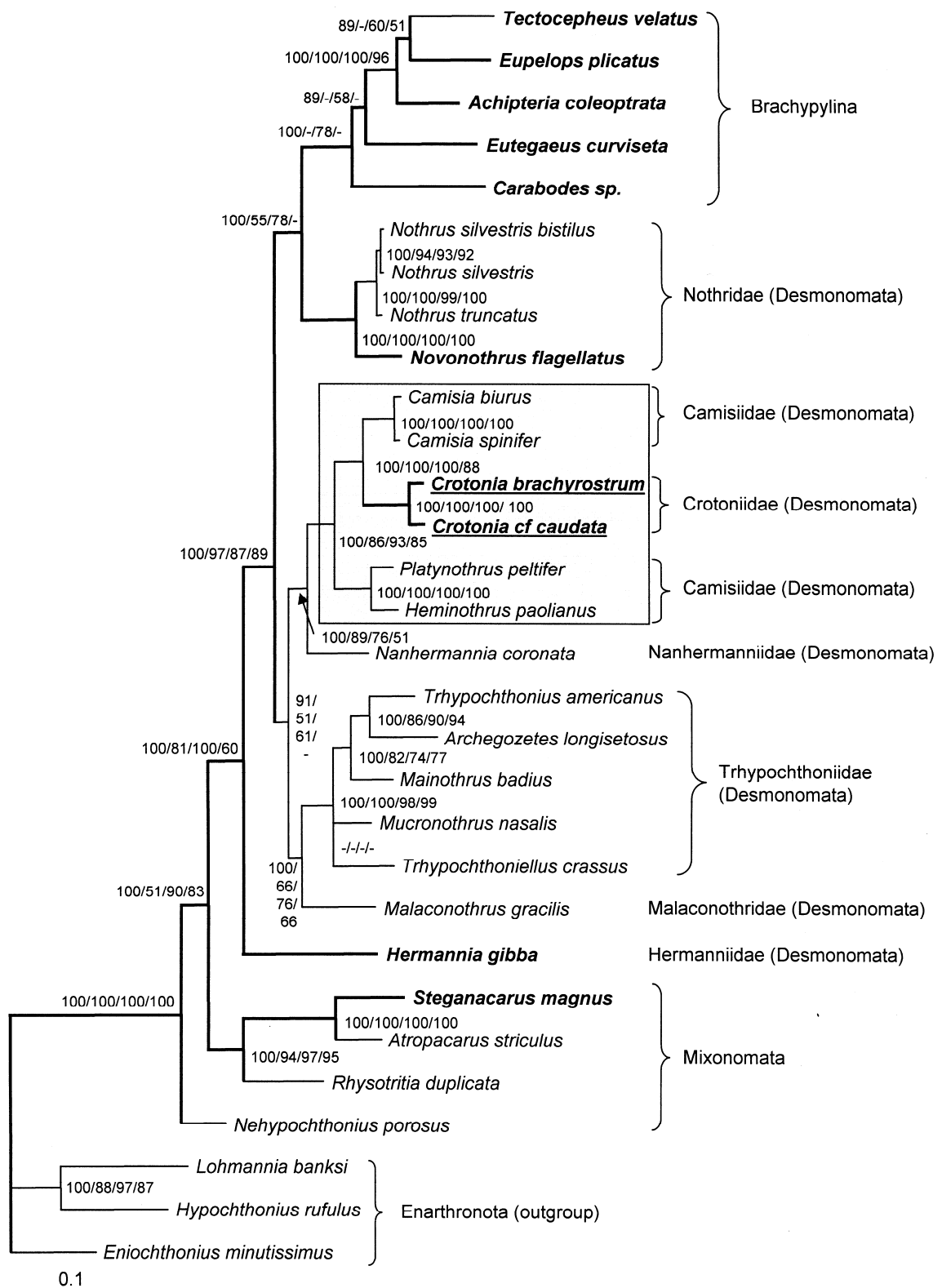
Figure 3.1 Bayesian tree of combined sequences of the ribosomal 18S region, the heat shock protein 82 and the elongation factor 1 alpha of 30 oribatid mite taxa. Enarthronota are used as outgroup. Numbers at nodes represent posterior probabilities for Bayesian analyses and bootstrap support values for neighbour-joining, maximum likelihood and maximum parsimony analyses. Sexual lineages are in bold line and font; species that likely re-evolved sexual reproduction are bold and underlined.

By contrast, the sexual genus *Crotonia* clustered within Camisiidae, a large parthenogenetic family of about 80 species, with *Camisia* being its sister-taxon. In this topology, four successive outgroups of *Crotonia* – two inside and two outside Camisiidae – are entirely parthenogenetic. Monophyly of Camisiidae/Crotoniidae was supported by high bootstrap and posterior probability values (Fig. 3.1). Maximum likelihood and maximum parsimony analyses of character evolution assigned parthenogenesis as the ancestral reproductive mode of the Camisiidae/Crotoniidae clade (Fig. 3.2a, b). Maximum likelihood analysis estimated the rates of loss and regaining of sex to be 0.12 under a symmetrical model of character evolution; under the asymmetrical model the rate of loss was three times that of regaining sex (0.18 and 0.06, respectively). More biased assumptions for the loss of sex (5:1, 10:1) gave similar results (data not shown).

Results from phylogenetic analyses and the reconstruction of the ancestral states of reproduction support the hypothesis that Crotoniidae re-evolved sexual reproduction from parthenogenetic ancestors which contradicts Dollo's Law. Therefore, the loss of the complex process of sexuality likely is not irreversible in evolution.

Figure 3.2 Cladogram of the Desmonomata based on maximum likelihood. Ancestral state of nodes is analysed by (a) maximum likelihood based on a symmetrical model with equal rates for the loss and regain of sex and (b) maximum parsimony. Black dots indicate sexual reproduction, white dots indicate parthenogenetic reproduction. Sexual species are in bold; species that likely re-evolved sexual reproduction are bold and underlined.
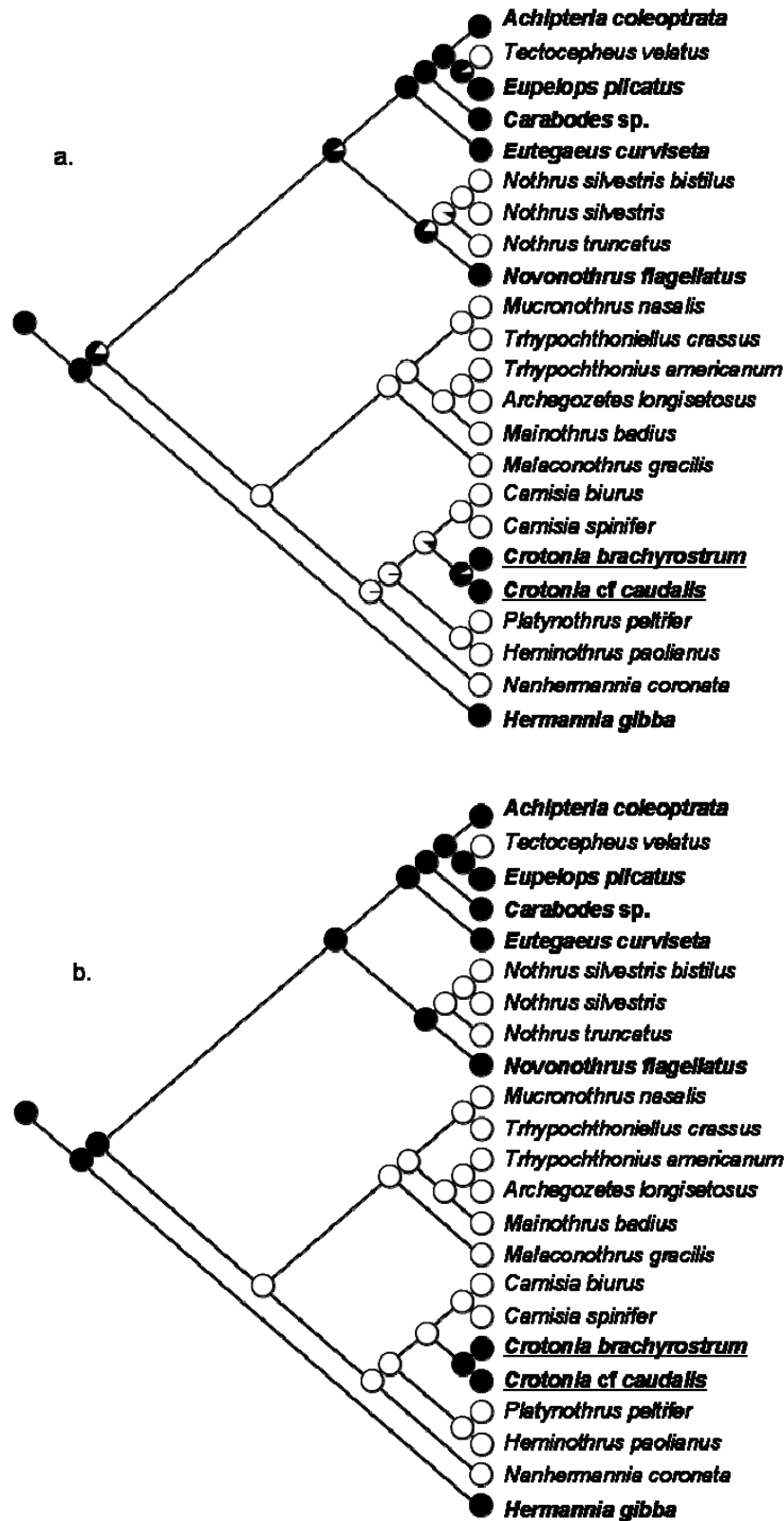
3.4 Discussion

The atavistic resurrection of complex ancestral traits – contrary to Dollo's Law – appears to be more frequent than commonly thought (Rainkov et al. 1979, Marshall et al. 1994, Collin and Cipriani 2003, Pagel 2004, Whiting et al. 2003, Collin et al. 2007, Cruickshank and Paterson 2006, Verhulst 1996). Morphological examples include the re-evolution of shell coiling in Gastropoda after 10 myr of absence (Collin and Cipriani 2003, Pagel 2004), the re-appearance of wings in several lineages of stick insects (Whiting et al. 2003) and regaining of ancestral muscles in bowerbirds (Rainkov et al. 1979). Life history examples include the re-evolution of feeding larvae within a group of direct-developing species in the gastropod *Crepipatella* (Collin et al. 2007) and reversal to a free-living state in several parasites (Cruickshank and Paterson 2006). Atavisms are also present in humans (Verhulst 1996). Another example relates to reproductive biology; the plant *Hieracium pilosella* (Chapman et al. 2003) re-evolved sexuality, but from a recent and narrow parthenogenetic lineage. The re-evolution of sexuality in ancient parthenogenetic clusters of oribatid mites as suggested by this study is, to our knowledge, unique in the animal kingdom.

Much of what has been written about large parthenogenetic clusters in oribatid mites has focused on Desmonomata (Maraun et al. 2004, Schaefer et al. 2006, Norton et al. 1993), especially Camisiidae, Malaconothridae and Trhypochthoniidae. Our data support monophyly of species-rich parthenogenetic taxa within Desmonomata and therefore that parthenogenetic lineages of oribatid mites are not evolutionary "dead-ends"; they have persisted and radiated to form clusters, e.g. the parthenogenetic genus *Nothrus* with 67 species. These lineages of oribatid mites join bdelloid rotifers and darwinulid ostracods (Martens et al. 1998, Mark Welch and Meselson 2000, Butlin et al. 1998) as "ancient asexual" groups, which challenge the view that sexual reproduction is indispensable for long-term survival and radiation of lineages.

Adding to the "scandal" of ancient asexuals (Maynard Smith 1978) results of our study suggest that Crotoniidae have re-evolved sex from parthenogenetic ancestors. Re-evolution of sex likely resulted from changes in evolutionary forces. In contrast to Camisiidae which typically colonize soil organic layers, Crotoniidae species frequently colonize trees; soil collections may prove to be accidental for many species (Olszanowski 1999). Generally, parthenogenesis predominates in oribatid mite communities in soil, whereas the bark of trees and mosses are colonized almost exclusively by sexual species (Cianciolo and Norton 2006, Erdmann et al. 2006). This suggests that sexual reproduction is necessary for coping with the

more heterogeneous environment (Tangled Bank hypothesis; Ghiselin 1974) or increased exposure to antagonists (Red Queen hypothesis; Van Valen 1973) in aboveground habitats. Further, the reproductive mode is affected by the availability of resources. In soil, the permanent availability of resources (litter material and detritus) may explain the widespread occurrence of parthenogenesis (Scheu and Drossel 2007). While *Crotonia* has changed to a tree-dwelling life cycle the mode of reproduction may have changed accordingly.

  How reversion from parthenogenetic to sexual reproduction occurred remains unclear. In animal taxa with cyclical parthenogenesis (intermittent mixis), pure bisexual reproduction can re-evolve by the abandonment of the parthenogenetic part of the life cycle, but cyclical parthenogenesis is unknown in oribatid mites (Norton et al. 1993). Reversion to sexual reproduction may be facilitated since transitions between different modes of reproduction exist in higher mite taxa (Cruickshank and Thomas 1999). The studied parthenogenetic oribatid mite species reproduce by automictic thelytoky in which the meiotic maturation division is followed by fusion of haploid nuclei to restore diploidy (Wrensch et al. 1994). In automictic species, the reversion to sexual reproduction requires that the ability to produce males has been maintained during long evolutionary periods of parthenogenesis. Many parthenogenetic species of oribatid mites are known to produce rare non-functional ("spanandric") males (Palmer and Norton 1991), as is common for parthenogenetic animals in general (Lynch 1984) including the "ancient asexual" darwinulid ostracods (Smith et al. 2006). In parthenogenetic oribatids non-functionality of males is caused by incomplete spermatogenesis and females ignore spermatophores if they are formed (Taberly 1988). Non-functionality has also been indicated by population genetic studies since populations are unaffected by male presence (Palmer and Norton 1992). Why spanandric males persist despite the costs to produce them is unclear. Presumably, such males form as developmental "accidents" as in other parthenogenetic species (Groot and Breeuwer 2006) and, being rare events, the costs of non-functional males may be negligible. Irrespective of the mechanisms involved, the occasional formation of spanandric males in parthenogenetic Camisiidae presumably facilitated the capture of functionality of ancestral genes for male production over long evolutionary timescales and therefore the re-evolution of sex in Crotoniidae. Knowledge of the genetic and epigenetic mechanisms controlling developmental cascades that lead to male production will answer these questions.

3.4.1 Conclusions

In summary, parthenogenetic radiations are infrequent events in evolution and the re-evolution of sexual reproduction from parthenogenetic ancestors, as suggested in this study for the first time, is even more rare. In general, most parthenogenetic taxa have close sexual relatives and contain few species; in Desmonomata, especially in Camisiidae/Crotoniidae, the pattern is reversed with few sexual taxa within a large cluster of parthenogenetic species. Results of the present study suggest that Crotoniidae indeed re-evolved sex which is a spectacular case of breaking Dollo's Law implying that parthenogenesis is not necessarily an evolutionary dead-end. The re-evolution of sexual reproduction in Crotoniidae within the ancient clade of parthenogenetic Camisiidae suggests that sexual reproduction is indispensable at certain environmental conditions. Oribatids are an ideal model group to explore these conditions and therefore unravel the enigma of the evolution of sexual reproduction, and the conditions under which these reproductive modes prevail.

The Complete Mitochondrial Genome of the Sexual Oribatid Mite *Steganacarus magnus*: Genome Rearrangements and Loss of tRNAs

Complete mitochondrial (mt) genomes and the gene rearrangements therein are increasingly used as molecular markers for investigating phylogenetic relationships, especially for elucidating deep splits. We have determined the first complete mitochondrial genome of a sarcoptiform mite, the sexually reproducing oribatid mite *Steganacarus magnus* (Acari, Oribatida) by sequencing long PCR products. This sequence contributes to the set of available arthropod mt genomes, and in particular helps to fill gaps in the coverage of mites. The mt genome of *S. magnus* lacks 16 tRNAs; only those for leucine, histidine, proline, tryptophan, glutamine and serine are present. Within those tRNAs only tRNA-His and tRNA-Pro have kept their original position, the others are translocated. Furthermore, the mt genome of *S. magnus* consists of 13,818 bp and it is composed of 13 protein-coding genes and two genes for the ribosomal RNA subunits that are typically found in metazoan mt genomes. The gene order in *S. magnus* differs from the hypothetical ancestral chelicerate arrangement as conserved in *Limulus polyphemus*: instead of *nad1-rrnL-rrnS*-LNR-*nad2* (tRNAs excluded) *S. magnus* has *nad2-rrnL-nad1-rrnS*-LNR. Phylogenetic analyses of a concatenated amino acid dataset of all mt protein-coding genes of 28 arthropod species suggest a sister-group relationship of sarcoptiform and prostigmatid mites (*S. magnus* and *Leptotrombidium*).The mt gene arrangement of *S. magnus* differs from the hypothetical ground plan of arthropods and from that of other mites further contributing to the variety of mt gene arrangements found in Arachnida. The unexpected lack of tRNAs is enigmatic, probably showing that the loss of mt genes is an ongoing evolutionary process. For solving phylogenetic relationships of oribatid mite lineages and their position within Acari further complete mt genomes are needed.

4.1 Introduction

Mitochondria are maternally inherited cell organelles that contain a circular genome of about 14-19 kb in bilaterian animals; the mitochondrial (mt) DNA in metazoans usually codes for 13 proteins, 22 transfer RNAs (tRNA), two ribosomal RNAs (rRNA; large (*rrnL*) and small (*rrnS*) ribosomal subunit) and contains a non-coding control region (LNR) of variable length (Wolstenholme 1992, Boore 1999). The loss of genes in mitochondria is a commonly recognized and ongoing process in eukaryotes (Blanchard and Lynch 2000). Eukaryotic mt genomes generally contain fewer genes than their free-living bacterial ancestors since the majority of the original mt proteins are now encoded in the nucleus; this is either caused by the transfer of the original mt gene to the nucleus or by the replacement of its function by a preexisting nuclear gene (Adams and Palmer 2003). The protein-coding genes which have been retained in mt genomes are mainly those involved in electron transport and phosphorylation, e.g., cytochrome b (*cob*) and the cytochrome oxidases (*cox1, cox2, cox3*) (Adams and Palmer 2003), but their number is variable ranging from three in the malaria parasite *Plasmodium falsciparum* (Apicomplexa) (Feagin 1994) to 67 in *Reclinomonas americana*, the earliest branching aerobic protist (Chlorophyta) (Lang et al. 1997).

In addition to gene loss, the positions of genes relative to each other exhibit frequent rearrangement. While the arrangement of mt genes is conserved in some lineages of arthropods (Boore et al. 1998, Shao et al. 2004), it is highly variable in others (Covacin et al. 2006, Shao et al. 2006, Cameron et al. 2007). In particular, the positions of the relatively small genes for tRNAs frequently vary within and among taxa. The arrangement of the hypothetical ancestor of arthropods is conserved in the horseshoe crab *Limulus polyphemus* (Lavrov et al. 2000), whereas most insect genomes differ from the ancestral state by the location of one tRNA (Boore et al. 1998).

Oribatid mites (Acari, Oribatida) are soil-dwelling animals that occur in high numbers in almost all terrestrial ecosystems (Walter and Proctor 1999). A characteristic feature of this group is the unusually high percentage of parthenogenetic taxa (~ 10 % of all species) and the co-occurrence of sexuality and parthenogenesis in the same habitat (Palmer and Norton 1991). Oribatid mite fossils date back at least 360 million years (Krivolutsky and Druk 1986, Norton et al. 1988) and therefore a number of parthenogenetic lineages of oribatid mites join bdelloid rotifers as "ancient asexual scandals" (Mark Welch and Meselson 2000, Martens et al. 2003, Judson and Normark 1996, Heethoff et al. 2007a). Since oribatid mites provide insights into the evolution and maintenance of sex, recently much attention has been paid to their

phylogeny and radiation (Maraun et al. 2003, 2004, Schaefer et al. 2006, Domes et al. 2007b, c). However, studies based on single genes such as the ribosomal 18S region (18S), the heat shock protein 82 (*hsp82*), the elongation factor 1α (*ef1α*) or *cox1* could neither satisfactorily resolve phylogenetic relationships (Domes et al. 2007c) nor clarify the number of parthenogenetic radiations (Maraun et al. 2003) nor delineate the age of the group (Heethoff et al. 2007a). Since oribatid mites apparently are among the first terrestrial animals (I. Schaefer, unpublished data) and species exhibit different evolutionary mutation rates, lineages are probably vulnerable to long-branch attraction in phylogenetic reconstruction. Therefore, further markers, such as gene rearrangements in mt genomes, are needed to resolve phylogenetic relationships of oribatid mite lineages, among mite taxa (Acari) and among chelicerates in general.

Until now no complete mt genome of an oribatid mite species was available although mt genomes have become invaluable phylogenetic markers during the last few years. Complete mt genome sequences are now known for about 150 arthropods, including 26 chelicerates with 15 species of Acari. These 15 acarine genomes represent ten species of ticks (Ixodida, Parasitiformes), two mesostigmate mites (Mesostigmata, Parasitiformes) and three species of the genus *Leptotrombidium* (Prostigmata, Acariformes). In contrast to other arthropods and Metazoa in general (Boore 1999), the arrangement of the mt genes differs markedly within and among taxa of Acari. While the ancestral state of arthropods is retained in soft- and prostriate-hard ticks (Shao et al. 2004, Black and Roehrdanz 1998), there is a major rearrangement shared by all metastriate hard ticks (Black and Roehrdanz 1998, Shao et al. 2005), and there are numerous, lineage specific rearrangements in the mesostigmate species, such as *Varroa destructor* (Navajas et al. 2002) and *Metaseiulus occidentalis* (Jeyaprakash and Hoy 2007), and also in the prostigmate mite genus *Leptotrombidium* (Shao et al. 2006). Since the deep phylogeny of Acari, especially within the large subgroup of Acariformes, is still controversial, synapomorphic rearrangements of mt genes, if present, would likely allow new insights into phylogenetic relationships.

We report the first mt genome for an oribatid mite, *Steganacarus magnus* (Nicolet, 1855). The mt genome of *S. magnus* is compared with that of other mites and with basal chelicerates to clarify phylogenetic relationships within Acari and Chelicerata in total. We show that the mt genome of *S. magnus* is slightly rearranged and lacks 16 tRNA genes.

4.2 Materials and Methods

4.2.1 DNA Processing

Specimens of *S. magnus* were collected from the Kranichstein forest located about 8 km northeast of Darmstadt, Germany. Animals were extracted from leaf litter by heat using a modified Kempson extractor (Kempson et al. 1963), preserved in 75% ethanol and stored at -20°C until usage. Total DNA was extracted from single specimens using the DNeasy Tissue kit (Qiagen) following the manufactures' protocol (but final elution of DNA was in 40 µl instead of 200 µl). Polymerase chain reactions (PCR) were performed for the small ribosomal subunit (*rrnS*), the cytochrome b (*cob*) and cytochrome c oxidase I (*cox1*) genes using the primers 12SA and 12SB, CB3 and CB4 and COIarch1 and COIarch2, respectively (Table 4.1). PCR reaction mixtures contained 12.5 µl HotStarTaq MasterMix (Qiagen), 0.7 µl of each primer (100 pmol/µl), and 4 µl DNA (unquantified) in a total volume of 25 µl. Amplification conditions included an initial activation step at 95°C for 15 min followed by 34 cycles of 95°C for 45 s; 44°C (*rrnS*), 50°C (*cob*) or 51°C (*cox1*) for 1 min; 72°C for 55 s and a final elongation at 72°C for 10 min. PCR products were visualized on a 1% agarose gel, purified using the QIAquickPCR Purification kit (Qiagen) and directly sequenced by Macrogen (South Korea).

Long PCR amplifications were performed using 1 µl Elongase (Invitrogen), 1 µl buffer A, 4 µl buffer B, 2.5 µl dNTPs (25 mM), 1 µl of each primer (10 mM) and 1-2 µl of DNA (unquantified) with the following conditions: 92°C for 2 min; 40 cycles of 92°C for 30 s, 50°C for 30 s, 68°C for 12 min and a final extension step of 68°C for 20 min. Initial primers for long PCR were designed from the previously obtained sequences of *rrnS*, *cob* and *cox1* (Table 3.1). PCR products were visualized on 1% agarose gels and purified using Millipore Montage vacuum purification plates. Sequencing was performed using ABI BigDye 3.1 dye terminator technology with an ABI capillary sequencer at the John Curtin Medical School sequencing centre (Australian National University). Cycle sequencing reactions contained 1 µl BigDye, 0.5 µl of the primer (25 mM) and 0.5-1.0 µl template (in a total volume of 3 µl) and amplification conditions were 28 cycles of 94°C for 10 s, 50°C for 5 s and 60°C for 4 min. Within each long PCR product the complete double-stranded sequence was determined by primer walking (a list of all primers is given in Appendix 3). Since PCR amplifications derived from DNA extracted from different specimens the final genome sequence is a consensus from a pool of individuals.

Table 4.1 Initial primers for PCR amplifications of the mitochondrial genome of *Steganacarus magnus*.

region	primer pair	orientation	primer sequence (5'-3')	reference
rrnS	12SA	forward	TACTATGTTACGACTTAT	Skerratt et al. 2002
	12SB	reverse	AAACTAGGATTAGATACCC	
cob	CB3	forward	GAGGAGCAACTGTAATTACTAA	Pons 2006
	CB4	reverse	AAAAGAAARTATCATTCAGGTTGAAT	
cox1	COIarch1	forward	GGTCAACAAATCATAAAGAYATYGG	Folmer et al. 1994, modified by R.H. Thomas
	COIarch2	reverse	TAAACTTCAGGGTGACCAAAAAATCA	
rrnS-cob	Steg1	reverse	AAATCAGGTCAATGTTCGG	this paper
	Steg6	forward	CAATTAGAAATTACCCAG	
cob-cox1	Steg5	reverse	AAAAATCGGTTTAGAGTGG	this paper
	Steg8	forward	AAAGAAACTCCTTTTGG	
	Steg10*	forward	AAGTTATGATTGTATACC	

rrnS=small ribosomal subunit; *cob*=cytochrome b; *cox1*=cytochrome c oxidase subunit I
* used with Steg 5

4.2.2 Analysis and Annotation

Data were assembled into contigs using Sequencher™ version 4.7 (Gene Codes Corperation 2006). Protein-coding genes (PCG) were identified by the comparison of their amino-acid sequences using the blastx search BLAST algorithm implemented at the NCBI website (http://www.ncbi.nlm.nih.gov/) and by eye-comparison with other chelicerate sequences. Annotation of the N- and C-terminal ends of each PCG was checked by comparison with the translated amino acid sequences of homologous mt genes for other chelicerates in MEGA ver. 3.1 (Kumar et al. 2004); MEGA ver. 3.1 was also used for nucleotide composition analyses.

tRNA genes were initially identified by tRNAScan-SE (Lowe and Eddy 1997) using both the generalized mitochondrial and the specific nematode mitochondrial tRNA settings and by the program ARWEN (Laslett and Canback 2008). Genes found were adjusted by eye to identify structures more similar to those found in other chelicerates (Masta and Boore 2008). Non-coding regions were also searched by eye for stem-loop motifs which could form part of plausible tRNA-like structures but none were found. While it is possible that additional tRNAs are present but unannotated in the *Steganacarus* mt genome they cannot be identified at this time.

Candidate rRNA genes were identified by blastn searches and aligned with homologous rRNA genes from other chelicerates and insects. A secondary structure model for each gene was elucidated by comparison to the published rRNA secondary structures for *Apis* (Gillespie et al. 2006), *Manduca* (Cameron and Whiting 2008) and *Leptotrombidium* (Shao et al. 2006).

For phylogenetic analyses we used the concatenated amino acid dataset of all protein-coding genes previously used by Fahrein et al. (2007); amino acid sequences were choosen since a more conservative and more unambiguous alignment was possible. Sequences were aligned using the default settings in ClustalX 1.81 (Thompson et al. 1997); the gene order was adapted from Fahrein et al. (2007). Since most parts of the aligned amino acid sequences were unambiguous all parts were included in tree construction. Bayesian phylogenetic analysis was performed with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) using the mitochondrial genetic code for Metazoa and a haploid ploidy level with two independent runs of 1,000,000 generations and four chains each; trees were sampled every 500 generations. A majority consensus tree was generated using a burn-in of 200. Phylogenetic trees were also constructed using neighbour-joining (NJ) based on uncorrected p-distances as implemented in PAUP* 4b10 (Swofford 1999). Reliability of the branches was ascertained by bootstrap analyses with 10,000 replicates.

4.3 Results and Discussion

4.3.1 Mitochondrial Genome Organization

The mt genome of *S. magnus* is the first genome published for the large mite group of Sarcoptiformes. The complete mt genome is circular and consists of 13,818 bp (GenBank: EU935607). It encodes 13 protein-coding genes, two rRNA genes, six tRNA genes and includes a large non-coding control region as well as several size-variable intergene spacer regions (Fig. 4.1, Table 4.2). Genes are encoded on both strands which is typical for arthropods. Compared to the mt arrangement of the ancestral arthropod, which is conserved in *Limulus polyphemus* (Lavrov et al. 2000), the genome of *S. magnus* is slightly rearranged: instead of *nad1*-*rrnL*-*rrnS*-LNR-*nad2* (tRNAs excluded, underlined genes are coded on the minority strand) the gene arrangement is *nad2*-*rrnL*-*nad1*-*rrnS*-LNR (Fig. 4.1); all translocated genes have kept their original orientation. Furthermore, the genes for tRNA-Leu, -Trp, -Gln and -Ser are translocated to new positions with tRNA-Ser, -Leu, and -Trp also being inverted relative to the ground plan; only tRNA-His and -Pro remained in their ancestral location.

Table 4.2 Annotation of the mitochondrial genome of *Steganacarus magnus*

gene	location	size (bp)	size (aa)	A	C	G	T	AT skew[1]	AT/4*	CG skew	CG/4*	start codon	stop codon	anti codon	intergenic nucleotides
cox1	1-1534	1534	511	25.2	13.2	16.3	45.3	-0.29	-0.35	-0.11	-0.70	ATG	T	-	-
cox2	1535-2203	669	223	28.0	13.3	14.2	44.5	-0.23	-0.28	-0.03	0.17	ATG	stem loop	-	-
atp8	2204-2353	150	50	35.3	8.0	9.3	47.3	-0.15	-0.29	-0.08	-	ATT	TAG	-	-
atp6	2350-2999	650	216	26.2	12.6	7.5	53.7	-0.34	-0.57	0.25	0.00	ATA	TA	-	-
cox3	3000-3785	786	262	25.7	12.6	14.4	47.1	-0.29	-0.23	-0.07	-0.33	ATG	TAA	-	39
nad3	3825-4161	337	112	27.9	8.0	14.5	49.6	-0.28	-0.28	-0.29	-0.43	ATT	T	-	127
tRNA-Leu	4289-4339	51	-	-	-	-	-	-	-	-	-	-	-	TAA	-
nad5	4340-5927	1588	529	32.0	12.1	12.2	43.7	-0.15	0.02	-0.004	0.36	ATT	T	-	13
tRNA-His	5941-5997	57	-	-	-	-	-	-	-	-	-	-	-	GTG	-
nad4	5998-7260	1263	421	28.2	12.8	12.0	47.0	-0.25	-0.18	0.03	-0.22	ATT	TTT	-	-
nad4L	7256-7522	267	89	26.6	12.7	10.5	50.2	-0.31	-0.06	0.09	1.00	ATG	TAA	-	3
tRNA-Pro	7526-7581	56	-	-	-	-	-	-	-	-	-	-	-	AGG	2
nad6	7584-7988	405	135	32.8	13.1	9.9	44.2	-0.15	-0.16	0.14	0.20	ATT	TAA	-	-
cob	7988-9050	1063	354	29.6	15.9	12.8	41.7	-0.17	-0.15	0.11	-0.06	ATG	T	-	-
tRNA-Trp	9051-9106	56	-	-	-	-	-	-	-	-	-	-	-	TCA	26
nad2	9133-10089	957	319	37.2	12.5	10.1	40.1	-0.04	0.15	0.11	0.00	ATC	TA	-	46
tRNA-Gln	10136-10202	67	-	-	-	-	-	-	-	-	-	-	-	TTG	45
tRNA-Ser	10248-10304	57	-	-	-	-	-	-	-	-	-	-	-	TGA	-
rrnL	10305-11296	992	-	34.7	9.3	14.2	41.8	-0.1	-	-0.21	-	-	-	-	-
nad1	11297-12190	894	299	22.9	11.2	15.5	50.4	-0.38	-0.59	-0.16	0.16	ATG	TAA	-	-
rrnS	12191-12799	609	-	30.0	10.5	15.1	44.3	-0.19	-	-0.18	-	-	-	-	-
LNR	12800-13818	1019	-	37.3	12.0	12.4	38.4	-0.01	-	-0.02	-	-	-	-	-

[1] AT skew=(A-T)/(A+T) [2] CG skew=(C-G)/(C+G) * skews at 4-fold degenerated sites
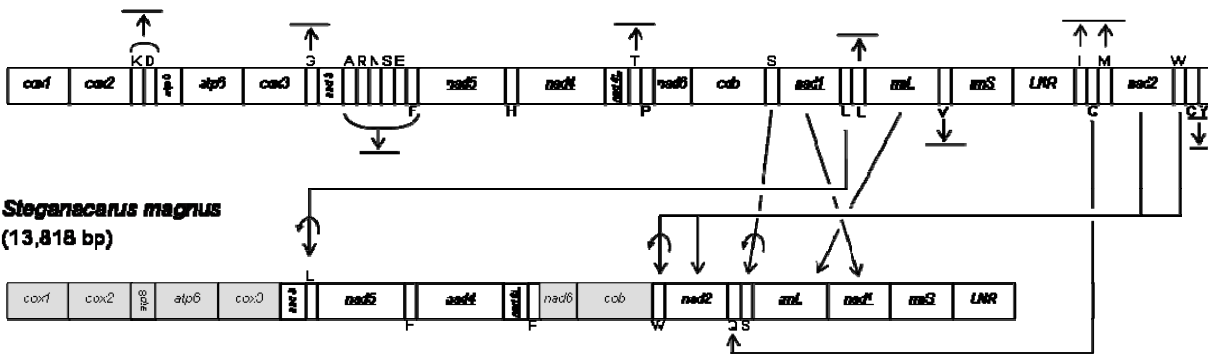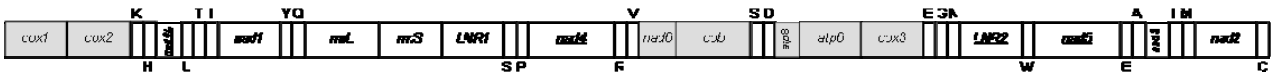
Figure 4.1 Mitochondrial genome organization of *Limulus polyphemus* (Lavrov et al. 2000)*, Steganacarus magnus* and two *Leptotrombidium* (Shao et al. 2006) species. Arrows indicate gene rearrangements (translocations, inversions and loss). Underlined genes and tRNAs with letters below are encoded in the opposite strand. Shaded boxes indicate shared gene boundaries between *S. magnus* and *Leptotrombidium*. Gene abbreviations: *cox1-3*: cytochrome oxidase subunits 1-3; *atp6,8*: ATP synthase subunit 6,8; *nad1-6, 4L*: NADH dehydrogenase subunit 1-6, 4L; *cob*: cytochrome b; *rrnL*: large ribosomal subunit; *rrnS*: small ribosomal subunit; LNR: large non-coding control region. tRNA genes are symbolised by the one-letter code of their amino acid: I=Isoleucine; Q=Glutamine; M=Methione; W=Tryptophan; C=Cysteine; Y=Tyrosine; K=Lysine; D=Aspartate; G=Glycine; A=Alanine; R=Arginine; N=Asparagine; S=Serine; E=Glutamate; F=Phenylalanine; H=Histidine; T=Threonine; P=Proline; L=Leucine; V=Valine.

Rearrangements of genes in mt genomes are useful markers for deep splits within phylogenies (Boore 1999) although the relative frequency of rearrangements varies among lineages (Dowton et al. 2002). For example, *Drosophila* (Insecta) and *Daphnia* (Crustacea) share the same mt rearrangement although they diverged 400-500 million years ago (Dowton et al. 2002) and the mt genome of soft ticks remained unchanged for a similar time period (Shao et al. 2004). In contrast, gene arrangements in mt genomes of lice are highly variable (Covacin et al. 2006, Cameron et al. 2007), and in the mite genus *Leptotrombidium* they even differ between species (Shao et al. 2006). In general, in mt genomes of Acari a number of rearrangements have occurred. While the ground plan is retained in soft- and prostriate-hard ticks (Black and Roehrdanz 1998), the arrangement of mt genes in Mesostigmata (Parasitiformes), especially *Metaseiulus occidentalis*, is strongly derived. Notably, the *Metaseiulus* mt genome is the largest within chelicerates, even though *nad3* and *nad6* were lost, due to the duplication of many of the remaining genes (Jeyaprakash and Hoy 2007).

Fossils of oribatid mites are known from Devonian sediments and molecular studies suggest that their origin may predate this record by ~180 mya (I. Schaefer, unpublished data). Further,

oribatids probably diverged from other acariform mites, such as *Leptotrombidium*, about 570 mya ago (I. Schaefer, unpublished data). The mt genomes of *Leptotrombidium* and *S. magnus* differ markedly; they only share the gene boundaries *cox1-cox2*, *nad6-cob* and *atp8-atp6-cox3* (Fig. 4.1). However, since these boundaries are also present in the ground plan of chelicerate mt genomes there are no derived arrangements supporting the common ancestry of Acariformes.

4.3.2 Protein-coding Genes and Nucleotide Composition

All 13 protein-coding genes typically present in arthropods could be identified in the mt genome of *S. magnus*. They start with the mt start codons ATT, ATG, ATA and ATC (Table 4.2). Six genes (*cox1, atp6, nad3, nad5, cob, nad2*) terminate with incomplete stop codons (T or TA; Table 4.2) while all others terminate with either TAA, TAG or TTT (*atp8, cox3, nad4, nad4L, nad6, nad1*; Table 4.2). In *cox2*, which is flanked by two other protein-coding genes (*cox1* and *atp8*), no stop codon is present; as shown for sets of protein-coding genes in *Anabrus simplex* (Orthoptera, Insecta) a stem-loop formation in the secondary structure of the transcribed polycistronic mRNA probably functions as terminator (Fenn et al. 2007).

The percentage nucleotide composition of the mt (+)-strand is A=36.5, C=13.2, G=12.2 and T=38.1. Therefore, there are approximately equal numbers of each complementary nucleotide pairs (A:T, G:C) but a strong AT-bias is present. The pattern for all protein-coding genes is also strongly AT-biased but with a much higher T than A content (Table 4.2). Skews calculated for neutral fourfold degenerate sites do not indicate consistent asymmetric strand bias (Table 3.2). Genes encoded on the (+)-strand show either neutral (*atp6, nad2*), positive (*cox2, nad6*) or negative CG-skew (*cox1, cox3, nad3, cob*). The majority of genes encoded on the (-)-strand are positive CG-skewed (*nad5, nad4L, nad1*) (Table 4.2). The AT skew at fourfold degenerate sites is only positive for *nad2* and *nad5*, but negative for all other protein-coding genes (Table 4.2).

A reversal of the strand bias is usually explained by an inversion of the control region (LNR) which contains the origin of replication and translation (Boore 1999, Zhang and Hewitt 1997). During replication the two different strands ((-)- and (+)-strand) are exposed to different mutational pressures, typically causing distinct skews since one strand remains longer in the single-stranded state than the other (Hassanin et al. 2005). Therefore, the LNR likely functions as a key region for determining strand bias and an inversion results in a complete reversal of the strand nucleotide composition over time (Hassanin et al. 2005). In *S.*

magnus most genes encoded on the (+)-strand show a negative CG-skew at fourfold degenerate third codon positions which is inverted to the common pattern and probably indicates a reversal of the LNR. On the other hand, the presence of neutral or positive skewed genes may indicate that this reversal is of recent origin and consequently the process of inverting nucleotide skew is not completed so far.

The absence of a distinct strand bias can also be explained by the recent inversion of single genes which homogenize general patterns of asymmetry (Hassanin et al. 2005, Hassanin 2006). However, since no inversions of protein-coding genes were found in *S. magnus*, the absence of a distinct asymmetrical skew in the genome awaits explanation.

4.3.3 Putative Control Region

The major non-coding region (LNR), which presumably functions as the mitochondrial control region, is 1019 bp in length and located between *rrnS* and *cox1* (Fig. 4.1). There are additional non-coding intergenic regions ranging in size from 2-127 bp. These regions were blasted and checked for tRNA genes but could not be assigned to any functional gene.

The relative location of the LNR varies greatly among invertebrates with the ancestral pattern of arthropods being *rrnS*-LNR-tRNA-Ile (Wolstenholme 1992, Boore 1999). It also varies in length mostly due to different numbers of sequence repeats, and length heteroplasmy within individuals has also been recorded (Zhang and Hewitt 1997). There was no length heteroplasmy in *S. magnus* but two inverted sequence repeat regions, each with a length of 190 bp, were present at positions 133-322 (repeat 1) and 830-1019 (repeat 2). The region before repeat 1 contains four stem-loop structures at positions 3-26, 32-56, 60-76 and 82-129 (Fig. 3.2); the region between the two repeats contains 10-12 stem-loop structures depending on differences in folding. None of the hairpin structures is associated with a poly-A or poly-T stretch which would mark the origin of replication (OR) in insect mt genomes (Zhang and Hewitt 1997) or with a TATA- or GA(A)T-motif as present in other arthropods (Black and Roehrdanz 1998). Since the OR typically is close to the gene of *rrnS* and repeat regions are posterior to it (Zhang and Hewitt 1997), we assume the first region to be the OR of the mt genome of *S. magnus*.
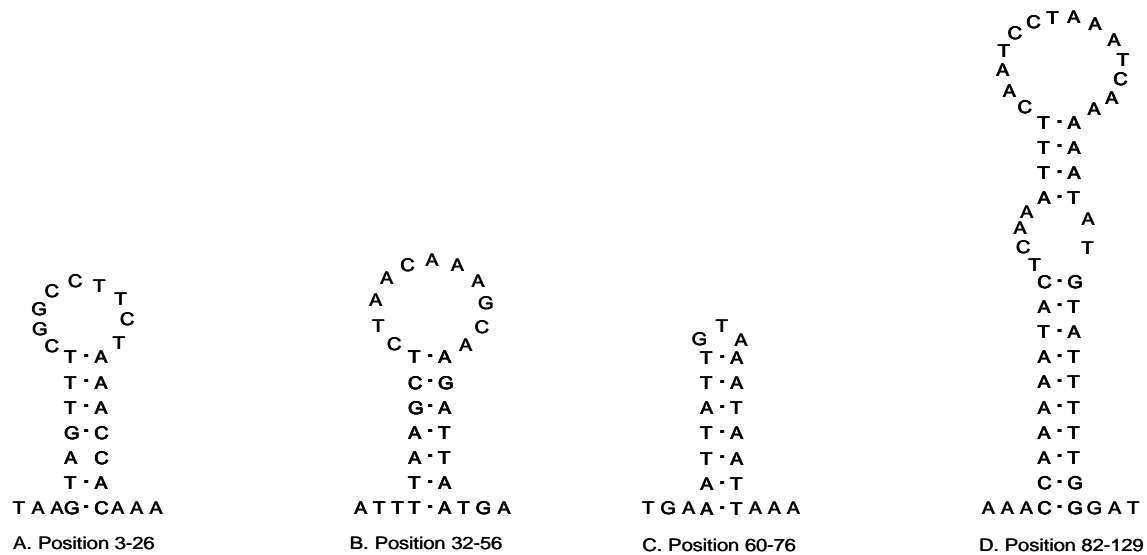
Figure 4.2 Possible stem-loop structures of the origin of replication in the putative control region within the mitochondrial genome of *Steganacarus magnus*.

In the mesostigmate mite *M. occidentalis* the stem-loop structure which probably represents the OR comprises only AT nucleotides but does not have any similarity to sequences from other chelicerates (Jeyaprakash and Hoy 2007). The LNR of the mite *V. destructor* includes several repetitions of a 157-bp motif and eleven sites of potential stem-loop structures have been identified close to it (Navajas et al. 2002). In the genus *Leptotrombidium*, the closest relative to *S. magnus* for which an mt genome is sequenced, the LNR is duplicated (and one is inverted) in *L. akamushi* and *L. deliense* and four copies are present in *L. pallidum* (Shao et al. 2005, 2006). Although mt LNR possess several distinct structural features (e.g., high AT-content, concerted evolution of tandem repeats, stem-loop structures), their use for evolutionary studies is limited by the high variability of the sequence and the possibility of length heteroplasmy within individuals (Zhang and Hewitt 1997).

4.3.4 rRNA Genes

The large subunit of the rRNA (*rrnL*) is 992 bp in length (Table 3.2) which is a bit shorter than in other mite species (e.g., about 1,014 bp in *Leptotrombidium* (Shao et al. 2006), 1,212 bp in *Carios capensis*, Shao et al. 2004). The 5`-end starts three nucleotides apart from *nad1* (encoded on the (-)-strand). The 3`-end was difficult to assign since parts of the last stem-loop structure can be included in the gene for tRNA-Ser (Fig. 4.3A). The gene for the small subunit (*rrnS*) is 609 bp in length and located between *nad1* and the control region (Table 4.2, Fig. 4.3B). Both ribosomal subunits have a similar AT content to the protein-coding genes and both are encoded on the (-)-strand as in most species of arthropods and chelicerates (e.g.,

Limulus, Araneae, Ricinulei, Pycnogonida, Scorpiones and Ixodidae) (Black and Roehrdanz 1998, Lavrov et al. 2000, Dávila et al. 2005, Podsiadlowski and Braband 2006, Fahrein et al. 2007). In contrast, in the mt genome of the closely related genus *Leptotrombidum* as well as in *M. occidentalis* both ribosomal RNA genes are encoded on the (+)-strand (but *L. pallidum* has a duplicated *rrnL* gene on the (-)-strand) (Shao et al. 2005, 2006, Jeyaprakash and Hoy 2007).
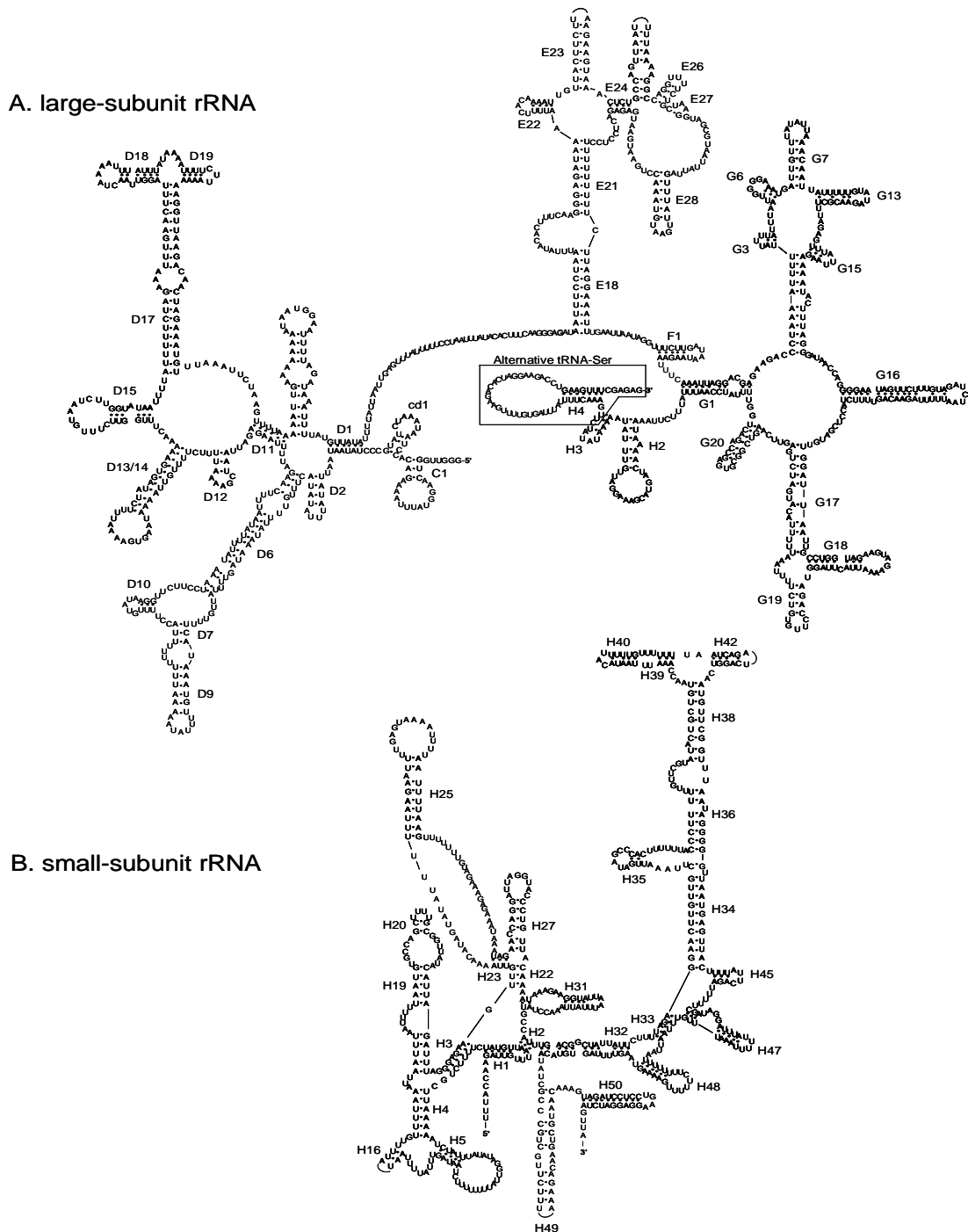

Figure 4.3 Putative secondary structure of the large- (A) and small-subunit ribosomal RNA (B) of *Steganacarus magnus*. Dots indicate complementary nucleotide bonds.

The secondary structures of the rRNA genes in *Steganacarus* differ from those published for *Leptotrombidium* (Shao et al. 2006) but are more similar to those published for insects (e.g., Gillespie et al. 2006, Cameron and Whiting 2008). The *Leptotrombium rrnS* gene lacks helices 1, 2, 4, 5, 7, 8 and 22, and the compound helices 19-20-21 and 39-40-42 as depicted have a very different secondary structure from that found in other arthropods (Shao et al. 2006). In contrast, most of the helices found in arthropod *rrnS* genes are present in *Steganacarus*, but helices 7 and 8 are absent. However, helix 5 has a large loop such that there is limited difference in sequence length in this region between *Steganacarus* and arthropods which possess these helices; this is similar to the structure found in Hymenoptera (Gillespie et al. 2006). Helix 16 is greatly shortened relative to other species, consisting of just 2 paired bases compared to up to 8 in *Leptotrombidium* (Shao et al. 2006). The loop regions between helices 38 and 39-40-42 are also greatly reduced consisting of just 4 bp on the 5' side and 2 bp on the 3'; this is in contrast to insects where these loops consist of a dozen or more bases on each side.

Similarly, the *rrnL* gene of *Leptotrombidium* entirely lacks domain I (helices B12, B20), helices C1, cd1, D1 and H3 and the structure of the compound helices D17-D18-D19 is unique to *Leptotrombidium*. The *Steganacarus rrnL* secondary structure is again more similar to that of other arthropods; domain I is absent but helices C1, cd1 and D1 are present and D17-D18-D19 has a more canonical structure. Helix G3 is greatly reduced, consisting of just 2 paired stem bases and 3 loop bases, relative to both *Leptotrombidium*, 6 stem and 3 loop bases, and insects, up to 20 stem and 22 loop bases in *Manduca* (Cameron and Whiting 2008). The 3' end of the *rrnL* molecule is ambiguous, bases 10346-10290 either form the tRNA gene for Serine or they form helices H3 and the 3' side of H2. Without an analysis of the mature transcribed genes it is not possible to determine which form is more likely in the mature molecule. Helix H3 is not present in all arthropod *rrnL* genes found in Hymenoptera and Lepidoptera but is absent from Coleoptera and *Leptotrombidium* and its function in the mature rRNA is unclear. Accordingly, we present both possibilities, helices H2 and H3 are included in Figure 4.3 with the region which potentially forms the tRNA shown in a box, while tRNA-Ser is included in Figure 4.1 and Table 4.2.

4.3.5 tRNA Genes

Out of the 22 tRNA genes typically present in arthopods only six are present in *S. magnus*. Out of these six tRNAs, only two have kept their original position (tRNA-His between *nad5*

and *nad4*, tRNA-Pro between *nad4L* and *nad6* with tRNA-Thr missing) while all others are translocated relative to the ground plan. Further, tRNA-Leu overlaps the *nad5* gene by four nucleotides and tRNA-Trp overlaps with the 3'-end of *cob* by 16 nucleotides (Table 3.2); as described above, tRNA-Ser presumably forms part of the *rrnL* gene at the 3'-end (Fig. 3.3A). Remarkably, although 16 tRNA genes have been lost, the mt genome size of *S. magnus* (13,818) is comparable to those of *Leptotrombidium deliense* (13,731) and *L. akamushi* (13,698) (Shao et al. 2005, 2006) which is due to a larger LNR and more intergenic spacer regions.

All present tRNAs differ remarkably from the typical cloverleaf structure: in tRNA-Leu and –Ser the D-stem and -loop are missing and the TψC-stem is short with only two complementary base pairs (Fig. 3.4). In contrast, the homologous tRNA for leucine in *L. pallidum* and *M. occidentalis* lacks the TψC- instead of the D-arm (Shao et al. 2005, Jeyaprakash and Hoy 2007); while the structure of tRNA-Ser in *L. pallidum* is similar to that of *S. magnus*, it differs in *M. occidentalis* which lacks the TψC-arm.

The tRNAs for histidine, proline, tryptophan and glutamine lack the TψC-stem and -loop but possess a complete DHU-stem with a smaller loop in tRNA-Trp and a larger in tRNA-Gln (Fig. 4); this lack of the TψC-arm is also present in *L. pallidum* (Shao et al. 2005) and *M. occidentalis* (Jeyaprakash and Hoy 2007).

All tRNAs present in *S. magnus* are shorter than the average length of arthropod tRNAs (about 66 bp) and are highly modified. The loss of the TψC-arm and the replacement by a size-variable loop was first recognized in nematode tRNAs (Wolstenholme et al. 1987) and can also be found in *M. occidentalis* (Jeyaprakash and Hoy 2007), *L. pallidum* (Shao et al. 2006) and other chelicerates including scorpions (Dávila et al. 2005) and spiders (Masta and Boore 2004). Further, a study on truncated tRNAs in Arachnida revealed that the tRNAs for proline, histidine and glutamine have experienced the greatest number of independent TψC-arm losses in arachnids whereas TψC-arm loss in genes for arginine, lysine and methione have occurred only once and is synapomorphic for opisthothele spiders (Masta and Boore 2008). However, arachnids seem to have a compensatory mechanism that allows truncated tRNAs to function during translation and the interaction with the ribosome (Masta and Boore 2008).
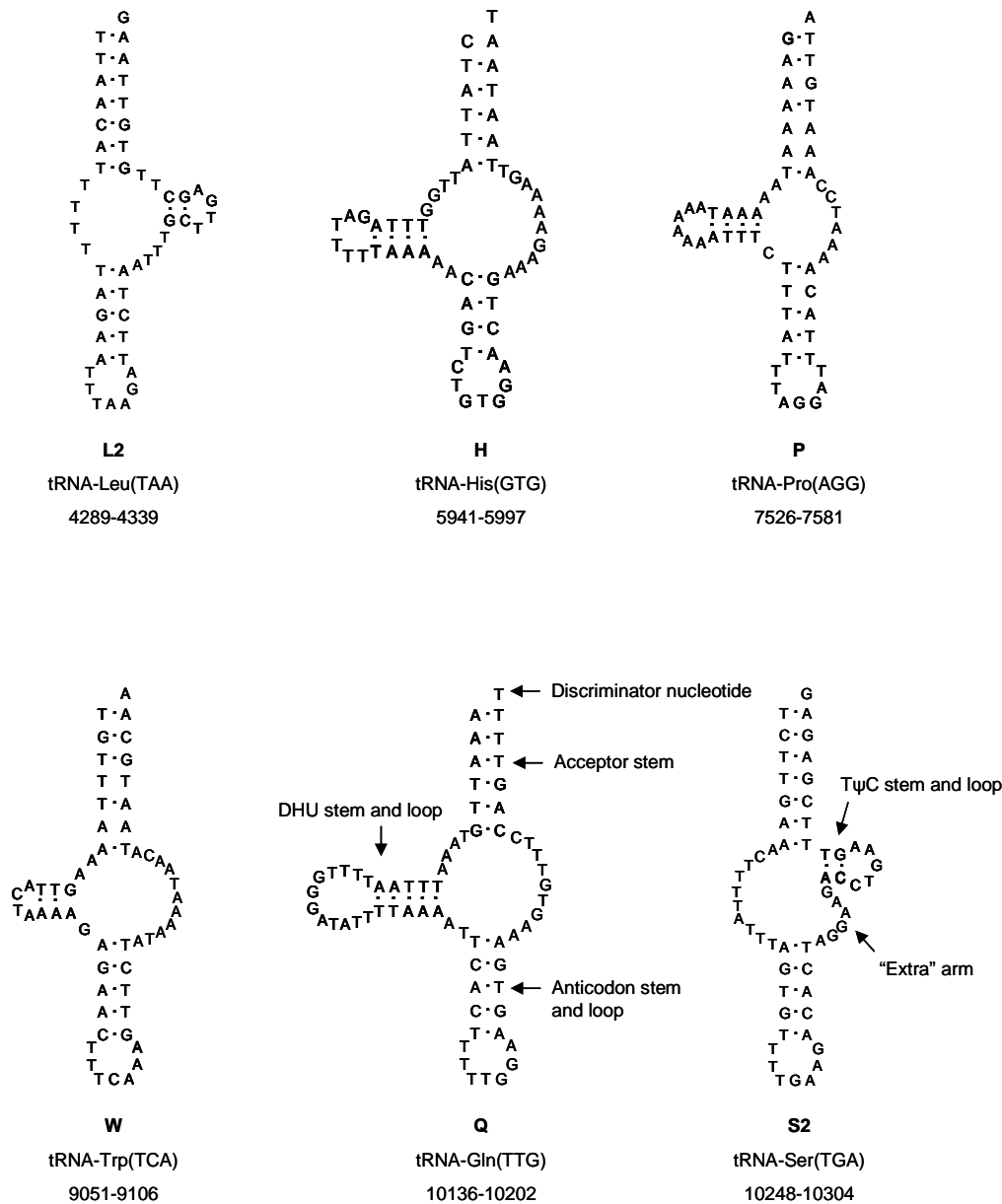
Figure 4.4 tRNA secondary structures of *Steganacarus magnus*. tRNAs are labelled with the abbreviations of their corresponding amino acids. Numbers indicate their genomic position.

While the two rRNA genes are present in all eukaryotic genomes (Adams and Palmer 2003), the number of tRNA genes varies markedly among taxa. No tRNAs are present in the protists *Plasmodium falciparum* (Apicomplexa) and *Trypanosoma brucei* (Kinetoplastida) (Gray et al. 1998) but up to 27 are present in *Reclinomonas americana* (Chlorophyta) (Lang et al. 1997). The loss of mt tRNA genes may be facilitated by the fact that the proteins with which they interact during translation in the mitochondria are encoded in the nucleus (Clayton 1992). Furthermore, the mutation rate of mt encoded tRNA genes is about five-fold higher than that of nuclear genes which experience strong purifying selection (Lynch 1996, 1997). Since

tRNAs are encoded in the nucleus anyway and selective pressure favours the reduction of mt genome size (Adams and Palmer 2003), it is likely that *S. magnus* simply lost its tRNA genes instead of transferring them to the nucleus. However, the differences in the genetic code between nucleus (universal code) and mitochondria (invertebrate mt code) would probably argue for two sets of tRNAs or alternatively some modifications of the tRNA-amino acyl transferases are needed to treat each tRNA isotype differently in the two different compartments.

4.3.6 Phylogenetic Analysis

Phylogenetic analyses performed on a concatenated dataset of all protein-coding genes (amino acid sequences) included three outgroup species (*Daphnia pulex* (Crustacea), *Penaeus monodon* (Crustacea), *Lithobius forficatus* (Myriapoda)), one species of Solifugae, Xiphosura, and Ricinulei, two Scorpiones species, four species of Araneae and Acariformes and twelve species of Parasitiformes (Fig. 4.5). All major groups, notably Parasitiformes, Acariformes, Araneae and Scorpiones, were monophyletic and supported by moderate (Araneae) to high (Acariformes, Scorpiones) support values. The sister-group relationship of Scorpiones and Araneae was only supported by posterior probablities (Fig. 4.5).

The newly sequenced *S. magnus* formed the closest relative of the prostigmate mite genus *Leptotrombidium* which was expected following previous studies (Walter and Proctor 1999). As in previous studies (Fahrein et al. 2007) the ricinuleid species *Pseudocellus pearsei* clustered as sister-group of Acariformes but was only supported by Bayesian posterior probabilities (Fig. 4.5). In contrast to the study of Fahrein et al. (2007) the Acariformes/ Ricinulei clade did not form the sister-group of Araneae but of Parasitiformes; however, the support for both possibilities is similarly weak (see Fahrein et al. 2007).

For a broader investigation of chelicerate phylogeny mt DNA data of key taxa such as Opiliones, Pseudoscorpiones, Palpigradi, Uropygi and Amblypygi are missing. Even for a complete study of Acari phylogeny many taxa remain to be sampled; no complete mt genome is available for Astigmata, Endeostigmata or Opilioacarida.

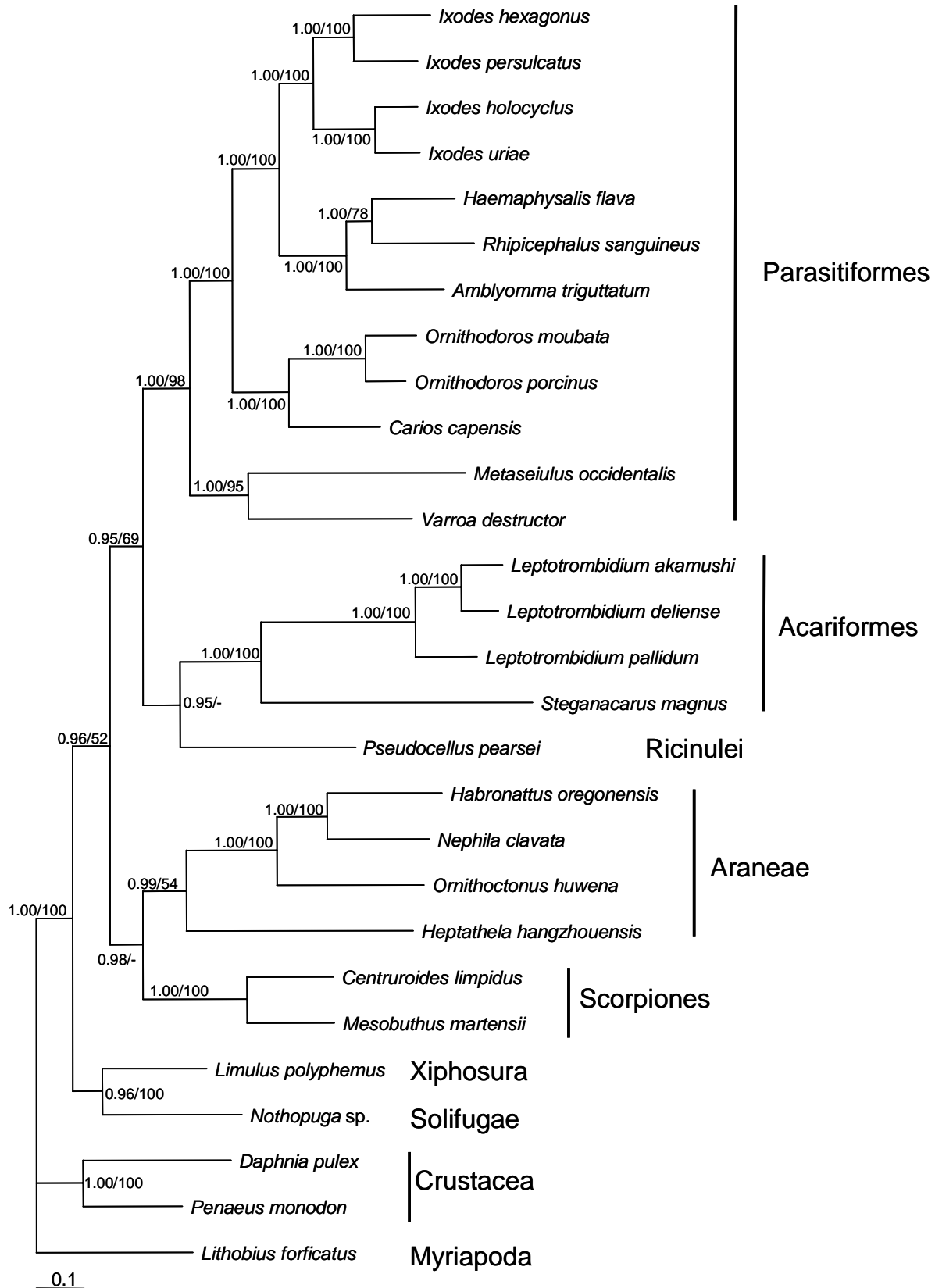**Figure 4.5** Bayesian tree phylogeny. Bayesian tree of a concatenated amino acid dataset of all mitochondrial protein-coding genes in the order *atp8, atp6, cox1, cox2, cox3, cob, nad1, nad2, nad3, nad4, nad4L, nad5* and *nad6* of 28 arthropod species. Myriapoda are used as outgroup. Numbers at nodes represent posterior probabilities for Bayesian analyses and bootstrap support values for neighbour-joining.

While using mt DNA for phylogenetic studies, a reversal of nucleotide strand bias or a reversal of nucleotide bias of single genes (caused by gene inversion or the inversion of the control region) can be misleading for phylogenetic relationships by causing long-branch attraction artifacts (Hassanin 2006). Further, since mt genomes evolve at higher rates than the nuclear genome (Li 1997), saturation of the phylogenetic signal can also be problematic in deep split phylogenies. Species exhibiting unusual genomic features such as complicated gene arrangements or multiple control regions as well as species with small body sizes or parasitic lifestyles are also vulnerable to long branches (Hassanin 2006). Collectively, Acari exhibit all these features and so a heavy sampling effort will be necessary to reliably use mt genomics in mite phylogenetic studies.

4.3.7 Conclusions

The first complete mt genome for an oribatid mite (*S. magnus*, Oribatida, Acariformes) is a typical circular molecule and comprises all protein-coding genes typically present in Metazoa and two genes for the ribosomal subunits. Compared to the putative ground pattern of arthropods the genome is rearranged, affecting the genes *nad1, rrnL, rrnS*, and *nad3*. Further, the genome of *S. magnus* lacks all but six tRNAs, but is comparable in size with genomes of the closely related genus *Leptotrobidium*; the close relationship of both of these acariform mites was confirmed by phylogenetic analyses using all mt protein-coding genes. Since mt gene arrangements vary strongly among mite species and no full mt genomes are available for Astigmata, Endeostigmata and Opilioacarida, the use of mtDNA rearrangements for phylogenetic studies is limited at present, especially since gene rearrangements in the studied Acari species do not show a distinct phylogenetic pattern. However, the growing number of published genomes and the better understanding of rearranging mechanisms make mt genomes promising markers for resolving phylogenetic relationships of acarine lineages and Chelicerata in general.

Is the Complete Mitochondrial Genome of the Parthenogenetic Oribatid Mite *Platynothrus peltifer* Heteroplasmic?

$\mathbb{G}$enes encoded in the circular DNA molecule of mitochondria are witnesses of the bacterial ancestry of this organelle. Compared to the nuclear genome mitochondrial (mt) genes are characterized by a higher mutation rate due to their vicinity to the respiratory chain and the occurrence of free radicals, making them useful markers for investigating recent evolutionary changes. In contrast, the arrangement of mitochondrial genes can elucidate deep split speciations and is commonly used in phylogenetic studies. Further, the gene for the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) is often used as barcoding marker for the molecular identification of species. High intraspecific variation in the partheogenetic oribatid mite *Platynothrus peltifer* argues against the use of *cox1* as molecular barcoding gene in oribatids. To exclude that the high intraspecific *cox1* variation in *P. peltifer* is due to gene duplication the complete mt genome was sequenced and the presence of small-scale heteroplasmy, i.e. the intraindividual presence of different haplotypes, was investigated. Further, the local genetic variability of *cox1* at one forest site was investigated and the sequences were compared to those previously published. We expected that the genome of *P. peltifer* is heteroplasmic due to gene duplication of *cox1* and that different haplotypes co-occur at a single location. The complete mt genome of *P. peltifer* consists of 14,891 bp and it is composed of all genes typically found in Metazoa except nine tRNAs (13 protein-coding genes, 13 tRNA genes, two rRNA genes). The gene arrangement differs to the hypothetical ground plan as conserved in *Limulus polyphemus* (Xiphosura) and to the arrangement in *Steganacarus magnus* (Oribatida) mainly concerning the positions of *nad1* and *nad2*. In contrast to our hypothesis, no gene duplication of *cox1* was found but single nucleotide polymorphisms (SNPs) caused a low level of intraindividual heteroplasmy. However, different *cox1* haplotypes could be sequenced at a single location indicating that different clone lineages coexist and migration events are rare but detectable.

5.1 Introduction

Mitochondria still contain DNA reflecting their former existence as free-living bacteria (Wolstenholme 1992). The mitochondrial DNA (mtDNA) typically encodes for 13 proteins, 22 transfer RNAs (tRNA), two ribosomal RNAs (rRNA; large (*rrnL*) and small (*rrnS*) ribosomal subunit) and contains a non-coding control region (LNR) of variable length (Wolstenholme 1992, Boore 1999). Due to their vicinity to the respiratory chain within the mt membrane and the loss of repair mechanisms mitochondrial (mt) genes are characterized by a higher mutation rate as compared to nuclear genes which makes them useful markers for investigating recent evolutionary changes, i.e. in population genetic studies (Taanman 1999). On the other hand, mt gene rearrangements can also be used for elucidating deep phylogenetic splits and therefore are commonly used for phylogenetic studies (Boore and Brown 2000, Fahrein et al. 2007, Domes et al. 2008).

The terminal complex of the mt respiratory chain located in the inner membrane of the organelle is build by the cytochrome c oxidase complex (Kadenbach et al. 1983). Three subunits of this holoenzyme (Cox1-3) are encoded in the mtDNA, ten more are encoded in the nucleus (Wu et al. 2000). Cytochrome c subunit I (Cox1) is the largest subunit of the complex consisting of 12 transmembrane helices and three redox centers (Kadenbach et al. 1987, Capaldi 1990). Usually, for molecular barcoding and phylogenetic analyses about 650 basepairs (bp) of the 5`-end of the *cox1* gene are used (Folmer et al. 1994, Hajibabaei et al. 2007). The interspecific divergence of this segment is usually higher than 2%, the intraspecific divergence lower than 2% (Avise 2000); the limit for separating species is defined as 2.7% (Hebert et al. 2004a, b). In contrast to this rule, the parthenogenetic oribatid mite *Platynothrus peltifer* showed a genetic diversity of about 2% within clades collected at 16 sites in North America, Europe and Asia, but an average corrected genetic diversity of 56% between clades (Heethoff et al. 2007a). This high intraspecific variability of the partial *cox1* gene was explained by the high age of the species (~ 100 million years) and by several cryptic speciations (Heethoff et al. 2007a).

However, the unusually high *cox1* diversity within the species *P. peltifer* may also be explained by heteroplasmy due to gene duplication or the presence of a (nuclear) pseudogene (Bensasson et al. 2001). Heteroplasmy reflects the presence of a mixture of more than one type of an organellar genome within a cell or individual. Since eukaryotic cells contain many hundreds of mitochondria with hundreds of copies of mtDNA, it is possible and indeed very

common that mutations affect only some of the copies while the remaining ones remain unaffected (Van Leeuwen et al. 2008).

Soil-dwelling oribatid mites are ubiquitous microarthropods playing an important role in the decomposition of litter materials in temperate ecosystems (Lussenhop 1992). Previous molecular studies explored the genetic variability among and within taxa, molecular phylogenies and the age of the group (Salomone et al. 1996, 2002, Maraun et al. 2003, 2004, Schaefer et al. 2006, Domes et al. 2007b, c, Heethoff et al. 2007a, Laumann et al. 2007). Established genes for studies on oribatid mites include the mt *cox1* gene, the elongation factor 1 alpha (*ef1α*), the heat shock protein 82 (*hsp82*) and the ribosomal 18S as well as the D3 region of the 28S rDNA. So far, for oribatid mites only the complete mt genome of the sexually reproducing species *Steganacarus magnus* is available (Domes et al. 2008). This genome is characterized by several gene rearrangments and the unusual loss of 16 tRNA genes, but there is no evidence for gene duplication or recombination events.

In order to elucidate if gene duplication causes the high genetic diversity of the mt *cox1* in the parthenogenetic oribatid mite species *P. peltifer* the complete mt genome was sequenced using long-PCRs. We further investigated 18 clones of a partial *cox1* fragment to test for heteroplasmy in a single individual. In addition, we sequenced 540 bp of the mt *cox1* gene of eight specimens of *P. peltifer* from the Kranichstein forest and compared those with available *cox1* sequences from GenBank published by Heethoff et al. (2007a) to clarify if different haplotypes are present at a restricted location.

5.2 Materials and Methods

5.2.1 DNA Processing

Specimens of *P. peltifer* were collected from the Kranichstein forest located about 8 km northeast of Darmstadt, Germany. Animals were extracted from leaf litter by heat using a modified Kempson extractor (Kempson et al. 1963), preserved in 75% ethanol and stored at -20°C until usage. Total DNA was extracted from single specimens using the DNeasy Tissue kit (Qiagen) following the manufactures' protocol (but final elution of DNA was in 40 µl instead of 200 µl). Polymerase chain reactions (PCR) were performed for the small ribosomal subunit (*rrnS*), the cytochrome b (*cob*) and cytochrome c oxidase I (*cox1*) genes using the primers 12SA and 12SB, CB3 and CB4 and COIarch1 and COIarch2, respectively (Table 5.1). PCR reaction mixtures contained 12.5 µl HotStarTaq MasterMix (Qiagen), 0.7 µl of each primer (100 pmol/µl), and 4 µl DNA (unquantified) in a total volume of 25 µl.

Amplification conditions included an initial activation step at 95°C for 15 min followed by 34 cycles of 95°C for 45 s; 44°C (*rrnS*), 50°C (*cob*) or 51°C (*cox1*) for 1 min; 72°C for 55 s and a final elongation at 72°C for 10 min. PCR products were visualized on a 1% agarose gel, purified using the QIAquickPCR Purification kit (Qiagen) and directly sequenced by Macrogen Inc. (Seoul, South Korea).

For sequencing the complete mitochondrial genome, initial primers for long PCR were designed from the previously obtained sequences of *rrnS*, *cob* and *cox1* (Table 5.1). Long PCR amplifications were performed using 1 µl elongase (Invitrogen), 1 µl buffer A, 4 µl buffer B, 2.5 µl dNTPs (25 mM), 1 µl of each primer (10 mM) and 1-2 µl of DNA (unquantified) with the following conditions: 92°C for 2 min; 40 cycles of 92°C for 30 s, 50°C for 30 s, 68°C for 12 min and a final extension step of 68°C for 20 min. PCR products were visualized on 1% agarose gels and purified using Millipore Montage vacuum purification plates. Sequencing was performed using ABI BigDye 3.1 dye terminator technology with an ABI capillary sequencer at the John Curtin Medical School sequencing centre (Australian National University). Cycle sequencing reactions contained 1 µl BigDye, 0.5 µl of the primer (25 mM) and 0.5-1.0 µl template (in a total volume of 3 µl) and amplification conditions were 28 cycles of 94°C for 10 s, 50°C for 5 s and 60°C for 4 min. Within each long PCR product the complete double-stranded sequence was determined by primer walking (a list of all primers is given in Appendix 4).

To test for intraindividual heteroplasmy, a 540 bp fragment of *cox1* obtained with primers COIarch1 and COIarch2 was cloned using the Qiagen PCR Cloning Kit (Qiagen, Hilden, Germany). The sticky-end ligation mixture contained 0.5 µl pDrive Cloning Vector (50 ng/µl), 2 µl purified PCR amplification product and 2.5 µl ligation mastermix (2x) in a total volume of 5 µl. 2 µl of the ligation mixure were transformed to *E. coli* Qiagen EZ Competent Cells via heatshock. Positive clones were selected by blue/white screening, plasmids were purified using the FastPlasmid™ Mini Kit (Eppendorf, Hamburg, Germany) and sequenced by Macrogen Inc. (Seoul, South Korea).

Table 5.1 Initial primers for PCR amplifications of the mitochondrial genome of *Platynothrus peltifer*

region	primer pair	orientation	primer sequence (5'-3')	reference
rrnS	12SA	forward	TACTATGTTACGACTTAT	Skerratt et al. 2002
	12SB	reverse	AAACTAGGATTAGATACCC	
cob	CB3	forward	GAGGAGCAACTGTAATTACTAA	Pons 2006
	CB4	reverse	AAAAGAAARTATCATTCAGGTTGAAT	
cox1	COIarch1	forward	GGTCAACAAATCATAAAGAYATYGG	Folmer et al. 1994
	COIarch2	reverse	TAAACTTCAGGGTGACCAAAAAATCA	mod. by RH Thomas

rrnS=small ribosomal subunit; *cob*=cytochrome b; *cox1*=cytochrome c oxidase subunit I

5.2.2 Analysis and Annotation of the Mitochondrial Genome

Data were assembled into contigs using Sequencher^{TM} version 4.7 (Gene Codes Corperation 2006). Protein-coding genes (PCG) were identified by the comparison of their amino-acid sequences using the blastx search BLAST algorithm implemented at the NCBI website (http://www.ncbi.nlm.nih.gov/) and by eye-comparison with other chelicerate sequences. Annotation of the N- and C-terminal ends of each PCG was checked by comparison with the translated amino acid sequences of homologous mt genes for other chelicerates in MEGA ver. 3.1 (Kumar et al. 2004); MEGA ver. 3.1 was also used for nucleotide composition analyses.

tRNA genes were initially identified by tRNAScan-SE (Lowe and Eddy 1997) using both the generalized mitochondrial and the specific nematode mitochondrial tRNA settings and by the program ARWEN (Laslett and Canback 2008). Genes found were adjusted by eye to identify structures more similar to those found in other chelicerates. Candidate rRNA genes were identified by blastn (http://www.ncbi.nlm.nih.gov/) searches and aligned with homologous rRNA genes from other chelicerates and insects; the secondary structure analysis remains to be done.

5.3 Results
5.3.1 Mitochondrial Genome

The complete mt genome of the parthenogenetic oribatid mite *P. peltifer* is circular and consists of 14,891 bp. Thirteen protein-coding genes, two rRNA genes, 13 tRNAs and a large non-coding control region (LNR; not finally annotated yet but located between *rrnS* and *cox*) are present; the genome further contains several size-variable intergene spacer regions (Fig. 5.1, Table 5.2). Genes are encoded on both strands and no gene duplication was found.

Compared to the hypothetical ground plan present in *Limulus polyphemus* (Lavrov et al. 2000) the genome of *P. peltifer* differs at the position of *nad2*: instead of <u>*nad1*</u>-<u>*rrnL*</u>-<u>*rrnS*</u>-LNR-*nad2* (tRNAs excluded, underlined genes are coded on the minority strand) the gene arrangement of *P. peltifer* is <u>*nad1-nad2*</u>-<u>*rrnL*</u>-<u>*rrnS*</u>-LNR (Fig. 5.1). Furthermore, the tRNA genes for leucine, tryptophan, cysteine, isoleucine and glutamine are translocated to new positions and the genes for serine and glutamate switched positions (Fig. 5.1, Table 5.2).
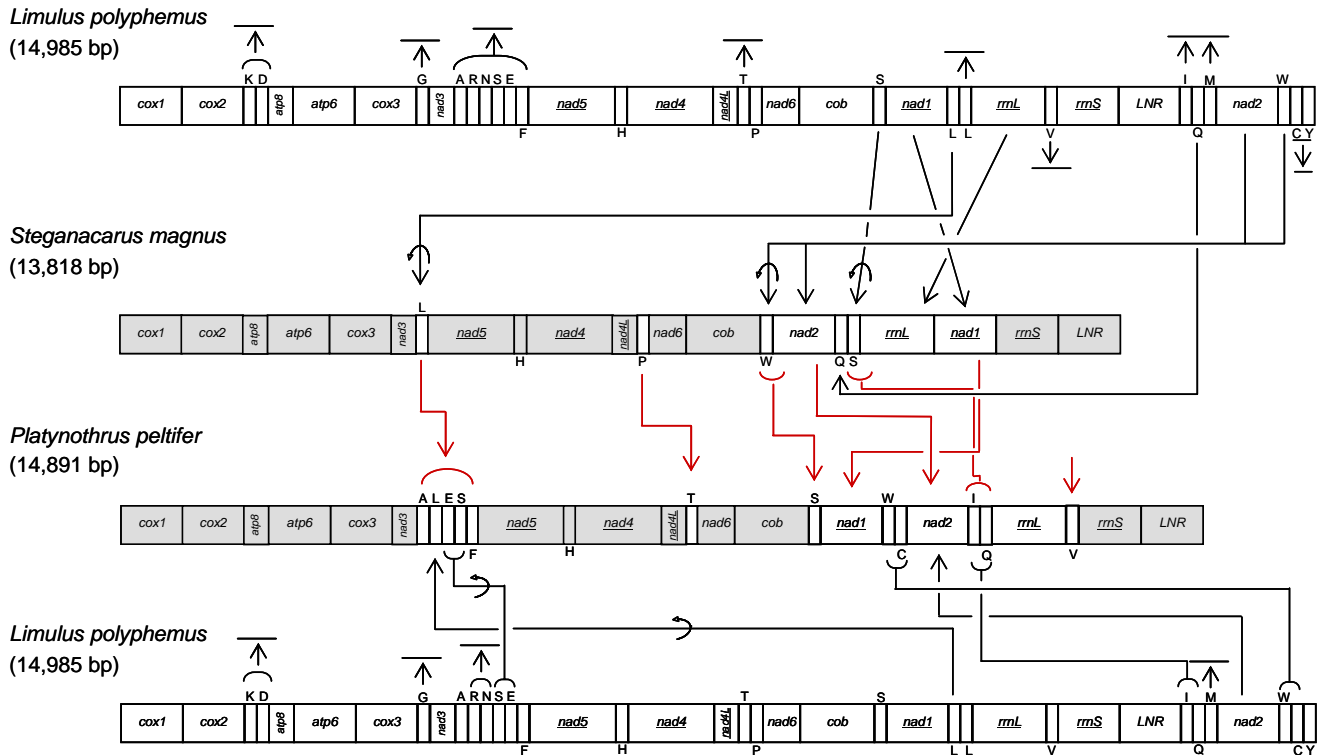
Figure 5.1 Mitochondrial genome organization of *Platynothrus peltifer*, *Limulus polyphemus* and *Steganacarus magnus*. Arrows indicate gene rearrangements (translocations, inversions and loss). Underlined genes and tRNAs with letters below are encoded in the opposite strand. Shaded boxes indicate shared gene boundaries between the oribatid mite species *P. peltifer* and *S. magnus*. Gene abbreviations: *cox1-3*: cytochrome oxidase subunits 1-3; *atp6, 8*: ATP synthase subunit 6, 8; *nad1-6, 4L*: NADH dehydrogenase subunit 1-6, 4L; *cob*: cytochrome b; *rrnL*: large ribosomal subunit; *rrnS*: small ribosomal subunit; LNR: large non-coding control region. tRNA genes are symbolised by the one-letter code of their amino acid: I=Isoleucine; Q=Glutamine; M=Methione; W=Tryptophan; C=Cysteine; Y=Tyrosine; K=Lysine; D=Aspartate; G=Glycine; A=Alanine; R=Arginine; N=Asparagine; S=Serine; E=Glutamate; F=Phenylalanine; H=Histidine; T=Threonine; P=Proline; L=Leucine; V=Valine.

The 13 protein-coding genes in *P. peltifer* which are typically found in arthropods feature all possible start codons for mt genes (ATC, ATG, ATA and ATT; Table 5.2). While the majority of genes terminates with TAA, the stop codon for *nad4L* and *nad5* is TAG; *nad2*, *nad3* and *nad4* have a single T as incomplete stop codon (Table 5.2). There are several genes overlapping each other by four (*atp6*/*cox3* and <u>*nad4*</u>/<u>*nad4L*</u>), seven (*atp8*/*atp6*) or 15 bp (<u>tRNA-Thr</u>/<u>*nad5*</u> and <u>tRNA-Cys</u>/<u>*nad2*</u>).

Table 5.2 Annotation of the mitochondrial genome of *Platynothrus peltifer*.

gene	location	size (bp)	size (aa)	proportion of nucleotides				AT skew[1]	AT/4*	CG skew[2]	CG/4*	start codon	stop codon	anti codon	intergenic nucleotides
				A	C	G	T								
cox1	1-1545	1545	514	27.1	23.3	17.3	32.2	-0.09	0.40	0.15	0.38	ATC	TAA	-	7
cox2	1553-2218	665	221	34.6	25.1	12.8	27.5	0.11	0.25	0.32	0.55	ATG	TAA	-	54
atp8	2273-2428	156	51	41.7	24.4	9.0	25.0	0.25	0.20	0.46	0.11	ATA	TAA	-	-
atp6	2422-3090	669	222	31.7	26.2	11.7	30.5	0.02	0.41	0.38	0.57	ATG	TAA	-	-
cox3	3087-3875	789	262	31.6	23.4	16.6	28.4	0.05	0.51	0.17	0.31	ATA	TAA	-	44
nad3	3920-4259	340	113	33.6	23.0	12.7	30.7	0.05	0.44	0.29	0.33	ATA	T	-	-
tRNA-Ala	4259-4306	48	-	-	-	-	-	-	-	-	-	-	-	TGC	-
tRNA-Leu	4306-4368	63	-	-	-	-	-	-	-	-	-	-	-	TAA	28
tRNA-Glu	4397-4450	54	-	-	-	-	-	-	-	-	-	-	-	GTG	-
tRNA-Ser(GCT)	4450-4501	52	-	-	-	-	-	-	-	-	-	-	-	GTC	11
tRNA-Phe	4523-4564	42	-	-	-	-	-	-	-	-	-	-	-	GAA	1
nad5	4566-6191	1626	541	15.7	12.2	24.8	47.3	-0.50	0.08	-0.34	0.43	ATG	TAG	-	8
tRNA-His	6200-6253	54	-	-	-	-	-	-	-	-	-	-	-	GTG	-
nad4	6253-7549	1297	432	15.9	11.4	25.3	47.3	-0.50	-0.59	-0.38	-0.52	ATA	T	-	-
nad4L	7546-7824	279	92	17.2	10.0	21.9	50.9	-0.49	0.11	-0.37	0.64	ATA	TAG	-	-
tRNA-Thr	7824-7872	68	-	-	-	-	-	-	-	-	-	-	-	TGT	2
nad6	7877-8299	423	140	37.8	27.7	9.7	24.8	0.21	0.65	0.48	0.47	ATC	TAA	-	14
cob	8314-9390	1077	358	32.1	28.6	14.2	25.1	0.12	0.71	0.34	0.55	ATG	TAA	-	-
tRNA-Ser(TGA)	9391-9442	52	-	-	-	-	-	-	-	-	-	-	-	TGA	2
nad1	9445-10338	894	297	22.7	11.2	15.6	50.5	-0.38	0.27	-0.16	0.80	ATC	TAA	-	302
tRNA-Trp	10641-10702	62	-	-	-	-	-	-	-	-	-	-	-	TCA	-
tRNA-Cys	10694-10760	67	-	-	-	-	-	-	-	-	-	-	-	GCA	-
nad2	10747-11706	961	320	31.7	25.0	15.0	28.3	0.06	0.45	0.25	0.25	ATT	T	-	-
tRNA-Ile	11707-11764	58	-	-	-	-	-	-	-	-	-	-	-	GAT	96
tRNA-Gln	11764-11815	52	-	-	-	-	-	-	-	-	-	-	-	TTG	-
rrnL	11912-13014	1103	-	25.1	10.1	21.3	43.3	-	-	-	-	-	-	-	-
tRNA-Val	13015-13060	46	-	-	-	-	-	-	-	-	-	-	-	TAC	-
rrnS	13061-13780	720	-	27.3	11.4	19.2	42.0	-	-	-	-	-	-	-	-
LNR	13781-14891	1111	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] AT skew=(A-T)/(A+T)
[2] CG skew=(C-G)/(C+G)
* skews at 4-fold degenerated sites

The percentage nucleotide composition of the mt (+)-strand is A=37.9, T=24.4, C=24.2 and G=13.5; numbers of each complementary nucleotide pairs (A:T, G:C) are not approximately equal, rather, adenine and cytosin outnumber their respective partner. Overall, the genome is AT-biased. The pattern of nucleotide composition for the protein-coding genes is mixed up. For all genes (except *cox1*) that are encoded on the (+)-strand (*cox2, atp8, atp6, cox3, nad3, nad6, cob,* and *nad2*) the percentage of nucleotides follows A>T>C>G, for all genes on the (-)-strand (*nad5, nad4, nad4L* and *nad1*) it is T>G>A>C; in *cox1* the percentage nucleotide composition is T>A>C>G (Table 5.2). Skews calculated for neutrally evolving fourfold degenerated sites do not indicate a consistent asymmetric strand bias, instead, almost all genes show a positive AT-skew (except *nad4*) as well as a positive CG-skew (except *nad4* and *nad2*) (Table 5.2).

The large ribosomal RNA subunit (*rrnL*) is 1,103 bp in length and located between tRNA-Gln and tRNA-Val, all three are encoded on the (-)-strand (Fig. 5.1, Table 5.2). The gene for the small subunit of the rRNA (*rrnS*) is 720 bp and also encoded on the (-)-strand; the arrangement in *P. peltifer* (tRNA-Val-*rrnS*-LNR) is similar to that of *L. polyphemus* (Fig. 5.1). The secondary structures for both ribosomal RNA subunits need further investigation and comparison to those of *Steganacrus magnus* (Domes et al. 2008)*, Leptotrobidium* (Shao et al. 2006) and insects.

The mt genome of *P. peltifer* further includes 13 of 22 tRNA genes typically found in arthropods (Fig. 5.1, Table 5.2); the genes for lysine, aspartate, glycine, arginine, asparagine, proline, leucine(TAG), methione and tyrosine are lost. Among the present genes those for alanine, phenylalanine, histidine, threonine, serine(TGA) and valine have kept their original position as conserved in *L. polyphemus* (Fig. 5.1). The tRNA genes for glutamate and serine(GCT) also remained in their ancestral position but in reversed order, the gene for leucine(TAA) translocated between tRNA-Ala and -Glu. Further, tRNA-Trp and -Cys concertedly changed their position, but kept their original orientation; instead of *nad2*-tRNA-Trp-tRNA-Cys-tRNA-Tyr in *P. peltifer nad1*-tRNA-Trp-tRNA-Cys-*nad2* is found with tRNA-Trp/tRNA-Cys overlapping by 9 bp (Table 5.2). tRNA-Ile and -Gln also translocated pairwise in their original orientation; instead of LNR-tRNA-Iso-tRNA-Gln-*nad2* in *L. polyphemus* the arrangement in *P. peltifer* is *nad2*-tRNA-Iso-tRNA-Gln-*rrnL* (Fig 5.1).
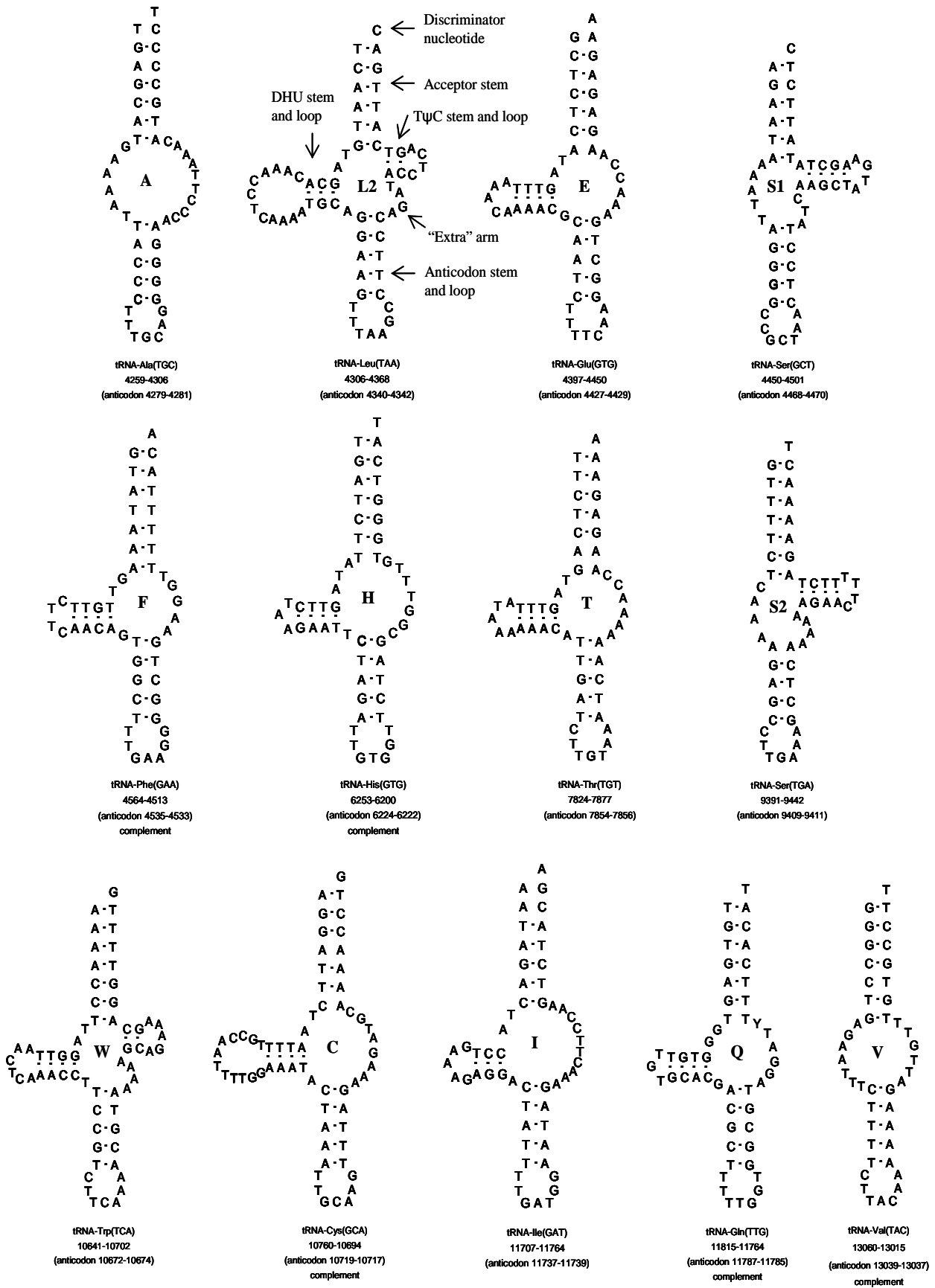
Figure 5.2 tRNA secondary structures of *Platynothrus peltifer*. tRNAs are labelled with the abbreviations of their corresponding amino acids.

Almost all tRNAs in *P. peltifer* differ from the typical cloverleaf structure; only tRNA-Leu(TAA) and -Trp have both D-stem and -loop as well as TψC-stem and -loop forming the common cloverleaf structure (Fig. 5.2). While the tRNA-Glu, -Phe, -His, -Thr, -Cys, -Ile and -Gln lack the TψC-stem and -loop, both tRNAs for serine lack the D-stem and -loop. Further, tRNA-Ala and -Val are only composed of the acceptor and anticodon stem including the anticodon loop (Fig. 5.2). The length of tRNA genes in *P. peltifer* ranges from 42 (tRNA-Phe) to 67 nucleotides (tRNA-Cys) with an average of 54.4 nucleotides.

5.3.2 Heteroplasmy

While sequencing the complete mt genome of the parthenogenetic oribatid mite *P. peltifer* there was no evidence for a gene duplication of *cox1* nor of any other mt gene. However, sequencing long-PCR fragments by primer-walking and designing new primers was sometimes complicated by ambiguous results indicated by "dirty" chromatograms, especially at the *cox1* region. Therefore, we investigated if small-scale heteroplasmy is present in a partial *cox1* fragment by sequencing 16 clones for individual PPKW29_ex5, belonging to consensus group 1a (see next section for explanation).

Thirteen clones (haplotype 1) were identical to each other and identical to the partial sequence of consensus group 1a (Table 5.3); two haplotypes (haplotype 2 and 4) varied at one position (position 234 and 453, respectively) causing a synonymous mutation. A mutation at position 290 (A→G) resulted in an amino acid substitution from histidine to arginine in haplotype 3 (Table 5.3). In general, genetic variation between clones was low.

Table 5.3 Clonal haplotypes, mutational differences and consequences for the amino acid sequence of a single individual of *Platynothrus peltifer*.

Name	Difference to consensus group1a	Mutation	Amino acid sequence	Number of clones
Haplotype 1	identical	none		13
Haplotype 2	pos. 234, 3. cdp	G→A	synonymous	1
Haplotype 3	pos. 290, 2. cdp	A→G	histidine → arginine	1
Haplotype 4	pos. 453, 3. cdp	A→G	synonymous	1

cdp=codon position

.   **5.3.3 Intraspecific Diversity**

We sequenced 540 bp of the mitochondrial *cox1* gene of eight specimens of *P. peltifer* from the Kranichstein forest and compared those with available *cox1* sequences from GenBank published by Heethoff et al. (2007a); *S. magnus* was used as outgroup (GenBank: EU935607). Identical, respectively similar sequences were pooled to eight groups for further analyses (Fig. 5.3, Table 5.4).

Table 5.3 Cytochrome c oxidase 1 sequence haplotypes of *Platynothrus peltifer* from different locations pooled to eight consensus groups.

Identifier of consensus	Sequence name	Origin	Nucleotide compostion (%)			
			A	T	C	G
Group1a	PPKW27_ex5		this study			
	PPKW31_ex5		this study			
	PPKW33_ex5		this study			
	PPKW36_ex5		this study			
	PPKWa		DQ381197			
	PPKWb		DQ381198			
	PPKWc	Germany	DQ381199	25.7	31.5	25.9	16.9
Group1b	PPKW25_ex5		this study			
	PPKW32_ex5		this study			
	PPKW34_ex5		this study			
	PPKWd	Germany	DQ381200	25.6	31.3	26.3	16.9
Group2	PPINa		DQ381222			
	PPINb	Italy	DQ381223	25.2	30.9	26.7	17.2
Group3a	PPKW2_ex4		this study			
	PPOGa		DQ381210			
	PPOGe	Austria	DQ381214	24.8	31.7	25.7	17.8
Group3b	PPISa		DQ381218			
	PPIMa	Italy	DQ381221	25.0	32.0	25.6	17.4
Group4	PPUWa		DQ381176			
	PPUWb		DQ381177			
	PPUWc	USA	DQ381178	23.3	34.1	23.9	18.7
Group5	PPUHa		DQ381183			
	PPUHb		DQ381184			
	PPUHc	USA	DQ381185	26.1	30.7	26.9	16.3
Group6	PPJFa		DQ381171			
	PPJFb		DQ381172			
	PPJFc	Japan	DQ381173	25.0	31.9	25.6	17.6

Seven of eight *cox1* sequences from the Kranichstein forest were similar or identical to those previously published by Heethoff et al. (2007a) (Fig. 5.3, Table 5.4); the sequences were pooled to group 1a and 1b with both groups differing by only two nucleotides. In contrast, the sequence PPKW2_ex4 was similar to haplotypes previously obtained from individuals

sampled in Austria (PPOGa and PPOGe; Fig. 5.3, Table 5.4) and was therefore included in group 3a; the remaining five groups consisted exclusively of sequences from the study of Heethoff et al. (2007a) (Fig. 5.3, Table 5.4). The nucleotide composition differed slightly between the groups, but all consensus sequences had the T>C>A>G order (Table 5.3).
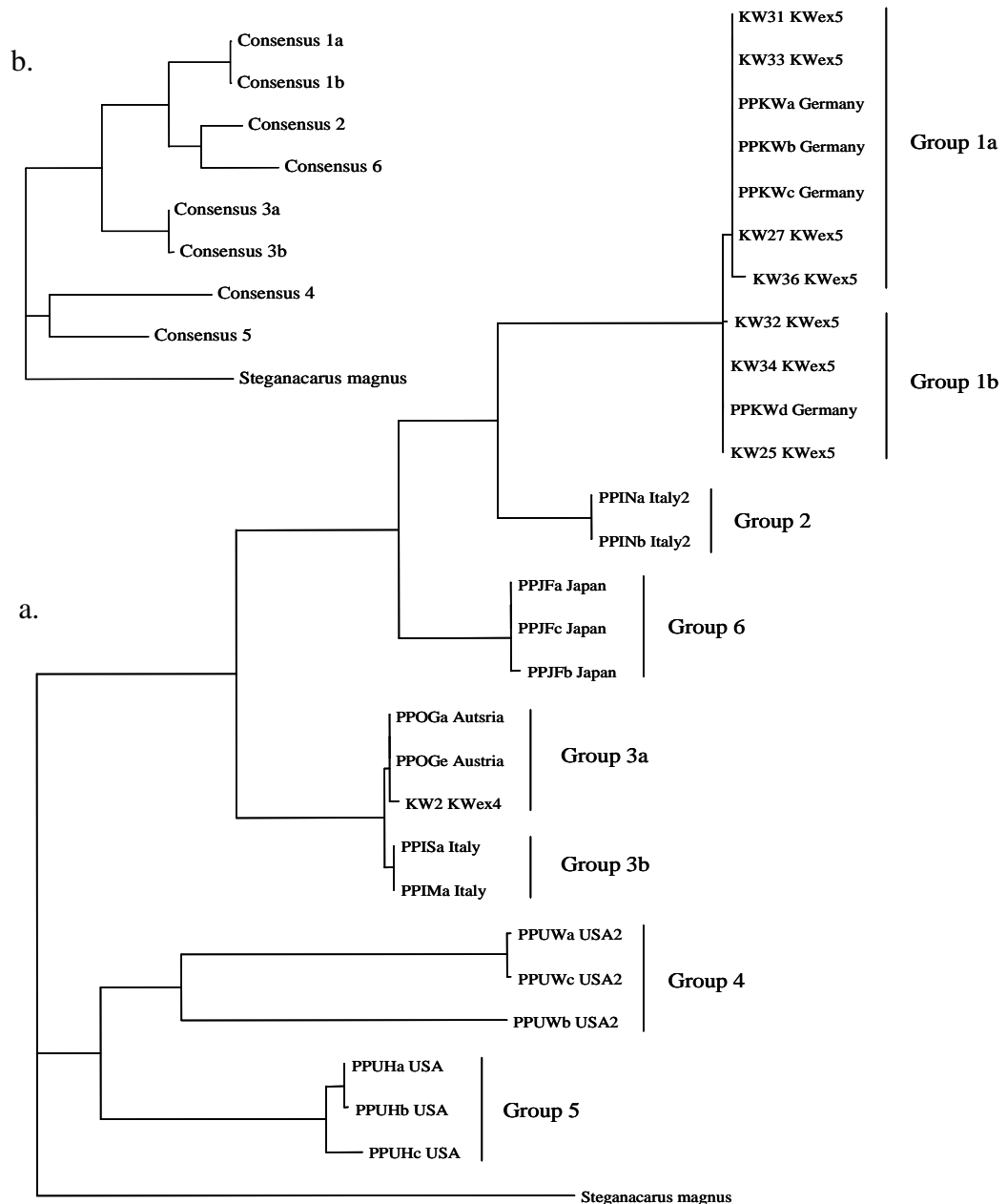
Figure 5.3 Neighbour-joining phylogeny based on uncorrected p-distances of (a) 27 cytochrome c oxidase 1 sequences of *Platynothrus peltifer* from different locations and (b) the eight consensus groups. *Steganacarus magnus* was used as outgroup.

Both uncorrected intraspecific p-distances and corrected distances among the eight consensus *cox1* groups of *P. peltifer* were lowest between group1a and 1b (0.4%) and highest between group 4 and 6 (23.3% and 167.3%, respectively; Table 5.5A). In total, 66 transitions

and 30 transversions were detected; while 84.9% of the transitions occurred at the third and 15.2% on the first codon position, all transversions affected the third codon position. The amino acid composition of the eight *cox1* groups was highly conserved; while group 3b and 5 were characterized by a single specific amino acid substitution, all others were identical (Table 5.5B).

Table 5.5 Genetic diversity between eight consensus groups of the cytochrome c oxidase 1 gene of *Platynothrus peltifer*. (A.) Numbers above the midline are corrected by the evolutionary model HKY+G (see text for values), numbers below are uncorrected p-distances. (B.) Genetic diversity of protein sequences.

A.	Group1a	Group1b	Group2	Group3a	Group3b	Group4	Group5	Group6
Group1a	-	0.0037	0.3692	1.0177	1.0938	1.6265	1.4450	0.6328
Group1b	0.0037	-	0.33454	1.0109	1.0864	1.5677	1.3922	0.6180
Group2	0.1352	0.1315	-	0.7332	0.7639	1.3649	1.5388	0.3126
Group3a	0.1963	0.1981	0.1815	-	0.0057	1.3814	1.4186	0.6644
Group3b	0.2019	0.2037	0.1833	0.0056	-	1.4129	1.4760	0.6930
Group4	0.2185	0.2167	0.2056	0.2148	0.2148	-	1.0751	1.6735
Group5	0.2148	0.2130	0.2204	0.2019	0.2037	0.1815	-	1.5282
Group6	0.1722	0.1722	0.1222	0.1685	0.1704	0.2333	0.2222	-

B.	Group1a	Group1b	Group2	Group3a	Group3b	Group4	Group5	Group6
Group1a	-							
Group1b	0.0000	-						
Group2	0.0000	0.0000	-					
Group3a	0.0000	0.0000	0.0000	-				
Group3b	0.0056	0.0056	0.0056	0.0056	-			
Group4	0.0000	0.0000	0.0000	0.0000	0.0056	-		
Group5	0.0056	0.0056	0.0056	0.0056	0.0111	0.0056	-	
Group6	0.0000	0.0000	0.0000	0.0000	0.0056	0.0000	0.0056	-

5.4 Discussion

5.4.1 Mitochondrial Genome

The mt genome of the parthenogenetic oribatid mite *P. peltifer* comprises all genes typically expected for arthropods except nine tRNA genes. Its arrangement differs only slightly from the hypothetical ancestor as represented by *L. polyphemus* (Lavrov et al. 2000); only *nad2* and several tRNAs translocated to new positions. The *nad1/nad2* arrangement also differs compared to the mt genome of the closely related sexual oribatid mite species *S. magnus* (Domes et al. 2008): while *nad1-nad2-rrnL-rrnS*-LNR (tRNAs excluded) is found in *P. peltifer*, *S. magnus* is *nad2-rrnL-nad1-rrnS*-LNR (Fig. 5.1). All genes except *cox2* and *nad1* are slightly longer in the parthenogenetic than in the sexual species (Table 5.6). Compared to *Leptotrombidium akamushi* (Shao et al. 2006), a related mite species belonging to Prostigmata

(Acariformes), both oribatid mites have slightly longer genes which causes their overall larger mt genome (*P. peltifer*: 14,891, *S. magnus*: 13,818, *L. akamushi*: 13,698; Table 5.6) although *S. magnus* lacks several tRNA genes (Domes et al. 2008) and *L. akamushi* has a duplicated control region (Shao et al. 2006).

Table 5.6 Comparison of the mitochondrial gene length of *Platynothrus peltifer*, *Steganacarus magnus* and *Leptotrobidium akamushi*. Number in brackets resembles complete genome size.

	P. peltifer (14,891)	*S. magnus* (13,818)	*L. akamushi* (13,698)		*P. peltifer* (14,891)	*S. magnus* (13,818)	*L. akamushi* (13,698)
	length (bp)				length (bp)		
atp6	669	650	624	*nad3*	340	337	303
atp8	156	150	147	*nad4*	1,335	1,263	1,230
cob	1,077	1,063	1,056	*nad4L*	279	267	255
cox1	1,545	1,534	1,530	*nad5*	1,625	1,588	1,536
cox2	665	669	648	nad6	423	405	426
cox3	789	786	777	*rrnL*	1,103	992	1,014
nad1	894	894	879	*rrnS*	720	609	608
nad2	963	957	882	LNR	1.111	1.019	262/259

Compared to *S. magnus*, which comprises only six tRNA genes (Domes et al. 2008), only the position of histidine is completely identical in both oribatid mite species. Leucine(TAA) and glutamine are located at the same place, but they do not have the same gene boundaries; the genome of *S. magnus* lacks the tRNAs that are the immediate neighbours in *P. peltifer*. In contrast, the genes for serine(TGA) and tryptophan are translocated and are encoded on different strands. While serine is on the (+)-strand between tRNA-Glu and -Phe in *P. peltifer*, it is on the (-)-strand between tRNA-Gln and *rrnL* in *S. magnus*; for tryptophan the arrangement is *nad1*-tRNA-Trp-tRNA-Cys in *P. peltifer* and *cob*-tRNA-Trp-*nad2* in *S. magnus*. In contrast to the diverse tRNA pattern in oribatid mites, in three species of the prostigmate mite genus *Leptotrombidium* the tRNA arrangements are very similar; while the arrangement of *Leptotrombidium deliense* and *L. akamushi* are identical, that of *L. pallidum* differs only by the position of tRNA-Gln (Shao et al. 2006).

Almost all tRNAs of both oribatid mite species are highly degenerated and do not show the typical cloverleaf structure (Fig. 5.2; Domes et al. 2008), a characteristic which is also found in *L. pallidum* (Shao et al. 2005) and *Metaseiulus occidentalis* (Jeyaprakash and Hoy 2007); while in *L. palldium* also either the D- or the TψC-stem is missing, tRNAs in *M. occidentalis* always lack the TψC-arm. While the average length of tRNA genes in arthropods is 66 nucleotides, almost all genes in *P. peltifer* are shorter due to their high modification; this also

applies for *S. magnus* (Domes et al. 2008). Generally, strong modifications of tRNA secondary structures are common in chelicerates and therefore no derived feature supporting any ancestry of species (Dávila et al. 2005, Shao et al. 2005, Jeyaprakash and Hoy 2007, Masta and Boore 2008, Domes et al. 2008).

5.4.2 Heteroplasmy

Heteroplasmy, i.e. the presence of different haplotypes in an individual, is probably fostered by the high number of copies in mitochondrial genes. The mt genome of *P. peltifer* is characterized by the presence of single nucleotide polymorphisms (SNPs) in several genes such as *cox1*, *cox2* and *nad2* (K. Domes, unpublished data). However, the intraindividual variation was not as high as between individuals (i.e. intraspecific variation).

SNPs are also commonly reported in other studies, e.g., in humans where they are involved in certain diseases and aging (Richter 1995, Ozawa 1997). Further, heteroplasmy caused insecticide resistance in the arthropod pest *Tetranychus urticae* because of point mutations at four sites in the *cob* gene (Van Leeuwen et al. 2008); the heteroplasmy was inferred from a double peak in the sequencing chromatograph at a consistent position and the frequencies of wildtype and resistant haplotype were quantified by the relative peak hights. Frey and Frey (2004) describe the intraindividual *cox1* variation in *Thrips tabaci* (Thripidae); while the most abundant haplotype was described as "original" *cox1* gene, also heteroplasmatic haplotypes and mutations introduced by the amplification process could be detected.

Mutations, especially point mutations, can also represent experimental artefacts which may be introduced by ethidium bromide staining and UV radiation and the culturing of clonal products in *E. coli* cells (Cariello et al. 1988, Chen and Hebert 1999). However, the level of mutants due to the overnight culture in bacterial host cells is extremely low and outnumbered by the original wildtype (Chen and Hebert 1999); in addition, ethidium bromide and UV exposure were reduced to a minimum during DNA processing.

If the frequency of heteroplasmy in *P. peltifer* affects mtDNA-based molecular studies remains to be shown by a more extensive clone screening; however, it seems unlikely to be responsible for the high intraspecific variation as reported by Heethoff et al. (2007a).

5.4.3 Intraspecific Diversity

Since the frequency of intraindividual heteroplasmy is rather low in the parthenogenetic oribatid mite *P. peltifer*, the high intraspecific variability of the *cox1* gene sequence previously reported by Heethoff et al. (2007a) and confirmed in this study remains puzzling. Since the complete mitochondrial genome was sequenced, the presence of a gene duplication can be excluded. Presence of a nuclear pseudogene, though possible, also is unlikely as all sequences obtained were translatable in proteins without stop codons.

In general, the intraspecific variability of the *cox1* gene usually is higher in parthenogenetic than in sexual species (e.g., Arnaud et al. 2000, Hebert et al. 2003b, Navajas and Boursot 2003, Ros et al. 2008); for example, while parthenogenetic *Bryobia kissophila* (Acari) has an intraspecific diversity of 8.8 % (Ros et al. 2008), the diversity in sexual *Pinctada mazatlanica* (Gastropoda) varies between 0.12 and 1.3 % (Arnaud et al. 2000). Many parthenogenetic species show a higher genetic diversity of the *cox1* gene than the 2.7 % species threshold as proposed by Hebert et al. (2004a, b). Further, contrasting levels of *cox1* variability were reported for sexually reproducing species, such as the mite *Stereotydeus mollis* (Penthalodidae) and the springtail *Gomphiocephalus hodgsoni* (Hypogastruridae; Stevens and Hogg 2006); while the intraspecific variation for *G. hodgsoni* is ≤ 2 %, up to 18 % variation / divergence exists in *S. mollis*.

While the corrected nucleotid diversity of *cox1* in *P. peltifer* averages 56 % (Heethoff et al. 2007a), the amino acid sequences are identical in specimens from different locations (A. Weigand, unpublished data). This contrasts the pattern in the sexual oribatid mite *S. magnus*; here, the intraspecific *cox1* variability is unusually high for a sexual species (up to 30%) and also the protein sequences exhibit a high intraspecific diversity resulting in different proteins between specimens of *S. magnus* from different locations (M. Rosenberger, unpublished data). These discrepancies between *P. peltifer* and *S. magnus* may be explained by the different reproductive modes in combination with different types of mutations (A. Weigand, unpublished data).

Since synonymous mutations in mtDNA, i.e. caused by replication errors or free radicals, do not affect the protein sequence and therefore are selectively neutral, they accumulate equally in the mt genepool of sexual and parthenogenetic species. In contrast, non-synonymous mutations that alter the coded amino acids are under negative selection. Subunits of the cytochrome c complex (COXI, II, III) that are encoded in the mtDNA interact with numerous proteins encoded in the nucleus and this interaction must be optimized to enable an efficient

energy production by the respiratory chain. Non-synonymous mutations likely affect these interactions and modify the efficiency of cell respiration. In parthenogens, both mt and nuclear DNA are uniparentally inherited and the optimized interaction has to be conserved due to the lack of outcrossing and recombination since mutations in the mtDNA cannot be buffered by genetic diversity of nuclear DNA. Therefore, effects of non-synonymous mutations in parthenogens are more severe and likely cause strong negative selection resulting in fast elimination of mutated less effective genotypes. The close coupling of mt and nuclear DNA therefore may explain the uniformity of the COXI amino acid sequence in parthenogenetic species such as *P. peltifer* (A. Weigand, unpublished data). In contrast, in sexuals mt and nuclear DNA are uncoupled; while mitochondria are uniparentally inherited, the nuclear DNA is reshuffled and recombined in every generation. This results in permanent adaptation of mt and nuclear gene interactions of the cytochrome c complex; this "co-evolution" was even shown to be accelerated in mt-coded protein subunits that are in vicinity to nuclear encoded amino acids (Hughes 1992, Wu et al. 2000, Schmidt et al. 2001).

Non-synonymous mutations that do not change amino acid sequences of proteins are under less negative selection and can even become manifested in parthenogens if they do not influence the protein function significantly. In *P. peltifer* the high intraspecific *cox1* variability is close to saturation at the third codon position with predominantly synonymous mutations suggesting high age of the species (Heethoff et al. 2007a). At the same time, the amino acid sequence remains conserved ensuring functionality in cell respiration.

5.4.4 Conclusions

Although inconsistencies occurred while sequencing the complete mitochondrial genome of *P. peltifer*, no direct evidence for explaining the high intraspecific diversity of the *cox1* gene could be found; the gene arrangement differs only slightly from known genomes of other chelicerates, no evidence for a gene duplication was found and only little small-scale heteroplasmy was present. However, different haplotypes of *cox1* could be sequenced at a single location indicating that different clone lineages coexist and migration events are rare but detectable. Further studies using population genetic markers such as microsatellites will gain a more detailed insight into intraspecific haplotype distribution in populations of *P. peltifer*.

Resources and Sex: Soil Re-colonization by Sexual and Parthenogenetic Oribatid Mites

$\mathbb{F}$actors responsible for the dominance of sexual reproduction in Metazoa are controversial. Generally, the mode of reproduction is correlated with ecological factors: under resource-limited conditions sexual taxa dominate, whereas theoretically parthenogenetic species prevail in stable habitats. To investigate if sexual or parthenogenetic taxa are more affected by environmental conditions we analysed (1) the effect of resource depletion on the density of sexual and parthenogenetic oribatid mite taxa and (2) the re-colonization of defaunated soil and litter by sexual and parthenogenetic taxa. In both experiments the number of eggs in parthenogenetic and sexual species was counted. For the first experiment, laboratory microcosms were established where resources declined with time; for the second experiment, microcosms were defaunated by heat and inoculated with fresh soil and litter material, respectively, as resource for re-colonization by oribatid mites. We hypothesized that parthenogenetic species will suffer more from resource limitation compared to sexual taxa, and that they will colonize habitable space faster due to their faster mode of reproduction. In agreement with our hypothesis, parthenogenetic taxa suffered more from resource limitation than sexual species. In contrast, in the re-colonization experiment the proportion of parthenogenetic taxa remained constant in the treatment re-colonised from soil as well as from litter, indicating that parthenogenetic species are not faster colonizers. In general, egg numbers were higher in sexual species than in parthenogenetic species.

6.1 Introduction

A large number of theories have been proposed to explain why sexual reproduction prevails in most animal taxa (e.g., Muller 1964, Williams 1975, Maynard Smith 1978, Bell 1982), but the question is still not satisfactorily answered (Birky 1996, Vrijenhoek 1998, Barton and Charlesworth 1998, Birky et al. 2005). Both sexual and parthenogenetic reproduction have advantages and disadvantages. Parthenogens do not invest resources in male production which doubles the population growth rate compared to sexual taxa (Williams 1975, Maynard Smith 1978, Bell 1982, Butlin et al. 1998). On the other hand, non-recombining parthenogens should accumulate mutations and therefore should be doomed to extinction in the long-term (Muller 1964, Kondrashov 1988, Paland and Lynch 2006). In contrast, sexual species profit from mixis and recombination allowing them to react faster to changing environments (Bell 1982), but the dilution and mixing of their genome is disadvantageous from a selfish gene point of view (Williams 1975, Maynard Smith 1978). Overall, there is a plethora of different views on the advantages and disadvantages of reproductive modes which do not necessarily exclude but may even complement each other (West et al. 1999).

Despite theoretical considerations and the overwhelming evidence for the prevalence of sexual reproduction in the field there are few taxa that appear to have survived evolutionary periods of time without sexual reproduction. Those famous "ancient asexual scandals" (Maynard Smith 1978, Judson and Normark 1996) comprise bdelloid rotifers (Mark Welch and Meselson 2000), darwinulid ostracods (Martens et al. 1998, but see Smith et al. 2006) and several taxa of oribatid mites (Norton and Palmer 1991, Maraun et al. 2004, Schaefer et al. 2006). Oribatid mites are the most species rich and probably the oldest of these three taxa. They are about 375 million years old since fossils are known from the Devonian (Shear et al. 1984, Norton et al. 1988, Labandeira et al. 1997); molecular studies suggest an age of about 390 million years (I. Schaefer, unpublished data).

The reproductive mode of most animal species is correlated with ecological factors. Theory predicts sexuality to be superior to parthenogenesis in unstable habitats since higher genetic diversity allows a faster reaction to changing environmental conditions (Williams 1975, Hamilton 1980). Consequently, parthenogenetic taxa should dominate in stable habitats because there is no need to adapt to changing environmental conditions. Forests appear to be rather stable habitats. In fact, the percentage of parthenogenetic taxa (e.g., mites, collembolans, enchytraeids, and nematodes) in forest soils is high compared with other habitats (Norton and Palmer 1991, Siepel 1994, Niklasson et al. 2000, Bloszyk et al. 2004).

Resource quality and quantity are important ecological factors for animal communities and reproductive modes may also change with changing resource availability (Korpelainen 1990). Resource availability correlates with parthenogenetic reproduction whereas resource shortage is often associated with sexuality (Redfield 1999), as shown in cyclical parthenogens such as monogonont rotifers, aphids and cladocerans (Bell 1982, Innes and Singleton 2000).

For the re-colonization of habitats sexual species need at least one male and one female whereas parthenogens only need a single individual. Therefore, parthenogenetic species are usually faster colonisers (Williams 1975, Bell 1982, Scheu and Schulz 1996, Lindberg and Bengtsson 2005). However, colonization success does not depend only on reproductive mode but also on other factors, such as mobility, fertility, resource availability and habitat characteristics (Debouzie et al. 2002).

We investigated the effect of resource depletion on the density and community structure of sexual and parthenogenetic oribatid mites. Laboratory microcosms were established where the availability and quality of resources declined with time. After ten months, the density and community structure of oribatid mite species, and the sex ratios and numbers of eggs per female were measured. We hypothesized that due to resource depletion (1) the number of specimens will decline with time and (2) sexual taxa will be favoured over parthenogenetic taxa, since greater genetic diversity allows sexual species to react more flexibly to changes in the amount and quality of resources.

In a second experiment we investigated the re-colonization of defaunated soil and litter by sexual and parthenogenetic oribatid mite taxa. After defaunation, soil columns were inoculated with soil or litter, respectively, as sources for the re-colonization by microarthropods. We hypothesized that parthenogenetic oribatid mite species will colonize empty habitats faster than sexual species, but that sexual species will replace parthenogenetic taxa over the course of the experiment.

6.2 Materials and Methods

6.2.1 Study Site

Soil samples were taken from the Kranichsteiner forest, located about 8 km northeast of Darmstadt (Germany). The Kranichsteiner forest is dominated by beech (*Fagus sylvatica*) interspersed with ca. 190 y old oak (*Quercus robur*) and hornbeam (*Carpinus betulus*). The herb layer is dominated by *Luzula luzuloides, Milium effusum, Anemone nemorosa* and

Polytrichum formosum. Parent rock is Rotliegend covered with sand; the humus form is moder (FAO-UNESCO classification)**.**

6.2.2 Resource Depletion Experiment

Five soil cores (Ø 21 cm; L, F, H layer and the upper 3 cm of the Ah layer) were taken from the Kranichsteiner forest and placed in laboratory microcosms. The microcosms were closed with plastic at the bottom and with gauze on top and kept in darkness at 15°C; loss of water was evaluated and replaced by adding about 100 ml distilled water every week. After 2, 11, 21 and 44 weeks soil cores (Ø 5 cm) were taken from the microcosms (holes were filled with sand), separated into litter and soil (0-3 cm depth) and oribatid mites were extracted by heat (Macfadyen 1961, Kempson et al. 1963).

Oribatid mites were counted and determined to species level. Further, adult individuals were sexed, and in females the number of eggs was counted to compare egg production between parthenogenetic and sexual species. Species were considered to be sexual when there were more than 5% males. Brachychthoniidae, Phthiracaridae and Suctobelbidae were difficult to sex; females could only be identified if they carried eggs. The mode of reproduction in these taxa was inferred from the literature (Cianciolo and Norton 2006).

6.2.3 Re-colonization Experiment

Fifteen soil cores (Ø 21 cm; see above) were taken from the field and placed in laboratory microcosms. Before the start of the experiment, the soil cores were defaunated by drying at 60°C for eight weeks. Then, five of the soil cores were re-inoculated with fresh soil and another five with litter material. Soil and litter for each microcosm were taken from three pooled soil samples (Ø 5 cm) from the study site. Additionally, five control microcosms without inoculation were established (defaunated control). The microcosms were closed with plastic on the bottom and with gauze on top and kept at 15°C in darkness; loss of water was evaluated and replaced as described above. Samples were taken at the same time intervals as in the resource depletion experiment and the same dependent variables were measured. At the end of the experiment the defaunated control was free of microarthropods indicating that drying at 60°C effectively killed microarthropods including eggs.

6.2.4 Statistical Analysis

Abundances of oribatid mites and percentage of parthenogenetic individuals were analysed by multivariate repeated measures analysis of variance (RM-ANOVA) for the re-colonization experiment, and by multivariate analysis of variance (MANOVA) for the resource depletion experiment in SAS 9.13 (SAS Institute Inc., Cary, USA) with the fixed factors time and treatment (Scheiner and Gurevitch 2001). Numbers of eggs per female (including females without eggs) of the first two (resource depletion experiment) and of the first three (re-colonization experiment) sampling dates were pooled and compared to those of the later sampling dates using RM-ANOVA. A Principal Components Analysis (PCA) was performed for the re-colonization experiment for ordinating oribatid mite species using CANOCO 4.5 (Ter Braak and Šmilauer 1998).

Abundances of oribatid mites and numbers of eggs per female were log(x+0.1) transformed and percentages of parthenogenetic individuals were arcsin-transformed prior to statistical analysis to increase homogeneity of variances. Species with fewer than five individuals in total and species which occurred in fewer than three samples were excluded from the statistical analyses.

6.3 Results

6.3.1 Resource Depletion Experiment

In total, 1,784 oribatid mites were found; 530 individuals were juvenile and not determined further. The 1,254 adult oribatid mites represented 40 species; of these, 1,234 individuals from 24 species were included in the statistical analysis.

Total abundances of juvenile and adult oribatid mites decreased significantly with time (MANOVA: $F_{3,12}=16.1$, P=0.0002; $F_{3,12}=9.37$, P=0.002, respectively; Fig. 6.1). The decrease of adult oribatid mites was mainly caused by *Microppia minus* and *Oppiella nova* (ANOVA: $F_{3,16}=3.79$, P=0.031 and $F_{3,16}=3.13$, P=0.05, respectively).

On average 70% of oribatid mite individuals inhabited the litter layer, and this did not vary with time (MANOVA: $F_{3,12}=1.42$, P=0.284). In addition, the fraction of parthenogenetic oribatid mites in the litter and soil layer remained constant with time (MANOVA: $F_{1,4}=3.19$, P=0.149); in the litter layer 60% and in the soil layer 80% of the individuals were from parthenogenetic species. Since the number of individuals of parthenogenetic species did not differ significantly and most of the individuals were found in the litter layer, data of both

layers were pooled for further analyses. The fraction of individuals from parthenogenetic species continously decreased from week two to week 21, but slightly increased to week 44 with the changes in time being significant (RM-ANOVA: $F_{3,12}=3.27$, P=0.05 Fig. 6.2).
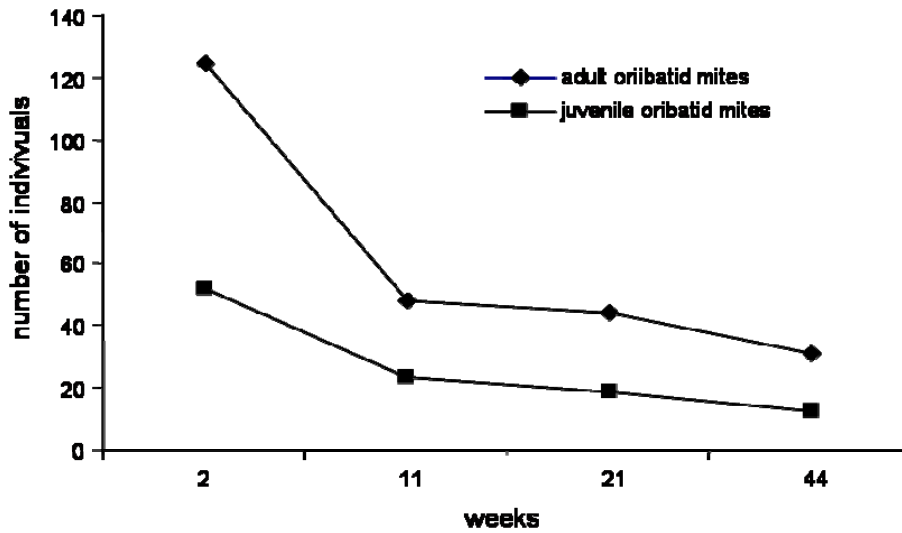

Figure 6.1 Total abundances of adult and juvenile oribatid mites 2, 11, 21 and 44 weeks after initiation of the resource depletion experiment.
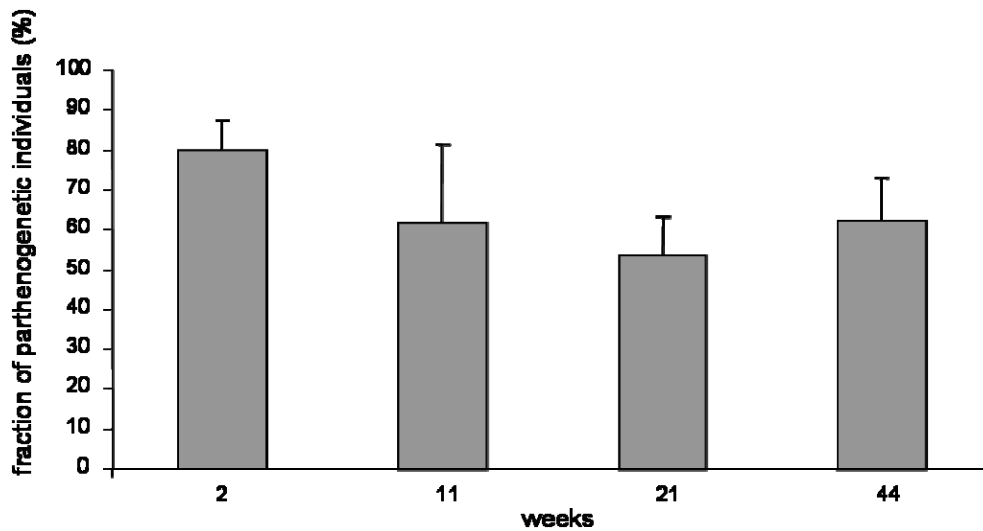

Figure 6.2 Percentage of parthenogenetic oribatid mite individuals 2, 11, 21 and 44 weeks after initiation of the resource-depletion experiment.

Among the 24 oribatid mite species 15 were parthenogenetic and nine were sexual (Table 6.1). Parthenogenetic species had significantly lower numbers of eggs per female (including females without eggs) than sexual species (0.7 and 1.6, respectively; ANOVA: $F_{1,21}=7.40$, P=0.013). This was mainly true for small parthenogenetic species such as *Tectocepheus minor*, *Oppiella nova, Microppia minus*, *Eniochthonius minutissimus, Hypochthonius rufulus* and Suctobelbidae. Members of parthenogenetic Desmonomata with a body size of about 600-800 µm usually had more than one egg per female and sexual Poronota had up to 4.5 eggs per female (Table 6.1). The average egg number per female (including females without eggs) significantly decreased in parthenogenetic species from 1.2 to 0.6 (RM- ANOVA: $F_{1,4}=9.4$, P= 0.037). In sexual species the number of eggs per female also decreased (from 1.8 to 1.1), but the decrease was not significant (RM-ANOVA: $F_{1,4}=1.26$, P=0.325).

Table 6.1 Sex ratio and numbers of eggs of oribatid mite species after resource depletion.

Species	female	male	sex unkown	total	total sexed	% females$	eggs+	eggs/female#	eggs/all females§
Enarthronota									
Brachythoniidae									
Enichthoniidae									
Enichthonius minutissimus (Berlese, 1903)	0	0	8	8	0	-	-	-	-
Hypochthoniidae									
Hypochthonius rufulus Koch, 1835	21	0	61	82	21	100	15	1.07	0.71
Mixonomata									
Euphthiracaridae									
Rhysotritia duplicata (Grandjean, 1953)	2	0	3	5	2	100	3	1.50	1.50
Phthiracaridae	8	0	71	79	8	100	18	2.25	2.25
Desmonomata									
Malaconothridae									
Malaconothrus gracilis v.d. Hammen, 1952	75	0	18	93	75	100	36	1.00	0.48
Nanhermanniidae									
Nanhermannia coronata Berlese, 1913	26	0	0	26	26	100	22	1.47	0.85
Nanhermannia nana (Nicolet, 1855)	7	0	0	7	7	100	6	1.20	0.86
Nothridae									
Nothrus silvestris Nicolet, 1855	35	0	0	35	35	100	49	1.69	1.40
Pycnonotic Apheredermata									
Suctobelbidae									
Suctobelbella subcornigera (Forsslund, 1941)	5	0	97	102	5	100	5	1.00	1.00
Suctobelbella subtrigona (Oudemans, 1900)	1	0	31	32	1	100	1	1.00	1.00
Tectocepheidae									
Tectocepheus minor (Berlese, 1903)	16	0	0	16	16	100	9	1.00	0.56
Tectocepheus sarekensis Trägardh, 1910	18	0	0	18	18	100	3	3.00	0.17
Tectocepheus velatus (Michael, 1880)	13	0	0	13	13	100	2	2.00	0.15
Oppiidae									
Oppiella nova (Oudemans, 1902)	265	0	0	265	265	100	54	1.17	0.20
Micrappia minus (Paoli, 1908)	192	2	0	194	194	99	4	1.00	0.02
Disshorina ornata (Oudemans, 1900)	67	40	7	114	107	62.6	53	1.56	0.79
Medioppia subpectinata (Oudemans, 1900)	31	10	0	41	41	75.6	29	2.23	0.94
Thyrisomidae									
Banksinoma lanceata (Michael, 1888)	5	8	1	14	13	38.5	7	1.75	1.40
Poronta									
Achipteriidae									
Achipteria coleoptrata (Linnaeus, 1758)	16	10	1	27	26	61.5	16	1.60	1.00
Oribatellidae									
Ophidiotrichus tectus (Michael, 1884)	2	2	1	5	4	50	2	1.00	1.00
Oribatulidae									
Oribatula tibialis (Nicolet, 1855)	14	6	1	21	20	70	23	3.83	1.64
Phenopelopidae									
Eupelops plicatus (Koch, 1836)	2	3	0	5	5	40	9	4.50	4.50
Punctoribatidae									
Minunthozetes semirufus (Koch, 1841)	15	7	2	24	22	68.2	22	2.00	1.47

6.3.2 Re-colonization Experiment

In the re-colonization experiment a total of 1,035 oribatid mites were found; 157 were juvenile and not determined further. The 878 adult oribatid mites represented 35 species; of these, 839 individuals from 16 species were included in the statistical analysis.

Density of adult oribatid mites in the treatment re-colonized from litter exceeded that in the treatment re-colonized from soil throughout the experiment, and significantly increased with time (RM-ANOVA: $F_{1,8}=5.05$, $P=0.05$; $F_{3,24}=9.87$, $P=0.0002$, respectively). The number of juvenile oribatid mites also increased significantly (RM-ANOVA: $F_{3,24}=6.84$, $P=0.002$; Fig. 6.3). The increase of adult oribatid mites was mainly due to *Suctobelbella subcornigera, Oppiella nova* and *Disshorina ornata* (ANOVA: $F_{3,24}=4.43$, $P=0.013$; $F_{3,24}=27.23$, $P<0.0001$; $F_{3,24}=13.46$, $P<0.0001$, respectively). The sexual species *D. ornata* and *Medioppia subpectinata* had significant higher densities in the treatment re-colonized from litter (ANOVA: $F_{1,8}=10.77$, $P=0.011$; $F_{1,8}=20.42$, $P=0.002$, respectively).
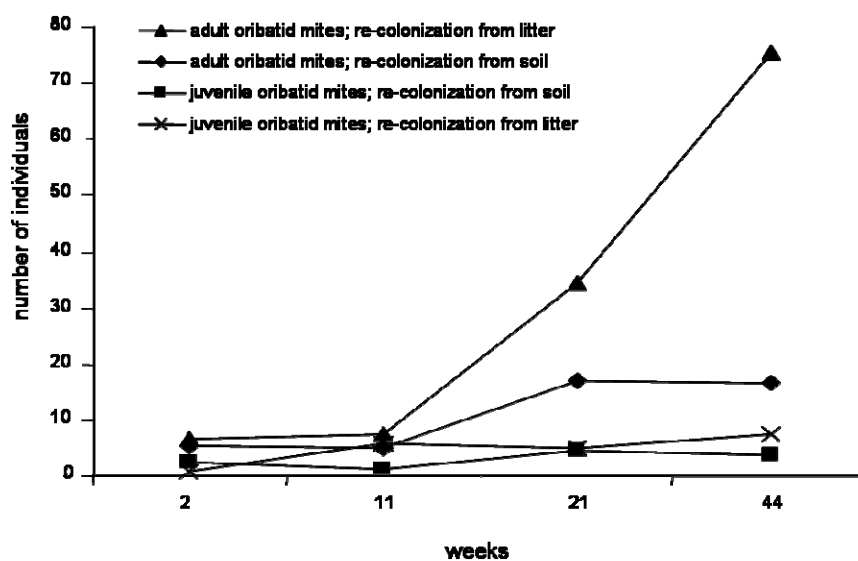

Figure 6.3 Total abundances of adult and juvenile oribatid mites in the treatment re-colonized from litter and re-colonized from soil (see text for further details).

The percentage of adult oribatid mite individuals in the litter layer changed neither with time nor between treatments (RM-ANOVA: $F_{3,18}=0.97$, $P=0.429$ and $F_{1,6}=0.01$, $P=0.921$). On average 77% and 76%, respectively, of the individuals in the treatment re-colonized from soil and litter were present in the litter layer. Since the number of individuals of parthenogenetic species did not differ significantly with time and most of the individuals occured in the litter layer, data of both layers were pooled for further analyses.

The fraction of parthenogenetic individuals did not change with time (RM-ANOVA: $F_{3,18}$=0.81, P=0.51) but significantly differed between the treatments, with an average of 79% parthenogenetic individuals in the treatment re-colonized from soil and 55% in the treatment re-colonized from litter (ANOVA: $F_{1,6}$=5.61, P=0.05). PCA reflects that parthenogenetic species, such as *Eniochthonius minutissimus, Tectocepheus minor*, *Malaconothrus gracilis* and *Microppia minus,* were more strongly associated with the first two sampling dates, while sexual taxa, such as *Minunthozetes semirufus*, *Medioppia subpectinata*, *Dissorhina ornata*, *Banksinoma lanceata, Oribatella quadricornuta and Ophidiotrichus tectus,* were more strongly associated with the third and fourth sampling date (Fig. 6.4). This indicates that parthenogenetic species responded faster than sexual taxa.

Among the 16 oribatid mite taxa, eight were parthenogenetic and eight were sexual (Table 6.2). Females with eggs were rare, especially for sexual species in the treatment re-colonized from soil, but parthenogenetic species had on average fewer eggs than sexual species. Small parthenogenetic species of Oppiidae, Suctobelbidae and *Eniochthonius minutissimus* had about one egg per female whereas sexual species had up to eight eggs per female, e.g. in *Oribatula tibialis* (Table 6.2). The average number of eggs per female (including females without eggs) slightly increased in both treatments; in the treatment re-colonized from litter the number of eggs per female (including females without eggs) of parthenogenetic species increased from 0.6 to 1.1, in sexual species from 1.2 to 2.4, but the increase was only significant in the treatment re-colonized from soil for parthenogenetic species (from 0.1 to 1.4; RM-ANOVA: $F_{1,4}$=22.25, P=0.009).
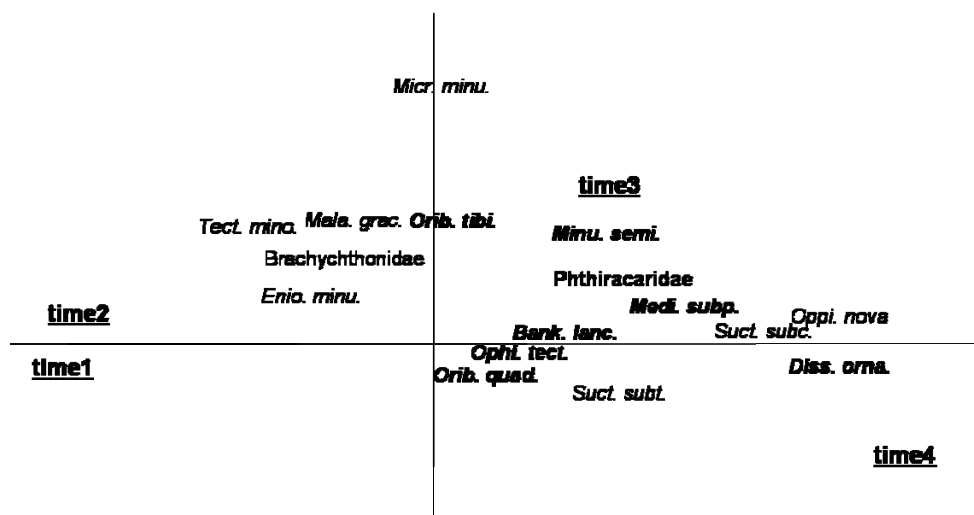

Figure 6.4 Principal Components Analysis of oribatid mite species in the re-colonization experiment. Bold names indicate sexual reproduction. *Bank. lanc.=Banksinoma lanceata, Diss. orna.=Disshorina ornata, Enio. minu.=Eniochthonius minutissimus, Mala. grac.=Malaconothrus gracilis, Medi. subp.=Medioppia subpectinata, Micr. minu.=Microppia minus, Minu. semi.=Minunthozetes semirufus, Ophi. tect.=Ophidiotrichus tectus, Oppi. nova=Oppiella nova, Orib. tibi.=Oribatula tibialis, Orib. quad.=Oribatella quadricornuta, Suct. subc.=Suctobelbella subcorniger, Suct. subt.=Suctobelbella subtrigona, Tect. mino.=Tectocepheus minor.* Eigenvalues axis 1: 0.57, axis 2: 0.14.

Table 6.2 Sex ratio and numbers of eggs of oribatid mite species after re-colonization from soil and litter.

Species	female	male	sex unkown	total	total sexed	% females$	eggs+	eggs/ female#	eggs/ all females§
Enarthronota									
Brachythoniidae	0	0	5	5	0	-	-	-	-
Enichthoniidae									
Enichthonius minutissimus (Berlese, 1903)	9	0	11	20	9	100	4	1.00	0.44
Mixonomata									
Phthiracaridae	8	0	19	27	8	100	15	1.88	1.88
Desmonomata									
Malaconothridae									
Malaconothrus gracilis v.d. Hammen, 1952	7	0	4	11	7	100	3	1.50	0.43
Pycnonotic Apheredermata									
Suctobelbidae									
Suctobelbella subcornigera (Forsslund, 1941)	5	0	72	77	5	100	5	1.00	1.00
Suctobelbella subtrigona (Oudemans, 1900)	1	0	8	9	1	100	1	1.00	1.00
Tectocepheidae									
Tectocepheus minor (Berlese, 1903)	10	0	0	10	10	100	5	1.00	0.50
Oppiidae									
Oppiella nova (Oudemans, 1902)	318	0	0	318	318	100	135	1.24	0.42
Microppia minus (Paoli, 1908)	73	3	1	77	76	96.1	1	1.00	0.01
Disshorina ornata (Oudemans, 1900)	117	90	0	207	207	56.5	117	1.56	1.00
Medioppia subpectinata (Oudemans, 1900)	15	10	0	25	25	60	18	2.25	1.20
Thyrisomidae									
Banksinoma lanceata (Michael, 1888)	3	7	1	11	10	30	4	2.00	1.33
Poronta									
Oribatellidae									
Ophidiotrichus tectus (Michael, 1884)	1	3	1	5	4	25	0	0.00	0.00
Oribatella quadricornuta (Michael, 1880)	4	2	0	6	6	66.7	8	2.67	2.00
Oribatulidae									
Oribatula tibialis (Nicolet, 1855)	9	5	0	14	14	64.3	24	8.00	2.67
Mycobatidae									
Mimnuthozetes semirufus (Koch, 1841)	8	9	0	17	17	47.1	2	2.00	0.25

6.4 Discussion

6.4.1 Resource Depletion Experiment

The aim of this experiment was to investigate if resource depletion in the soil system affects oribatid mite communities. Oribatid mites feed on a wide range of food resources indicating that they are generalist feeders (Maraun et al. 2003) although in the field trophic niche differentation is pronounced (Schneider et al. 2004a, b). In the resource depletion experiment density of oribatid mites significantly decreased with time indicating that resource availability indeed declined.

Within oribatid mites parthenogenetic species (*O. nova*, *M. minus*) were more affected by resource depletion than sexual species, indicating lower ecological flexibility of parthenogenetic taxa. Higher ecological flexibility of sexual species is presumably related to higher genetic variability due to meiotic recombination and amphimixis. Genetically diverse populations may exploit a wider range of resources and therefore suffer less from resource depletion than sexual species (Williams 1975, Glesener and Tilman 1978). In addition, sexually produced offspring with new genotypes may be able to use different resource combinations than the parent generation and therefore depletion of resources favours those individuals which switch to a currently underutilized resource (Ghiselin 1974). In parthenogenetic species, genetic diversity and the production of new genotypes depends on the mechanism of parthenogenesis. Although the parthenogenetic oribatid mite species which have been studied reproduce via thelytokous automixis (with terminal fusion of the meiotic products), the species are not genetically uniform but have similar diversity as compared to sexual oribatid mite species (Schaefer et al. 2006, but see Palmer and Norton 1992). Therefore, sexual and parthenogenetic oribatid mites may respond less differently than we had hypothesized.

The results suggest that parthenogenetic oribatid mite taxa adjust investment in reproduction by reducing egg production with declining availability of resources. In general, the number of eggs per female in parthenogenetic species was lower than in sexual species.

About 8-9% of oribatid mite species reproduce by parthenogenesis, and parthenogenetic species generally dominate reaching 60-80% of the total number of oribatid mites in litter and soil (Norton and Palmer 1991, Norton et al. 1993, Maraun et al. 2003, Cianciolo and Norton 2006). The generation time of oribatid mites varies from two to three weeks in Oppiidae (under lab conditions with high temperature; reviewed in Norton and Palmer 1991) up to 950 days in *Steganacarus magnus* (Travé et al. 1996) and up to five years in *Ameronothrus*

lineatus (Søvik and Leinaas 2003). Fecundity also differs between species; some taxa produce eggs during the whole year, in others egg production depends on season. Egg number as well as egg size is also variable among taxa (Travé et al. 1996). Enarthronota, a group with small specimens in deeper soil layers and thelytokous reproduction, lay only few eggs that develop slowly (Forsslund 1942, 1957). Also, in Phthiracaridae, a group with large (~2 mm) saprophagous species and mainly sexual reproduction, egg development is slow (longer than 120 days) (Luxton 1981, Travé et al. 1996). In Oppiidae and Suctobelbidae thelytoky is widespread and eggs develop quickly within 40 days (Luxton 1981, Travé et al. 1996). In contrast to these groups with small species (about 300 µm), in thelytokous Desmonomata egg development takes longer than 120 days (Luxton 1981, Travé et al. 1996).

As indicated by results of this study, small parthenogenetic species, such as *Suctobelbella subcornigera*, *Oppiella nova*, *Hypochthonius rufulus* and *Eniochthonius minutissimus,* carry one egg per female on average whereas sexual species, such as *Eupelops plicatus* and *Oribatula tibialis,* produce on average more than two eggs per female. Interestingly, sexual oppiids, e.g. *Medioppia subpectinata,* produce more eggs than parthenogenetic oppiids, e.g. *O. nova,* despite their similar body size. Unfortunately, the causal relationships between life history parameters, such as generation time, mode of reproduction and numbers of eggs produced, are little understood.

Overall, the results supported our hypothesis that parthenogenetic taxa are more sensitive to resource limitation. By investigating the response of oribatid mites to disturbances, Maraun et al. (2003) also concluded that parthenogenetic oribatid mite species are more sensitive to environmental changes than sexual species.

6.4.2 Re-colonization Experiment

In the re-colonization experiment density of oribatid mites increased with time indicating that following the defaunation resources were in ample supply. In the treatment re-colonized from litter, abundances of oribatid mites generally exceeded those in the treament re-colonized from soil indicating more rapid colonization by litter living species. Generally, about 77% of the oribatid mites colonized the litter layer suggesting more favourable conditions in the litter as compared to soil. Indeed, in early successional stages in the field oribatid mites predominantly colonize the litter layer (Scheu and Schulz 1996).

The percentage of parthenogenetic oribatid mite species remained constant in the treatment re-colonized from soil but slightly decreased in the treatment re-colonized from litter;

suggesting that re-colonization of sexual taxa was faster than that of parthenogenetic taxa, which contradicts theory. Re-colonization from soil resulted in a higher percentage of parthenogenetic oribatid mites (79%) as compared to re-colonization from litter (55%) indicating that parthenogenetic taxa predominate the fauna of the mineral soil layer (Norton and Palmer 1991, Norton et al. 1993).

The results of this study did not confirm that parthenogenetic species are faster colonizers although PCA analysis reflects that parthenogenetic taxa are correlated with the first two sampling dates. Small Oppiidae (*Oppiella nova*) and Brachychthoniidae (*Liochthonius sellnicki, Mixochthonius laticeps*) are fast colonizers of soils following disturbances (Luxton 1982, Ryabinin and Ran'kov 1987, Scheu and Schulz 1996). Furthermore, parthenogenetic oribatid mite taxa prevail in disclimax habitats but they are also present in later succesional stages indicating that they do not disappear with habitat development (Norton and Palmer 1991, Cianciolo and Norton 2006).

Sexuality should be favoured under conditions of unpredictable biological interactions which are pronounced in environmental stable habitats (Ghiselin 1974, Glesener and Tilman 1978). On the other hand, parthenogenetic reproduction may prevail in unstable habitats due to less specialized genotypes in parthenogenetic species (general-purpose genotype, Lynch 1984). Sexual as well as parthenogenetic oribatid mite taxa of different phylogenetic lineages (isolated lineages within Brachypylina and large parthenogenetic clusters within Desmonomata or Enarthronota) occupy similar niche breadth and co-occur in forest soil and litter habitats (Cianciolo and Norton 2006).

Similar to the resource depletion experiment, results of the re-colonization experiment also suggest that in parthenogenetic oribatid mite taxa egg production depends on the environmental conditions. In contrast to our expectations, the number of eggs per female was lower in parthenogenetic than in sexual taxa, most pronounced in small parthenogenetic and large sexual species. This pattern remains enigmatic since theory suggests that parthenogenetic species reproduce faster. Norton and Palmer (1991) hypothesized that body size and numbers of eggs may be poorly correlated in most Oribatida except in the more derived Brachypylina; no conclusion about the speed of egg deposition can be made since ovipostion rates can vary greatly and are not determinable from the number of eggs in a female body; egg numbers per female and egg development need further investigation.

In conclusion, the results suggest that parthenogenetic and sexual oribatid mite species differ little in the speed they recover from disturbances and re-colonize litter and soil, with body size and generation time having greater impact than reproductive mode. Furthermore,

parthenogenetic species are more affected by resource limitation although their genetic diversity presumably is comparable with that of sexual species. Parthenogenetic species changed their egg production quicker according to the environmental conditions since they are independent from mating and insemination. Generally, resource availability seems to affect parthenogenetic and sexual taxa in different ways; while resource depletion has a greater impact on parthenogenetic species, parthenogenetic and sexual taxa react similar to resource accessibility. This indicates that parthenogenetic species may depend on stable resource conditions while sexual species can adapt more easily.

Does Improved Resource Quality Promote Oribatid Mites?
A Laboratory Experiment

The availability of high quality resources is an important factor for community structure and reproductive mode of animals. Parthenogenetic reproduction prevails when resources are available in excess, whereas sexuality correlates with resource shortage. In this study we investigated the effect of resource availability on the community structure of oribatid mites in a laboratory experiment. The amount and quality of food resources were increased by addition of glucose to leaf litter and reduced by leaching of nutrients from leaves. Experimental systems were incubated at three different temperatures (10, 15, 20°C) to investigate if resource exploitation is accelerated at higher temperatures. The community structure of oribatid mites and the number of eggs per female were measured over a period of ten months. We expected the number of specimens to decline in the reduced litter quality treatment but to increase in the glucose treatment. Both effects were assumed to be more pronounced at higher temperatures due to faster exploitation of resources (reduced litter quality treatment) and accelerated reproduction (glucose treatment). We hypothesized that sexual species will be less affected than parthenogens by reduced resource quality due to higher genetic diversity allowing more efficient exploitation of limited resources, but that sexuals will be outnumbered by parthenogens in case of resource addition since parthenogens reproduce faster. In contrast to our hypotheses, the number of specimens declined in each treatment, and sexual and parthenogenetic oribatid mite species in general responded similarly. The parthenogenetic Brachychthoniidae and *Tectocepheus* dominated early in the experiment but were replaced later by parthenogenetic Desmonomata and *Rhysotritia*. In parthenogens the number of eggs per female increased during the experiment while the number of eggs in sexual females remained constant or decreased slightly; in general, egg numbers were higher in sexuals than in parthenogens. Overall, our data indicate that for sustaining oribatid mite populations other resources (e.g., mycorrhizal fungi) than easily available carbon or saprotrophic microorganisms are needed. The results also indicate that there are two groups of parthenogens in oribatid mites: exploiters of easily available resources and consumers of leaf litter associated resources, with the latter being unable to respond quickly to the availability of high quality resources.

7.1 Introduction

The amount and availability of resources is one of the most important factors structuring animal communities. In addition to changing population density and community structure it also influences the reproductive mode. Temporary or permanent parthenogenetic reproduction dominates when resources are available in excess whereas sexuality correlates with resource shortage (Bell 1982, Scheu and Drossel 2007). Theoretical models explaining the prevalence of sex in the animal kingdom, such as the Tangled Bank (Ghiselin 1974) or Lottery Models (Williams 1975), imply that the response of individuals with different genotypes to environmental heterogeneity differs. Sexual species are likely to occupy a wider range of environmental conditions and to exploit a wider range of resources as they include diverse genotypes due to outcrossing and recombination (Bell 1982). Consequently, theory predicts the dominance of sexuality in unstable habitats with varying environmental conditions (Williams 1975, Glesener and Tilman 1978, Hamilton 1980). On the other hand, parthenogenesis should be favoured in stable habitats (such as the litter layer or deep soil) with constant availability of resources (Scheu and Drossel 2007). Fluctuations in resource quantity and quality therefore are likely to influence the predominance of sexual or parthenogenetic taxa (Korpelainen 1990); that sexuality in fact often is associated with resource shortage is exemplified by cyclical parthenogens such as aphids and cladocerans (Bell 1982, Redfield 1999).

A recent model predicts that sex prevails when structured resources are in short supply, implying that exploited resources will not be available to the same extent and same quality to the consumer in the next generation and that only few genotypes can be present at one location at the same time (Scheu and Drossel 2007). In this model parthenogens are favoured when death rates are high, resources are little structured, resources regenerate fast or several genetically diverse parthenogens cover the entire spectrum of resources. According to this model the higher percentage of parthenogenetic species in forest soils as compared to other terrestrial ecosystems is likely due to constant availability of resources (Scheu and Drossel 2007), with resources being provided seasonally via litter material (Fioretto et al. 2003, Bardgett et al. 2005, McNaughton et al. 1989) and plant roots (Pollierer et al. 2007). The variety of food resources available, including leaf and root litter, bacteria, fungi, algae and lichens, allows coexistence of a large number of soil meso- and macrofauna species (Schneider et al. 2004a, b, Ruess et al. 2005). However, it is puzzling why in many soil taxa

sexually and parthenogenetically reproducing species of close taxonomic affiliation and assumed similar trophic niche coexist in the same habitat.

An important soil-dwelling group of microarthropods are oribatid mites (Oribatida, Acari). They play a key role in the decomposition of litter and consume a great variety of food resources (Schneider et al. 2004a). A remarkable feature of the speciose group of oribatid mites is the high incidence of parthenogenesis; about 9% of all oribatid mite species have abandoned sex (Norton and Palmer 1991) and in forest soils of the temperate and boreal zone typically 60-80% of the individuals reproduce by parthenogenesis (Norton and Palmer 1991, Norton et al. 1993, Maraun et al. 2003, Cianciolo and Norton 2006, Domes et al. 2007a). Based on the high percentage of parthenogenesis and the high age (Shear et al. 1984, Norton et al. 1988, Hammer and Wallwork 1979, Heethoff et al. 2007), some taxa of oribatid mites join bdelloid rotifers (Mark Welch and Meselson 2000) and darwinulid ostracods (Martens et al. 2003) as "ancient asexual scandals" (Maynard Smith 1978, Judson and Normark 1996), challenging evolutionary theories that predict the extinction of parthenogens in the long-term.

One possible explanation for the coexistence of sexual and parthenogenetic oribatid mite species in forests is that they colonize different microhabitats. Indeed, oribatid mite species on the bark of trees are mainly sexual (Erdmann et al. 2006) whereas parthenogenetic species predominantly live in soil (Cianciolo and Norton 2006, Domes et al. 2007a). This correlates with the assumption that sex prevails in unstable habitats (the bark of trees) while parthenogenesis is favoured when conditions are stable as it is the case in soil; recent studies on oribatid mites have shown that parthenogenetic taxa indeed suffer more from resource limitation than sexual species (Domes et al. 2007a).

The present study investigates the influence of changes in the availability of resources on oribatid mite density, communitiy structure and reproductive mode at different temperatures in a laboratory microcosm experiment. Therefore, treatments with (a) untreated litter material, (b) with litter of reduced quality and (c) with glucose-enriched high quality litter material were established. Microcosms were incubated at different temperatures as we expected effects to be more pronounced at higher temperatures due to faster exploitation of resources and accelerated reproduction. In general, we expected the number of specimens to decline in the reduced litter quality treatment due to declining resources but to increase in the glucose enriched treatment. We hypothesized that parthenogenetically reproducing species will be favoured in the high quality litter treatment due to faster reproduction caused by the avoidance of searching mating partners and investing in the production of males. Consequently, when resources become limiting we expected sexuals to become more

dominant at the expense of parthenogens since sexual populations presumably comprise more diverse genotypes allowing more complete resource exploitation.

7.2 Materials and Methods

7.2.1 Study Site

Soil samples were taken from the Kranichsteiner forest, located about 8 km northeast of Darmstadt (Germany). The Kranichsteiner forest is dominated by beech (*Fagus sylvatica*) interspersed with ca. 190 y old oak (*Quercus robur*) and hornbeam (*Carpinus betulus*). The herb layer is dominated by *Luzula luzuloides, Milium effusum, Anemone nemorosa* and *Polytrichum formosum.* Parent rock is Rotliegendes covered with sand; the humus form is moder (FAO-UNESCO classification)**.**

7.2.2 Experimental Setup

Soil cores (45 in total; Ø 21 cm L, F, H layer and the upper 3 cm of the Ah layer) were taken from the Kranichsteiner forest and placed in laboratory microcosms. The litter material was removed, mixed and weighted. Subsequently, equivalent amounts of litter were placed back into the microcosms establishing three treatments (five replicates each): (a) control treatment with untreated litter material, (b) reduced litter quality treatment with litter kept in 60°C water for 24 h to reduce food quality by leaching of nutrients, and (c) high quality litter treatment with untreated litter sprinkled with glucose solution as additional carbon source at regular intervals. Microcosms were incubated at constant 10, 15 and 20°C in darkness. The microcosms were closed with plastic at the bottom and with gauze (45 µm) at the top; loss of water was evaluated gravimetrically and replaced by distilled water every week for the control and reduced litter quality treatment or by adding glucose solution for the glucose enriched treatment. Microbial respiration was measured as CO_2 produced and mineralization of carbon was calculated as mg C microcosm^{-1} week^{-1}. CO_2 evolved in the microcosms was trapped in 2 ml alkali (1 M NaOH) in vessels placed on the leaf litter. Trapped CO_2 was measured in an aliquot by titration with 0.1 M HCl after precipitation of carbonate with saturated $BaCl_2$ (Macfadyen 1970). Microbial C mineralization was used to calculate the amount of carbon produced and subsequently replenished by adding the 2-fold amount of glucose to the glucose enriched treament.

After 2, 10, 20 and 44 weeks soil cores (Ø 5 cm) were taken from the microcosms, separated into litter and soil (0-3 cm depth) and oribatid mites were extracted by heat (Macfadyen 1961, Kempson et al. 1963). Holes subsequently were filled with sand. Oribatid mites were counted and determined to species level if possible. In addition, adults were sexed, and in females the number of eggs was counted to compare egg production between parthenogenetic and sexual species. Species were considered to be sexual when there were more than 5% males. Brachychthoniidae, Phthiracaridae and Suctobelbidae were difficult to sex; females could only be identified if they carried eggs. The mode of reproduction in these taxa was inferred from the literature (cf. Cianciolo and Norton 2006).

7.2.3 Aggregation of species

Species of adult oribatid mites were aggregated into ten subgroups according to phylogenetic relationships and the inferred mode of reproduction: (1) Brachychthoniidae (parthenogenetic, including undetermined Brachychthoniidae, *Brachychthonius berlesei*, *Liochthonius* sp. and *Sellnickochthonius honestus*), (2) Enarthronota (parthenogenetic, comprising only *Eniochthonius minutissimus;* excluding Brachychthoniidae), (3) Phthiracaridae (undetermined species, mainly sexual, except *Rhystotritia duplicata*), (4) *Rhystotritia duplicata* (parthenogenetic), (5) Desmonomata (parthenogenetic, comprising *Malaconothrus gracilis, Nanhermannia coronata, N. nana* and *Nothrus silvestris*), (6) *Tectocepheus* (parthenogenetic, comprising *T. minor, T. sarekensis* and *T. velatus*), (7) Suctobelbidae (parthenogenetic, including undetermined Suctobelbidae, *Suctobelbella subcornigera* and *S. subtrigona*), (8) parthenogenetic Oppiidae (including *Oppiella nova* and *Microppia minus*), (9) sexual Oppiidae (including *Berniniella sigma, Disshorina ornata* and *Medioppia subpectinata*) and (10) other Circumdehiscentiae (sexual, including *Achipteria coleoptrata, Cultroribula bicultrata, Carabodes femoralis, C. ornatus, Galumna lanceata, Ophidiotrichus tectus, Oribatula tibialis, Minunthozetes semirufus* and *Banksinoma lanceata*).

7.2.4 Statistical Analysis

For statistical analyses specimens of the litter and soil layer were combined; the great majority of inviduals occurred in the litter layer. Changes in time of micorbial C mineralization, density of total oribatid mites, density of subgroups of oribatid mites, percentages of parthenogenetic individuals, and numbers of eggs per female were analysed by

repeated measures multivariate analysis of variance (RM-ANOVA) or repeated measures general linear models (RM-GLM) in SAS 9.13 (SAS Institute Inc., Cary, USA), with time as repeated and treatment and temperature as categorical factors (Scheiner and Gurevitch 2001).

Further, changes in oribatid mite community composition were analysed by non-metric multidimensional scaling (NMDS; Kruskal 1964) implemented in Statistica (StatSoft Inc., Tulsa, USA). NMDS reduced the number of meaningful dimensions to three, and therefore stress values of three dimensions were used as independent factors in discriminant function analysis (DFA) to explore effects of time. In addition, data on oribatid mite species were analysed by detrended correspondence analysis (DCA) using CANOCO 4.5 (Ter Braak and Šmilauer 1998).

Abundances of individuals, numbers of eggs per female and data on microbial C mineralization were log(x+1) transformed and percentages of parthenogenetic individuals were arcsin square root transformed prior to statistical analysis to increase homogeneity of variances. Species with fewer than ten individuals in total and species which occurred in less than three samples were excluded from the statistical analyses (Table 7.1).

7.3 Results

7.3.1 Microbial C Mineralization

The average daily microbial C mineralization per microcosm was at a maximum at 20°C (ANOVA, $F_{2,36}=141.59$, P<0.0001 for temperature) and increased by glucose addition (ANOVA, $F_{2,36}=101.46$, P<0.0001 for treatment; Fig. 7.1a). Temperature had the strongest effect in the reduced litter quality treatments showing the steepest increase from 10°C and 15°C to 20°C (ANOVA, $F_{4,36}=2.14$, P=0.096 for the interaction between temperature and treatment). The cumulative microbial C mineralization per microcosm exponentially increased from week two to 12 and then leveled off to week 35 (Fig. 7.1b). However, the pattern changed significantly with time in the control treatments at 10°C and 20°C and the reduced litter quality treatment at 20°C with the lowest increase after week 18 (RM-ANOVA, $F_{60,540}=2.35$, P<0.0001 for the interaction between time, temperature and treatment).
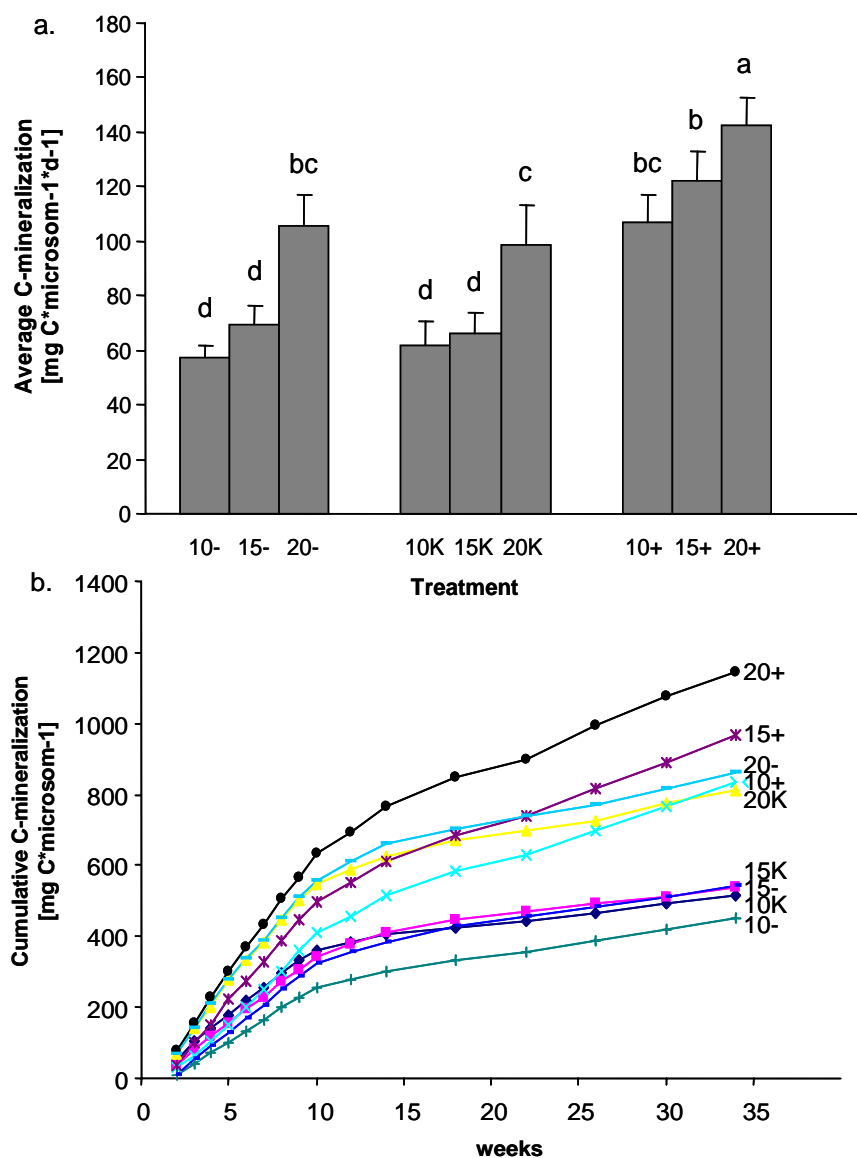
Figure 7.1 Daily microbial C mineralization per microcosm as affected by treatment (control, reduced litter quality and glucose addition) and temperature (10, 15 and 20°C) (a) and cumulative microbial C mineralization during 34 weeks of incubation in the respective treatments (b). "-" = reduced litter quality, "+" = glucose additon, K =control.

7.3.2 Density and Community Composition of Oribatid Mites

In total, 25,639 oribatid mites were inspected; 12,224 individuals were juvenile and not determined further. The 13,415 adult oribatid mites represented 33 species and three pooled taxa (undetermined Brachychthoniidae, Phthiracaridae and Suctobelbidae); of these, 13,401 individuals from 28 species and the three pooled taxa were included in the statistical analysis (Table 7.1).

Table 7.1 Sex ratio and numbers of eggs of oribatid mite species. $=Species are excluded from statistical analysis (see text for details), °=from sex-determinable specimens, +=total number, #=only females with eggs, § =total number of females including those without eggs.

Group	Species	female	male	sex unkown	total	total sexed	% female°	eggs+	eggs/ female#	eggs/ population§
Enarthronota										
Brachythoniidae	*Brachychthonius berlesei* Willmann, 1936	54	0	298	352	54	100	49	1.00	0.91
	Brachychthoniidae Thor, 1934	7	0	140	147	7	100	5	1.00	0.71
	Lichochthonius sp. v.d. Hammen, 1959	1	0	13	14	1	100	1	1.00	1.00
	Selnickochthonius honestus (Moritz, 1976)	8	0	85	93	8	100	8	1.00	1.00
Enichthoniidae	*Enichthonius minutissimus* (Berlese, 1903)	382	0	600	982	382	100	210	1.00	0.55
Hypochthoniidae	*Hypochthonius rufulus* Koch, 1835$	4	0	5	9	4	100	1	1.00	0.25
Mixonomata										
Euphthiracaridae	*Rhysotritia duplicata* (Grandjean, 1953)	367	0	10	377	367	100	420	1.71	1.14
Phthiracaridae	Phthiracaridae Petry, 1841	244	0	291	535	244	100	429	1.81	1.76
	Steganacarus magnus (Nicolet, 1955)$	2	0	1	3	2	100	5	5.00	2.50
Desmonomata										
Malaconothridae	*Malaconothrus gracilis* v.d. Hammen, 1952	242	0	31	273	242	100	50	1.02	0.21
Nanhermanniidae	*Nanhermannia coronata* Berlese, 1913	41	0	0	41	41	100	30	1.36	0.73
	Nanhermannia nana (Nicolet, 1855)	548	0	0	548	548	100	390	1.30	0.71
Nothridae	*Nothrus silvestris* Nicolet, 1855	737	0	0	737	737	100	1169	2.08	1.59
Circumdehiscentiae										
Achipteriidae	*Achipteria coleoptrata* (Linnaeus, 1758)	171	127	3	313	310	55.2	311	2.27	1.82
Astegistidae	*Cultroribula bicultrata* (Berlese, 1905)	38	4	0	42	42	90.5	11	2.20	0.29
Carabodidae	*Carabodes areolatus* Berlese, 1916$	0	2	2	4	2	0	0	-	-
	Carabodes femoralis (Nicolet, 1855)	4	5	6	14	8	50	7	3.50	1.75
	Carabodes ornatus Storkamm 1925	2	5	4	11	7	28.6	1	1.00	0.50
Galumnidae	*Galumna lanceata* Oudemans, 1900	10	10	4	24	20	50	7	2.33	0.70
Oppiidae	*Berniella sigma* (Strenzke, 1951)	49	51	0	105	105	46.7	2	1.00	0.04
	Disshorina ornata (Oudemans, 1900)	88	75	0	169	169	52.1	98	1.61	1.11
	Medioppia subpectinata (Oudemans, 1900)	13	10	0	23	23	56.5	16	2.00	1.23
	Micreppia minus (Paoli, 1908)	3209	0	22	3231	3209	100	22	1.00	0.01
	Oppiella nova (Oudemans, 1902)	1812	35	6	1855	1849	98	414	1.19	0.23
Oribatellidae	*Ophidiotrichus tectus* (Michael, 1884)	11	11	0	22	22	50	8	2.67	0.73
	Oribatella quadricornuta Michael, 1880$	1	1	0	3	3	33.3	4	4.00	4.00
Oribatulidae	*Oribatula tibialis* (Nicolet, 1855)	244	93	16	353	337	72.4	434	3.50	1.78
Phenopelopidae	*Eupelops plicatus* (Koch, 1836)$	3	0	1	4	3	100	0	-	-
Punctoribatidae	*Minunthozetes semirufus* (Koch, 1841)	53	39	3	95	92	57.6	23	2.09	0.43
Suctobelbidae	Suctobelbidae Jacot, 1938	3	0	180	183	3	100	3	1.00	1.00
	Suctobelbella subcornigera (Forsslund, 1941)	17	0	595	612	17	100	17	1.00	1.00
	Suctobelbella subtrigona (Oudemans, 1900)	7	2	156	163	7	100	7	1.00	1.00
Tectocepheidae	*Tectocepheus minor* (Berlese, 1903)	289	0	0	291	291	99.3	115	1.15	0.40
	Tectocepheus sarekensis Trägardh, 1910	451	0	0	451	451	100	52	1.24	0.12
	Tectocepheus velatus (Michael, 1880)	1146	0	1	1147	1146	100	103	1.11	0.09
Thyrisomidae	*Banksinoma lanceata* (Michael, 1888)	86	76	27	189	162	53.19	45	1.50	0.52

Among the 28 species included in the analysis 12 were sexual with percentages of females ranging from 29% in *Carabodes ornatus* to 90% in *Cultroribula bicultrata* and 16 were parthenogenetic comprising 100% females except *Oppiella nova* (98%) and *Tectocepheus minor* (99%; Table 7.1). The percentage of total parthenogenetic individuals neither changed with time (RM-GLM, $F_{3,81}=1.34$, P=0.266) nor differed between treatments (RM-GLM, $F_{2,27}=2.87$, P=0.0744) or was affected by temperature (RM-GLM, $F_{2,27}=1.22$, P=0.311); on average it was 84%.

Total abundance of adult oribatid mites decreased significantly with time (RM-ANOVA, $F_{3,132}=93.35$, P<0.0001), mainly between week 10 and 20 (RM-ANOVA, $F_{1,44}=37.78$, P<0.0001) and 20 and 44 (RM-ANOVA, $F_{1,44}=102.68$, P<0.0001; Fig. 7.2a). It was neither significantly affected by treatment (RM-ANOVA, $F_{2,36}=0.02$, P=0.985) nor by temperature (RM-ANOVA, $F_{2,36}=0.62$, P=0.542). Parthenogenetic Oppiidae, *Tectocepheus* and Desmonomata were most abundant while Brachychthoniidae, *Rhysotritia*, sexual Oppiidae and Phthiracaridae were least abundant (Table 7.1, Fig. 7.3). Total numbers of juvenile oribatid mites also significantly decreased with time at 10 and 15°C but remained constant at 20°C (RM-ANOVA, $F_{3,108}=30.49$, P<0.0001 for time and $F_{6,108}=4.74$; P=0.0003 for the interaction between time and treatment; Fig. 7.2b).
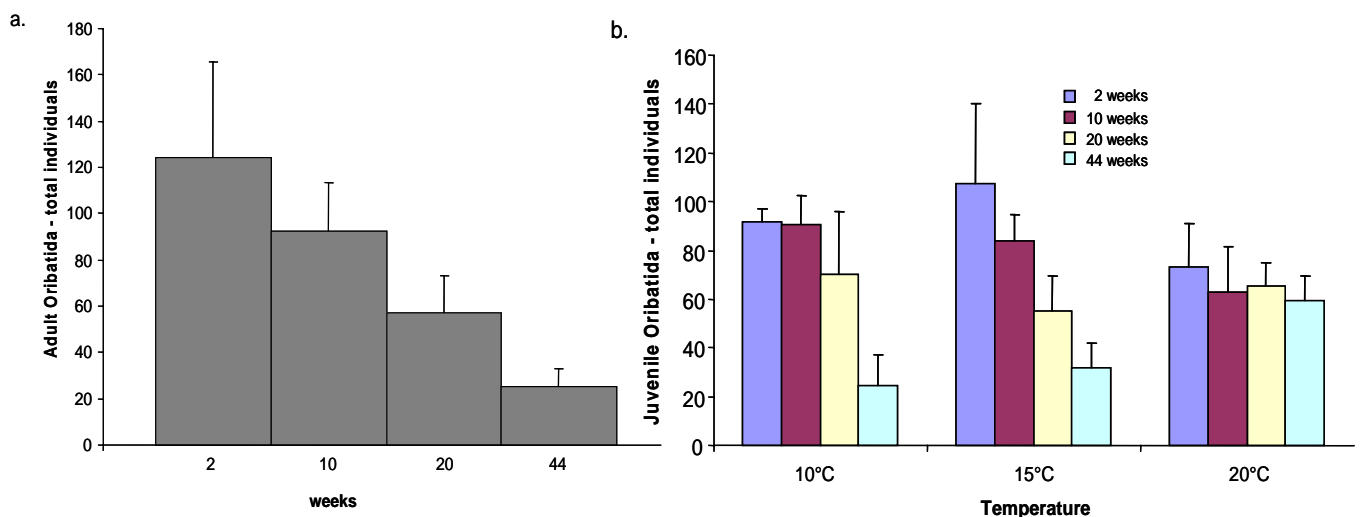
Figure 7.2 Changes in the abundance of (a) adult oribatid mites in total (pooled for temperature and treatment, see text for details) and (b) juvenile oribatid mites at 10, 15 and 20°C (pooled for treatment).

Subgroups of oribatid mites also changed significantly with time but responded differently to treatment and temperature (Table 7.2, Fig. 7.3). In sexuals, Circumdehiscentiae and Phthiracaridae responded similar to the treatments at each of the temperatures; while total abundance of Circumdehiscentiae significantly decreased with time, total numbers of

Phthiracaridae slightly increased. Sexual Oppiidae responded significantly to time, temperature and treatment. Abundances generally declined in the control treatment while they increased until week 10 and declined thereafter in the glucose enriched treatments at 10 and 15°C but not at 20°C. The response in the reduced litter quality treatment varied with temperature; while abundances increased until week 20 and declined thereafter at 10°C, they continuously declined at 15°C, but recovered to week 44 at 20°C.

Table 7.2 Table of F- and P-values on the effect of time (2, 10, 20 and 44 weeks), temperature (10, 15 and 20°C) and treatment (control, reduced litter quality and glucose addition) of the density of ten pooled oribatid mites groups analysed by repeated measures analysis of variance.

	df	Brachychthonidae F	P	Enarthronota F	P	Rhysotritia F	P	Desmonomata F	P
temp	2, 36	3.72	**0.0340**	0.48	0.6241	4.77	**0.0145**	1.92	0.1612
tr	2, 36	0.42	0.0551	0.33	0.7239	8.29	**0.0011**	2.35	0.1100
temp x tr	4, 36	3.92	**0.0097**	0.19	0.9430	0.72	0.5850	1.26	0.3051
time	3, 108	35.08	**<0.0001**	33.94	**<0.0001**	3.7	**0.0139**	3.84	**0.0118**
time x temp	6, 108	2.95	**0.0105**	2.01	0.0711	1.34	0.2439	3.79	**0.0018**
time x tr	6, 108	0.48	0.8194	3.02	**0.0091**	1.45	0.2014	2.9	**0.0116**
time x temp x tr	12, 108	1.36	0.1968	1.11	0.3569	0.84	0.6063	1.2	0.2953

	df	Tectocepheus F	P	Suctobelbidae F	P	Oppiidae (parth) F	P	Oppiidae (sex) F	P
temp	2, 36	6.51	**0.0039**	1.2	0.3121	11.57	**0.0001**	2.64	0.0850
tr	2, 36	0.53	0.5931	0.79	0.4625	1.37	0.2682	0.21	0.8147
temp x tr	4, 36	0.74	0.5686	0.5	0.7358	3.12	**0.0265**	1.15	0.3498
time	3, 108	194.55	**<0.0001**	10.75	**<0.0001**	76.48	**<0.0001**	14.02	**<0.0001**
time x temp	6, 108	2.93	**0.0109**	3.36	**0.0045**	7.23	**<0.0001**	3.51	**0.0048**
time x tr	6, 108	1.58	0.1601	1.45	0.2032	0.38	0.8587	1.01	0.4213
time x temp x tr	12, 108	0.67	0.7738	1.17	0.3162	1.39	0.1798	2.43	**0.0106**

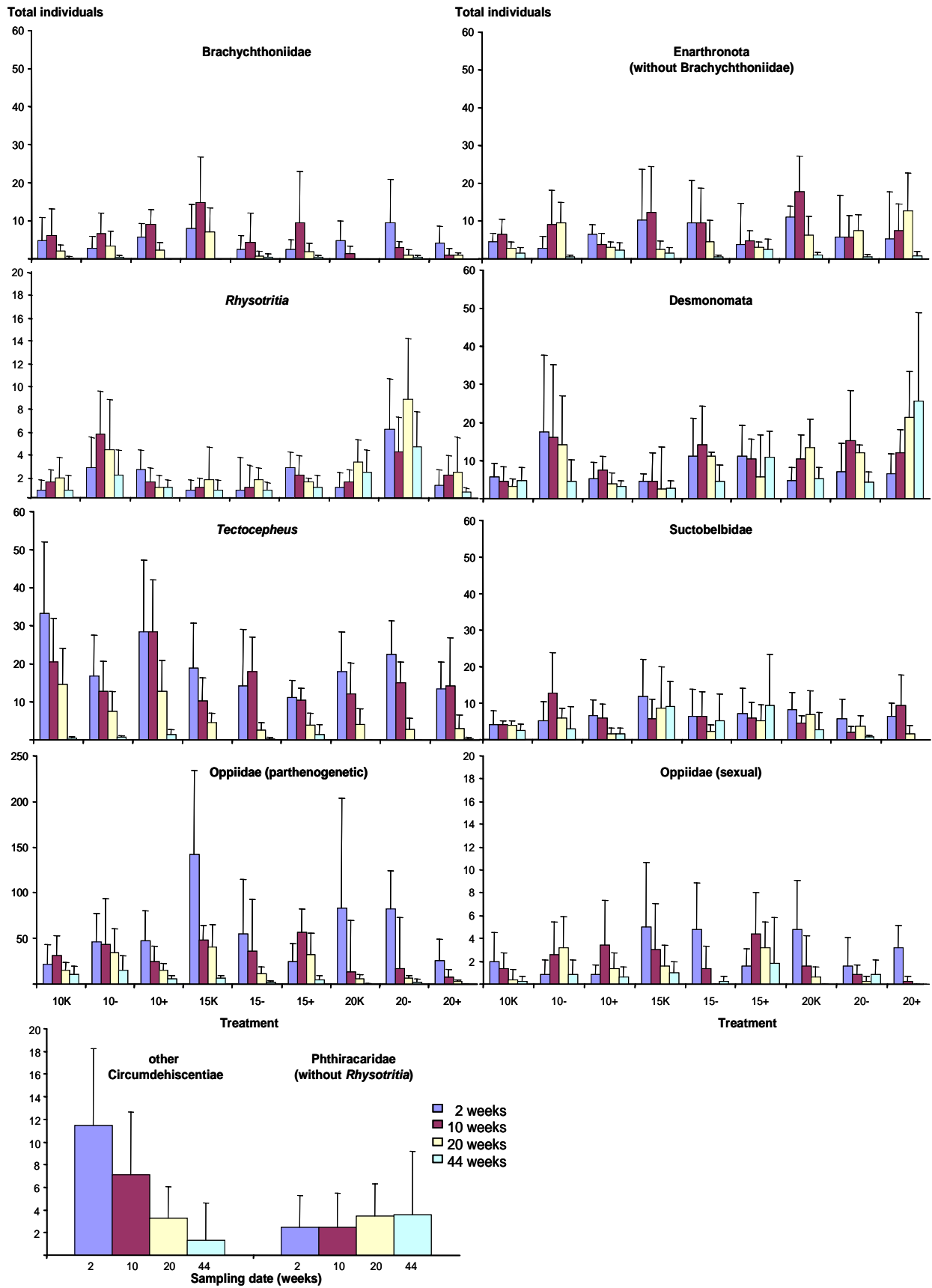|	df	Circumdehiscentiae F	P	Phthiracaridae F	P
temp	2, 36	1.45	0.2489	2.36	0.1088
tr	2, 36	1.74	0.1899	2.86	0.0704
temp x tr	4, 36	0.72	0.5846	0.55	0.6990
time	3, 108	91.06	**<0.0001**	2.71	**0.0489**
time x temp	6, 108	1.22	0.3028	1.56	0.1653
time x tr	6, 108	1.05	0.4001	0.51	0.7993
time x temp x tr	12, 108	1.54	0.1206	1.16	0.3219

Figure 7.3 Changes in density of ten oribatid mite subgroups as affected by treatment (control, reduced litter quality and glucose addition) and temperature (10, 15 and 20°C). Note different scales. Subgroups are in phylogenetic order (except Phthiracaridae). "-"=reduced litter quality, "+"=glucose addition, "K"=control.

In parthenogens, the total number of individuals declined with time. Brachychthoniidae, *Tectocepheus,* Suctobelbidae and parthenogenetic Oppiidae were strongly affected at higher temperatures as indicated by fast decline in total individuals; overall, while densities of Brachychthoniidae, Suctobelbidae and parthenogenetic Oppiidae were at a maximum at 15°C, the density of *Tectocepheus* was at a maximum at 10°C (Fig. 7.3). In contrast, the densities of Enarthronota, *Rhysotritia* and Desmonomata were highest at 20°C, with Desmonomata continuously increasing in the glucose treatment. Enarthronota responded differently to time and treatment. While densities increased from week 2 to 10 but decreased thereafter in control treatments, the decrease was retarded (after week 20) in the reduced litter quality and glucose treatments.

Discriminant function analysis (DFA) proved significant changes in community structure between week 10 and 20 (F=3.60, P=0.017, df=4,29) and week 20 and 44 (F=5.68, P=0.002, df=4,29; Fig. 7.4). Ordination by detrended correspondence analysis (DCA) further showed a shift in oribatid mite subgroups; while parthenogenetic Brachychthoniidae (e.g., *Brachychthonius berlesei, Sellnickochthonius honestus*) and the genus *Tectocepheus* were associated with early sampling dates (Fig. 7.5), parthenogenetic Desmonomata (e.g., *Malaconothrus gracilis, Nanhermannia nana, N. coronata*) and the parthenogenetic genus *Rhysotritia* were associated with later samplings dates. Sexual Phthiracaridae and *M. subpectinata* also occurred late while sexual Circumdehiscentiae (e.g., *Minunthozetes semirufus, Achipteria coleoptrata, Ophidiotrichus tectus*) were associated with early to mid sampling dates.
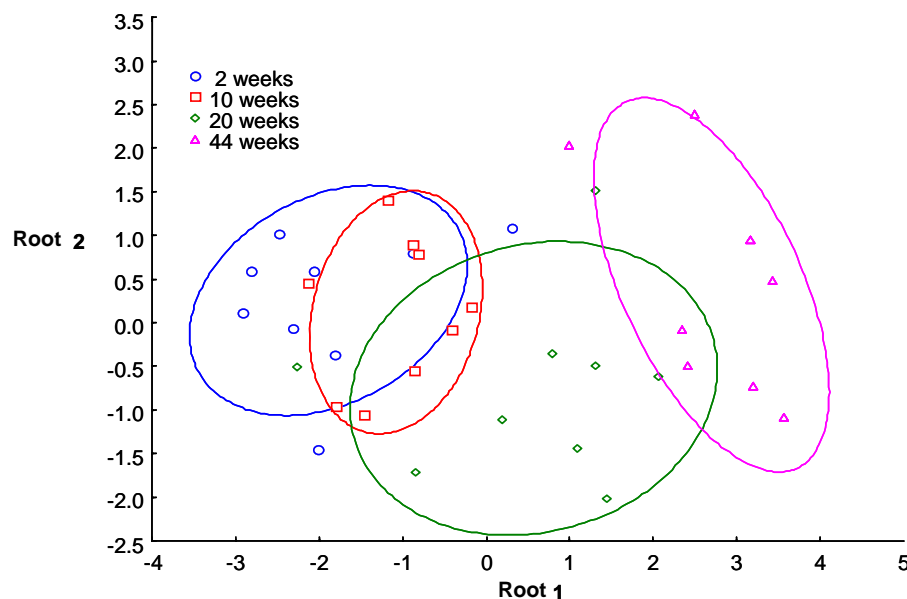
Figure 7.4 Changes of the oribatid mite community over time (2, 10, 20 and 44 weeks) analysed by non-metric multidimensional scaling; number of species were reduced to meaningful dimensions of three, stress values of three dimensions were subsequently used as independent factors in discriminant function analysis to explore effects of time.
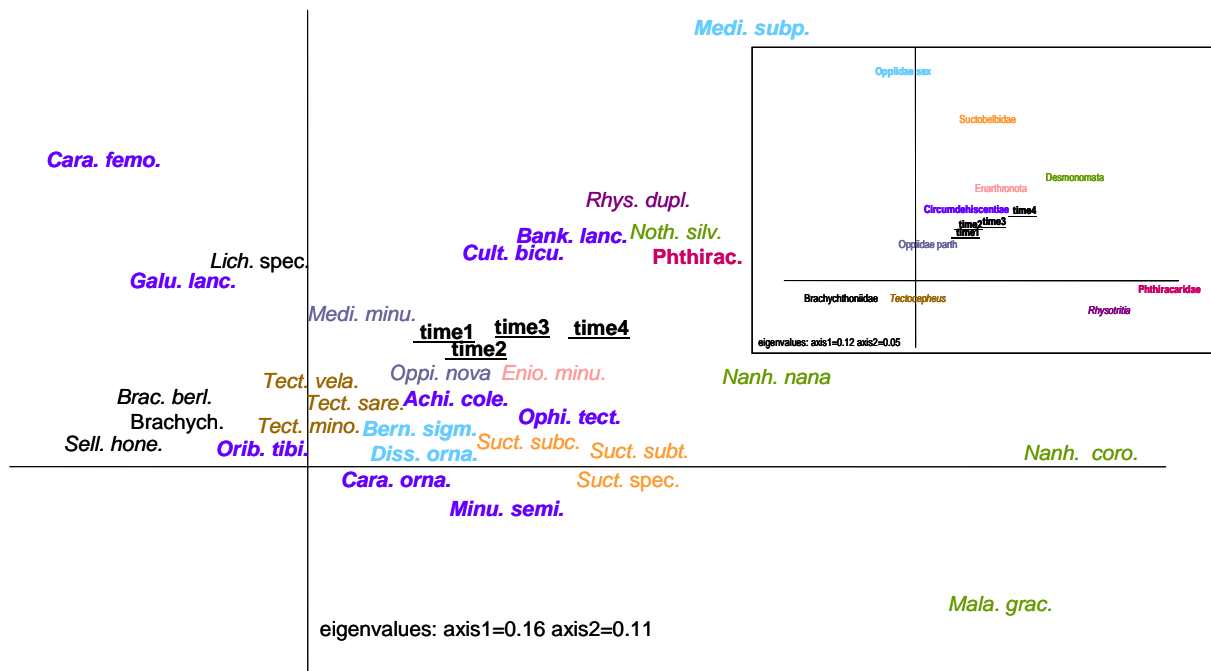
Figure 7.5 Detrended correspondence analysis of oribatid mite species and subgroups with sampling time included as supplementary variable not affecting the ordination (time 1=2 weeks, time 2=10 weeks, time 3=20 weeks, time 4=44 weeks). Bold names indicate sexual reproduction. *Ache. cole.=Achipteria coleoptrata*, *Bank. lanc.=Banksinoma lanceata*, *Bern. sigm.= Berniella sigma*, *Brac. berl.=Brachychthonius berlesei*, Brachych.=Brachychthoniidae, *Cara. femo.=Carabodes femoralis*, *Cara. orna.=Carabodes ornatus*, *Cult. bicu.=Cultroribula bicultrata*, *Diss. orna.=Disshorina ornata*, *Enio. minu.=Eniochthonius minutissimus*, *Galu. lanc.=Galumna lanceata*, *Lich.* spec.*=Lichochthonius* sp., *Mala. grac.=Malaconothrus gracilis*, *Medi. subp.=Medioppia subpectinata*, *Medi. minu.=Microppia minus*, *Minu. semi.=Minunthozetes semirufus*, *Nanh. coro.=Nanhermannia coronata*, *Nanh. nana=Nanhermannia nana*, *Noth. silv.=Nothrus silvestris*, *Ophi. tect.=Ophidiotrichus tectus*, *Oppi. nova=Oppiella nova*, *Orib. tibi.=Oribatula tibialis*, Phthirac=Phthiracaridae, *Rhys. dupli.=Rhysotritia duplicata*, *Sell. hone.=Sellnickochthonius honestus*, *Suct.* spec.*=Suctobelbella* sp., *Suct. subc.=Suctobelbella subcorniger*, *Suct. subt.=Suctobelbella subtrigona*, *Tect. mino.=Tectocepheus minor*.

7.3.3 Numbers of Eggs

Sexual individuals on average carried significantly more eggs per gravid female (2.21) than parthenogenetic species (1.23; RM-GLM, $F_{1,88}$=183.79, P<0.0001). The same pattern applied when related to all females including those without eggs, on average (respective data of 1.36 and 0.46; RM-GLM, $F_{1,64}$=168.48, P<0.0001; Fig. 7.6a). While the average number of eggs per female (including those without eggs) in parthenogenetic species slightly increased with time, it remained constant in sexual species (RM-GLM, $F_{3,192}$=4.04, P=0.008 for the interaction between time and reproductive mode; Fig. 7.6a). However, the number of eggs changed significantly with time, temperature and treatment (RM-GLM, $F_{12,108}$=2.54, P=0.006 and $F_{9,45}$=2.70, P=0.013 for the interaction between time, temperature and treatment in parthenogenetic and sexual species, respectively). In parthenogenetic species the number of eggs generally increased with time except in the glucose enriched treatment at 15°C (RM-

ANOVA, $F_{3,108}=37.81$, P<0.0001; Fig. 7.6b); the increase was faster and resulted in higher egg numbers at 20°C (RM-ANOVA, $F_{6,108}=4.22$, P=0.0007; for the interaction between time and temperature; Fig. 7.6b). In contrast to parthenogenetic species, the number of eggs per female in sexual species (including those without eggs) was higher at lower temperatures with the maximum being at 10°C (RM-GLM, $F_{2,15}=26.53$, P<0.0001; Fig. 7.6c). Generally, the number of eggs either remained constant (e.g., reduced litter quality treatment at 20°C) or decreased with time (e.g., control at 15°C and 20°C; RM-GLM, $F_{2,15}=4.49$, P=0.03 for treatment, $F_{3,13}=3.22$, P=0.052 for the interaction between temperature and treatment; Fig. 7.6c).
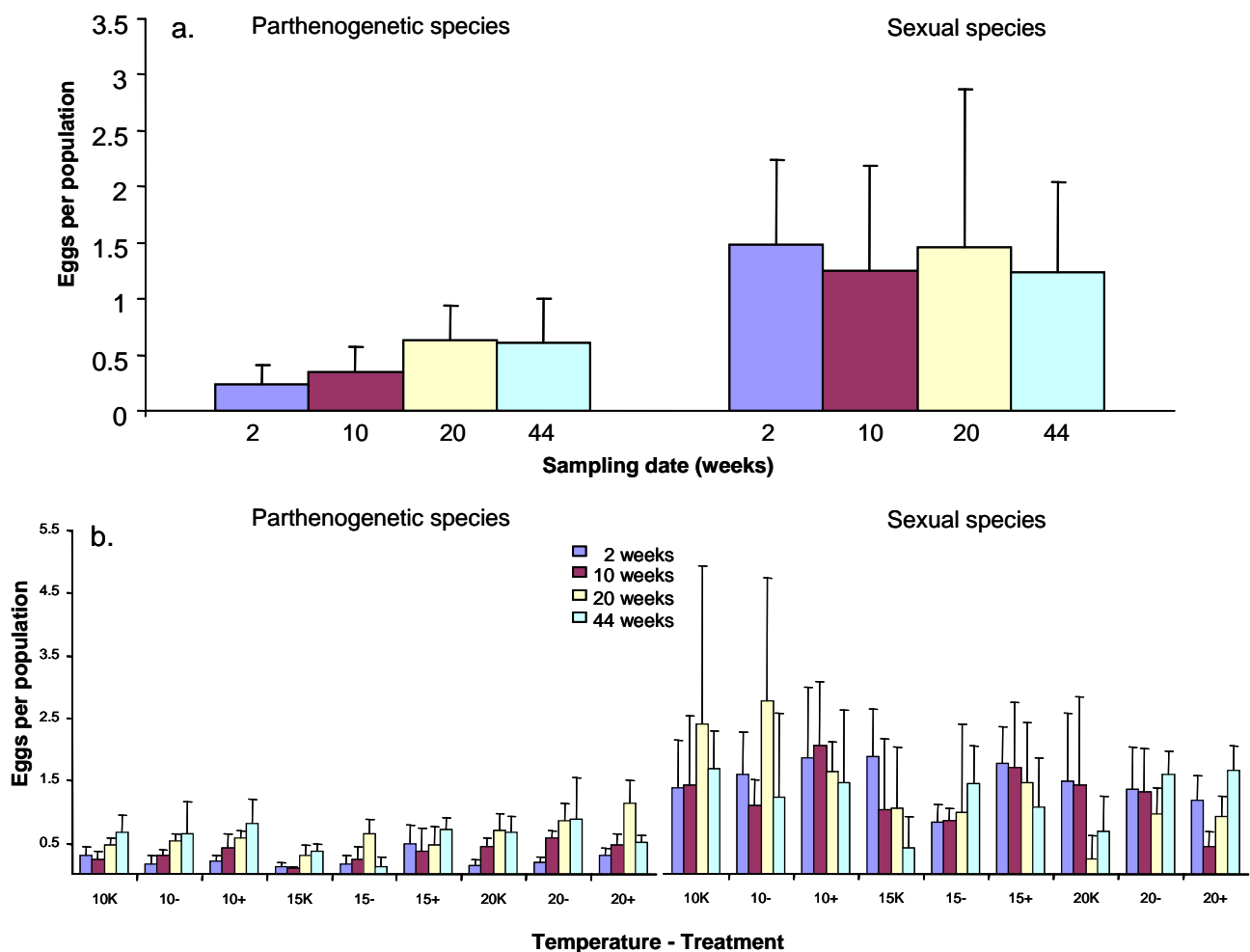
Figure 7.6 (a) Changes with time in the average number of eggs per female (calculated on the basis of all females including those without eggs) in parthenogenetic and sexual oribatid mite species (pooled for control, reduced litter quality and glucose addition treatment) and (b) changes with time in the number of eggs per female in control, reduced litter quality and glucose enriched treatments at 10, 15 and 20°C in parthenogenetic and sexual oribatid mites.

7.4 Discussion

7.4.1 Density and Community Structure of Oribatid Mites

In general, almost all parthenogens comprised exclusively of females, whereas sexuals had a least 10% males. However, although sexuals are thought to have a balanced sex ratio of approximately 1:1 (as in *Achipteria coleoptrata* and *Disshorina ornata*), some taxa had significantly higher numbers of females (e.g., 90.5% in *Cultroribula bicultrata* and 72.4% in *Oribatula tibialis*). These values may result from sampling artefacts or from different lifespan or limitited mobility of males. Low percentages of females in *Carabodes ornatus* and *Oribatella quadricornuta* are probably an artifact resulting from the low number of individuals analysed.

In the parthenogenetic species *Oppiella nova* and *Tectocepheus minor* 2% and 0.7% males were present, respectively. These rare males probably represent non-functional "spanandric" relics of former sexuality (Taberly 1988). Spanandric males in oribatid mites have been reported in several parthenogenetic species within the parthenogenetic species clusters of Enarthronota, Desmonomata and Circumdehiscentiae, e.g., *Hypochthonius rufulus*, *Nothrus silvestris*, *Platynothrus peltifer, Trhypochthonius tectorum* and *T. velatus*, (Grandjean 1941, but see Fujikawa 1988a). In contrast to *T. velatus*, no males have been found in *T. minor* but their presence is plausible since rare males also occur in other species of this genus. In *O. nova* males have been reported by Fujikawa (1988b) with the density varying with season probably due to changes in the availability of food resources. In the present study 24 of the 35 males of *O. nova* occurred in the treatment with reduced litter quality, seven in the untreated control and only four males in the high quality litter treatment. This agrees with the findings of Fujiaka (1988b) and indicates that the community structure of *O. nova* is influenced by resource presence or resource shortage similar to species reproducing by cyclical parthenogenesis, such as cladocerans (Young 1979, Lynch and Spitze 1994) and aphids (Rhomberg et al. 1985, De Barro et al. 1995); in cyclical parthenogens the sexual phase typically is induced when resources become limiting (Williams 1975).

Overall, we expected oribatid mite density to change parallel to resource quality, i.e. to decline in the untreated and reduced litter quality treatment but to increase in the glucose addition treatment in particular at higher temperatures. In contrast to these expectations, the density of oribatid mites uniformly declined in each treatment at each of the temperatures indicating that the addition of glucose did not increase resource availability. Furthermore, sexual and parthenogenetic species responded similarly, i.e. parthenogens did not outnumber

sexuals in the glucose treatment and the number of sexuals declined parallel to the number of parthenogens with increasing resource shortage. This contradicts our assumption that sexual species outcompete parthenogenetic species if resources become limiting.

In theory, higher genetic diversity due to outcrossing and recombination allows sexuals to exploit a wider range of resources making them less sensitive to environmental fluctuations (Williams 1975, Glesener and Tilman 1978, Scheu and Drossel 2007). On the other hand, parthenogens prevail in stable habitats; if there is no need to adapt to changing conditions they can leverage the advantages of their faster reproduction due to avoiding the investment in producing males (Williams 1975, Scheu and Drossel 2007). Previous results suggested that parthenogenetic species indeed suffer more from resource shortage than sexual species (Domes et al. 2007a). This agrees with theory and may explain the high abundance of parthenogenetic oribatid mite species in soil (with abundant resources) and the prevalence of sexual taxa on the bark of trees (with resources being in short supply; Erdmann et al. 2006, Cianciolo and Norton 2006, Domes et al. 2007a, Scheu and Drossel 2007). However, results of the present study do not support the assumption that parthenogenetic species are more sensitive to resource shortage as sexuals and parthenogens responded in a similar way. Parthenogenetic oribatid mite species probably have a similar genetic diversity as sexual oribatid mite species, perhabs either captured from sexual ancestors since their phylogenetic split or accumulated as clonal diversity over evolutionary time; the specific mode of parthenogenesis (terminal fusion automixis; Taberly 1987, Wrensch et al. 1994, Heethoff et al. 2006a) which still includes a meiotic processes with intrachromosomal genetic recombination may also has an impact on the genetic diversity of parthenogenetic oribatid mite populations.

The fact that the density of virtually all taxa declined in each of the treatments at each of the temperatures indicates that resources uniformly declined suggesting that oribatid mites rely on other resources than those replenished by the addition of glucose. This is in agreement with results of Pollierer et al. (2007) suggesting that most decomposers including most oribatid mite species in fact rely on root derived rather than leaf litter resources. Potentially, many oribatid mite species feed on mycorrhizal fungi. Supporting this conclusion Salamon et al. (2006) showed that the addition of large amounts of litter resources to the forest floor of a deciduous forest only little affected the density and community structure of oribatid mites and other microarthropods even after 17 months. Despite the rather uniform response of most species in the present study, the community structure of oribatid mites shifted over time: small parthenogenetic species, such as Brachychthoniidae and the genus *Tectocepheus*, dominated

early in the experiment while the larger parthenogenetic Desmonomata and *Rhysotritia* became more dominant later. Presumably, this reflects different trophic niches of these oribatid mite groups with species of the first group predominantly exploiting easily available food resources whereas species of the latter also exploiting recalcitrant litter resources.

7.4.2 Numbers of Eggs

In agreement with previous studies (Domes et al. 2007a), the number of eggs per female in sexual taxa on average exceeded that in parthenogenetic taxa and this was true throughout the experiment. In sexual species most eggs were produced at low temperatures, indicating that harsh environmental conditions initiate the production of more offspring. In contrast, in parthenogenetic species the average number of eggs per female significantly increased during the experiement and this was independent of the quality of resources. In general, the number of eggs in parthenogenetic species was at a maximum at high temperature indicating increased reproduction. The increase in egg production during the experiment further indicates that parthenogenetic taxa adjusted the investment in reproduction to alterations in the availability of resources which is consistent with earlier results (Domes et al. 2007a). The fact that the density of parthenogenetic species declined parallel to that of sexual species despite the number of eggs per female in parthenogens increased probably indicates that parthenogens suffered from higher death rates than sexuals.

Small parthenogenetic species, such as Enarthronota and *Microppia minus*, carried one egg per female whereas sexual species often carried at least two eggs, which is consistent with observations of Domes et al. (2007a). Unfortunately, life history strategies in oribatid mites, such as the investment of resources in the production of eggs and egg size, are little investigated. The patterns observed in this study suggest that oribatid mites may contribute significantly in refinement of existing theories.

7.4.3 Conclusions

Results of the present study indicate that the addition of glucose as high quality resource does not promote oribatid mite performance suggesting that oribatid mites depend on other food resources than detritus and/or saprotrophic fungi, presumably mycorrhiza. Furthermore, parthenogenetic and sexual taxa appear to respond in a similar way to changes in resource shortage; potentially, this is due to similar genetic diversity or adaptation to stable

environmental conditions in forest soils allowing parthenogens to persist over time and to coexist with sexuals. Interactions between the number of eggs, the ability to adjust the investment in reproduction by changing egg production, the deposition of eggs and other life history parameters, such as generation time, are little understood but may be key factors for the success of parthenogens.

General Discussion

"Each mite represents a world in us, a world possibly not born until they arrive, and it is only

by this meeting that a new world is born."

(unknown)

"Nothing is predestined: The obstacles of your past can become the gateways that

lead to new beginnings."

(unknown)

8.1 Sex and Parthenogenesis

The widespread distribution of sex despite its high costs caused by the dilution of the genome through meiosis and the production of males puzzled evolutionary biologists over decades and is known as the "queen of problems in evolutionary biology" (Bell 1982). Parthenogens are thought be short-lived and doomed to extinction since they cannot overcome the negative effects of accumulating mutations (Maynard Smith 1978, Bell 1982, Kondrashov 1993, Butlin et al. 1999, Butlin 2002, Schön et al. 2008). Until today the existence of "ancient asexual scandals", lineages that persist over evolutionary timescales (Judson and Normark 1996), namely bdelloid rotifers (Mark Welch and Meselson 2000), darwinulid ostracods (Martens et al. 2003) and some groups of oribatid mites (Maraun et al. 2003, Heethoff et al. 2007a), are not fully accepted. Skeptics believe in the presence of rare males that can easily be overlooked but are still functional; if covert sex is present, a little bit of mixis may be just as efficient as a lot (Lynch 1991, Green and Noakes 1995, Little and Hebert 1996, Hurst and Peck 1996). To leave no doubts on their existence, facts have to prove the presence of ancient parthenogenesis in the three putative candidates. The following aspects have been investigated for oribatid mites in the present study: (a) What is the phylogenetic pattern of sex and parthenogenesis in oribatid mites? (b) Is there genomic or other molecular evidence for the long-term absence of recombination? (c) Are males present in parthenogenetic species? (d) Is the prevalence of sex and parthenogenesis influenced by ecological factors such as the availability of resources?

The phylogenetic distribution of sexual and parthenogenetic taxa usually is distinct; sexuals radiate and form clusters, sometimes comprising single parthenogenetic offshoots that disappear over time due to their genetical burden thereby remaining scattered in the phylogenetic tree (Barraclough et al. 2003, Birky 1996, Birky et al. 2005, but see Schwander and Crespi 2009). A speciality of oribatid mites is the presence of at least two large parthenogenetic clusters that have radiated while being parthenogenetic, Enarthronota and Desmonomata (Maraun et al. 2004). Even more amazing than the radiation of parthenogens is the assumed re-evolution of sex within one of these clusters: sexual Crotoniidae likely orginated within parthenogenetic Desmonomata being the sister-group of *Camisia* (Domes et al. 2007b; Chapter 3). This reversal was titled "breaking Dollo`s law" as it implies that a complex character (i.e., sexual reproduction) has been re-evolved (Gould 1970; see 8.3.1.1). Re-evolution of sex suggests that genes involved in mating and spermatogenesis must have remained functional over millions of generations since Desmonomata are thought to be 330

million years old (I. Schaefer, unpublished data); Marshall et al. (1994) estimated that unused protein-coding genes can keep their function for up to 6 million years. Presumably, meiotic processes retained in automictically reproducing parthenogenetic oribatid mites are key for the regain of sexuality in Crotoniidae. Transitions between different reproductive modes also exist in higher mite taxa (Cruickshank and Thomas 1999) and the occasional formation of spanandric males in parthenogenetic Desmonomata (see below and 8.2.1) may keep ancestral genes for male production functional. A putative second reversal to sex could not be confirmed; although morphological data suggest sexual Astigmata to originate within parthenogenetic Desmonomata (Norton 1998), molecular phylogenetic analyses placed them outside oribatids as sister-group to Endeostigmata (Domes et al. 2007c; Chapter 2).

Since the long-term absence of outcrossing and recombination should affect the genome by accumulation of mutations (Muller`s ratchet, Kondrashov`s hatchet; see 1.1.1), molecular approaches can be used for testing long-term avoidance of sex. For example, the so-called "Meselson effect" has been proposed for proving ancient parthenogenesis (Mark Welch and Meselson 2000). Although the Meselson effect has an asymmetrical significance (its presence confirms ancient parthenogenesis, but its absence means nothing; Butlin 2002, Schaefer et al. 2006, Schön et al. 2008), all three potential ancient parthenogens have been tested and in none of the groups the Meselson effect could be confirmed. The first proof in bdelloid rotifers, which reproduce by thelytokous apomixis (Mark Welch and Meselson 2000), was due to the tetraploidy of the inspected gene (Mark Welch et al. 2008); in darwinulid ostracods, which are also apomicts (Martens et al. 2003), mitotical mechanisms such as gene conversion and DNA repair may take place (Schön and Martens 1998, Butlin 2002) and in oribatid mites automictic reproduction presumably prevents the evolution of the Meselson effect (Schaefer et al. 2006). Overall, the investigation of molecular effects of the long-term absence of sex is hindered by the presence of different types of parthenogenesis, all having different genetical consequences and making general conclusions disputable. Focussing on other molecular markers than those encoded in the nucleus, i.e. those present in the mitochondrial genome which are maternally inherited irrespective of the reproductive mode, may help in avoiding the difficulties caused by the different types of parthenogenesis (Chapters 4 and 5; see 8.3).

Other molecular approaches which may allow proving the presence of parthenogenesis comprise the investigation of DNA-repair mechanisms such as mitotic recombination (McVean et al. 2002, Gandolfi et al. 2003, Schön and Martens 2003, Schaefer et al. 2006, Schön et al. 2008), the investigation of proteins included in meiosis and male production (Schön et al. 2008) and the search for transposable elements (Schön and Martens 2000,

Arkhipova and Meselson 2005a, b). For future studies on oribatid mites focussing on the presence of transposable elements (TE`s) is most promising. TE`s are separated into two groups: (1) retrotransposons [either with long terminal repeats (LTRs) or without (non-LTR retrotransposons or LINE-like elements)] that spread as RNA intermediate by reverse transcription and (2) transposons which transpose as DNA by a cut-and-paste mechanism mediated by an element-encoded transposase (Capy et al. 1998, Wright and Finnegan 2001). The abundance of TE`S in Metazoa differs remarkebly among species; while the human genome comprises almost 50% TE`s, *Caenorhabditis elegans* has less than 5% (Kidwell and Lisch 2000, Arkhipova 2001, Wright and Finnegan 2001). TE`s are thought to spread in sexual populations from one host to another although this may introduce deleterious mutations (Hickey 1982, Wright and Finnegan 2001). In parthenogens they are restricted to single individuals since outcrossing is absent; this distributional pattern of TE`s has been proved by several studies (Zeyl et al. 1996, Arkhipova and Meselson 2000, Arkhipova 2001). In parthenogenetic bdelloid rotifers retrotransposons are absent while in Monogononta with mixed reproduction they are present; in contrast, several families of transposons can also be found in bdelloids (Arkhipova and Meselson 2000, 2005a, b). In the putative ancient asexual species *Darwinula stevensoni* two different LINE-like elements were screened which has been explained by an unusually low mutation rate in the genome but which needs further investigation (Schön and Martens 2003, Schön and Arkhipova 2006, Schön et al. 2008). Studies searching for TE`s in oribatid mites may be promising for proving the long-term presence of parthenogenesis and primers adapted from those for bdelloid rotifers (kindly provided by D. Mark Welch) must be optimized to search for different transposable element families in future.

The absence of males in recent populations (and in the fossil record) strongly indicates absence of sexual reproduction of an organism. While no males have ever been reported for bdelloid rotifers, only three males were found in the darwinulid species *Vestalenula cornelia* (Smith et al. 2006) with their functionality awaiting confirmation. In oribatid mites, spanandric males exist in several parthenogenetic species (e.g., *Platynothrus peltifer*, *Hypochthonius rufulus*; Grandjean 1941) and were found during this thesis in *Tectocepheus minor* and *Oppiella nova* (see 8.2.1). However, the spermatophores of spanandric males were proven to be sterile and ignored by females, at least for *Trhypochthonius tectorum* and *P. peltifer* (Grandjean 1941, Taberly 1988, Palmer and Norton 1992) but final molecular evidence for their non-functionality is lacking. Population genetic data (e.g., tests for Hardy-Weinberg or linkage equilibrium) are promising to reveal if outcrossing is present or absent in

case sex and recombination events in parthenogenetic populations are not too rare. If parthenogenetic populations are regularly punctuated by sexual reproduction, recombination and segregation can maintain genotype frequencies close to the Hardy-Weinberg equilibrium (Hughes 1989), which has even been shown for lake-dwelling cladocerans at stable habitat conditions (Mort and Wolf 1985). The investigation if rare sex is present in oribatid mites has already been started; the development of microsatellites for the parthenogenetic species *P. peltifer* and the sexual species *Steganacarus magnus* and their use for population genetics are in progress (I. Schaefer, unpublished data).

Ecological factors, such as the quality and quantity of resources, are important for the prevalence and distribution of sex and parthenogenesis in nature. Theory predicts sex to be associated with resource shortage since higher genetic diversity enables sexuals to exploit a wider resource spectrum than parthenogens (Williams 1975, Glesener and Tilman 1978, Scheu and Drossel 2007). In contrast, parthenogenesis is advantageous in stable habitats with resources in ample supply; parthenogens with well adapted genotypes can take advantage of their faster mode of reproduction by the production of all-female progeny (Williams 1975, Glesener and Tilman 1978). These theoretical predictions are realized in cyclical parthenogens such as cladocerans (Lynch and Spitze 1994) and aphids (De Barro et al. 1995, Simon et al. 2002) in which parthenogenesis is present during summer when resources are plenty while sexuality is induced by resource shortage in autumn/winter (Williams 1975). Cyclical parthenogenesis is unkown in oribatid mites (Norton et al. 1993) but the small parthenogenetic oppiid species *O. nova* responds to resource fluctuations by the production of males (Fujikawa 1988b, K. Domes, unpublished data; Chapter 7, see 8.2.1); males occured in long-term organic farming systems and in laboratory systems where litter quality had been reduced.

Overall, parthenogenetic oribatid mite species are as successful as their sexual congeners in forest soils over evolutionary timescales. Presumably, the frequency of sexual and parthenogenetic species in different habitats (e.g., soil vs. bark of trees) changes until the advantages of both reproductive modes are balanced (Norton and Palmer 1991, Palmer and Norton 1991, Domes et al. 2007a, Cianciolo and Norton 2006, Cianciolo 2009). Numerous hypotheses aim at explaining the maintenance and prevalence of sex (see 1.1.1) with several being not necessarily exclusive (West et al. 1999). In oribatid mites neither biotic uncertainty (mediated by multiple species interactions in complex habitats) nor general purpose genotypes (due to clonal selection among parthenogenetic lineages that keep benefical mutations) are able to explain the success of parthenogenetic species (Cianciolo and Norton 2006, but see

Heethoff et al. 2000). A recent model links the prevalence of parthenogenesis in oribatid mites (which is one to two magnitudes higher than in other animal groups) and in soil-dwelling invertebrates in general with the fast replenishment of a narrow resource spectrum in soil (Scheu and Drossel 2007). Laboratory microcosm experiments showed that parthenogenetic oribatid mite taxa suffered more from resource shortage than sexual taxa (Domes et al. 2007a, Chapter 6) which may explain the prevalence of parthenogens in habitats with abundant resources (e.g., in forest soil) and the prevalence of sexuals on the bark of trees with resources being in short supply (Scheu and Drossel 2007). That parthenogenetic and sexual oribatid mite taxa reacted less differently than expected (Chapter 7) either points to similar genetic diversity or to strong adaptation to stable environmental conditions in forest soils. Further laboratory and field experiments are needed to clearly quantify the role of resource availability in triggering the distribution of sex and parthenogenesis in oribatid mites.

8.2 Oribatida
8.2.1 Diversity and Reproductive Pattern

Due to slow reproduction of most species, oribatid mites are less suited as model organism for laboratory experiments as e.g. *Drosophila* or bdelloid rotifers. However, one of the advantages of oribatid mites is the coexistence of sexually and parthenogenetically reproducing species in the same habitat (Norton and Palmer 1991, Cianciolo and Norton 2006, Domes et al. 2007a) which facilitates the performance of experiments where environmental factors are manipulated to compare the development of sexual vs. parthenogenetic species.

For this thesis about 16,500 adult oribatid mite specimens were determined to species-level, sexed and in females the numbers of eggs were counted. Females were determined by the presence of the ovipositor which differs slightly in shape and size among species (Fig. 8.1); respectively, males were determined by the presence of male genitales (Fig. 8.2).

In total, oribatid mite communities in four experiments were studied (Table 8.1): community 1 originated from a field experiment which aimed to study the vertical mobility of microarthropods, community 2 and 3 were obtained from the resource-depletion and re-colonisation study, respectively (Domes et al. 2007a; Chapter 4), and community 4 was extracted from the microcosm experiment described in Chapter 5.
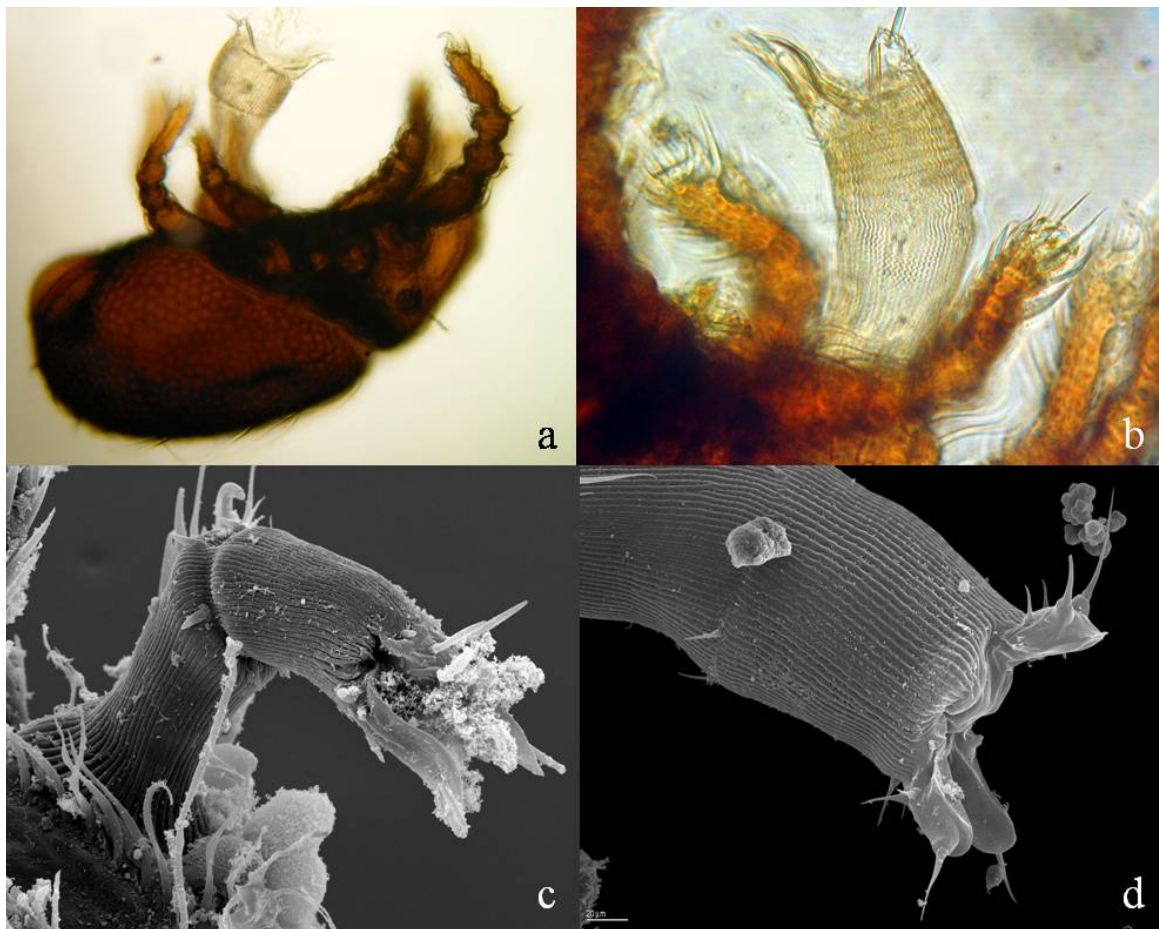
Figure 8.1 Ovipositor of parthenogenetic females in (a) *Nanhermannia nana*, (b) *Nothrus silvestris,* (c) *Nanhermannia coronata* and (d) *Platynothrus peltifer*. A and b are taken through the ocular of a light microscope, c and d are scanning electron microscope pictures.

Overall, the percentage of females in sexual species ranged from 11.8% (*Banksinoma lanceata*, community 1) to 75.6% (*Medioppia subpectinata*, community 2) with the lowest average in *B. lanceata* (46.6%) and the highest average in *Oribatula tibialis* (72.2%) suggesting that sexual species comprise at least 20% males. Sex ratio values of these studies are similar to those reported by Palmer and Norton (1991) who focussed on the large group of Desmonomata; therein, sexuals had at least 30% males. Since sexual Desmonomata species inhabit the southern hemisphere (following a Gondwanian distribution; Hammer and Wallwork 1979), only parthenogens (e.g., *P. peltifer*, *Nothrus silvestris*) were found in the present studies. All of them were highly abundant and comprised 100% females; no single male was found which is in agreement to the findings of Palmer and Norton (1991) and Cianciolo and Norton (2006). However, in other studies spanandric males have been reported for parthenogenetic Desmonomata species, including *P. peltifer, Archegozetes magna, N. silvestris, N. palustris* and *Trhypochthonius tectorum* (Grandjean 1941, Taberly 1988).

Table 8.1 Number of specimens and percentages of females in four oribatid mite communities (com 1, 2, 3 and 4). Species in bold reproduce by parthenogenesis.

	Species	com 1 n	com 1 %females	com 2 n	com 2 %females	com 3 n	com 3 %females	com 4 n	com 4 %females	total n	average %females
Enarthronota											
Enichthoniidae	***Enichthonius minutissinus* (Berlese, 1903)**	46	100	21	100	9	100	382	100	458	100
Desmonomata											
Malaconothridae	***Malaconothrus gracilis* v.d. Hammen, 1952**	29	100	75	100	7	100	242	100	353	100
Nanhermanniidae	***Nanhermannia coronata* Berlese, 1913**	15	100	25	100	-	-	41	100	81	100
	***Nanhermannia nana* (Nicolet, 1855)**	9	100	7	100	-	-	548	100	564	100
Nothridae	***Nothrus silvestris* Nicolet, 1855**	15	100	35	100	-	-	737	100	787	100
Circumdehiscentiae											
Achipteriidae	*Achipteria coleoptrata* (Linnaeus, 1758)	17	41.2	26	61.5	-	-	310	55.2	353	55.0
Oppiidae	*Disshornia ornata* (Qudemans, 1900)	41	48.8	107	62.6	207	56.5	169	52	524	55.7
	Medioppia subpectinata (Qudemans, 1900)	49	61.2	41	75.6	25	60	23	56.5	138	64.5
	***Oppiella nova* (Qudemans, 1902)**	275	100	265	100	318	100	1855	**98**	2713	**98.6**
Oribatellidae	*Ophidiotrichus tectus* (Michael, 1884)	-	-	4	50	4	25	22	50	30	46.7
	Oribatella quadricornuta Michael, 1880	21	66.7	-	-	6	66.7	3	33.3	30	63.3
Oribatulidae	*Oribatula tibialis* (Nicolet, 1855)	42	73.8	20	70	14	64.3	337	72.4	413	72.2
Punctoribatidae	*Minunthozetes semirufus* (Koch, 1841)	34	17.6	22	68.2	17	47.1	92	57.6	165	49.7
Tectocepheidae	***Tectocepheus minor* (Berlese, 1903)**	8	**87.5**	16	100	10	100	291	**99.3**	325	**99.1**
	Tectocepheus sarekensis Trägardh, 1910	21	100	18	100	-	-	451	100	490	100
	***Tectocepheus velatus* (Michael, 1880)**	14	100	13	100	-	-	1146	100	1173	100
Thyrisomidae	*Banksinoma lanceata* (Michael, 1888)	34	11.8	13	38.5	10	30	162	53.1	209	46.9

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Similar to the pattern in Desmonomata almost all parthenogenetic species of Enarthronota and Circumdehiscentiae consisted exclusively of females, but for *O. nova* and *T. minor* several males were found (Table 8.1, Fig. 8.2). *Oppiella nova* was highly abundant; of the inspected 2,713 specimens 35 were males. In *T. minor* only three of the 325 specimens investigated were males. Morphologically these males resembled the females and could easily be determined to species level. For both genera the occurrence of rare males has already been reported (Grandjean 1941, Fujikawa 1988a, b); the ones in *Tectocepheus* belonged to *T. velatus* (Grandjean 1941). Overall, the small thyelytokous oppiid species *O. nova* seems to be sensitive against changing environmental conditions with the production of males being increased when food resources fluctuate (Fujikawa 1988b). In the present study the majority of males were found in treatments with reduced litter quality (Chapter 7), indicating that parthenogenetic reproduction is favoured at high and constant resource availability. Unfortunately, no males have been reported in previous resource depletion experiments (Domes et al. 2007a; Chapter 6); therefore, the reproductive biology and the development of *O. nova* need further investigation.

Other studies did not find any males in parthenogenetic oribatid mites (e.g., Luxton 1981 and references therein), suggesting that males only occur at ceratin environmental conditions. If spanandric males indeed are non-functional has only been proven for *P. peltifer* and *T. tectorum* in which the spermatogenesis stops at late developmental stages rendering them sterile (Taberly 1988). Future studies need to adopt molecular methods to investigate if male genes in fact do not add to the genepool of parthenogenetic populations.
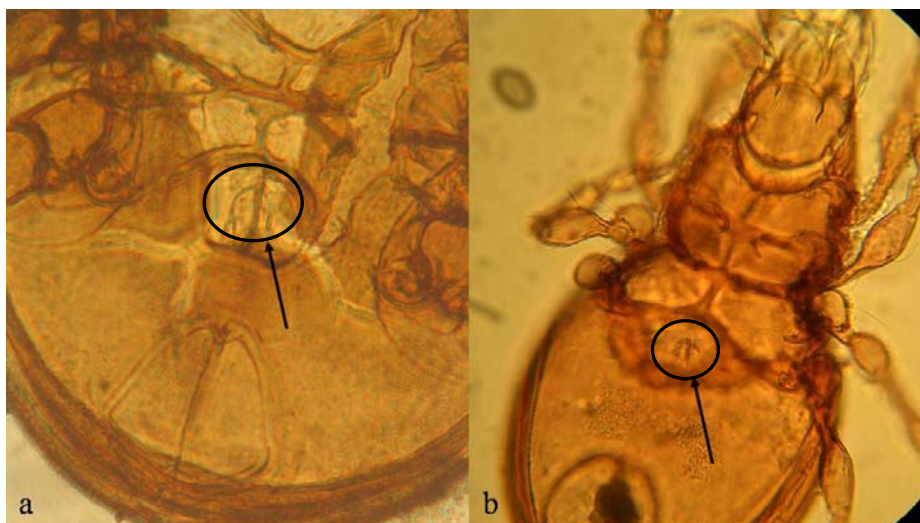

Figure 8.2 Males in the parthenogenetic oribatid mite species (a) *Tectocepheus minor* and (b) *Oppiella nova*. Male genitales are marked by an arrow.

8.2.2 Numbers of Eggs

Numbers of eggs as well as egg size varied among oribatid mite species which is in agreement to Travé et al. (1996). Generally, while eggs in sexuals were small, parthenogens had large eggs often filling the whole body (Fig. 8.3). Further, small parthenogenetic species, such as *Suctobelbella subcornigera*, *O. nova*, *H. rufulus* and *Eniochthonius minutissimus,* carried one egg per female on average whereas sexual species, such as *Eupelops plicatus* and *O. tibialis,* usually produced more than two eggs per female. Small thelytokous species living in deeper soil layers (e.g., Enarthronota) and members of Desmonomata lay only few eggs that develop slowly (Forsslund 1942, 1957, Luxton 1981, Travé et al. 1996). Similarly, small thelytokous Oppiidae and Suctobelbidae species produce few eggs, but egg development is much faster (within 40 days; Luxton 1981, Travé et al. 1996). Most eggs were present in Phthiracaridae and Circumdehiscentiae (e.g., *O. tibialis*).

Figure 8.3 Eggs in parthenogenetic females of (a) *Eniochthonius minutissimus*, (b) *Oppiella nova* and (c) *Nothrus silvestris*.

In general, sexual species had more eggs on average than parthenogens (only eggs visable by light microscopy were counted, more eggs are present in the ovary and oviducts; Heethoff et al. 2007b), most pronounced in small parthenogenetic and large sexual species which is in contrast to our original hypothesis (Chapters 6 and 7). This pattern remains puzzling since theory suggests parthenogenesis to be the faster mode of reproduction implying higher egg numbers in parthenogens. However, the speed of egg deposition remains unknown; oviposition rates can vary greatly and depend on season (Luxton 1981, Travé et al. 1996). The complexity of generation time, reproductive mode, numbers of eggs produced, egg development and egg deposition rates is little understood and needs further investigation. Nevertheless, results of experiments with conditions of resource shortages (Domes et al. 2007a; Chapters 6 and 7) suggest that in parthenogenetic oribatid mite taxa egg production

depends on the environmental conditions and that they adapt their egg production quicker than sexuals since they are independent from mating and insemination.

8.3 Molecular Markers

8.3.1 Phylogeny

Phylogenies are the basis for numerous studies. Their principle aim is to correctly depict relationships of species and supraspecific taxa, i.e. groups at different taxonomic levels (Whelan et al. 2001, Sanderson and Shaffer 2002). Achieving this goal offers new opportunities for further evolutionary analyses, such as evaluating the ancestral state of certain characters. An important assumption for combining morphological or life history characters with molecular phylogenies is that the phylogenetic tree indeed is correct. Not surprisingly numerous phylogenetic trees are still controversially discussed since molecular phylogenies can easily be affected by analytical artefacts such as long-branch attraction describing that taxa with long branches are attracted by each other or by the long branch of the outgroup (Felsenstein 1978, Domes et al. 2007c; Chapter 2). Molecular phylogenies significantly depend on the chosen gene, the performed alignment and are vulnerable to different substitution rates among taxa, base composition biases and the relation to the outgroup (Sanderson and Shaffer 2002). On the other hand, morphological phylogenies often suffer from an insufficient number of characters, by not adequately defined characters and by symplesiomorphies (characters shared by a number of groups, but inherited from ancestors older than the last common ancestor; Sanderson and Shaffer 2002). Discrepancies between morphological and molecular phylogenies are still reported (Halanych 2004); e.g., the phylogenetic position of Astigmata in respect to Oribatida differs remarkably considering morphological vs. molecular data, and even the latter gave different results (Norton 1998, Maraun et al. 2004, Murrell et al. 2005, Domes et al. 2007c; Chapter 2). For improving reliability of phylogenies a number of issues need to be considered.

First, phylogenies need to be based on an adequate taxon sampling. This can often be problematic, especially if the group under investigation comprises of 10,000 species, as it is the case in oribatid mites, or if taxa are difficult to sample. Further, the taxon list is often subjectively choosen by the authors. The sensitivity of molecular phylogenies to the taxa included is obvious comparing the phylogenetic position of Astigmata in two studies on the ribosomal 18S region; while the single astigmatid mite species *Rhizoglyphus* sp. clustered within oribatid mites as sister-group of *N. silvestris* and *Steganacarus magnus* (Murrell et al.

2005), the inclusion of eight astigmatid species resulted in a monophyletic astigmatid clade forming the sister-taxon of oribatids (Domes et al. 2007c; Chapter 2). Further, not only the taxon sampling but also the tree construction algorithm impacts phylogenetic relationships (see below), and this was also the case in the reconstruction of the phylogenetic position of Astigmata.

Second, while morphological phylogenies often suffer from a small number of characters, molecular phylogenists have to pay attention to the choosen gene. Due to different levels of conservations different genes are appropriate for answering different questions; highly conserved genes are used for investigating deep-splits, variable genes can elucidate recent events. For example, in oribatid mites the D3 region of the ribosomal 28S rDNA gene was appropriate for elucidating relationships within genera (Laumann et al. 2007), but inappropriate for reconstructing large-scale phylogenies (Maraun et al. 2004); while the relationships among three species of *Tectocepheus* could satisfactorily resolved, the complete oribatid mite phylogeny was not reliably reconstructed. In contrast, the ribosomal 18S region (which belongs to the same nuclear ribosomal gene cluster as the 28S rDNA) reliably reconstructed the phylogeny of all oribatid mite taxa (Domes et al. 2007b, c, I. Schaefer, unpublished data, P. Pachl, unpublished data). The choice of the gene used as molecular marker has accurately been phrased as "all of them [molecular markers] have their problems, but by choosing the marker most appropriate for the task these problems can be minimized before any sequencing is done, saving both time and money" (Cruickshank 2002).

While some authors argue for using longer genes and more taxa, others vote for simply using more genes (Whelan et al. 2006, de Queiroz and Gatesy 2006). The trend in molecular phylogenetics points to analyses based on combined super-matrices consisting of several genes for as many taxa as possible. These studies are often supported by high statistical values as seen for the phylogeny of Desmonomata/Crotoniidae based on a combined dataset of the ribosomal 18S region and the single-copy genes *ef1α* and *hsp82* (Domes et al. 2007b; Chapter 3). In addition, not only the choice of the gene influences the structure of phylogenetic trees but also the methodology used for the analysis. Unlike the parsimony approach used in morphological studies, a number of algorithms are used for analysing molecular data. These approaches in part are parsimony- and model-based whereas the choice of an evolutionary model introduces another component adding uncertainty.

Many phylogenetic studies are based on mitochondrial (mt) data (see 8.3.3); either concatenated datasets of the protein-coding genes (either DNA or amino acid sequences) or the arrangement of all mt genes are used (Nardi et al. 2001, Fahrein et al. 2007, Domes et al.

2008; Chapter 4). Although the latter apporach is not fully accepted, many mt-based phylogenies are congruent with the classical arthropod phylogeny (Boore et al. 1995).

Overall, molecular studies have changed the interpretation of metazoan phylogeny in many aspects (Halanych 2004), and DNA data are often considered more objective. Nevertheless, care is necessary especially if contradicting morphological phylogenies with strong support exist. These contradictions need to be faced as accurate phylogenies are essential for making progress in uncovering character evolution and therefore in understanding the evolutionary history of species.

8.3.1.1 Dollo`s Law

As described above, phylogeny-based inferences need to be based on robust and reliable phylogenies. Many studies on Dollo`s law use phylogenies and further analyses of character evolution to emphasize that a character has been lost and re-evolved during evolution (e.g., Whiting et al. 2003, Collin and Cipirani 2003, Igic et al. 2006, Domes et al. 2007b, Collin and Miglietta 2009; Chapter 3). In a recent study, Goldberg and Igic (2008) identified two major causes of errors in studies on Dollo`s law: incorrect assignment of root state frequencies and neglect of the effect of the character state on rates of speciation and extinction. They argue for the use of more general model selection methods such as the Akaike or Bayesian information criterion and point to a possible dependence of the diversification rate of species on the present character state (Goldberg and Igic 2008); a model that incorporates state-specific rates of speciation and extinction has recently been developed by Maddison et al. (2007). Goldberg and Igic (2008) tested their assumptions using two case studies, the one on stick insects by Whiting et al. (2003) and the study on oribatid mites by Domes et al. (2007b; Chapter 3). For stick insects, the model allowing reversals from wingless to winged states received considerably less support than the irreversible model. For oribatid mites, both models were equally supported, indicating that there is presently no definitive evidence for regain of sexual reproduction, but also no evidence against it. Goldberg and Igic (2008) further state that powerful tests of Dollo`s law require data beyond phylogenies and character states of extant taxa, i.e. the inclusion of fossil records. However, phylogenies cannot display if the regain of a lost character only includes the recovery of a genetical pathway or if exactly the same nucleotides are re-established. If the same genetic pathway or genes need to be involved to match "Dollo´s law" or if exactly the same nucleotides must be regained needs further definition. Collin and Miglietta (2009) even discuss if Dollo`s law is useless as an

evolutionary principle since several restrictions occur: (1) pleiotropic effects (i.e. the involvement of a single gene in multiple pathways) can retain functionality of genes coding for lost characters, so only certain genes are actually affected by Dollo`s law and (2) similar characters can also be developed convergently. Sexual reproduction in Crotoniidae is unlikely to have evolved convergently since meiotic sex evolved early in the eukaryote history rendering sex the ancestral reproductive mode (Williams 1975, Hurst and Peck 1996, Cavalier-Smith 2002). Genes involved in male production may have pleiotropic effects supporting their conservation over time.

8.3.2 Barcoding

  Although barcoding is controversially discussed, the use of gene sequences for species taxonomy in groups that lack sufficient morphological characters is helpful (Ben-David et al. 2007). Further, it may allow to determine juvenile stages in species in which laval stages of different species resemble each other (Huang et al. 2007). Almost all genes investigated in oribatid mites can be used for molecular barcoding in its narrower sense, i.e. the identification of a species by its DNA sequence. The ribosomal internal transcribed spacer region 1 (ITS1), which is part of the 5.8/18/28S complex, is only available for three species (*P. peltifer*, *N. silvestris*, *Heminothrus thori*) but those could easily be distinguished (Heethoff et al. 2000); however, the intraindividual variation of the ITS1 region in *P. peltifer* is high (4.1%) rendering the threshold of 2.7% for distinguishing species unsuitable (Hebert et al. 2003a, 2004a). The D3 domain of the ribosomal 28S RNA region was explicitely tested as species marker and used in a number of studies (Maraun et al. 2003, 2004, Laumann et al. 2007). Although there was no intraspecific or intraindividual variation in the D3 domain in the species studied, it was identical in two closely related species of different genera (*Nanhermannia nana* and *N. coronata*, *Eupelops hirtus* and *E. torulosus*; Maraun et al. 2003). While Maraun et al. (2004) describe the D3 region as good species marker, Laumann et al. (2007) point to its high saturation and therefore its limited use for investigating recent splits; since identical sequences were obtained for closely related species, care is necessary with interpreting D3 sequences for oribatid mites. The genes for *ef1α*, *hsp82* and 18S have been used frequently for reconstructing phylogenies and for investigating the genetic variability among and within oribatid mite taxa (Schaefer et al. 2006, Domes et al. 2007b, c, Laumann et al. 2007; Chapters 2 and 3, see 8.3.1). Even closely related species are characterized by different DNA sequences indicating that each of these genes may be used for molecular

barcoding. An additional advantage of the ribosomal 18S sequence is its secondary structure (Fig. 8.4). While stem-regions are identical among almost all oribatid mite species sequenced so far, variable loop regions are often characteristic for certain taxa only.

The mitochondrial cytochrome c oxidase I (*cox1*) gene is commonly used as barcoding marker (Hebert et al. 2003b, 2004a, b, Hogg and Hebert 2004, Ball et al. 2005, Hajibabaei et al. 2007). However, using this gene for barcoding oribatid mites is problematic due to its high intraspecific variability, as shown in the parthenogenetically reproducing *P. peltifer* and the sexually reproducing *S. magnus* (Heethoff et al. 2006, K. Domes, unpublished data, M. Rosenberger, unpublished data; Chapter 7, see 8.3.3.2). Similar to oribatid mites, extensive *cox1* polymorphism was found in the spider mite *Tetranychus urticae* (Navajas et al. 1998) suggesting that *cox1* may not be suited as barcoding marker in mites in general.
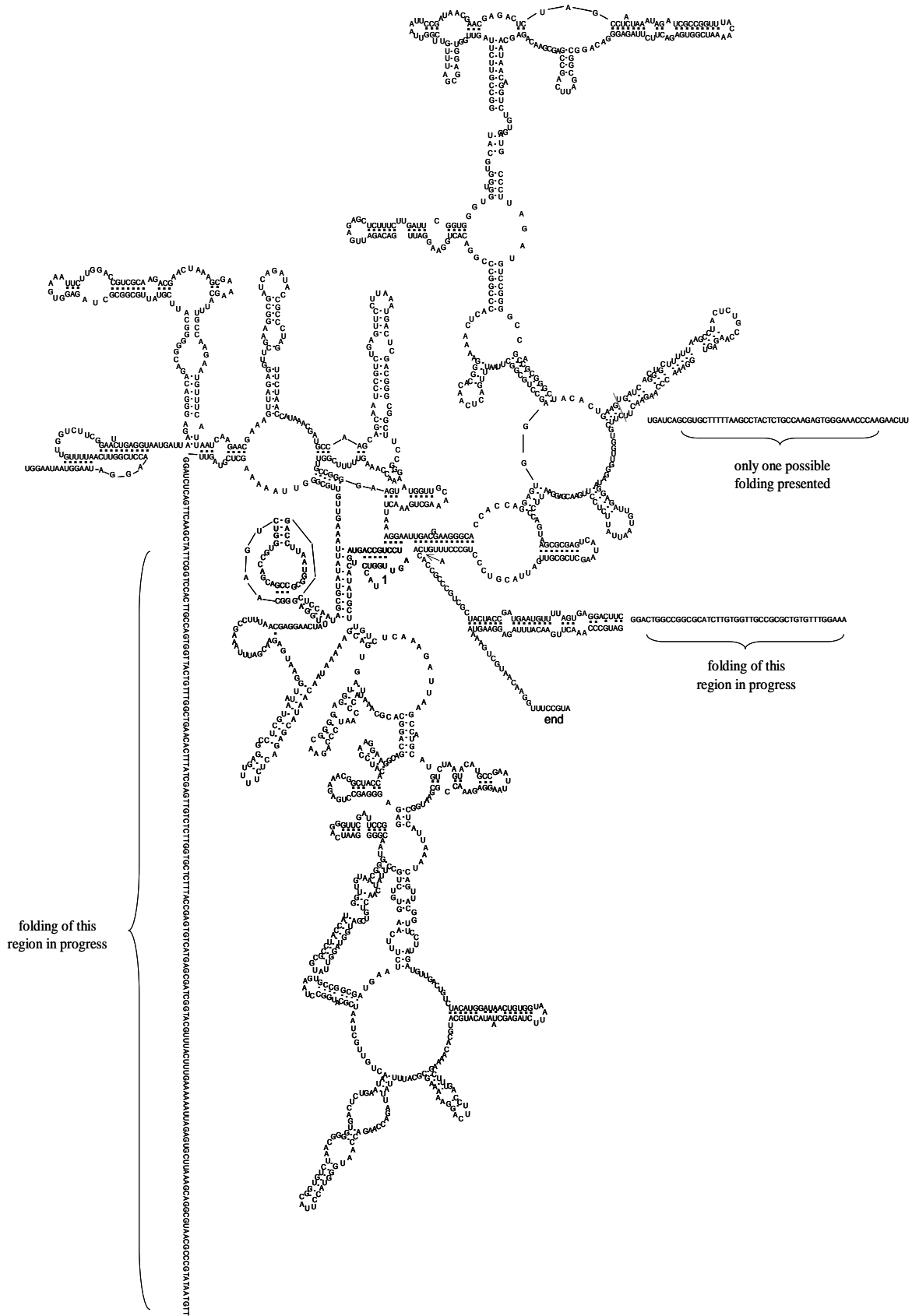
Figure 8.4 Secondary structure of the ribosomal 18S region of the sexual oribatid mite species *Steganacarus magnus*. The sequence was folded by eye considering the secondary 18S structure of *Daphnia pulex* (The European Ribosomal RNA database).

8.3.3 Mitochondria

8.3.3.1 Gene Arrangements

The evolution and biology of mitochondria and their DNA (mtDNA) are well understood (Wolstenholme 1992, Avise 1994, Lane 2005). A number of complete genomes have been sequenced, including about 150 arthropods, 30 chelicerates and 17 species of Acari. The great interest in mitochondria and their gene sequences in part relate to their involvement in human diseases, such as Parkinson, Diabetes mellitus and cancer, and in aging in general (Scheffler 2001, Lane 2005, Wallace 2005). Using mtDNA for molecular studies is facilitated by a number of features. First, large amounts of DNA are present in animal tissue as several hundred mitochondria are present in a single cell each containing several circular DNA molecules. Second, results can easily be compared with other organisms since almost all Metazoa have kept the same set of genes. Finally, although similar in size and gene content, the arrangement of mitochondrial genes may differ markedly among taxa, thereby providing a unique tool for reconstructing phylogenetic relationships (Wolstenholme 1992, Boore 1999, Lane 2005).

The rearrangement of genes dramatically changes the mitochondrial genome structure and therefore rearrangements of genes presumably are rare events in mitochondrial genome evolution (Boore 1999). Consequently, similar or identical gene arrangements in different taxa unlikely developed convergently but rather suggest common ancestry (Boore 1999). However, although generally rare, a number of gene rearrangements occurred during the evolution of Metazoa (Boore 1999, Dowton et al. 2002). The hypothetical ground plan of the mitochondrial gene arrangement of arthropods is conserved in the horseshoe crab *Limulus polyphemus* (Lavrov et al. 2000) as well as e.g. in soft- and prostriate-hard ticks (Black and Roehrdanz 1998). In contrast, in other lineages, such as lice (Siphonaptera, Insecta) or Mesostigmata (parasitiforme Acari), the mitochondrial genome is highly variable (Covacin et al. 2006, Cameron et al. 2007, Jeyaprakash and Hoy 2007).

The complete mitochondrial genomes of the sexually reproducing oribatid mite *S. magnus* and the parthenogenetic species *P. peltifer* contribute to the set of available arthropod mitochondrial genomes and fill the gap in mite mitochondrial genomes by providing the first for oribatid mites (Domes et al. 2008; Chapters 4 and 5). Both genomes differ from the hypothetical ground plan, from other mites and slightly from each other; both add to the variability in mitochondrial gene arrangements in arthropods, especially arachnids (Chapter 5). The main differences of the arrangement of protein-coding genes between *S. magnus* and

P. peltifer concern the *nad1/nad2/rrnL* complex; while these genes are in a different order (*nad2*-<u>*rrnL*</u>-<u>*nad1*</u> in *S. magnus* and <u>*nad1*</u>-*nad2*-<u>*rrnL*</u> in *P. peltifer*, underlined genes are encoded on the (-)-strand), all others share the same gene boundaries (except the tRNA genes; see below). To clarify if the rearrangements of protein-coding genes, or DNA or protein sequences can be used for reconstructing the phylogeny of oribatid mites and related groups such as Astigmata and Endeostigmata, the mitochondrial genome of further mite species need to be sequenced.

Remarkebly, *S. magnus* lacks 16 tRNA genes and those present differ markedly from the typical cloverleaf structure (Domes et al. 2008; Chapter 4). Similarly, *P. peltifer* lacks nine tRNAs and the remaining are also highly degenerated in their secondary structure (Chapter 5). While the degeneration of tRNAs frequently occurred in arthropods (Masta and Boore 2004, Dávila et al. 2005, Shao et al. 2006, Jeyaprakash and Hoy 2007), loosing entire tRNAs remains enigmatic. If the loss of tRNAs may be used for reconstructing oribatid mite phylogeny or the phylogeny of mites in general awaits further investigation and needs sequencing the mitochondrial genome of further taxa.

8.3.3.2 Intraindividual and Intraspecific Diversity

Mitochondria are non-Mendelian inherited since there is (mostly) no recombination during meiosis and the transmission to offspring is predominantly uniparental from the mother (Lane 2005, Van Leeuwen et al. 2008). The evolution of new mtDNA haplotypes causing heteroplasmy (i.e. the presence of different haplotypes in one individual) in the offspring implies mutations in germline cells of the mother, resulting in at least one heteroplasmic gamete (Van Leeuwen et al. 2008). Since mitochondria are rapidly and unequally segregated in the female germline, the heteroplasmic stage is either of short duration or transferred to the progeny ("genetic bottlenecking"). Therefore, genotype frequencies in heteroplasmic specimens can shift rapidly during transmission from mother to offspring caused by either selective mtDNA replication or unequal partioning (Jenuth et al. 1996, Clayton 1997). In general, homoplasmy is usually reset through genetic bottlenecking in early oogenesis which provides a uniform genetic background for the investigation of somatic mutations (Jenuth et al. 1996, Chen and Hebert 1999).

Nevertheless, heteroplasmy frequently occurs in arthropods (e.g., *Tetrodontophora bielanensis* (Collembola), Nardi et al. 2001; *Tetranychus urticae* (Parasitiformes), Van Leeuwen et al. 2008) and plays an important role in human diseases (Richter 1995, Ozawa

1997). The level of heteroplasmy varies between single nucleotide polymorphism (SNP) and the presence of variable gene sequences, i.e. due to gene duplications which may strongly affect intraspecific diversity of mitochondrial genes. For example, the mitochondrial *cox1* gene is commonly used as barcoding marker (see 8.3.2) implying that the intraspecific gene variation is less than 2.7%, the variation used as threshold for separating species (Hebert et al. 2004a, b). The genetic *cox1* diversity in the parthenogenetic *P. peltifer* markedly exceeds this threshold; the corrected genetic diversity between clades from different locations averaged 56% (Heethoff et al. 2007a). No gene duplication and only small amounts of heteroplasmy were found which could have explained the high variability (K. Domes, unpublished data, A. Weigand, unpublished data; Chapter 5). In parthenogens the intraspecific *cox1* variability often exceeds that in sexuals, with the variability frequently exceeding the threshold level for species of 2.7% (Arnaud et al. 2000, Hebert et al. 2003b, Navajas and Boursot 2003, Stevens and Hogg 2006, Ros et al. 2008). In contrast to the variability at the nucleotide level, amino acid sequences of COX1 are identical in the parthenogenetic *P. peltifer*, but differ between specimens of the sexual *S. magnus* (M. Rosenberger, unpublished data, A. Weigand, unpublished data).

Different reproductive modes combined with different kinds of mutations may explain discrepancies in nucleotide and amino acid variability in sexual and parthenogenetic species (Chapter 5). While the nuclear and mitochondrial genomes in parthenogenetic species are coupled (since outcrossing is absent), in sexuals nuclear genes are reshuffled in every generation while the mitochondrial genome is only maternally inherited. Therefore, in parthenogens the matching between nuclear and mitochondrial gene products involved in cell respiration is maintained by conserving the protein function, i.e., allowing for only synonymous mutations as is the case in *P. peltifer*. In contrast, continuous recombination of nuclear genes in sexual species may allow coevolutionary changes in nuclear and mitochondrial proteins interacting in e.g. cell respiration. Thereby, sexual reproduction may function as reshuffling mechanism that ensures that the interaction of proteins coded by nuclear and mitochondrial genes remains functional.

8.4 Conclusions and Implications for Future Studies

Results of the present thesis suggest that oribatid mites are ideal model organisms for the investigation of evolutionary consequences of sex vs. parthenogenesis. A plethora of evolutionary and phylogenetic questions can be investigated: (a) morphological and molecular

characaters can be combined to resolve uncertainties in the affiliation of taxa, to reconstruct oribatid mite phylogeny and the phylogeny of mites in general, (b) effects of the high age of oribatid mites on genome structure and genetic diversity can be investigated, (c) the re-evolution of sex allows insight into unusual evolutionary pathways, and (d) overall, the coexistence of sexual and parthenogenetic species may help understanding the factors responsible for the maintenance and prevalence of reproductive modes.

Topics for future studies need to include the investigation of population structures using microsatellites as neutral markers. Since in some taxa spanandric males are present, e.g. in *T. minor* and *O. nova*, their functionality needs closer investigation by studying Hardy-Weinberg and linkage equilibrium evaluating if "little bit of sex", i.e. occasional amphimixis, is present. In those taxa were no spanandric males have been found so far, such as *P. peltifer*, proving the functionality (or non-functionality) of genes involved in meiosis, sperm synthesis and ejaculate proteins form interesting research subjects. Further, investigating transposable elements, as done in bdelloid rotifers, is promising for proving ancient parthenogenesis in oribatid mites; at least some types of transposable elements should be absent in "true" parthenogens as they are thought to be bound to sexual reproduction. For further elucidating the role of resource quality and quantity in triggering the distribution of sex and parthenogenesis in soil microarthopods (and animals in general) further laboratory experiments with varying amounts of resources need to be set up and followed by respective experiments in the field. Overall, the full power of oribatids as model organisms in evolutionary biology awaits to be exploited.

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Appendix

Appendix 1 Primer sequences for PCR of the heat shock protein 82 (*hsp82*), the elongation factor 1 alpha (*ef1α*) and the ribosomal 18S region (18S).

gene	primer name	sequence (5'-3')	references
hsp82	hsp8x hsp1.2	ACGTTCTAGARTGRTCYTCCCARTCRTTNGT TGCTCTAGAGCACARTTYGGTGTNGGTTTYTA	Schön and Martens (2003)
ef1α	40.71F 52.RC	TCNTTYAARTAYGCNTGGGT CCDATYTTRTANACRTCYTG	Klompen (2000)
18S (PCR)	18Sforward 18Sreverse	TACCTGGTTGATCCTGCCAG TAATGATCCTTCCGCAGGTTCAC	modified after R.H. Thomas
18S (sequencing)	18S554f 18S1282r 18S1150f 18S614r	AAGTCTGGTGCCAGCAGCCGC TCACTCCACCAACTAAGAACGGC ATTGACGGAAGGGCACCACCAG TCCAACTACGAGCTTTTTAACC	

Appendix 2 PCR conditions for the heat shock protein 82 (*hsp82*), the elongation factor 1 alpha (*ef1α*) and the ribosomal 18S region (18S).

	hsp82		*ef1α*		18S	
	temperature (°C)	time	temperature (°C)	time	temperature (°C)	time
initial denaturation step	95	15 min	95	15	95	15
denaturation	95	50 sec	95	50 sec	95	45 sec
annealing	50	50 sec	46	70 sec	57	1 min
elongation	72	2 min	72	2 min	72	1 min
number of cycles	9		9		34	
denaturation	95	50 sec	95	50 sec	-	-
annealing	55	50 sec	50	70 sec	-	-
elongation	72	2 min	72	2 min	-	-
number of cycles	34		34		-	
final elongation	72	10 min	72	10 min	72	10 min

Appendix 3 Primer sequences for the mitochondrial genome of *Steganacarus magnus*. Gene abbreviations: *cox1-3*: cytochrome oxidase subunits 1-3; *atp6*: ATP synthase subunit 6; *nad1-6, 4L*: NADH dehydrogenase subunit 1-6, 4L; *cob*: cytochrome b; *rrnL*: large ribosomal subunit; *rrnS*: small ribosomal subunit; LNR: large non-coding control region, tRNA-Gln, -Ser: tRNAs for glutamine and serine; rev: reverse, for: forward.

primer name	primer sequence (5'-3')	genomic position	gene	orientation	primer name	primer sequence (5'-3')	genomic position	gene	orientation
Steg1	AAATCAGGTCAATGTTCGG	12408-12426	rrnS	rev	Steg42	TATAATAGTAGGATGAGACG	5654-5673	nad5	rev
Steg2	TTGGTTACAGCAGTATACG	12453-12472	rrnS	for	Steg43	ACTTTAATTTTATTCGTCGG	5713-5732	nad5	rev
Steg5	AAAAATCGGTTTAGAGTGG	8529-8547	cob	rev	Steg45	AAGATACATTAACTAGATCC	12224-12243	nad1	for
Steg6	CAATTAGAAATTACCCAG	8736-8753	cob		Steg46	TTTTAGAGGAACTTGTTGC	12509-12527	rrnS	rev
Steg8	AAAGAAACTCĊTTTTGG	823-839	cox1	for	Steg47	TTCTTTTGTGGACAAGG	378-395	cox1	rev
Steg9	AAATTGAACCAATTTGTCC	157-175	cox1	rev	Steg48	TGAATAATATAAGTTTTTGG	323-342	cox1	for
Steg10	AAGTTATGATTGTATACC	561-578	cox1	for	Steg50	AAGATGTTACTAAACCTCG	2009-2027	cox2	rev
Steg11	ATATCTTTAAATTCCAGGCC	13619-13638	LNR	rev	Steg51	TAATGGAATAGAAGTTGC	6865-6882	nad4	for
Steg12	TCCTCAACATTTTCTTGG	1310-1328	cox1	for	Steg53	ATTTCACTTTTTCTTAGC	4552-4569	nad5	for
Steg14	TTTTTGTGGTTATGGTTCC	11926-11945	nad1	rev	Steg54	TTTCCGTTTTATAAGTGG	5035-5052	nad5	rev
Steg15	AACACTAAGTTTAATGAGG	9420-9438	nad2	for	Steg55	AATAATACCAGTTAATCC	1087-1104	cox1	rev
Steg16	ATTAAAACCGAACATTGACC	12402-12420	rrnS	for	Steg56	AAGTTTTTGGTTCTTCC	333-350	cox1	for
Steg19	ATTCGTTTAATTCATTCC	8243-8260	cob	for	Steg57	TTCTACAGGTTCATTCC	9023-9040	cob	rev
Steg20	TAATTGAAAATGGAGTAGG	8072-8090	cob	rev	Steg58	AAATATCTTATTGAGGAGC	8421-8439	cob	for
Steg21	TCATACTTTACTCTAAACTCG	13544-13564	LNR	rev	Steg59	ACTCTTTCATTCATATGC	4164-4181	nad3	rev
Steg22	TCAGATGTAATTCATTCTTGG	2027-2047	cox2	for	Steg60	AATAATCACATGAATTCC	3654-3671	cox3	rev
Steg24	TAACCCTTATTTAAAACTGG	10167-10185	tRNA-Gln	for	Steg61	AAGATTCTCGGTCTACATCC	3218-3237	cox3	rev
Steg27	TTACACAGAATAAAAATGG	7650-7668	nad6	rev	Steg62	TACCAAGATTACAAAGAACC	11081-11100	rrnL	for
Steg28	TAAGATATTTGACCTCAAGG	8411-8430	cob	for	Steg63	AAAGATCCCGTAAATTCC	6640-6657	nad4	for
Steg29	TTCTGCTATTCCTTACATTGG	8461-8481	cob	for	Steg64	AAAGTCCTTATATATCG	7148-7165	nad4	for
Steg30	ACCCCTACTAAATATATGC	11294-11312	nad1	rev	Steg66	AAATTAGGACGAGAAGACC	10666-10684	rrnL	rev
Steg31	TTGAATTCKGTTAAATAGG	11334-11352	nad1	rev	Steg67	TGTCATTTCTAACATTTGG	7559-7577	nad4l	for
Steg32	AAAAGTAACTCATTAACC	12380-12397	rrnS	for	Steg68	AATCTGTTTCTACAATGG	5225-5242	bad5	for
Steg33	TCTTAGTAGCAAAATAAACC	6921-6940	nad4	rev	Steg69	TTGAAACTACAAAAATGC	5798-5815	nad5	for
Steg34	TTCCTTATTTTATAGTAGG	2738-2756	atp6	for	Steg70	AAATCCAGTGTTTGATCC	6445-6462	nad4	for
Steg35	TATATAGTTTTATCTCACG	6414-6432	nad2	rev	SNP3rev	TGTCAATACTTCTATAAGG	2224-2242	cox2	rev
Steg36	TTTGATCCTATGAGAGTTC	3411-3430	cox3	for	SNP3for	AAGAAAAATAGGAGTTCC	2773-2790	atp6	for
Steg37	TTTCGAGAGTAATTCTGC	10281-10298	tRNA-Ser	rev	SNP2rev	TCATCTGATGTAACAATAGG	3360-3379	cox3	for
Steg38	AAATCATTGATGTAAAGG	9481-9498	nad2	rev	SNP5rev	AATAGCAAAAGAGATACC	11787-11804	nad1	for
Steg39	TAACAAAGTAGATCCTCC	12251-12268	rrnS	rev	SNP4rev	ACTTGTCTTTAAAGAACG	12271-12288	rrnS	for
Steg40	TAAAAGATGTTGAAAACC	13136-13153	LNR	for	SNP4for	TTATACATTAGATTTAGG	12693-12710	rrnS	rev

Appendix 4 Primer sequences for the mitochondrial genome of *Platynothrus peltifer*. Primers on the right were designed on "dirty" PCR products, but do not match the actual complete mitochondrial genome sequence. Gene abbreviations: *cox1-3*: cytochrome oxidase subunits 1-3; *atp6*: ATP synthase subunit 6; *nad1-6, 4L*: NADH dehydrogenase subunit 1-6, 4L; *cob*: cytochrome b; *rrnL*: large ribosomal subunit; *rrnS*: small ribosomal subunit; LNR: large non-coding control region, tRNA-Trp: tRNA for tryptophan, tRNA-Ile: tRNA for isoleucine; rev: reverse, for: forward.

primer name	primer sequence (5'-3')	genomic position	gene	orientation	primer name	primer sequence (5'-3')
Plat1	TATTTATACGAGGGAAGCC	276-295	*cox1*	rev	Plat3	AGTGTAGAGAGAAAACCGG
Plat2	ACGGTCAGATTTTACTCCGG	769-788	*cox1*	for	Plat4	CAAATTCAATCCATCCTTCAC
Plat5	TACCCTAGGGATAACAGCGC	12186-12205	*rrnL*	rev	Plat6	AGTTAAACCCTCGTGTGGCC
Plat7	TTGGGCTCCAATTATTAACGG	250-270	*cox1*	rev	Plat8	ATAAGCTTGGACTCCATCC
Plat16	ACAATTGTACTTTATAGG	14417-14434	LNR	rev	Plat9	GTCTAACCGCGACGGCTGG
Plat20	TGTTGCTGCTGTAAAGTATGC	907-927	*cox1*	rev	Plat10	TCTAACCGCGACGGCTGGC
Plat21	TATCTATAGGAGCTGTATTCG	11358-11378	*nad2*	rev	Plat11	AATCACGGAATTCTGGATCCG
Plat24	TTATGTTAAGGGTCATATTGG	10155-10176	*nad1*	rev	Plat12	CTTCATCGTTTACGGCGTGG
Plat29	TTACATAAACAACTTCATAGC	10168-10189	*nad1*	for	Plat13	TTCTACTCGATATTGCTCCG
Plat30	TAGCTCACAGAATCTTAATCC	13238-13258	*rrnS*	for	Plat14	ATGATCACGGAAATGACTGC
Plat31	ATTGTAGCTAGATTTGGTTGG	12666-12686	*rrnL*	rev	Plat15	AGTCTACATTCTAATTCTACC
Plat33	ATATGTCAATATACCTCAACC	10971-10991	*nad2*	for	Plat17	AAGAAACCCTAAGAAAACC
Plat34	TTTAGGATAGATAGCTAAGG	12206-12225	*rrnL*	rev	Plat18	TAATGATAACTGAAGATGG
Plat37	TCCAAATGAACATTTTAGAGG	14454-14474	LNR	rev	Plat19	ACTGGTTGTTCAGTTTATCG
Plat39	TGGATTTAATGTCGAGTATGG	9692-9712	*nad1*	rev	Plat22	ATGAAGAGAATGCATGGACC
Plat41	AAAGAGCTAATATAATCACC	10533-10552	tRNA-Trp	for	Plat23	TACTAAACCATATGCTTGG
Plat42	TTGATGGAATAGAAACTTCC	11741-11761	tRNA-Ile	for	Plat25	AGAATGAATAAACAAACG
Plat43	AAAACYCAACTAGTAAAGG	7239-7257	*nad4*	for	Plat26	TTTATTTGGAGTCATACTGG
Plat46	ATGAAACGTTGTGTCATCC	1347-1365	*cox1*	for	Plat27	TTAACGCTTATGCCACTGG
Plat47	TTGGAAGGTTTTTATCG	7411-7428	*nad4*	rev	Plat28	TTTCGAAAATGCATATATCC
Plat49	TAAGGACGATTTTACGATGG	8336-8355	*cob*	rev	Plat32	TTTAGAAAGTACATATCG
Plat53	AATAGGATCTTCTTTAAGTGC	84-104	*cox1*	for	Plat35	AGATTATGAGTAGTATACC
Plat54	AAGTCTTTTGAGATTGAAGC	11923-11942	*rrnL*	rev	Plat36	ATAATGACAGTTTTCATCTCG
Plat55	AGATAATTAGCGTTTTTAGG	11305-11324	*nad2*	rev	Plat38	ATAAACCTAAATGGTACACC
Plat55a	TTGTTCGATTTGTATAGG	11369-11386	*nad2*	rev	Plat40	TCTTTTGATTGTGATTGC
Plat57	TTGCTTAAGCTTGATTAAGC	10377-10396	non-coding	rev	Plat44	AAAAAGATTGTTTGTRCC
Plat57a	ATATTAGCTCTTTCTAGTGG	10526-10545	non-coding	rev	Plat45	AAAACATAAAGACAAAGG
Plat57b	TTTTGGATTTCCTTTTGG	10318-10335	*nad1*	rev	Plat48	TCTTGTAAATCAAAAAACC
Plat58	AATAGGAAGTATTCCTTGG	9951-9969	*nad1*	rev	Plat50	ATTTGATTCCTACCCTTCC
Plat61	ATTATTGCTAAATCAACC	10371-10388	non-coding	for	Plat51	TCTATAAGAATGTGAACC
Plat62	AACATAACTATGTCAAGG	2237-2254	non-coding	for	Plat52	ATAAATCCTAAGGATCATAGC
Plat65	TAATATTAAACTTCATCG	11747-11764	tRNA-Ile	for	Plat56	TATATGGATATATGTAGG
Plat67	TTCATAACTTCAAAACGG	2084-2101	*cox2*	for	Plat57c	TTTTACATTGATGGAACG
Plat69	AATACATAGGCTTGAATGC	3032-3050	*atp6*	rev	Plat59	TACAATTTACCTTATAGG
Plat71	TATAGTAGGAATTGAGTGC	3782-3800	*cox3*	for	Plat60	ATGTTTGTCTTTTTGTTGG
Plat73	TAGAAATATGGGAGTACC	6510-6527	*nad4*	rev	Plat63	TCTATTGAGGAAACAAGG
Plat74	TATTCTAATAGTTCGAGG	5802-5819	*nad5*	rev	Plat64	AAGAAACTTAATTCGTCC
Plat76	TTTCTAAAAACCGTCACG	5770-5787	*nad5*	for	Plat66	ACTAATTTATTTTCCACC
Plat78	AACTAGAAACAGAAAACC	7246-7263	*nad4*	for	Plat68	TGAATATGATTAACATTCC
Plat81	AATCATTCCCGTTTTAAGTCC	3321-3341	*cox3*	rev	Plat70	ATTCGAATATATAGAAGC
Plat82	ATTGAGATAACCTTAGTCACG	2553-2573	*atp6*	rev	Plat72	TTTTATTCTCGTAGGCTGG
Plat83	TTATCAGTTTGGTGCTAAGG	5012-5031	*nad5*	rev	Plat75	TCATAGAAGAAGAAAGACC
Plat87	TTATGTTTCTTAAACTGG	2226-2243	non-coding	rev	Plat77	TAGCCCTAAAGATAAACC
coxfor	AAGAAAGAGGTTTTTCTCC	14642-14660	LNR	for	Plat79	TTGTTGGTCAAATGATGG
					Plat80	AATCCACATTCAAATTGG
					Plat84	TCATCTTAATCTACATCC
					Plat85	AGTCTACTTTTATTAACC
					Plat86	ATAAACAGAAAAAACAGC

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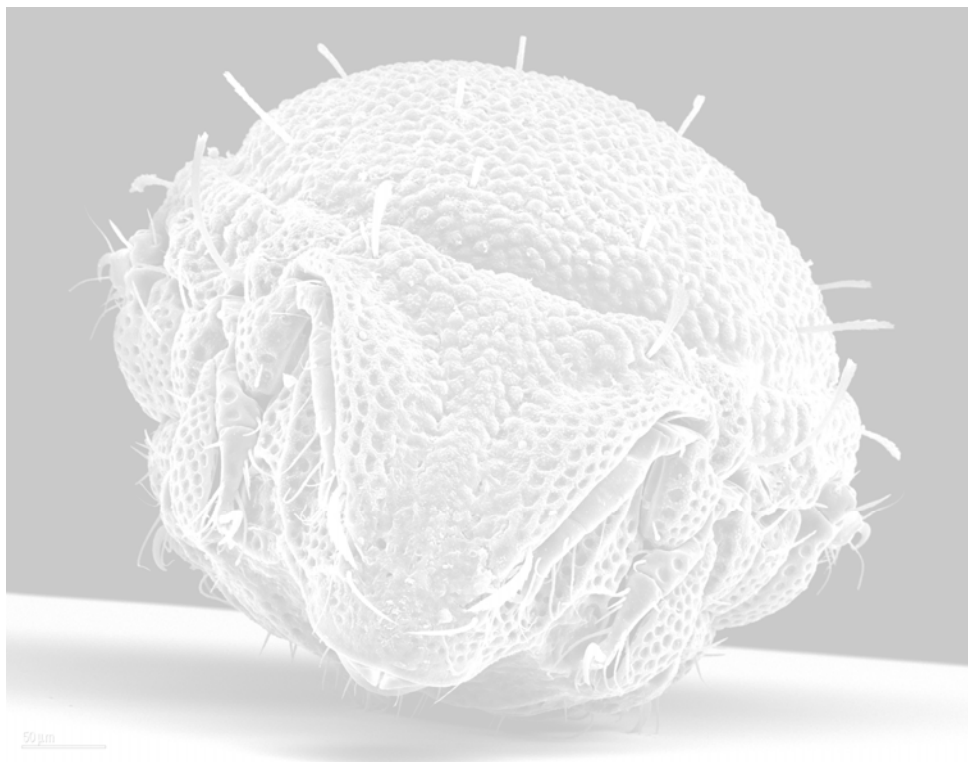
Eidesstattliche Erklärung

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation ohne fremde Hilfe angefertigt und mich keiner anderen als die von mir angegebenen Schriften und Hilfsmittel bedient habe.

Mörfelden, den 2. März 2009