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Strepsiptera and their host specialization

Řasníci (Strepsiptera) a jejich hostitelská specializace

Ph.D. Thesis

Supervisor
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DECLARATION

I hereby declare, that except for the individual papers in which the authorship is mentioned this Ph.D. thesis is exclusively my own work. I have used neither the whole thesis nor its substantial part to obtain any other academic degree. All publications and other sources used in the thesis have been properly cited.

In Prague, 30th August 2016

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Signature

PROHLÁŠENÍ

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 30.srpna 2016

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Podpis

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CONTENTS

AIMS OF THE STUDY AND METHODS

- The first aim was to create the phylogeny of the genus *Stylops* Kirby, 1802 using molecular-phylogenetic methods. Then, we were able to outline the approximate boundary for species delimitation and critically resolve the confusing situation in the species diversity of this genus. Finally, we aimed our attention to the degree of host specialization with alongside support of mapped host taxa onto the tree of the parasites. This aim was fulfilled in PAPER I:

  Jůzová, K., Nakase, Y., Straka, J., 2015. Host specialization and species diversity in the genus *Stylops* (Strepsiptera: Stylopidae), revealed by molecular phylogenetic analysis. Zoological Journal of the Linnean Society 174, 228–243.

- The taxonomy of the genus *Stylops* was conceived differently depending on the preferred species concept associated with the parasitic strategy of individual species. Such an approach created a large number of species names with uncertain validity. Thanks to the newly known phylogeny of the genus, we received essential information for a critical assessment of host specialization. Therefore, our aim was to evaluate the alpha taxonomy and nomenclature for particular species of the genus *Stylops* and propose a new division of this genus into species by means of an integrative taxonomic approach. The results of mentioned aim are presented in PAPER II:

  Straka, J., **Jůzová, K.**, Nakase, Y., 2015. Nomenclature and taxonomy of the genus *Stylops* (Strepsiptera): An annotated preliminary world checklist. Acta Entomologica Musei Nationalis Pragae 55, 305–332.

- Thanks to the current rediscovery of parasitized host species recorded only once almost a century ago, we were able to use modern methods for a more appropriate investigation. We applied an integrative taxonomic approach using DNA sequences and morphology, followed by a differential diagnosis of the females and first instars of genus *Stylops*. Our aim was to redescribe the species and reinterpret it's taxonomic status.
  To accomplish this aim we published PAPER III:

Straka, J., Alqarni, A.S., **Jůzová, K.**, Hannan, M.A., Hinojosa-Díaz, I.A., Engel, M.S., 2015a. Rediscovered parasitism of *Andrena savignyi* Spinola (Hymenoptera, Andrenidae) by *Stylops* (Strepsiptera, Stylopidae) and revised taxonomic status of the parasite. Zookeys 117–139.

- Although more than a little attention was given to the family Stylopidae, some new taxa remains undescribed. As a result of our foregoing studies of the family Stylopidae, we discovered a new genus and several new species. The last aim was to describe newly discovered autapomorphies and provide descriptions, diagnosis and key to species. Our effort resulted in PAPER IV:

  Straka, J., **Jůzová, K.**, Batelka, J., 2014. A new genus of Strepsiptera, *Rozenia* gen. n. (Stylopidae), a parasite of bee genera *Acamptopoeum* and *Calliopsis* (Andrenidae, Panurginae, Calliopsini). Zookeys 31–49.

„We have also seen that, as the specialisation of parts and organs is an advantage to each being, so natural selection will tend to render the organisation of each being more specialised and perfect.“

Charles Darwin

Male of *Xenos vesparum* Rossi, 1793 (Strepsiptera: Xenidae) emerging from *Polistes dominula* (Christ, 1791). Photo by Hubert Poláček.

INTRODUCTION

Strepsiptera as parasites of insects have to constantly interact with their hosts. The order as a whole is highly specialized with many adaptations to the parasitic lifestyle. Nevertheless, the pattern of their coevolution with hosts, especially on the interspecific level, is poorly known, neither is the degree of host specialization, species concept or host specificity, which are inextricably linked together.

The introduction in this thesis is divided into two parts. One, focused to the description of Strepsiptera without missing some of their peculiarities, and the second part – the foregoing one in fact, devoted to the description of phenomena and delimitation of the terms related to the host-parasitic relationship for the purpose of easier understanding of subsequent thoughts and contextualization of the topic in a more general framework.

HOST-PARASITE INTERACTIONS

THE PHENOMENON OF PARASITISM

According to the classic definition, a *parasite* is an organism that uses other organisms (hosts) as a food supply or living environment, thereby harming them (Combes, 2001). In view of such an ecological delimitation, encompassing, for example, also parasitic DNA stretches, all viruses or even phytoparasites, the parasitism is often inferred as the most common life strategy, since each species of organisms has its parasites (Thompson, 1994; Windsor, 1998). In many cases it is not easy to decide whether the relationship is parasitic or whether it is, for example, commensalism or predation. We can narrow the definition down a little and use one of the most common definitions as a framework for parasitism, which says that a parasite is such an organism which lives in or on the host against whom it is somehow adapted, feeding on it and causing some loss to it (Poulin, 2007) without causing immediate death (Begon et al., 2006; Townsend et al., 2008).

Providing that parasitic strategy leads to the death of the host, these organisms are called *parasitoids* (Eggleton and Gaston, 1990; Poulin, 2007). In such a conception it is possible to also include parasitic barnacles of the genus *Sacculina* between parasitoids, because they castrate their hosts, thus causing them an evolutionary death (Kuris, 1974). In a narrower sense, parasitoids are limited only to insects (Townsend et al., 2008). Even though the differences between definitions could be crucial, because their application results in including or excluding some

groups, and the differences between parasites and parasitoids are blurring. An attempt to introduce another term – *protelean organisms* intended for parasitoids or insects parasitic only in its larval stage (Askew, 1971; Godfray, 1994; Kopelman and Chabora, 1984; Legner, 1993; Zhang, 1992) complicated the situation even more. If we want to avoid inaccuracies, it is possible to use the umbrella term *parasitism* or set phrase *parasitic strategy*, even for parasitoids.

For obvious reasons it's impossible to fit the entire diversity of life into definitions. Therefore, it should be borne in mind that the defined terms always have their pitfalls.

COEVOLUTION BETWEEN PARASITE AND HOST

During evolution, parasites and their hosts interact with each other through selection pressure. Such a process is called *coevolution* (Clayton et al., 2015; Thompson, 1994; Townsend et al., 2008). According to the *Red Queen hypothesis*, the evolutionary line has to keep up with the pace of evolution of other lineages with which it interacts to prevent its extinction (Van Valen, 1973). As a consequence, the parasites and hosts are in a constant "*arms races*" in which the hosts are trying to develop mechanisms to defend against parasitism and, in contrary, parasites are developing ways to overcome these mechanisms (Bell et al., 1982; Dawkins and Krebs, 1979).

The coevolution of hosts and parasites may lead to the diversification of hosts, which are selected for the ability to avoid, escape or resist their parasites (Morand et al., 2015). The process does not necessarily have to continue by *cospeciation*, but, for example, it can lead to host-switches (Poisot et al., 2011a). Host-parasite coevolution, can result in many different scenarios (Schmid-Hempel, 2011). Possible examples are demonstrated in Figure 1.

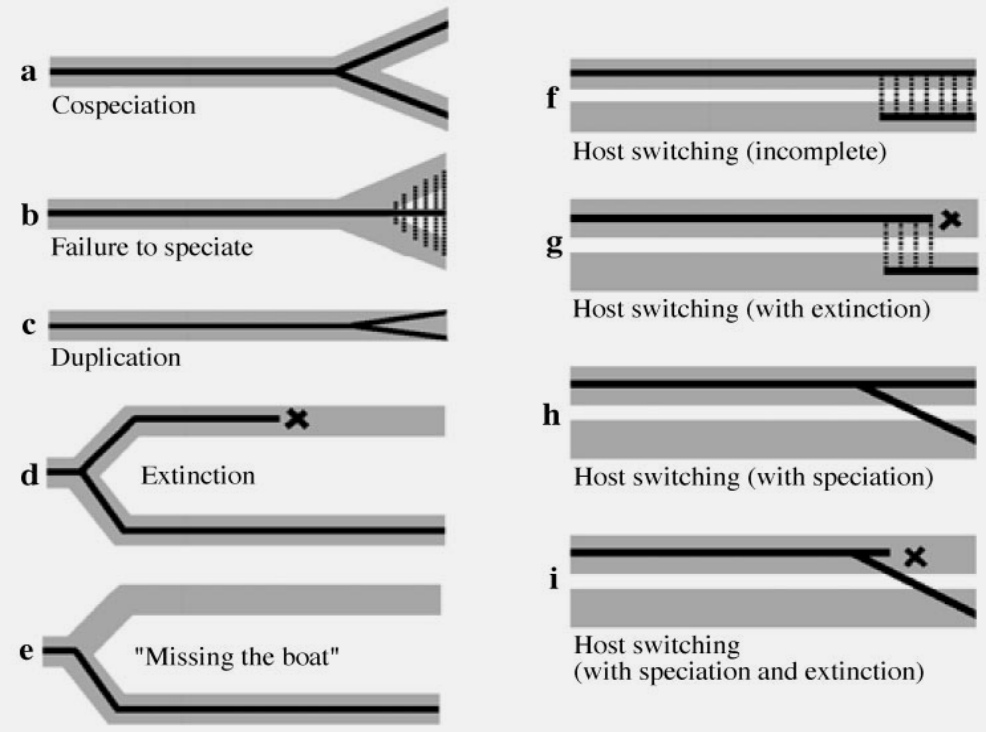

Fig. 1 Possible scenarios in host-parasite coevolutionary histories. Gray lines refers to a phylogenetic lineages of hosts, parasite lineages are black lines. Dashed lines correspond to the gene flow between different populations of the parasitic species. On the *left*: Evolution with host speciation events. *Right*: Evolution with parasitic host-switching.
According to Johnson et al., (2003).

Scenarios are following:

a. **Strict cospeciation** – host speciation followed by a speciation of parasite.
b. **Failure to speciate** – association, but host speciation is not linked to the speciation of parasite. Parasite uses two newly formed host species.
c. **Duplication** – speciation is restricted just to the parasite.
d. **Extinction** of parasite in one host.
e. **"Missing the boat"** – parasite follows just one of the hosts in cospeciation.
f. **Incomplete host switching** – parasite remains in both hosts.
g. **Host switching followed by extinction** in the former host.
h. **Host switching followed by speciation** of the parasite.
i. **Host switching with speciation** of the parasite and with the subsequent **extinction** in the former host.

Another situation can arise in the case of a highly specialized parasitic species when the parasite goes extinct together with its host – they *coextinct* (Colwell et al., 2012; Koh et al., 2004).

SPECIALIZATION OF PARASITES

Due to the coevolutionary processes, parasites are under the pressure to adapt to their hosts. By their nature, parasites usually have a certain degree of *specialization* (Thompson, 1994). It does not automatically mean that the parasites are always specialists. In nature we find both specialists and generalists (Poulin, 2007; Thompson, 1994). I will approach host specializations as a conceptual framework of an undefined degree of host-parasitic relationships going towards greater adaptation, or as a process of adaptation to an increasingly narrow subset of possible environments (Combes, 2001; Poisot et al., 2011a, 2011b; Schmid-Hempel, 2011).

Better specialization brings more than one advantage for the parasites. Narrower adaptation to a certain resource (host) enables its more effective use (Begon et al., 2006; Futuyma and Moreno, 1988). Thanks to this, the advantageous alleles in the population expand faster than they would in a population of generalists. In contrast, harmful mutations, which result in a more difficult use of resources, are rapidly eliminated (Whitlock, 1996). Specialists can also more easily adapt to resources that are difficult to use for generalists. Thus, the specialists face a lower level of competition and predation in their environment (Jaenike, 1990). Higher specialization may therefore be a preferable strategy in a stable environment where there is not such a problem to find and use resources, and if there is high trade-off between adaptations to particular resources. The reason for development of high specialization in species with limited mobility or low population might be higher probability of encounter of two individuals and thus increased number of opportunities for mating (Rohde, 1979). On the other hand, higher degree of specialization implies also some disadvantages, like in an unstable environment where conditions change frequently (Futuyma and Moreno, 1988) and therefore some of the organisms resorted to generalism. Generalists must retain a larger repertoire of traits from which other variants could possibly arise during evolution. Therefore, they have a greater evolutionary potential than specialists (Futuyma and Moreno, 1988). Since they are not strictly tied to only one resource, they are also less prone to extinction (Poulin et al., 2006) and, due to the higher amount of resources available, the time spent searching for a suitable resource is shorter than in the case of specialists (Begon et al., 2006; Jaenike, 1990).

Specializing to resources may not exclusively result in generalism or specialism. A species as a unit can utilize a wide range of resources and be commensurate to the level of generalists, but each individual (or group of individuals) may be individually specialized only to a particular source, irrespective of age, gender or affiliation to any morphotypes (Bolnick et al., 2003; Cloyed and Eason, 2016). This strategy is referred to as *individual specialization* or *specialization at the individual level* (Bolnick et al., 2003).

BLIND ALLEY OF SPECIALIZATION

Close adaptation of parasites to a particular resource is associated with a reduction in the number of traits that allow the use of other resources. Such a reduction could lead to a loss of evolutionary potential and thus "locking out" the flexibility to adapt to other resources (Futuyma and Moreno, 1988). In the traditional sense, this narrow specialization was understood as an evolutionary "*blind alley*" leading irreversibly into evolutionary dead ends and little diversification (Cope, 1904; Mayr, 1963; Poulin, 1998). This would mean that generalists could evolve into specialists, but not vice versa (Poulin et al., 2006). It could be also applied to morphological specialization when reduced morphological complexity of parasites would also march into dead ends (Noble and Noble, 1982). However, more recent studies have shown that the evolution of host specialization may, at least in some cases, run in the opposite direction (Klimov and OConnor, 2013; Poulin et al., 2006) and most specialized species do not necessarily have to be the most derived (Desdevises et al., 2002).

ECOLOGICAL FITTING

When studying coevolution and host-parasite interactions, we should bear in mind, that our interpretation could be contaminated by a variety of artifacts. For example, if we observe that a parasite circumvented the defenses of its host, it is very frequently and automatically presumed that it was evolutionarily produced by a coevolutionary process. Hovewer, it is possible, that some defense traits were produced through coevolution with former parasites. Also, when a parasite gets to a new habitat, it will use those species whose defense traits it can circumvent by the means it has available at that time. Therefore, not every feature of the organism must be an adaptation to the host and not every observed association among organisms is evidence of coevolution (Janzen, 1980). An alternative explanation offers the concept of "*ecological fitting*" (Agosta, 2006; Agosta and Klemens, 2008; Janzen, 1985), describing a situation in which organisms appear to indicate a shared coevolution, but

in fact their traits evolved elsewhere and in response to different conditions (Janzen, 1980). Of course, it is also difficult to distinguish ecological fitting from long-term coexistence. Using ecological fitting, it is also possible to explain the "*parasite paradox*" which arises from the fact that parasites are able to shift onto relatively unrelated hosts (Agosta et al., 2010), even though they are usually resource specialists with restricted host ranges and host-switches should be therefore rare (Ronquist, 2003). From the evidence in nature, host switching is more common than it was considered to be in the past (Agosta et al., 2010; Ronquist and Liljeblad, 2001).

ADAPTATION

An adaptation is a feature which evolved as a consequence of natural selection and which resulted in a selective advantage to its bearer (Poulin, 1994; Ridley, 1993). To assess an adaptive trait, such as the adaptive manipulation of a host's behavior, it should fulfill certain requirements: The trait has to be complex, it should show signs of a purposive design and has to increase the fitness of either the parasite or the host; and finally, it is more likely to be adaptation if the character has arisen independently in several lineages of parasites or hosts (Poulin, 1995). Only if these conditions are satisfied, should we talk about adaptive characters.

HOST SPECIFICITY

The degree of host-specificity is a very important characteristic in a parasite. It can give us evidence about the probability with which the parasite is able to jump on to a new host species, how it adapts to new hosts or how it is able to invade the new hosts when introduced to new geographical areas (Poulin and Mouillot, 2003; Schmid-Hempel, 2011).

According to the most common concept host specificity is the range in which the parasitic taxon is limited to a particular number of host species used in a certain stage of the life cycle. If a parasite has a narrow host range then it depends on its host far more than a parasite with a broader host range. Specificity then decreases with an increasing number of suitable hosts (Poulin, 2007). In this concept, specificity can be estimated as the sum of all the exploited host species. Insufficient sampling can bring a lot of artifacts. Regarding the fact that individuals of the population do not have to be able to use all of the possible hosts and host specificity can differ even among populations (Krasnov et al., 2004), this method of evaluation of the specificity isn't limited to distinguishing adaptation of parasites to locally available host species only. Ultimately the parasites would seem to be more

host-specific than their species would appear as a unit. Obviously, the incorrect identification of a species, unrecognized cryptic species and synonymization of species greatly influence the ability to determine host specificity (Poulin, 2007) Host range is therefore only a rough measure of host specificity.

The effort by scientists to express the degree of host specificity as accurately as possible has produced a number of indices. For example, a varying intensity of resource use, the so-called *Rohde's index* (Rohde, 1980) was defined. In addition to the ecological perspective, the indices can evaluate evolutionary perspective when the taxonomic distance of all of the host species is measured (Fallon et al., 2005; Poulin and Mouillot, 2003). Combining an evolutionary and ecological perspective has resulted in *index STD** (Poulin and Mouillot, 2005), which, among the average taxonomic distance between the exploited host lineages, also considers the prevalence of parasites in host species.

The host range and ability or readiness to parasitize the host can also be examined by different non-theoretical approaches. Via observation and field sampling, it is possible to assess the parasites' preferences in the hosts directly in the wild. We can also perform experiments. It is necessary to delimit against the uncritical application of laboratory experiments on wild species, as the methods may not yield the same results as in natural conditions. These methods include infectious experiments, the aim of which is to determine if a particular species of potential hosts is capable to act as a competent host or vector. In this type of experiment the transmissions are demonstrated in the wild to simulate the most natural state in order to find conclusive evidence of the host-parasite combination viability. Another option is using reciprocal cross leads to determine whether parasites are locally adapted to a host, and whether they would survive and multiply in the alternative host. Likewise, host choice experiments could be conducted in laboratory conditions when access to the potential hosts evaluated is provided and the parasite is observed to determine which host will it choose. Not far behind experimental methods are molecular genetic methods such as functional genomics, which enable a better understanding of gene expression during the host immune response and during parasite development (Morand et al., 2015).

It should be noted that if we take into consideration an ecological fitting, weighing the degree of host specificity does not necessarily reflect the degree of host specialization.

INVESTIGATING HOST-PARASITE INTERACTIONS

Host-parasite interactions can be studied at several levels using different methods. For example, we focus on the behavior of parasitized hosts and explore whether the changes are adaptive. It is also possible to evaluate the immune response of the host, morphology of parasites or hosts and its possible shifts or even (in)congruence of phylogenies of both actors with an interest in a closer examination of their possible coevolution, host-switches, and the level of host specialization.

Because species concept and presence of cryptic species change our view of host specificity, host range and the diversity of the group and the other important characteristics connected with the number of extant taxa (because the taxonomic unit is spp., we evaluate the traits as host specificity related to spp.). But there could be the opposite flow – synonymizing as an artifact in classification.

To conclude, there is no universal model that would describe, how the host-parasite relationship will develop in the course of evolution, and the original idea of gradual specialization within coevolution followed by cospeciation or coextinction has little support in current studies. It is therefore necessary to critically examine and assess each of these relationships separately.

STREPSIPTERA

CHARACTERISTICS

Strepsiptera, also known as twisted-wing parasites, are an endopterygote order of insects with obligatory mode of parasitism. They form about 600 species in nine extant families, which parasitize in Zygentoma, Blattodea, Mantodea, Orthoptera, Hemiptera, Diptera and Hymenoptera (Kathirithamby, 2016, 2009; Kinzelbach, 1971; Pohl and Beutel, 2013, 2008).

Fig. 2 *Polistes dominula* (Christ, 1791) (Hymenoptera) with *Xenos vesparum* Rossi, 1793 (Strepsiptera: Xenidae). *Left, right down*: two male's puparia. *top right*: two females.(Photos by Hubert Poláček.

Their uncertain phylogenetic position within the insect has been discussed for decades. Because of this the name "*the Strepsiptera problem*" became well known (Huelsenbeck, 1998; Kristensen, 1981; Whiting et al., 1997). Detailed study of history of phylogenetic placement of Strepsiptera is summarized in Pohl & Beutel (2013). Thanks to later multigene analyses (Ishiwata et al., 2011; Longhorn et al., 2010; Wiegmann et al., 2009), use of morphological characters (Beutel et al., 2011; Friedrich and Beutel, 2010) and phylogenomic analyses with use of transcriptomes unequivocally supported the hypothesis of phylogenetic relationship with Coleoptera (Boussau et al., 2014; Misof et al., 2014; Niehuis et al., 2012).

Strepsiptera are well known for their extreme sexual dimorphism (figure 1, 2). Adult males are free living with very short life span. After the emergence of the host, they can live just a few hours, during which they have to find and fertilize a female (figure 2; Kifune and Maeta, 1975; Linsley and MacSwain, 1957). The habitus of males is easily recognizable – they bear a pair of reduced forewings similar to halters, while hindwings are broad with simplified venation (Kinzelbach, 1971). Interesting feature are the eyes, which resemble by an ultrastructure of ommatidia the eyes of extinct trilobites. This is thought to be an ancestral adaptation to nocturnal way of life (Buschbeck, 2005; Buschbeck et al., 2003).

Except of two basal families, adult females are adapted to the endoparasitic lifestyle (ca. 97% of the known species). Morphologically, they are very simplified (Kathirithamby, 2009, 1989; Kinzelbach, 1978). Front part of the body of female so called *cephalothorax* (head, thorax and first segment of abdomen) is extruded from host's abdomen (figure 1, 2; Loewe et al., 2016). They release sex pheromone to attract the males (Cvačka et al., 2012; Kirkpatrick, 1937; Riek, 1970; Tolasch et al., 2012). Mating takes place through *traumatic insemination* (Peinert et al., 2016).

Fig. 3 *Andrena vaga* Panzer, 1799 (Hymenoptera) with *Stylops atter* Reicher, 1914 (Strepsiptera: Stylopidae). *Left*: Host with female. *Top right, down right*: Male mating with female. Photos by Pavel Krásenský www.macrophotography.cz.

Larvae of Strepsiptera develop in four stages. First larval instar is invasive and has to invade the host's larvae. Larvae of Strepsiptera, which parasitize hemimetabolan insects can reach their hosts relatively easily, as adults live in the same environment as immature stages. In case of parasiting Endopterygota, first instars have to actively get to places, where larvae of their hosts are. In that case they use phoretic transport (Linsley and MacSwain, 1957; Pohl and Beutel, 2008). The other larval instars are endoparasitic and their apolysis is not followed by ecdysis (Kathirithamby et al., 1984; Manfredini et al., 2007).

Specialization to the host

The amber findings suggest that Strepsiptera were specialized group already in the upper Mesozoic with many morphological autapomorphies (Pohl et al., 2005). In the evolution of Strepsiptera, attachment structures of first instars and males played an important role (Pohl and Beutel, 2004, 2008). Strepsiptera are parasitic castrators (Brandenburg, 1953; Salt, 1927; Solulu et al., 1998; Strambi and Girardie, 1973). Due to the fact that they cause to their hosts evolutionary death, Strepsiptera are sometimes considered parasitoids (Kathirithamby, 2009; Kuris, 1974; Thomas et al., 2009).

Strepsiptera are extraordinary in their ability to stay in the same host from larval to adult stages of hosts, even during host's hypermetamorphosis in case of Holometabolan hosts. It is a unique feature among parasites of insects (Hughes and Kathirithamby, 2005). The association with host can be very long-standing. Females of Strepsiptera, which overwinter with their hosts can associate with them for up to one year (Hughes et al., 2004).

It is not rare that Strepsiptera can change host's behaviour (for examples see Beani, 2006; Beani et al., 2011; Dapporto et al., 2007; Hughes et al., 2004, 2004; Kathirithamby and Hamilton, 1992; Linsley and MacSwain, 1957; Makino, 1993; Salt, 1927; Straka et al., 2014a, 2011; Westwood, 1877).

Strepsiptera as a model group

Insects in general are great model organisms for study of host specializations, because they are the most diversified taxa among animals. This helps us to compare the effect of the parasitic life on the evolution of specialization to a greater extent (Thompson, 1994).

Due to parasitism, Strepsiptera have many interesting interactions with their hosts. Thereby, enhancing of our knowledge about Strepsiptera will also increase our knowledge of the hosts. The family of our interest – Stylopidae – parasitize wild bees (Kinzelbach, 1978). Although wild pollinators are important for the ecosystem (Garibaldi et al., 2013), the impact of stylopization is not known. Simultaneously, many species and genera remain undescribed, even though the family is quite well explored.

CLASSIFICATION

The species concept of Strepsiptera depends on the opinion based concept for species recognition. Usually, morphology of adults and first instars were used, if available (Bohart, 1941; Kinzelbach, 1978, 1971), but not for family Stylopidae, which constitute of more than one quarter of known Strepsiptera species (Pohl and Beutel, 2008). There were over 110 available species names of genus *Stylops* Kirby, 1802 from this family, but with uncertain validity. Many of the species, especially in North America and Japan, were described on the principle of single host association (e.g. Hofeneder, 1924; Kifune, 1991; Pasteels, 1954; Perkins, 1918). Different concept used Bohart (1941, 1937, 1936) or Luna de Carvalho (1974), who took into consideration similarities of *Stylops* species from related hosts from the same subgenus. By contrast, Kinzelbach (1978) used "supergeneralistic" concept, in which all the recognized species of the Western Palaearctic were synonymized and placed them on the subspecies level. Since then, all the *Stylops* parasites in that area from *Andrena* Fabricius, 1755 hosts carried just one name *Stylops mellitae* Kirby, 1802 (e.g. Bleidorn et al., 2004; Pohl and Oehlke, 2003; Smit and Smit, 2005).

We decided to use this very family (and genus) for detailed investigation of their taxonomy and host-parasitic associations.

DISCUSSION AND CONCLUSION

Evaluation of specialization processes can be a little bit tricky. For example, not all beneficial consequences of a trait should be considered as adaptive. It should meet some requirements (for more info see Introduction, chapter Adaptation; Poulin, 1994; Ridley, 1993). The theory of ecological fitting offers one possible explanation. The same goes for an assessment of coevolution. Every association between parasites and hosts is not a proof of shared evolutionary history (Janzen, 1980). Not every cospeciation of parasites with their host is driven by the mechanism of coevolution (Gomulkiewicz and Thompson, 2000; Morand et al., 2015; Nuismer, 2006; Thompson, 1994). Without any knowledge of both host's and parasite's phylogenetical relationships it could be very difficult, even misleading, to assess any coevolution scenarios.

Strepsiptera are usually described as an extremely specialized insect order. They possess many adaptations to the parasitic lifestyle in morphology, behavior or physiology, and many complex adaptive traits; such as, the means to deal with the host's immune system or manipulate the host's behavior (Beani, 2006; Hughes et al., 2004; Kathirithamby and Hamilton, 1992; Manfredini et al., 2007; Pohl and Beutel, 2004, 2008). Despite the fact that our understanding in some area of knowledge is not poor, we are still lacking much important information, such as the comprehensive phylogeny of particular families of Strepsiptera. Due to such an ignorance, there is not any study focused on specialization events in response to coevolution. Furthermore, in the case of our model family Stylopidae, the host taxonomy needs revision and the comprehensive phylogeny is also missing. To change these conditions, we started to gradually reveal the particulars leading to a more comprehensive understanding of host-parasite interactions in Strepsiptera.

Our work started from a much needed phylogenetic study, where we tried to pick out a pattern of specialization of Strepsiptera, even without knowledge of the host's phylogeny. We continued through nomenclatural and taxonomic study, thanks to which we have examined a large number of potential species names, revised them and defined species concept. Our effort on taxonomic investigations also resulted in two descriptive articles. Fulfillment of our objectives and outcomes of relevant articles will be discussed individually. Detailed results are in appropriate papers.

PAPER I

Jůzová, K., Nakase, Y., Straka, J., 2015. Host specialization and species diversity in the genus *Stylops* (Strepsiptera: Stylopidae), revealed by molecular phylogenetic analysis. Zoololical Journal of the Linnean Society 174, 228–243.

We have provided the first molecular phylogeny of family Stylopidae on interspecific level. For this aim we chose 130 individuals from the genus *Stylops* associated with 70 host species of genus *Andrena* Fabricius, 1755 belonging to 25 subgenera. 20 individuals of Strepsiptera from other genera or even family were used as an outgroup. Six new primers for three genes were exclusively designed for that study. The monophyly of entire genus *Stylops* was well supported as well as monophyly of more than 30 crown groups. The distances in DNA base composition were usually between 3-9% from another crown group in the closest clade. The crucial problem is the definition of *Stylops* species, because the definition of species is problematic in general (de Queiroz, 2005; Nixon and Wheeler, 1990). The common threshold for DNA barcode sequence distances between species is usually 3% (Hebert et al., 2003). Evidence that this threshold can be different or exceed in well-defined species is known. Moreover, boundaries for interspecific and intraspecific differences in molecular markers can merge (Meyer and Paulay, 2005; Rubinoff et al., 2006). Therefore, there is no universal criterion for delimitation of *Stylops* species. For more accurate evaluation of number of species, morphological and taxonomical revision is needed. In all cases, it is obvious, that there is not just one species of *Stylops* in West Palearct, as was assumed according to Kinzelbach (1978).

Broad sampling of host species allowed testing three hypotheses of parasitic strategy: specificity to host species, specificity to host subgenus and specificity to host genus. The lineages, which should represent species lineages, were mostly clustered according to the host subgenus. Supergeneralistic concept as well as the concept of superspecialized *Stylops* species was rejected. The premise, that *Stylops* species are sorted according to host subgenera can be used as a support tool, but not as a strict one and it is necessary to approach to each lineage individually. Otherwise, it would bring a similar problem, as when a new association with a host led to description of a new species of parasite, only in our case it would be on different level. Our study laid the foundation for future studies on taxonomy and coevolution with strepsipteran hosts.

PAPER II

Straka, J., **Jůzová, K.**, Nakase, Y., 2015. Nomenclature and taxonomy of the genus *Stylops* (Strepsiptera): An annotated preliminary world checklist. Acta Entomologica Musei Nationalis Pragae 55, 305–332.

There has always been a large number of names for *Stylops* species (Kathirithamby, 2016; Kinzelbach, 1971). As a result of inconsistent use of species concepts, these names had uncertain validity – some species were considered as specialist and described on the principle of single host association (Perkins, 1918; Pierce, 1909), contrary to the supergeneralistic concept of Kinzelbach (Kinzelbach, 1978). Therefore, we re-evaluated nomenclature and taxonomy in that genus. We used morphological descriptions from literature, distances of DNA barcode sequences and previous phylogenetic study (paper I) as an auxiliary tool. When several names were proposed from the same host subgenus, synonymy was considered, but just as a guideline and not as a rule. Many species had to be synonymized as conspecific species. In case of species with unknown hosts (always in case of male findings), when we cannot decide about synonymy, no species were listed under synonyms. In lineages with very uncertain validity we recommended future resolving by morphology or population genetic tools. We also suggested using barcoding and sequencing the other genes to determine distances between DNA sequences. It is for a non-analytical method, which could be helpful even without making the phylogenetical analyses.

We prepared the preliminary nomenclatural list of all *Stylops* species and highlighted 67 valid species names for genus *Stylops*. The list is surely not definitive. This study should be just a first step towards comprehensive taxonomy of *Stylops*.

PAPER III

Straka, J., Alqarni, A.S., **Jůzová, K.**, Hannan, M.A., Hinojosa-Díaz, I.A., Engel, M.S., 2015a. Rediscovered parasitism of *Andrena savignyi* Spinola (Hymenoptera, Andrenidae) by *Stylops* (Strepsiptera, Stylopidae) and revised taxonomic status of the parasite. Zookeys 117–139.

A rediscovery of parasitized *Andrena savygnyi* Spinola, 1838 enabled to obtain DNA sequences and morphology of females and first instars of associated *Stylops* sp., originally described as *Stylops savygnyi* Hofeneder, 1924. Results were compared with DNA and morphology of related species. The parasite was redescribed as conspecific *S. nassonowi* Pierce, 1909, so we synonymized the former name.

Phylogenetic analysis of related species of *Stylops nassonowi* Kirby, 1802 and *Stylops aterrimus* Newport, 1851 (Jůzová et al., 2015) revealed, that these species form a species complex – they are close sibling species, but their morphology is almost indistinguishable. Intraspecific variance in DNA distances is below 2%, interspecific is on a 4% threshold, which is higher than in many other morphologically easily diagnosed species of *Stylops*. *S. nassonowi* and *S. atterimus* seem to be allopatric in Europe, therefore more material and phylogeographic study is needed. This paper was the first step in classification of *Stylops* by both morphological and molecular data.

PAPER IV

Straka, J., **Jůzová, K.**, Batelka, J., 2014. A new genus of Strepsiptera, *Rozenia* gen. n. (Stylopidae), a parasite of bee genera *Acamptopoeum* and *Calliopsis* (Andrenidae, Panurginae, Calliopsini). Zookeys 31–49.

In this paper we provided diagnosis and descriptions of three new species from new genus *Rozenia* gen. n. The key to species was attached for both females and first instar larvae. We have found, that first instars of *Rozenia* species possess some exceptional characters not know for any other species of Strepsiptera. These were extremely long caudal setae and the presence of additional row of setae on dorsal thoracic segments. We named that row an „interstitial row“. Until the discovery of *Rozenia*, the chaetotaxy of dorsal rows seemed to be reductive in evolution of the order. Possible adaptation of such long caudal setae is unknown. An easier attachment to the host is only a speculation.

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SUMMARY OF PAPERS

PAPER I

Host specialization is an important ecological characteristic of parasitic species. The identification of the parasitic strategy of the genus *Stylops* (Strepsiptera; Stylopidae) is, however, ambiguous. According to the number of recognized species based on existing taxonomy, highly specialized and supergeneralistic species exist in this genus. Our research aims to clarify the concept of host specialization in the genus *Stylops*, in which all of the members are parasites of *Andrena* bees. Based on the phylogenetic analysis of the parasites (mostly females) and the mapping of hosts onto the phylogenetic tree, we tested three hypotheses of host specialization: (1) each species of the genus *Stylops* is associated with a single host species; (2) *Stylops* species are specialized to a group of closely related hosts; and (3) a single *Stylops* species is a generalist, parasitizing all host *Andrena* species in this particular region. Our evidence clearly shows a close relationship between the parasite and the host: one species of *Stylops* attacks one or a few host species of *Andrena* bees, usually from a single subgenus. Moreover, a moderate generalistic strategy is also likely in a few *Stylops* species. According to our results, the species diversity of the strepsipteran parasites of bees must be reconsidered. A single European species of *Stylops* should be divided into a higher number of valid species.

PAPER II

Taxonomy and nomenclature of the genus *Stylops* (Strepsiptera) have been understood differently in different parts of world for a long time. Largest differences came from erroneous concept of host specialization of individual species. For this reason, we reevaluated taxonomy and nomenclature in all *Stylops* species based on distances of DNA barcode sequences (cytochrome c oxidase subunit I). Twenty six species (123 individuals) out of sixty six recognized *Stylops* species from all distribution range were DNA barcoded and their sequences compared. Taxonomy of all West Palaearctic species was restructured to be congruent with results of analysis of the genetic distances. Single European species *Stylops melittae* is divided into thirty species, whose species status is restituted. Nine names are recognized as nomina nuda and therefore unavailable in zoological nomenclature. Years of publications of the species names were corrected based on the original literature. Bee hosts are summarized for each species according to the new synonymies.

PAPER III

Parasitism of *Andrena* (*Suandrena*) *savignyi* Spinola (Hymenoptera: Andrenidae) by *Stylops* Kirby (Strepsiptera: Stylopidae) has been recorded only once, and from an individual collected in Egypt almost a century ago, with the parasite described as *Stylops savignyi* Hofeneder. The recent rediscovery of this *Stylops* from an individual of *A. savignyi* permits a reinterpretation of the species and its affinities among other *Stylops*. The bee was collected at flowers of *Zilla spinosa* (Turra) Prantl. (Brassicaceae) in Amariah, Riyadh, Kingdom of Saudi Arabia. Based on DNA barcode sequences from material sampled across Africa, Asia, and Europe, it is apparent that *S. savignyi* is conspecific with *S. nassonowi* Pierce, and we accordingly synonymize this name (**syn. n.**), with the latter representing the senior and valid name for the species. A differential diagnosis is provided for *S. nassonowi* and the morphology of the female is described, as well as the first instars.

PAPER IV

A new Strepsiptera genus from South America is described, *Rozenia* **gen. n.**, with three new species: *R. calliopsidis* **sp. n.** (type species), *R. peruana* **sp. n.** and *R. platicephala* **sp. n.** These three new species are parasites of bees belonging to the tribe Calliopsini (Andrenidae, Panurginae). *Rozenia calliopsidis* **sp. n.** is a parasite of the bee genus *Calliopsis* Smith, 1853 and *R. peruana* **sp. n.** and *R. platicephala* **sp. n.** are parasites of the bee genus *Acamptopoeum* Cockerell, 1905. Diagnoses and descriptions of female puparia are presented for all three species. Diagnoses and descriptions of first instars (triungulinids) are presented for *R. calliopsidis* **sp. n.** and *R. platicephala* **sp. n.** The first case of increased number of setae on the body of the first instars and augmentation of chaetotaxy of Strepsiptera are discussed.

PAPER I

Jůzová, K., Nakase, Y., Straka, J., 2015. Host specialization and species diversity in the genus *Stylops* (Strepsiptera: Stylopidae), revealed by molecular phylogenetic analysis. Zoololical Journal of the Linnean Society 174, 228–243.

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Host specialization and species diversity in the genus *Stylops* (Strepsiptera: Stylopidae), revealed by molecular phylogenetic analysis

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Host specialization is an important ecological characteristic of parasitic species. The identification of the parasitic strategy of the genus *Stylops* (Strepsiptera; Stylopidae) is, however, ambiguous. According to the number of recognized species based on existing taxonomy, highly specialized and supergeneralistic species exist in this genus. Our research aims to clarify the concept of host specialization in the genus *Stylops*, in which all of the members are parasites of *Andrena* bees. Based on the phylogenetic analysis of the parasites (mostly females) and the mapping of hosts onto the phylogenetic tree, we tested three hypotheses of host specialization: (1) each species of the genus *Stylops* is associated with a single host species; (2) *Stylops* species are specialized to a group of closely related hosts; and (3) a single *Stylops* species is a generalist, parasitizing all host *Andrena* species in this particular region. Our evidence clearly shows a close relationship between the parasite and the host: one species of *Stylops* attacks one or a few host species of *Andrena* bees, usually from a single subgenus. Moreover, a moderate generalistic strategy is also likely in a few *Stylops* species. According to our results, the species diversity of the strepsipteran parasites of bees must be reconsidered. A single European species of *Stylops* should be divided into a higher number of valid species.

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ADDITIONAL KEYWORDS: Andrenidae – bee parasites – generalist parasites – host–parasite systems – phylogenetics – Stylopidae.

INTRODUCTION

The Strepsiptera (twisted-wing insects) represent a small order of insect parasites (Brandenburg, 1953) or parasitoids (Kathirithamby, 2009) in which the hosts originate from the Zygentoma, Blattodea, Mantodea, Orthoptera, Hemiptera, Hymenoptera, and Diptera (Kinzelbach, 1971; Kathirithamby, 2009). Strepsipterans undergo dramatic remodelling of their body structures in the course of ontogeny (hypermetamorphic development). The first-instar larvae develop inside the mother's body and actively leave it through a fissure on the cephalothorax (Stylopiformia), through the opening of the single brood organ (Mengenillidae), or through the mouth opening of the female (Corioxenidae) (Pohl & Beutel, 2008). First-instar larvae possess three pairs of walking legs, live freely, and invade the host body. They moult into endoparasitic larvae that grow without shedding the old cuticles until reaching maturity (Kathirithamby *et al*., 1984; Kathirithamby, Ross & Johnston, 2003). Male larvae form puparia and mature males always leave the host, whereas adult females (except for the family Mengenillidae) are neotenic, legless, and remain inside the host body.

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Free-living males actively fly using their hindwings. They are also well known for their short lifespan of only several hours (Kathirithamby, 2009). Females release a powerful sex pheromone (called stylopsal in *Stylops* species) to attract males for mating (Cvačka *et al*., 2012; Tolasch, Kehl & Dötterl, 2012). In *Stylops* species, both sexes enhance the chance of finding a mate by manipulating their hosts to emerge earlier compared with non-parasitized conspecifics (Straka *et al*., 2011). When the eggs develop into first-instar larvae inside the female, manipulated hosts spread invasive first instars to reach the hosts of a new generation (Linsley & MacSwain, 1957; Kathirithamby *et al*., 2012b).

The list of Strepsipteran peculiarities would not be complete without mentioning the doubts about their phylogenetic position, called 'the Strepsiptera problem' (Kristensen, 1981; Whiting *et al*., 1997; Huelsenbeck, 1998; Pohl & Beutel, 2013). Twisted-wing parasites have been proposed to be the sister group of the Diptera or the Coleoptera, but most recently the latter is supported (Wiegmann *et al*., 2009; McKenna & Farrell, 2010; Beutel *et al*., 2011; Niehuis *et al*., 2012); however, this problem is not the only problem in the order. One of the pronounced problems is the absence of data on parasite specialization towards the hosts (Hayward, McMahon & Kathirithamby, 2011). There is inconsistent understanding and use of host specialization and species recognition in this order. Several cryptic species were discovered recently using molecular phylogenetic studies, resulting in an increase in the number of narrowly specialized species and a reduction in the number of generalistic species (Hayward *et al*., 2011; Nakase & Kato, 2013). For example, one species of the genus *Xenos*, known to be a parasite of several large hornet wasp species, was divided into two different specialized species (Nakase & Kato, 2013); however, close affinity to host species was not found in *Elenchus japonicus* Esaki & Hashimoto, 1931 (Matsumoto *et al*., 2011). In the case of the genus *Stylops*, the species are considered to be largely specialized, though not strictly, to host species in North America (Bohart, 1941) or Japan (Kifune & Hirashima, 1985; Kifune & Maeta, 1990; Kifune, 1991; Isaka, Ueda & Itino, 2012). In contrast, *Stylops melittae* Kirby, 1802 is a supergeneralistic species, and the only species to be recognized in Europe recently. It is known to be the parasite of tens of *Andrena* bee species from many subgenera (Kinzelbach, 1978). The existence of such a generalist parasite would be surprising because the evolutionary arms race between host and parasite drives specialization to a narrower source spectrum, which is more effective in source use (Van Valen, 1973; Futuyma & Moreno, 1988). Nevertheless, although host specificity is common, the generalist strategy also exists in nature. Generalists benefit from greater resource availability (Jaenike, 1990; Begon, Townsend & Harper, 2006); however, they are prone to the risk of deleterious mutations, inefficiency of source use, and other evolutionary costs (Kawecki, 1994). The generalistic strategy can also be explained by the ecological fitting theory (Janzen, 1985). Similarities between some unrelated potential host species can be very high, and thus they might not be recognized as differing and might occasionally be used by the parasite if reached by an invasive stadium.

We aim to contribute to this subject using phylogenetic methods, allowing us to reconstruct the relationships among particular *Stylops* individuals associated with their hosts. We gathered *Stylops* specimens from 70 host species collected throughout the entire range of their geographical distribution on four continents (Africa, Asia, Europe, and North America). We tested the following hypotheses of parasite specialization to hosts and overall *Stylops* species diversity: (1) each *Stylops* species is associated with a single host species; (2) *Stylops* species are specialized to a group of closely related hosts; (3) a single *Stylops* species is a generalist, parasitizing all host *Andrena* species in the particular region. These hypotheses were tested using phylogenetic information obtained from maternally transferred mitochondrial DNA and from nuclear DNA, which is transmitted by both sexes. Our ultimate aim is to critically review the particular host specificity that is exemplified by the genus studied, and to disentangle the confusing situation in the number of species of *Stylops* and its potential diversity in the world.

MATERIAL AND METHODS

Material and data sets

Our data set consists of 150 individuals of Strepsiptera. The voucher names, hosts, and collection localities are listed in Table 1. One hundred and thirty individuals (119 females and 11 males) are from the genus *Stylops* (in-group), and 20 individuals of the families Stylopidae and Xenidae are used as an out-group. *Stylops* specimens were extracted from metasoma of 70 *Andrena* host species belonging to 25 subgenera. Broad sampling of host species allows us to test three hypotheses of parasitic specificity of *Stylops* species: specificity to host species; specificity to host subgenus; and specificity to host genus.

The DNA sequences were newly acquired for our study, except for the *Eupathocera*, *Paraxenos*, and *Xenos* species used for the out-group (NCBI codes: JN082810, JN082844, JN082811, JN082845, JN082808, JN082843, JN082806, and JN082842) and a single in-group individual (JN082812), which were acquired from the first molecular phylogeny of the Strepsiptera (McMahon, Hayward & Kathirithamby, 2011). All of the sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov; National Center for Biotechnology

Table 1. List of specimens used in our study, with a list of host species and locality data

Voucher	Strepsiptera	Host	Country	Locality	*COI*	*NADH*	*EF1*
Out-group							
Eu	*Eupathocera*				JN082810	JN082844	NA
EuPo5	*Eupathocera*	*Podalonia* sp.	Mongolia	Arvaykheer 137 km NE, Överkhangay Province	KF803411	NA	NA
PaSp1	*Eupathocera*	*Ammophila heydeni* Dahlbom, 1845	Turkey	Salihli 30 km SEE, Manisa Province	KF803420	NA	NA
PaEr1	*Paraxenos*	*Bembecinus tridens* (Fabricius, 1781)	Hungary	Örkeny env.	KF803417	KF747735	NA
PaHo1	*Paraxenos*	*Stizus* sp.	Mongolia	100 km NW Dalanzadghad, Bayanzag	KF803418	NA	NA
PaIn1	*Paraxenos*	*Tachytes* sp.	Zambia	Serenje 62 km SW	KF803419	NA	NA
Ps	*Pseudoxenos*				JN082811	JN082845	NA
XDo1	*Xenos*	*Polistes dominula* (Christ, 1791)	CZ	Dubeček, Praha-Dubeč, Bohemia	KF803535	KF747749	NA
Xpe	*Xenos*				JN082808	JN082843	NA
Xve	*Xenos*				JN082806	JN082842	NA
CPa1	*Crawfordia*	*Panurgus pici* Pérez, 1895	Tunisia	coast, Sousse Province	KF803408	NA	NA
CPs1	*Crawfordia*	*Psaenythia bizonata* Friese, 1908	Argentina	Cachi env., Salta 85 km SWW, Salta Province	KF803409	NA	NA
EDu1	*Eurystylops*	*Dufourea dentiventris* (Nylander, 1848)	CZ	Buková hora, Malé Březno env., Bohemia	KF803410	NA	KF892823
HBr1	*Halictoxenos*	*Lasioglossum brevicorne* (Schenck, 1870)	Italy	Porto Pino, Sardinia	KF803412	NA	NA
HDve1	*Halictoxenos*	*Lasioglossum versatum* (Robertson, 1902)	USA	Kentucky	KF803413	NA	NA
HSi1	*Halictoxenos*	*Halictus simplex* Blüthgen, 1923	CZ	Mohelno env., Moravia	KF803414	NA	NA
HTu1	*Halictoxenos*	*Halictus tumulorum* (Linnaeus, 1758)	CZ	Dubeček, Praha-Dubeč, Bohemia	KF803415	NA	NA
MsHa1	*Melittostylops*	*Hesperapis rhodocerata* (Cockerell, 1897)	USA	Willcox 4mi E, Cochise Co., Arizona	KF803416	NA	NA
UCl1	*Kinzelbachus*	*Melitturga clavicornis* (Latreille, 1806)	Kyrgyzstan	Naryn N., Ak-Tal-Tchat	KF803534	NA	KF892879
YVa1	*Hylecthrus*	*Hylaeus variegatus* (Fabricius, 1798)	CZ	Boří les env., Moravia	KF803536	NA	NA
In-group							
SAc1	*Stylops*	*Andrena* sp. (*Aciandrena*)	Tunisia	Tamerza env.	KF803421	NA	KF892824
SAc2	*Stylops*	*A.* cf. *aciculata* Morawitz, 1886	Turkey	Burdur 20 km SW, Burdur Province	KF803422	NA	NA
SAc3	*Stylops*	*A. aciculata* Morawitz, 1886	CZ	Tasovice env., Moravia	KF803423	NA	NA
SAc4	*Stylops*	*A.* sp. (*Aciandrena*)	Turkey	Büyüksofulu env., Adana Province	KF803424	KF647676	KF892825
SAc5	*Stylops*	*A.* sp. (*Aciandrena*)	Turkey	Büyüksofulu env., Adana Province	KF803425	NA	KF892826
SAcg1	*Stylops*	*A.* sp. (*Aciandrena*)	Spain	Alcaníz 15 km W, Aragón Province	KF803426	NA	NA
SAcg3	*Stylops*	*A.* sp. (*Aciandrena*)	Spain	Alcaníz 13 km W, Aragón Province	KF803427	NA	NA
SAe1	*Stylops*	*A. aeneiventris* Morawitz, 1872	Turkey	Burdur 20 km SW	KF803428	NA	NA
SAg1	*Stylops*	*A. agilissima* (Scopoli, 1770)	Tunisia	Tamerza env.	KF803429	KF647686	NA
SBc1	*Stylops*	*A. bicolor* Fabricius, 1787	Spain	Mazarete 1.5 km SE, Castilla-La Mancha Province	KF803430	NA	KF892827
SBc3	*Stylops*	*A. bicolor* Fabricius, 1787	Spain	Muniesa 3 km S, Aragón Province	KF803431	NA	KF892828
SBi1	*Stylops*	*A. bisalicis* Viereck, 1908	USA	Chesterfield Co., South Carolina	KF803432	NA	NA
SCa1	*Stylops*	*A. carantonica* Pérez, 1902	CZ	Lednice env., Moravia	KF803433	NA	KF892829
SCa2	*Stylops*	*A. carantonica* Pérez, 1902	CZ	Divoká Šárka, Praha-Liboc, Bohemia	KF803434	NA	KF892830
SCa6	*Stylops*	*A. carantonica* Pérez, 1902	CZ	Dolní Věstonice env., Pálava, Moravia	KF803435	KF747736	NA
SCa9	*Stylops*	*A. carantonica* Pérez, 1902	CZ	Chvalské skály, Praha-H.Počernice, Bohemia	KF803436	KF647697	NA
SCi1	*Stylops*	*A. cineraria* (Linnaeus, 1758)	CZ	Chýnice, Bohemia	KF803437	NA	KF892831
SCi2	*Stylops*	*A. cineraria* (Linnaeus, 1758)	CZ	Tiché údolí, Roztoky env., Bohemia	KF803438	NA	KF892832
SCl1	*Stylops*	*A. clarkella* (Kirby, 1802)	CZ	Na Plachtě, Hradec Králové, Bohemia	KF803439	NA	NA
SCo1	*Stylops*	*A. commoda* Smith, 1879	USA	Pennington Co., South Dakota	KF803440	NA	NA
SCr4	*Stylops*	*A. carolina* Viereck, 1909	USA	Hancock Co., Maine	KF803441	KF647694	NA
SCs1	*Stylops*	*A. cressonii* Robertson, 1891	USA	Jackson Co., South Dakota	KF803442	KF647685	NA
SCs3	*Stylops*	*A. cressonii* Robertson, 1891	USA	Loudoun Co., Virginia	KF803443	NA	NA
SCw1	*Stylops*	*A. crawfordi* Viereck, 1909	USA	Denton	KF803444	NA	NA
SDi1	*Stylops*	*A. distinguenda* Schenck, 1871	CZ	Děvín, Pavlov env., Pálava, Moravia	KF803445	NA	KF892833
SDt nks03	*Stylops*	*A. dentata* Smith, 1879	Japan	Mt. Oohira Matsue Shimane pref.	KF803543	KF647670	KF892834
SEr2	*Stylops*	*A. erigeniae* Robertson, 1891	USA	Loudoun Co., Virginia	KF803446	KF647689	NA
SEr6	*Stylops*	*A. erigeniae* Robertson, 1891	USA	Pr. George's Co., Maryland	KF803447	KF647683	NA
SFc1	*Stylops*	*A. fulvicornis* Schenck, 1861	Turkey	Kütahya 28 km SSE, Kütahya Province	KF803448	NA	NA

SFc2	*Stylops*	*A. fulvicornis* Schenck, 1861	Austria	Poysdorf 1 km E, Lower Austria	KF803449	NA	NA
!SFc3a	*Stylops*	*A. fulvicornis* Schenck, 1861	CZ	Dolní Věstonice, Moravia	KP213296	NA	NA
!SFc3b	*Stylops*	*A. fulvicornis* Schenck, 1861	CZ	Dolní Věstonice, Moravia	KP213297	NA	NA
SFl2	*Stylops*	*A. flavipes* Panzer, 1799	CZ	Divoká Šárka, Praha-Liboc, Bohemia	KF803453	NA	KF892835
SFl4	*Stylops*	*A. flavipes* Panzer, 1799	CZ	Polabiny env., Pardubice, Bohemia	KF803455	NA	NA
SFl110	*Stylops*	*A. flavipes* Panzer, 1799	CZ	Dolní Dunajovice env., Moravia	KF803450	NA	NA
SFl11	*Stylops*	*A. flavipes* Panzer, 1799	CZ	Pouzdřanská step, Pouzdřany, Moravia	KF803451	NA	NA
SFl118	*Stylops*	*A. flavipes* Panzer, 1799	CZ	Ronna former mine, Kladno env., Bohemia	KF803452	NA	NA
SFl31	*Stylops*	*A. flavipes* Panzer, 1799	CZ	Záluží, Roudnice nad Labem env., Bohemia	KF803454	NA	KF892836
!SFl47	*Stylops*	*A. flavipes* Panzer, 1799	CZ	Čelákovice env., Bohemia	KP213295	NA	NA
SFlg1	*Stylops*	*A. flavipes* Panzer, 1799	Tunisia	Tamerza env.	KF803456	NA	NA
SFu1	*Stylops*	*A. fulva* (Müller, 1766)	CZ	Chýnice, Bohemia	KF803457	NA	KF892837
SFu2	*Stylops*	*A. fulva* (Müller, 1766)	CZ	Kamenný Přívoz, Jílové env., Bohemia	KF803458	NA	KF892838
SGr1	*Stylops*	*A. gravida* Imhoff, 1832	CZ	Polabiny env., Pardubice, Bohemia	KF803459	NA	NA
SGr4	*Stylops*	*A. gravida* Imhoff, 1832	Hungary	Budaörs, Budapest env.	KF803460	NA	NA
SGr5	*Stylops*	*A. gravida* Imhoff, 1832	Austria	Poysdorf 1 km E, Lower Austria	KF803461	NA	NA
SHe1	*Stylops*	*A. helvola* (Linnaeus, 1758)	CZ	Dolní Dunajovice env., Moravia	KF803462	NA	NA
SHo1	*Stylops*	A. sp. (*Hoplandrena*)	Turkey	Gözeldere 25 km E, Hakkari Province	KF803463	NA	NA
SHp1	*Stylops*	*A. hippotes* Robertson, 1895	USA	Upper and Lower Lakes WMA	KF803464	NA	NA
SHp2	*Stylops*	*A. hippotes* Robertson, 1895	USA	Lisle, Broome Co., New York	KF803465	NA	NA
SHug2	*Stylops*	A. sp. (*Chlorandrena*)	Spain	Alcaníz 15 km W, Aragón Province	KF803466	KF647674	KF892839
SHug3	*Stylops*	A. sp. (*Chlorandrena*)	Spain	Alcaníz 4 km W, Aragón Province	KF803467	KF647695	KF892840
SIm1	*Stylops*	A. sp. (*Graecandrena*)	Greece	Akrokorinthos, Korinth env., Korinthia Province	KF803468	NA	NA
SIm2	*Stylops*	A. sp. (*Graecandrena*)	Turkey	Mugla	KF803469	NA	NA
SIn2	*Stylops*	*A. intermedia* Thomson, 1870	Switzerland	Leuk env.	KF803470	NA	NA
SIt2	*Stylops*	*A. imitatrix* Cresson, 1872	USA	Columbus Co., North Carolina	KF803471	NA	NA
SKr1	*Stylops*	*A. krigiana* Robertson, 1901	USA	Chesterfield Co., South	KF803472	NA	NA
SLa1	*Stylops*	*A. labiata* Fabricius, 1781	Turkey	Budakli env., Kahramanmaraş Province	KF803473	NA	NA
SMc1	*Stylops*	A. sp. (*Micrandrena*)	Greece	Archaia Olympia, Ilis Province	KF803474	KF647690	KF892841
SMc4	*Stylops*	A. sp. (*Micrandrena*)	Turkey	between Kamişli and Findikli, Adana Province	KF803475	KF647693	KF892842
SMc8	*Stylops*	A. sp. (*Micrandrena*)	Spain	Muniesa 3 km S, Aragón Province	KF803476	F747737	KF892843
!SMc15	*Stylops*		CZ	Lysá nad Labem env., Bohemia	KP213292	NA	NA
!SMc16	*Stylops*		CZ	Dubeček, Praha-Dubeč, Bohemia	KP213293	NA	NA
SMi1	*Stylops*	*A. minutula* (Kirby, 1802)	CZ	Chýnice, Bohemia	KF803477	KF647671	KF892844
SMi6	*Stylops*	*A. minutula* (Kirby, 1802)	CZ	Dolní Věstonice env., Moravia	KF803479	KF647696	KF892845
SMi12	*Stylops*	*A. minutula* (Kirby, 1802)	Switzerland	Leuk env.	KF803478	NA	NA
!SMi17	*Stylops*	*A. minutula* (Kirby, 1802)	CZ	Praha-Žižkov, Bohemia	KP213294	NA	NA
SMi nks28	*Stylops*	*A. minutula* (Kirby, 1802)	Japan	Sakata Yamagata Pref.	KF803539	KF647665	KF892846
SMo1	*Stylops*	*A. minutuloides* Perkins, 1914	CZ	Lednice, Moravia	KF803480	KF647673	NA
SMo3	*Stylops*	*A. minutuloides* Perkins, 1914	CZ	Klánovický les, Praha-Klánovice, Bohemia	KF803481	KF647682	KF892847
SMr1	*Stylops*	*A. miranda* Smith, 1879	USA	Montague WMA, Franklin Co., Massachusetts	KF803482	NA	KF892848
SMr2	*Stylops*	*A. miranda* Smith, 1879	USA	Montague WMA, Franklin Co., Massachusetts	KF803483	NA	NA
SMs1	*Stylops*	*A. mitis* Schmiedeknecht, 1883	CZ	Polabiny env., Bohemia	KF803484	NA	KF892849
SMw1	*Stylops*	*A. milwaukeensis* Graenicher, 1903	USA	Cornwall, Black Rock Forest	KF803485	KF747738	NA
SNa1	*Stylops*	*A. nasonii* Robertson, 1895	USA	Howard Co., Maryland	KF803486	NA	NA
SNa2	*Stylops*	*A. nasonii* Robertson, 1895	USA	NW Virginia, Virginia	KF803487	KF647688	NA
SNi2	*Stylops*	*A. nigroaenea* (Kirby, 1802)	CZ	Prokopské údolí, Praha-Jinonice, Bohemia	KF803490	NA	KF892850
SNi4	*Stylops*	*A. nigroaenea* (Kirby, 1802)	France	St. Vincent deBarbeyrargues, Hérault	KF803491	KF747739	NA
SNi6	*Stylops*	*A. nigroaenea* (Kirby, 1802)	Greece	Kalogria env., Achaia Province	KF803492	KF747740	NA
SNi16	*Stylops*	*A. nigroaenea* (Kirby, 1802)	CZ	Prokopské údolí, Praha-Jinonice, Bohemia	KF803488	KF647691	NA
SNi19	*Stylops*	*A. nigroaenea* (Kirby, 1802)	Spain	Mazarete 1.5 km SE, Castilla-La Mancha Province	KF803489	F647668	NA
SNt9	*Stylops*	*A. nitida* (Müller, 1776)	CZ	Dolní Věstonice env., Moravia	KF803493	KF747741	NA
SNw nks27	*Stylops*	*A. nawai* Cockerell, 1913	Japan	Sakata, Yamagata Pref.	KF803538	NA	KF892851
SOp nks01	*Stylops*	*A. opacifovea* Hirashima, 1952	Japan	Mt. Oohira Matsue, Shimane pref.	KF803541	KF647678	KF892852

Table 1. *Continued*

Voucher	Strepsiptera	Host	Country	Locality	*COI*	*NADH*	*EF1*
SOv2	*Stylops*	*A.* aff. *ovatula* (Kirby, 1802)	Japan	Sapporo, Jozankei, Hokkaido	D	NA	NA
SOv3	*Stylops*	*A. albofasciata* Thomson, 1870	Spain	Maranchón 3 km NW, Castilla-La Mancha	KF803494	NA	KF892853
SOv nks07	Stylops	*A.* aff. *ovatula* (Kirby, 1802)	Japan	Kyogoku, Hokkaido	KF803544	NA	KF892860
SPr1	*Stylops*	*A. praecox* (Scopoli, 1763)	CZ	Chýnice, Bohemia	KF803495	NA	KF892854
SPr2	*Stylops*	*A. praecox* (Scopoli, 1763)	CZ	Klánovický les, Praha-Klánovice, Bohemia	KF803496	NA	KF892855
SPu1	*Stylops*	*A.* cf. *pusilla* Pérez, 1903	CZ	Orlice PP, Hradec Králové env., Bohemia	KF803497	KF647669	NA
SPx1	*Stylops*	*A. proxima* (Kirby, 1802)	CZ	Chýnice, Bohemia	KF803498	KF647666	NA
SPx2	*Stylops*	*A. proxima* (Kirby, 1802)	CZ	Kleneč NPP, Roudnice nad Labem env., Boh.	KF803499	KF647681	NA
SPx3	*Stylops*	*A. proxima* (Kirby, 1802)	CZ	Čakovice, Štětí env., Bohemia	KF803500	KF647679	KF892856
SPx6	*Stylops*	*A. proxima* (Kirby, 1802)	CZ	Čelákovice, Bohemia	KF803501	KF747742	KF892857
Sri1	*Stylops*	*A. lineolata* Warncke, 1968	Spain	Epina, Gomera isl., Canary Isl.	KF803502	KF647680	KF892858
SSg1	*Stylops*	*A. spinigera* (Kirby, 1802)	CZ	Dolní Věstonice, Pálava, Moravia	KF803503	NA	NA
Ssp1	*Stylops*	*A.* sp. (Hoplandrena)	Tunisia	Wadi Raml, 4.5 km E Douz	KF803504	NA	NA
Ssp2	*Stylops*	*A.* sp. (Poecilandrena)	UAE	Ras al Khainah, Wadi Shawga	KF803510	NA	NA
Ssp4	*Stylops*	*A.* sp. (Andrena)	USA	Cuyama 18mi WNW, St. Barbara Co., Calif.	D	NA	NA
Ssp10	*Stylops*	*A.* sp.	Canada	Ancaster, Ontario	KF803505	NA	NA
Ssp11	*Stylops*	*A.* sp. (*Trachandrena*)	USA	Dryden, Tompkins Co., New York	KF803506	NA	KF892859
Ssp12	*Stylops*	*A.* sp. (*Melandrena*)	USA	Shepherdstown, Jefferson Co., West Virginia	KF803507	NA	NA
Ssp13	*Stylops*	*A. sp.* (*Euandrena*)	Uzbekistan	Tshimgan Mt., Tshatkalskyi Mts	KF803508	NA	NA
Ssp15	*Stylops*	*A.* sp. (*Thysandrena*)	USA	Del Puerto Cnyn., Stanislaus Co., California	KF803509	NA	NA
Ssp nks24	*Stylops*	*A.* sp. (*Hoplandrena*)	Japan	Kyogoku, Hokkaido JPN	KF803540	NA	KF892861
SSr1	*Stylops*	*A. spiraeana* Robertson, 1895	USA	Prospect Park, Brooklin, Kings Co., NY	KF803511	NA	KF892862
SSt1	*Stylops*	*A. strohmella* Stöckhert, 1928	CZ	Chýnice, Bohemia	KF803512	KF747743	KF892863
SSt2	*Stylops*	*A. strohmella* Stöckhert, 1928	Switzerland	Leuk env.	KF803513	NA	NA
SSu2	*Stylops*	*A. subopaca* Nylander, 1848	CZ	Karlické údolí, Czech Karst, Bohemia	KF803514	KF747744	KF892864
SSu3	*Stylops*	*A. subopaca* Nylander, 1848	CZ	Vyžlovka, Praha 25 km E, Bohemia	KF803515	KF747745	KF892865
SSu nks14	*Stylops*	*A. subopaca* Nylander, 1848	Japan	Mt. Amakazari Otari Azumino, Nagano Pref.	KF803537	NA	KF892866
STa1	*Stylops*	*A. taraxaci* Giraud, 1868	Spain	Mazarete 1.5 km SE, Castilla-La Mancha	KF803516	KF747746	KF892867
STh1	*Stylops*	*A. thoracica* (Fabricius, 1775)	Turkey	Salihli 35 km SEE	KF803517	NA	NA
STi1	*Stylops*	*A. tibialis* (Kirby, 1802)	CZ	Sušice env., Bohemia	KF803518	KF647672	D
STi2	*Stylops*	*A. tibialis* (Kirby, 1802)	Hungaria	Örkeny env.	KF803519	NA	NA
STi3	*Stylops*	*A. tibialis* (Kirby, 1802)	CZ	Dolní Věstonice env., Moravia	KF803520	KF803545	NA
STig1	*Stylops*	*A. bimaculata* (Kirby, 1802)	Tunisia	Tamerza env.	KF803521	KF647684	NA
STig2	*Stylops*	*A. bimaculata* (Kirby, 1802)	Tunisia	Gafsa env.	KF803522	KF647677	NA
STr1	*Stylops*	*A. truncatilabris* Morawitz, 1877	Greece	Midea env., Argolis Province	KF803523	KF747747	NA
STr2	*Stylops*	*A. truncatilabris* Morawitz, 1877	Greece	Mykines env., Argolis Province	KF803524	KF647692	KF892868
St	*Stylops*		Sweden		JN082812	NA	NA
SUl1	*Stylops*	*A.* sp. (*Ulandrena*)	Greece	Mykines env., Argolis Province	KF803525	NA	KF892869
SUl2	*Stylops*	*A.* sp. (*Ulandrena*)	Greece	Midea env., Argolis Province	KF803526	KF647675	KF892870
SUl3	*Stylops*	*A.* sp. (*Ulandrena*)	Turkey	Mugla	KF803527	NA	KF892871
SVa1	*Stylops*	*A. vaga* Panzer, 1799	CZ	Čelákovice, Bohemia	KF803528	NA	KF892872
SVa2	*Stylops*	*A. vaga* Panzer, 1799	CZ	Prokopské údolí, Praha-Jinonice, Bohemia	KF803529	KF647667	KF892873
SVc3	*Stylops*	*A. vicina* Smith, 1853	USA	Shelton, Fairfield Co., Connecticut	KF803530	NA	KF892874
SVi1	*Stylops*	*A. viridescens* Viereck, 1916	Greece	Archaia Olympia, Ilis Province	KF803531	KF747748	KF892875
SVi2	*Stylops*	*A. viridescens* Viereck, 1916	Greece	Archaia Olympia, Ilis Province	KF803532	KF647667	KF892876
SVr1	*Stylops*	*A. varians* (Kirby, 1802)	CZ	Chýnice, Bohemia	KF803533	NA	KF892877
SYa nks02	*Stylops*	*A. yamato* Tadauchi & Hirashima, 1983	Japan	Mt. Oohira Matsue Shimane pref.	KF803536	KF647687	KF892878

All specimens were included in the phylogenetic analyses, excepting those used for male–female comparisons and those marked with '!'. The table includes GenBank association numbers for all three genes. CZ, Czech Republic; D, with data set available from Dryad; NA, not amplified.

Table 2. List of the applied primers for the polymerase chain reaction (PCR) and sequencing

Gene	Name of primer	Orientation	Sequence 5′ → 3′	Source
COI	CO122For	F	TCWACAAATCATAAAATAATTGG	This study
	CO1669Rev	R	TCCTCCTCCTAAAGGRTCRAA	This study
	Cox1LCO_DEG	F	TWTCWACHAAYCATAARGATATTGG	(Folmer *et al*., 1994)
	Cox1ALEX_DEG	R	TCAATTTCCAAAYCCYCCYAT	(McMahon, Hayward & Kathirithamby, 2009)
	Cox1NANCY_DEG	R	CCDGGTAAAATTAAAATATAAACTTC	(Simon *et al*., 1994)
NADH	ND1-143-F	F	GGTTATATTCADATTCGTAARRG	(McMahon *et al*., 2009)
	ND1-646-R	R	CWGAAACTAAYTCWGATTCHCC	(McMahon *et al*., 2009)
EF1	EF1_64_Styl_F20	F	YGGWTGGMATGGWGAYAAYA	This study
	EF1_960_Styl_R20	R	VCCRACHGGYACTGTTCCAA	This study
	EF1_A_F_Strepsipt	F	TGCCTTGGTTCAAGGGATGG	This study
	EF1_Z_R_Strepsipt	R	CCWCCYATTTTCTAGACATCCT	This study

F, forward; R, reverse.

Information, NCBI), except for three short fragments deposited at http://aculeataresearch.com, and the entire alignment is deposited at the same site (http://aculeataresearch.com) and at the Dryad data repository site (http://datadryad.com). The complete list of accession numbers is shown in Table 1. The vouchers of the newly studied specimens were deposited in J. Straka's collection held at Charles University, Prague.

Preparation of DNA sequences and basic statistics

Stylops material comprised female individuals removed from host bodies. The entire body was lysed in Proteinase K (Qiagen) and DNA was isolated using a DNA isolation kit (Qiagen), according to the manufacturer's protocol. Partial sequences of the following three genes were amplified by polymerase chain reaction (PCR): cytochrome *c* oxidase subunit I (*COI*), *nicotinamide adenine dinucleotide dehydrogenase 1* (*NADH*), and *elongation factor-1 alpha* (short copy) (*EF1*). Six new primers were developed using PRIMER 3 (Rozen & Skaletsky, 2000) based on sequences of the *EF1* gene from Strepsiptera available from the NCBI database. The list of primers is given in Table 2. We used 50 °C as an annealing temperature in all primer pairs used for PCR. The chromatograms were edited using CHROMAS LITE 2.01 (Technelysium Pty Ltd), then aligned in CLUSTAL W, and realigned manually in BIOEDIT 7.0.9 (Hall, 1999). Each sequence was checked for possible contamination by host DNA, and whether the sequences match the Strepsiptera order using BLAST (http://blast.ncbi.nlm.nih.gov). All of the alignments are available from the authors. Genetic distances were calculated using standard computational procedures in model F84 (Felsenstein, 1984), implemented in BIOEDIT 7.1.3.0 (Hall, 1999).

Phylogenetic analyses

One hundred and twenty-four *Stylops* individuals were used for the pyhlogenetic analysis. Six additional individuals were used only for comparison of male and female DNA sequences. The concatenated alignment was created using the web application FaBox 1.35 (Villesen, 2007), including sequences of all three genes for a total of 1521 nucleotide sites [605 base pairs (bp), for *COI*, 499 bp for *NADH*, and 417 bp for *EF1*, including a 254-bp intron].

Several alignment subsets were partitioned by gene, by codon position (first, second, and third codon position separately, and first and second codon position together), by exon–intron (in the *EF1* gene), or by any combination of these possibilities using the web application Sequence Manipulation Suite (Stothard, 2000). The list of partitioning schemes used was as follows: (1) *COI* – (i) without partitioning, (ii) partitioned by first and second codon position together, and (iii) partitioned by first, second, and third codon position separately; (2) *NADH* – (i) without partitioning, (ii) partitioned by first and second codon position together; (iii) partitioned by first, second, and third codon position separately; (3) *EF1* – (i) without partitioning, (ii) partitioned by exon and intron separately, (iii) partitioned by first and second codon position together, and the intron separately, and (iv) partitioned by first, second, and third codon position together, and the intron separately; (4) concatenated data set – (i) without partitioning, (ii) partitioned separately by gene, (iii) exon–intron partitioned separately, (iv) partitioned by gene and by exon–intron; (v) partitioned by first and second codon position together, and by the intron separately, with no gene separation, (vi) partitioned by first, second, and third codon position, and by the intron separately, with no gene separation, and (vii) by the best partitioning schemes for each gene.

For all of the partitioning schemes and each phylogenetic analysis, an appropriate DNA substitution model was selected by jModeltest 2 (Posada, 2008) using the Akaike information criterion value corrected for sample size (AICc; Akaike, 1974).

Both Bayesian and maximum-likelihood (ML) analyses were performed for all of the partitioning schemes. The ML analyses were conducted using the GARLI (Zwickl, 2006) implemented in BIOPORTAL (Kumar *et al*., 2009). Four search replicates were set for each analysis. The best partitioning schemes were chosen according to the lowest AICc value. To compute the branch support values, 1000 bootstrap replicates were performed. A consensus tree with bootstrap values was constructed from the bootstrap replicates prepared in Garli by PAUP 4.0b10 (Swofford, 1998).

All of the Bayesian analyses were calculated using MrBayes 3.2.1 (Huelsenbeck & Ronquist, 2001), implemented in Bioportal (Kumar *et al*., 2009). Four simultaneous Markov chains were run for 10 million generations for the *NADH* and the *EF1* alignments, for 25 million generations for the more extensive alignment of *COI*, and for 30 million generations for the concatenated alignment, with sampling every 1000 generations to ensure the independence of the samples. Two independent analyses starting from random trees were performed for each partitioning scheme. TRACER was used to examine the convergence of the Markov chains (Rambaut & Drummond, 2007). All of the parameters from the Markov chain Monte Carlo (MCMC) analyses were summarized using the sump command in MrBayes, and the first 25% were discarded as burn-in. The Bayes factor (BF) statistic was calculated as 2[ln(HM1) – ln(HM2)], in which HM1 is the harmonic mean of the posterior sample of likelihoods from the first partitioning scheme, and HM2 is the harmonic mean of the posterior sample of likelihoods from the second partitioning scheme. A more complex model was used only when it was significantly better than the simpler model: i.e. whether the value of 2ln(BF) of the compared models was higher than 2 (Brandley, Schmitz & Reeder, 2005).

RESULTS

Phylogenetic analysis

Bayesian phylogenetic analyses were performed without data-set partitioning because the difference in the results from the partitioned and non-partitioned models was not significant [all of the comparisons of 2ln(BF) between the models were < 2]. Thus, for the Bayesian method, the optimal DNA substitution model GTR + Γ + I was applied to the entire data set for *COI*, *NADH*, and the concatenation of all three genes, and the HKY + Γ substitution model was used for the *EF1* data set. All of the phylogenetic analyses based on the ML method were partitioned by codon position (first, second, and third) and intron (in the *EF1* gene), which were found to be the best partitioning schemes according to the AIC criterion [*COI* – AIC (no partitioning) = 24 892, AIC (partitioning scheme with first and second codon positions together) = 24 110, AIC (the most partitioned scheme) = 23 905; *NADH* – AIC (no partitioning) = 8753, AIC (partitioning scheme with first and second codon position together) = 7873, AIC (the most partitioned scheme) = 7834; *EF1* – AIC (no partitioning) = 7176, AIC (partitioning scheme with the exon and the intron separate) = 6976, AIC (partly partitioned scheme with the first and second codon positions together and the third codon position and the intron separate) = 6929, AIC (the most partitioned scheme) = 6925; concatenation of all three genes – AIC (no partitioning) = 41 419, AIC (partitioned by gene) = 40 718, AIC (partitioned by exon–intron) = 41 189, AIC (partitioned by gene and exon–intron) = 40 514, AIC (partitioning scheme with the first and second codon positions together, and the third position and intron for all of the genes together) = 40 263, AIC (partitioned by the first, second, and third codon positions, and by the intron with no gene separation) = 40 083, AIC (best partitioning scheme for each gene) = 39 181]. The following three models were found to be optimal for *COI* gene partitioning: TrN + Γ + I (first and third codon), TPM3uf + Γ (second codon). The following three models were found to be optimal for the *NADH* gene: HKY + Γ (first codon), F81 + Γ (second codon), and HKY + I (third codon). Four optimal models were found for the partitioning of the *EF1* gene: TrNef (first codon), TPM1 (second codon), GTR + I (third codon), and TVM + Γ (intron). Ten optimal models were found for the concatenated data set of all of the genes: *COI* – TrN + Γ + I (first and third codons), TPM3uf + Γ (second codon); *NADH* – HKY + Γ (first codon), F81 + Γ (second codon), HKY + I (third codon); *EF1* – TrNef (first codon), TPM1 (second codon), GTR + I (third codon), TVM + Γ (intron).

Phylogeny of *Stylops*

The phylogenetic analyses based on the individual genes (*COI*, *NADH*, and *EF1*) resulted in phylogenies with sufficiently high branch support for the target crown groups (Figs 1–3). Similar results are found from the concatenated sequence analysis (Fig. 4), in which support is consistently higher (both bootstrap support from the ML analysis as well as posterior probabilities from the Bayesian method). Trees resulting from the different analyses are congruent in determining most of the units, which can be understood as groups of individuals reproductively separated from other related lineages by attacking hosts with different evolutionary histories and biologies, thus representing tentative species lineages. Individuals from these lineages, considered as

tentative species, are usually 3–9% distant from individuals from the closest clade, or are up to 23% distant from the other groups in *COI* base composition (Table S1). Stem branching within the genus *Stylops* remains completely unresolved, but the monophyly of the entire genus is well supported (posterior probability – 1, bootstrap – 94; Fig. 4).

The results allow the recognition of more than 30 lineages of possible species level. Most of these lineages are associated with a single host subgenus; however, there are some exceptions (Fig. 4). Several lineages (nodes 1, 2, 3, 4, 6, 7, 9, and 10 in Fig. 4) consist of distinct taxa, most likely at the species level, but at least some individuals of different species were extracted from the same host subgenus. Additionally, the opposite is sometimes true, and some species lineages consist of individuals that were extracted from the hosts of different subgenera (lineages within nodes 1, 2, 5, and 10). At node 1, individuals from the subgenera *Aciandrena* and *Graecandrena* are mixed. At node 2, two lineages consist of individuals from the subgenera *Aciandrena* with *Ulandrena*, and from *Proxiandrena* and *Poecilandrena* with *Ulandrena*. At node 5, individuals from the subgenera *Melandrena* and *Zonandrena* are mixed. At node 10 there are three different lineages, and all of them consist of a few distinct subgenera: *Hoplandrena* with *Simandrena*, *Hoplandrena* with *Plastandrena* and *Agandrena*, and *Hoplandrena* with *Plastandrena*. Additionally, unrelated *Stylops* species can parasitize the same host subgenera of the genus *Andrena*. This is the case in the subgenus *Aciandrena* (some individuals from lineages 1 and 2), *Andrena s.s.* (nodes 6 and 7), and especially *Melandrena* (most individuals in lineages 5 and 8, and some from lineage 9). In these three cases, we found that a single *Andrena* species was a host of two different Strepsipteran species lineages. This is most evident in *Andrena* (*Melandrena*) *nigroaenea* (Kirby, 1802), which was found to be a host in distant *Stylops* lineages 5 and 8 (confirmed by sequences of *COI* as well as *EF1* genes). Most of the specimens of *Stylops* from the host *A.* (*M.*) *nigroaenea* are found within lineage 5. They are also from a broader distributional range compared with lineage 8, including Spain, France, the Czech Republic, and Greece. The second and third cases are *Andrena* (*Micrandrena*) *minutula* (Kirby, 1802) and *Andrena* (*Micrandrena*) *subopaca* Nylander, 1848, in which two *Stylops* species from lineage 3 are found. The different *Stylops* lineages from the same *Micrandrena* hosts were collected in Europe and Japan.

Our phylogenetic reconstruction shows that the strict application of any of the three hypotheses of species concepts based on host taxonomy are not useable as previously defined. Superspecialized *Stylops* with a single host association and a single generalist *Stylops* species in a strict sense can be easily rejected. The hypothesis that is closest to our results is the hypothesis that every *Stylops* species is associated with closely related species of the hosts grouped in subgenera; however, this cannot be strictly applied but must be individually evaluated for each case of *Stylops* species, because moderately generalistic species can also be recognized.

Comparison of *COI* sequences from infrequent material from males (11 individuals) show no, or insignificant, difference from females. In the case of seven males, which were collected with its host, is host association was identical to that known from the female (Table S2).

DISCUSSION

PHYLOGENETIC ANALYSIS AND *STYLOPS* MONOPHYLY

Our phylogenetic analyses were conducted in detail, but the enormous variability in sequences most likely influenced the resulting optimal data partitioning and the substitution models that were consequently found to be optimal for computing the phylogenetic trees. There was a very large difference in the data partitioning schemes between the Bayesian analyses and ML analyses, so the DNA substitution models used in these analytical approaches differed substantially. The simplest partitioning schemes were used in the Bayesian analyses, but the most partitioned data sets were analysed with ML. The crown groups, which should represent species lineages, were very similar and well supported with both methodical approaches, although partially differing topologies were found. Our phylogenetic study clearly confirmed the monophyly of the genus *Stylops*, including the fact that all of the *Stylops* species are parasites of bees of the genus *Andrena*. The most similar genus to *Stylops* is *Kinzelbachus* Özdikmen, 2009, with only one species, *Kinzelbachus friesei* (Hofeneder, 1949), which was described as *Stylops*, and its similarity to *Stylops* species was discussed by (Hofeneder, 1949). *Kinzelbachus* resembles *Stylops* in the shape of the cephalothorax and the long segmented brood canal of the female, but differs in the presence of possible remnants of metathoracal spiracles (Kinzelbach, 1978). Similarly to the host of *Stylops*, its host is an andrenid bee, *Melitturga clavicornis* (Latreille, 1808). *Kinzelbachus friesei* was also included in our study, and as expected it falls well within the out-group; however, its relationship to *Stylops* remains uncertain.

HOST SPECIALIZATION IN THE GENUS *STYLOPS*

Our molecular phylogenetic analysis convincingly rejected the existence of supergeneralist *S. melittae*, listed as hypothesis 3 within our aims. On the other hand,

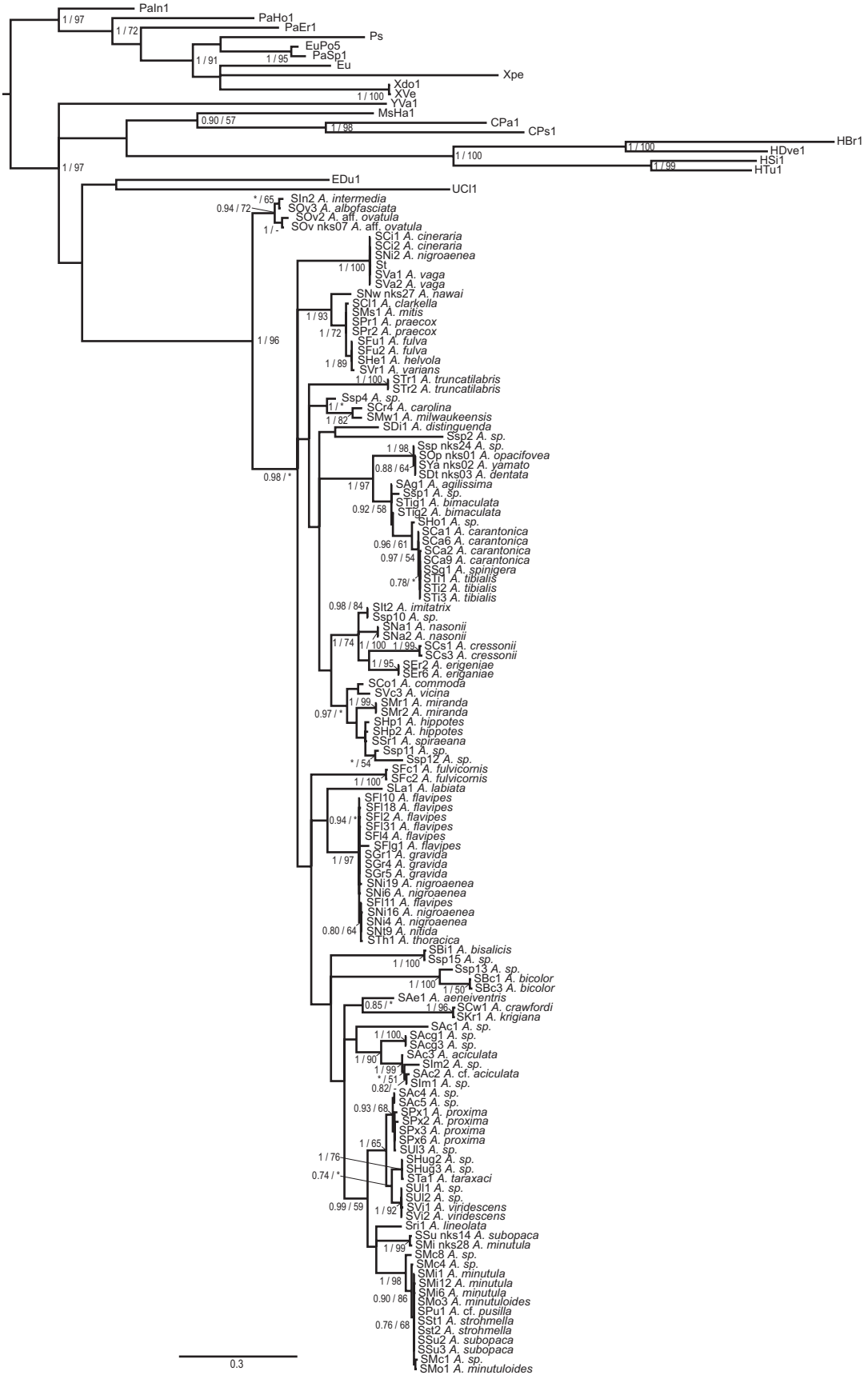

PaIn1
PaHo1
PaEr1
Ps
EuPo5
PaSp1
Eu
Xpe
Xdo1
XVe
YVa1
MsHa1
CPa1
CPs1
HBr1
HDve1
HSi1
HTu1
EDu1
UCl1
SIn2 A. intermedia
SOv3 A. albofasciata
SOv2 A. aff. ovatula
SOv nks07 A. aff. ovatula
SCi1 A. cineraria
SCi2 A. cineraria
SNi2 A. nigroaenea
St
SVa1 A. vaga
SVa2 A. vaga
SNw nks27 A. hawai
SCl1 A. clarkella
SMs1 A. mitis
SPr1 A. praecox
SPr2 A. praecox
SFu1 A. fulva
SFu2 A. fulva
SHe1 A. helvola
SVr1 A. varians
STr1 A. truncatilabris
STr2 A. truncatilabris
Ssp4 A. sp.
SCr4 A. carolina
SMw1 A. milwaukeensis
SDi1 A. distinguenda
Ssp2 A. sp.
Ssp nks24 A. sp.
SOp nks01 A. opacifovea
SYa nks02 A. yamato
SDt nks03 A. dentata
SAg1 A. agilissima
Ssp1 A. sp.
STig1 A. bimaculata
STig2 A. bimaculata
SHo1 A. sp.
SCa1 A. carantonica
SCa6 A. carantonica
SCa2 A. carantonica
SCa9 A. carantonica
SSq1 A. spinigera
STi1 A. tibialis
STi2 A. tibialis
STi3 A. tibialis
SIt2 A. imitatrix
Ssp10 A. sp.
SNa1 A. nasonii
SNa2 A. nasonii
SCs1 A. cressonii
SCs3 A. cressonii
SEr2 A. erigeniae
SEr6 A. erigeniae
SCo1 A. commoda
SVc3 A. vicina
SMr1 A. miranda
SMr2 A. miranda
SHp1 A. hippotes
SHp2 A. hippotes
SSr1 A. spiraeana
Ssp11 A. sp.
Ssp12 A. sp.
SFc1 A. fulvicornis
SFc2 A. fulvicornis
SLa1 A. labiata
SFl10 A. flavipes
SFl18 A. flavipes
SFl2 A. flavipes
SFl31 A. flavipes
SFl4 A. flavipes
SFlg1 A. flavipes
SGr1 A. gravida
SGr4 A. gravida
SGr5 A. gravida
SNi19 A. nigroaenea
SNi6 A. nigroaenea
SFl11 A. flavipes
SNi16 A. nigroaenea
SNi4 A. nigroaenea
SNi9 A. nitida
STh1 A. thoracica
SBi1 A. bisalicis
Ssp15 A. sp.
Ssp13 A. sp.
SBc1 A. bicolor
SBc3 A. bicolor
SAe1 A. aeneiventris
SCw1 A. crawfordi
SKr1 A. krigiana
SAc1 A. sp.
SAcg1 A. sp.
SAcg3 A. sp.
SAc3 A. aciculata
SIm2 A. sp.
SAc2 A. cf. aciculata
SIm1 A. sp.
SAc4 A. sp.
SAc5 A. sp.
SPx1 A. proxima
SPx2 A. proxima
SPx3 A. proxima
SPx6 A. proxima
SUl3 A. sp.
SHug2 A. sp.
SHug3 A. sp.
STa1 A. taraxaci
SUl1 A. sp.
SUl2 A. sp.
SVi1 A. viridescens
SVi2 A. viridescens
Sri1 A. lineolata
SSu nks14 A. subopaca
SMi nks28 A. minutula
SMc8 A. sp.
SMc4 A. sp.
SMi1 A. minutula
SMi12 A. minutula
SMi6 A. minutula
SMo3 A. minutuloides
SPu1 A. cf. pusilla
SSt1 A. strohmella
Sst2 A. strohmella
SSu2 A. subopaca
SSu3 A. subopaca
SMc1 A. sp.
SMo1 A. minutuloides
0.3

Figure 1. Phylogenetic tree resulting from a Bayesian analysis of the partial sequence from the mitochondrial *COI* gene. The names of the host *Andrena* bees are indicated with each *Stylops* voucher number. The posterior probabilities are given before the slash; the bootstrap values from the maximum-likelihood (ML) analysis are given after the slash. Posterior probability values lower than 0.9, and bootstrap values lower than 50, are considered as unsupported, and thus have been replaced by an asterisk (*); incongruent nodes between the two analyses are indicated by a dash (-). Branch support is omitted at the nodes that were unsupported in both the Bayesian and the ML analyses.

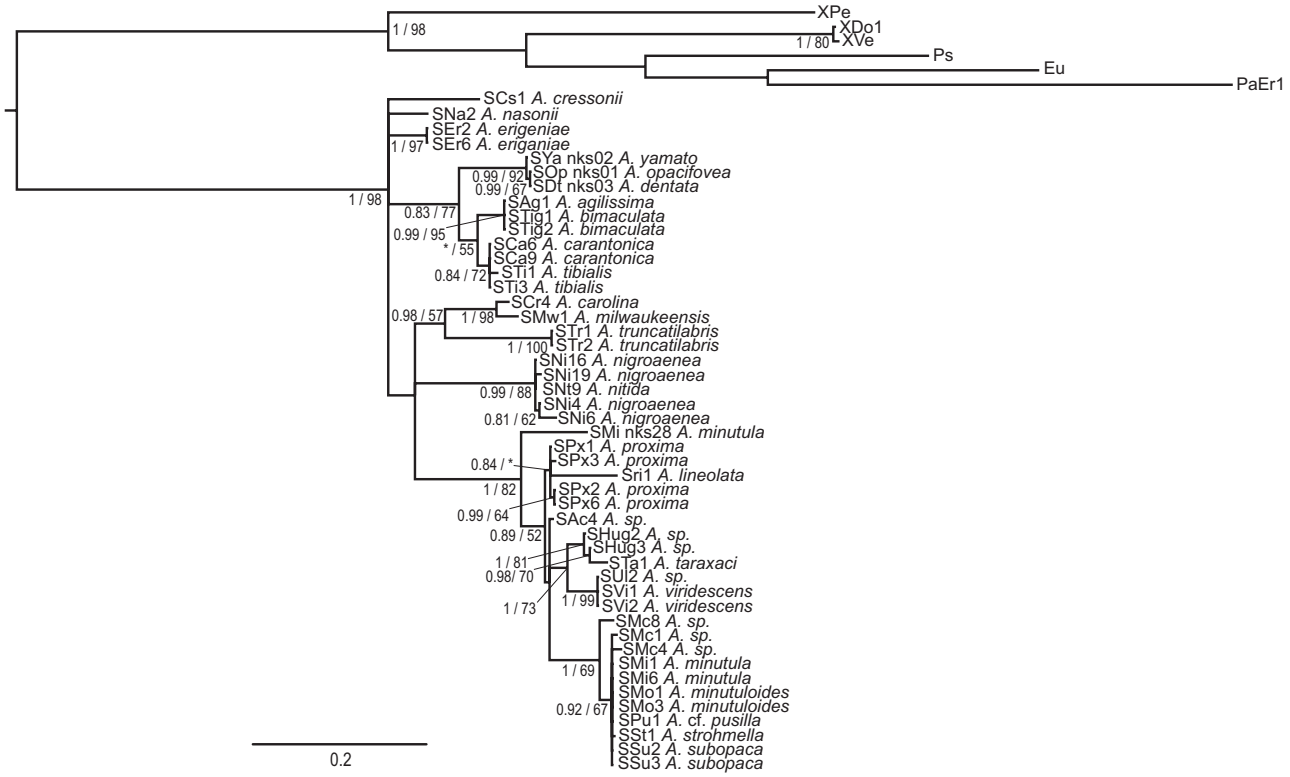


Figure 2. Phylogenetic tree resulting from a Bayesian analysis of the partial sequence from the mitochondrial *NADH* gene. The names of the host *Andrena* bees are indicated with every *Stylops* voucher number. The posterior probabilities are given before the slash; the bootstrap values from the maximum-likelihood (ML) analysis are given after the slash. Posterior probability values lower than 0.9, and bootstrap values lower than 50, are considered as unsupported and are thus replaced by an asterisk (*); incongruent nodes between the two analyses are indicated by a dash (-). Branch support is omitted at the nodes that were unsupported in both the Bayesian and the ML analyses.

hypotheses 1 (each species of the genus *Stylops* is associated with a single host species) and 2 (*Stylops* species are specialized to a group of closely related hosts) seem to be valid for some *Stylops* species. Moreover, it appears that the evolution of host specialization of *Stylops* parasites is more complex than we predicted. Excepting the evidence of species being specialized to a single species or to very few closely related species, there are also partially generalistic lineages (clades 2 and 10 in Fig. 4). The existence of specialist and generalist Strepsiptera parasites is interesting. Generalist parasites are not common in nature. Of course, such generalists exist, especially when considering ectoparasites (Graham *et al*., 2009; Johnson, Malenke & Clayton, 2009), but they can also occur among parasitoids (Steidle, Steppuhn & Ruther, 2003; Harris *et al*., 2012), cleptoparasites (Habermannová, Bogusch & Straka, 2013), or endoparasites (Sasal *et al*., 1999). The generalists considered are never defined as universal generalists, but they always use more than one species source (sometimes just two), sometimes with considerable variability in definitions within a study (Habermannová *et al*., 2013). Based on our results, we found some possible generalist *Stylops* species parasitizing seven different host species, or parasitizing host species from three different subgenera. Our results could be

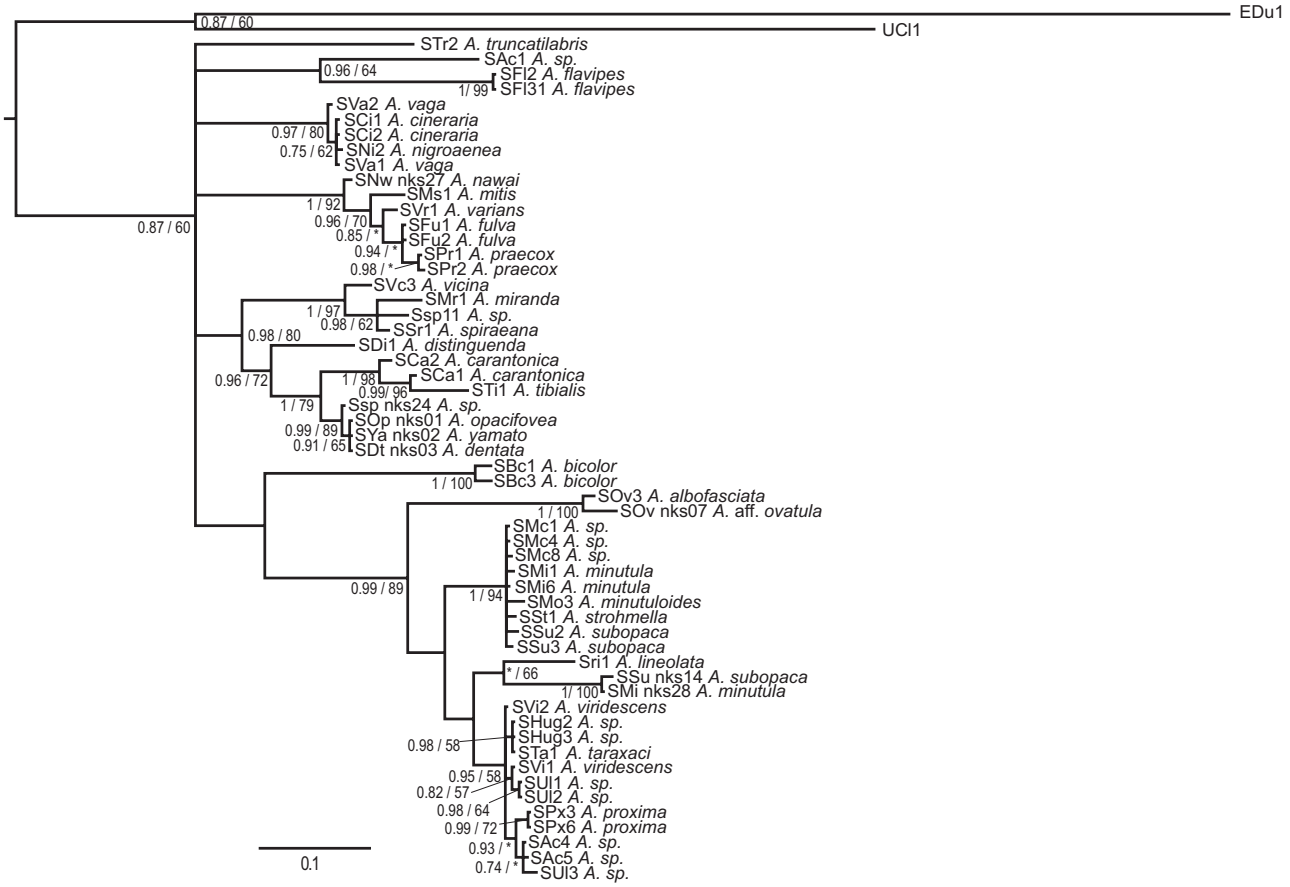


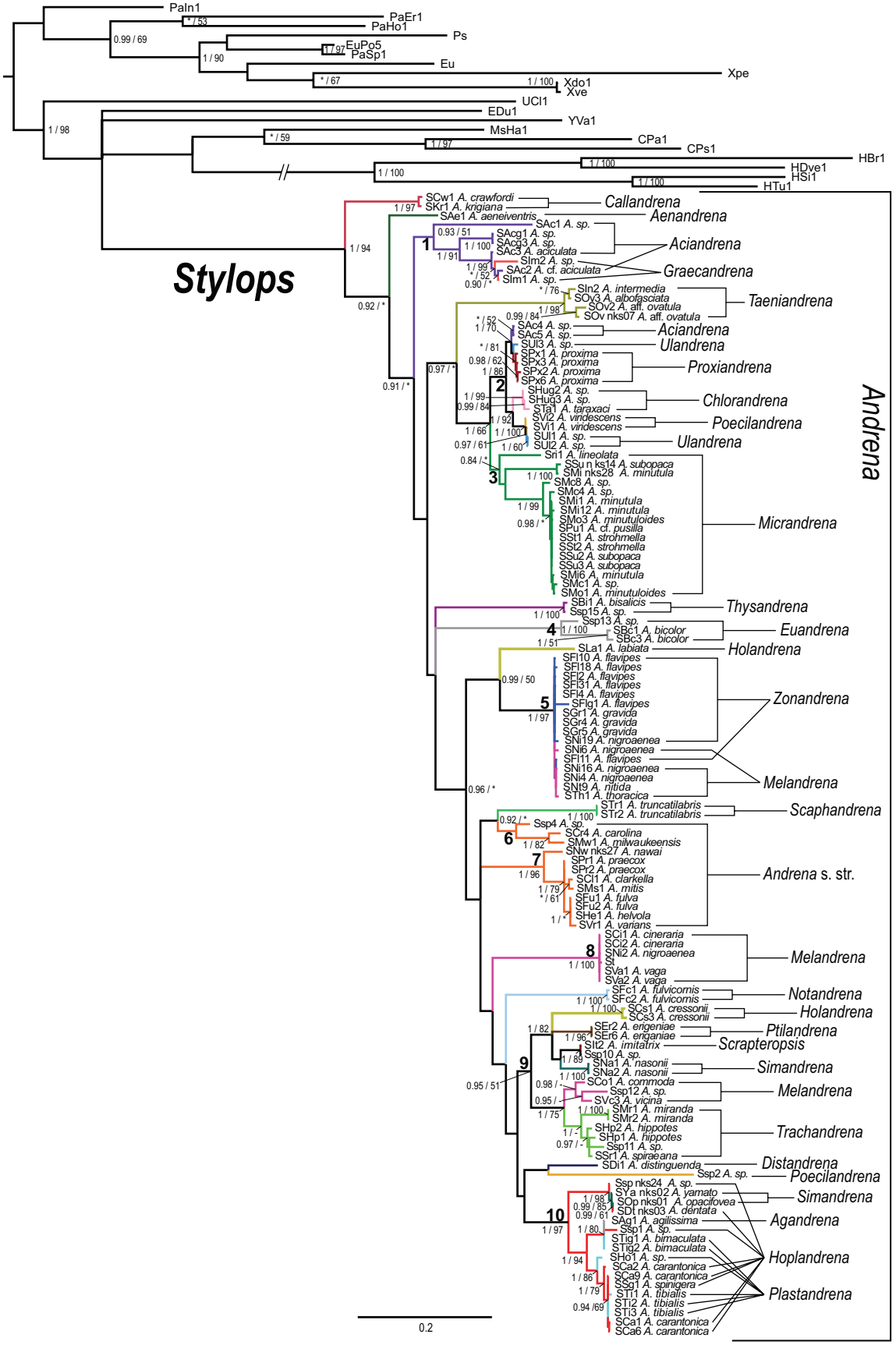
Figure 3. Phylogenetic tree resulting from a Bayesian analysis of the partial sequence from the nuclear *EF1* gene. Names of host *Andrena* bees are indicated at every *Stylops* voucher number. The names of the host *Andrena* bees are indicated with every *Stylops* voucher number. The posterior probabilities are given before the slash; the bootstrap values from the maximum-likelihood (ML) analysis are given after the slash. Posterior probability values lower than 0.9, and bootstrap values lower than 50, are considered as unsupported and thus replaced by an asterisk (*); incongruent nodes between the two analyses are indicated by a dash (-). Branch support is omitted at the nodes that were unsupported in both the Bayesian and the ML analyses.

Figure 4. Phylogenetic tree resulting from a Bayesian analysis of the concatenated data set from the mitochondrial *COI* and *NADH* genes, and from the nuclear *EF1* gene. The names of the host *Andrena* bees are indicated with every *Stylops* voucher number; the host subgenus and genus is indicated for recognized clades; the clade colours represent different host subgenera. The posterior probabilities are given before the slash; the bootstrap values from the maximum-likelihood (ML) analysis are given after the slash. Posterior probability values lower than 0.9, and bootstrap values lower than 50, are considered as unsupported and are thus replaced by an asterisk (*); incongruent nodes between the two analyses are indicated by a dash (-). Branch support is omitted at the nodes that were unsupported in both the Bayesian and the ML analyses.

hypothetically corroborated by the fact that only limited numbers of males were included in our study. Our comparison of DNA between males with a known host association and females (Table S2), as well as nuclear DNA (compare Figs 1 and 2 with Fig. 3), did not suggest any problem with using a limited number of male individuals.

HOST SWITCHES IN THE GENUS *STYLOPS*

There are several exceptions from the normal relationship between *Stylops* species and the host taxon, which suggests that *Stylops* parasites can switch between unrelated hosts on some occasions. Such cases may occur when the hosts frequently meet while visiting flowers at a specific time or season. Even more


Stylops
Andrena
PaIn1
PaEr1
PaHo1
Ps
EuPo5
PaSp1
Eu
Xpe
Xdo1
Xve
UCl1
EDu1
YVa1
MsHa1
CPa1
CPs1
HBr1
HDve1
HSi1
HTu1
Callandrena
Aenandrena
Aciandrena
Graecandrena
Taeniandrena
Aciandrena
Ulandrena
Proxiandrena
Chlorandrena
Poecilandrena
Ulandrena
Micrandrena
Thysandrena
Euandrena
Holandrena
Zonandrena
Melandrena
Scaphandrena
Andrena s. str.
Melandrena
Notandrena
Holandrena
Ptilandrena
Scrapteropsis
Simandrena
Melandrena
Trachandrena
Distandrena
Poecilandrena
Simandrena
Agandrena
Hoplandrena
Plastandrena
0.2

peculiar host switches are known in other Strepsipteran groups, e.g. species with heterotrophic heteronomy in the Myrmecolacidae, in which the males and the females parasitize two hosts from different insect orders (Kathirithamby, 2009), or in the Halictophagidae, in which multiparasitism by two different Strepsiptera species from different families (Halictophagidae and Elenchidae) was documented in a single host specimen (Riek, 1970; Kathirithamby *et al*., 2012a). For this reason, switches among unrelated hosts should not be surprising even for the genus *Stylops*. Several *Stylops* species are parasites of two or even three host subgenera, and thus possibly parasitize unrelated bees. A phylogeny of the genus *Andrena* with broad taxon sampling has not yet been published, however: there is only one publication with limited taxon sampling, but with a strong suggestion that some subgenera will not be natural taxa (Larkin, Neff & Simpson, 2006). For this reason, we cannot be certain about the relationships of the host species placed in different subgenera. Some subgenera of *Andrena* might be closely related, and the parasite is not able to determine that the bees are significantly different. Whether these host bees are related or not, we found a case where two unrelated *Stylops* species were found in the same host species [*A.* (*M.*) *nigroaenea*]. Our results suggest that partial coevolution between *Stylops* and *Andrena* is possible, but there will definitely be some exceptions because several host switches are evident from our results.

Definition of *Stylops* species

The crucial question is how can we define *Stylops* species. Defining species is problematic in general (Nixon & Wheeler, 1990; de Queiroz, 2005). For the result of phylogenetic study, we can apply a basic phylogenetic species concept (species as the smallest aggregation of populations or lineages diagnosable by a unique combination of character states in comparable individuals; Nixon & Wheeler, 1990). When we consider the results from analyses of all three genes, we can say that the result is highly comparable. Mitochondrial DNA data maintain female genetic information (female lineage), whereas nuclear DNA data provide more information about population structure. The information about gene flow among lineages of *Stylops* species is obtained from congruence in the tree topology results and branch lengths (or better DNA distances among lineages). There is obviously no gene flow among the crown lineages.

The possible species seem to be well defined, except in clades 2 and 10 of Figure 4, where distances within the crown group suggest that there are more than one species with uncertain division. The species delimitations seem to be especially clear for the mapping of information about the hosts (species, subgenus, and genus in Fig. 4) onto the tree. From a basic mapping of the hosts to the phylogenetic tree, it is evident that host species and subgenera cluster with *Stylops* clades in a common sense, usually irrespective of the collecting locality. Most *Stylops* species extracted from the same host species or subgenus are closely related and form crown groups. These lineages are well supported by high posterior probabilities (0.98–1.00) in the majority of cases, and they are also well supported by bootstrap values (80–100). Such good support for the monophyly of lineages that are regularly clustered according to the host species/subgenus support our understanding of these lineages as different species with different life strategies.

We can conclude that the delimitation of *Stylops* species seems to be quite sharp in most cases, and that the DNA distances of *COI* (DNA barcoding sequence) can by relatively easily applied. The DNA distances among species lineages seem to be quite distinct, i.e. 3–23% of different base pairs, but usually more than 7% in distance. Variability within reconstructed crown groups is usually much less than 2%, but sometimes exceeds this number. In such lineages, cryptic species and taxonomic problems can be expected. In *Stylops*, we found the standard variability in DNA barcode sequences found elsewhere in insects (Hebert, Ratnasingham & Waard, 2003). The common 3% threshold in DNA distances for barcode sequence distance (Hebert *et al*., 2003) can be applied for *Stylops* species in future studies; however, groups with lower variability can also consist of more than a single species, and such groups need to be studied by comprehensive population genetic methods.

Species diversity of *Stylops*

Our knowledge of the Strepsiptera diversity strongly depends on the taxonomic concept used for species recognition in the past. Until now, only an opinion-based concept was used, more or less supported by the morphology of adults and first-instar larvae (Bohart, 1941; Kinzelbach, 1971, 1978). The taxonomy of the genus *Stylops* is exceptional within this insect order. Tens of different species are recognized in North America (Bohart, 1941), but so far just a single species has been recognized in Europe (Kinzelbach, 1978); however, in the past not all authors understood *Stylops* taxonomy in such a narrow sense in Europe. There are over 50 names of *Stylops* described from Europe, and the authors considered *Stylops* to be a genus consisting of specialized species. The host–parasite association was commonly used for species identification, and finds of unknown parasitized hosts automatically led to the descriptions of new species (Pierce, 1909, 1911; Perkins, 1918; Luna de Carvalho, 1974). For this reason, for a long period of time we have not known whether the

genus *Stylops* includes a single species or hundred of species. Our study convincingly shows that neither of these extremes is applicable to the taxonomy of this genus. Although a detailed taxonomic and nomenclatoric study is needed, it seems evident that there will be tens of species in Europe instead of just one. On the other hand the number of species will remain roughly stable in North America and will be slightly reduced in Japan. To evaluate the actual number of species a comprehensive morphological revision of the genus *Stylops*, with definitions of the type material, is needed. Such a study should be performed in tandem with barcoding or other genetic studies, using an integrative taxonomic approach (Gibbs, 2009), to achieve certainty in the taxonomy of this peculiar group.

In conclusion, our data show that *Stylops* is a monophyletic genus specialized to parasitize *Andrena* bees. Most *Stylops* species are moderate parasite specialists to a host lineage of closely related species, but also strict specialists and moderate generalists can be found within this group. There is obviously no universal criterion for *Stylops* species discrimination, but the host association at approximately the subgeneric level can be used as a raw guideline for future taxonomic studies based on morphology, DNA, or preferably both to evaluate the number of existing species. Every *Stylops* species should be studied as a unique entity with its own specific biological characteristics, including specific host-use strategy, morphology, phenology, or biogeography. We also recommend using barcode sequences to better characterize each *Stylops* species.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website:

Table S1. Distance matrix of *COI* sequences.
Table S2. Comparison of DNA distances of *COI* sequences for male and female *Stylops*, showing a consistent DNA signal for males and females.

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Nomenclature and taxonomy of the genus *Stylops* (Strepsiptera): an annotated preliminary world checklist

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Abstract. Taxonomy and nomenclature of the genus *Stylops* Kirby, 1802 (Strepsiptera) have been understood differently in different parts of world for a long time. Largest differences came from erroneous concept of host specialization of individual species. For this reason, we re-evaluated taxonomy and nomenclature in all *Stylops* species based on distances of DNA barcode sequences (cytochrome c oxidase subunit I). Twenty six species (123 individuals) out of sixty six recognized *Stylops* species from all distribution range were DNA barcoded and their sequences compared. Taxonomy of all West Palaearctic species was restructured to be congruent with results of analysis of the genetic distances. Single European species *Stylops melittae* Kirby, 1802 is divided into thirty species, whose species status is restituted: *S. analis* Perkins, 1918; *S. andrenaphilus* Luna de Carvalho, 1974; *S. ater* Reichert, 1914; *S. aterrimus* Newport, 1851; *S. borcherti* Luna de Carvalho, 1974; *S. dalii* Curtis, 1828; *S. deserticola* Medvedev, 1970; *S. dinizi* Luna de Carvalho, 1974; *S. gwynanae* Günther, 1957; *S. hammella* Perkins, 1918; *S. ibericus* Luna de Carvalho, 1969; *S. kinzelbachi* Luna de Carvalho, 1974; *S. liliputanus* Luna de Carvalho, 1974; *S. lusohispanicus* Luna de Carvalho, 1974; *S. madrilensis* Luna de Carvalho, 1974; *S. maxillaris* Pasteels, 1949; *S. moniliaphagus* Luna de Carvalho, 1974; *S. nevinsoni* Perkins, 1918; *S. obenbergeri* Ogloblin, 1923; *S. obsoletus* Luna de Carvalho, 1974; *S. paracuellus* Luna de Carvalho, 1974; *S. pasteelsi* Luna de Carvalho, 1974; *S. praecocis* Luna de Carvalho, 1974; *S. risleri* Kinzelbach, 1967; *S. ruthenicus* Schkaff, 1925; *S. salamancanus* Luna de Carvalho, 1974; *S. spreta* Perkins, 1918; *S. thwaitesi* Perkins, 1918; *S. ventricosae* Pierce, 1909; and *S. warnckei* Luna de Carvalho, 1974. *Stylops hartfordensis* Pierce, 1909 is a single species from North America, whose status is restituted from

S. bruneri Pierce, 1909. Names of fifteen West Palaearctic species, fourteen East Palaearctic species and fifteen Nearctic species are supposed to be new junior subjective synonyms: *S. muelleri* Borchert, 1971 = *S. ater*; *S. dominiquei* Pierce, 1909 = *S. bimaculatae* Perkins, 1918 = *S. aterrimus*; *S. nitidiusculae* Poluszyński, 1927 = *S. hammella*; *S. esteponensis* Luna de Carvalho, 1974 = *S. maxillaris*; *S. flavipedis* Hofeneder, 1924 = *S. nitidae* Pasteels, 1954 = *S. giganteus* Luna de Carvalho, 1974 = *S. melittae*; *S. transversa* Pasteels, 1949 = *S. nevinsoni*; *S. duriensis* Luna de Carvalho, 1974 = *S. spreta*; *S. championi* Pierce, 1919 = *S. alfkeni* Hofeneder, 1939 = *S. albofasciatae* Günther, 1957 = *S. borealis* Kifune & Hirashima, 1985 = *S. thwaitesi*; *S. orientis* Kifune & Maeta, 1990 = *S. hirashimai* Kifune & Maeta, 1990 = *S. circularis* Kifune & Hirashima, 1985; *S. truncatus* Kifune & Hirashima, 1985 = *S. oblongulus* Kifune & Hirashima, 1985 = *S. truncatoides* Kifune & Hirashima, 1985 = *S. collinus* Kifune & Maeta, 1990 = *S. aburanae* Kifune & Maeta, 1990 = *S. japonicus* Kifune & Hirashima, 1985; *S. dentatae* Kifune & Maeta, 1990 = *S. aino* Kifune & Maeta, 1990 = *S. izumoensis* Kifune & Maeta, 1990 = *S. nipponicus* Kifune & Maeta, 1990 = *S. subcircularis* Kifune & Maeta, 1990 = *S. fukuiensis* Kifune, 1991 = *S. yamatonis* Kifune & Hirashima, 1985; *S. mandibularis* Pierce, 1911 = *S. moestae* Pierce, 1919 = *S. sinuatus* Pierce, 1919 = *S. advarians* Pierce, 1909; *S. oklahomae* Pierce, 1909 = *S. bipunctatae* Pierce, 1909; *S. neonanae* Pierce, 1919 = *S. duboisi* Bohart, 1937 = *S. bruneri*; *S. vicinae* Pierce, 1909 = *S. childreni* Gray & Westwood, 1832; *S. solidulae* Pierce, 1909 = *S. cornii* Pierce, 1909; *S. swenki* Pierce, 1909 = *S. crawfordi* Pierce, 1909; *S. salicifloris* Pierce, 1909 = *S. hippotes* Pierce, 1909; *S. grandior* Pierce, 1919 = *S. multiplicatae* Pierce, 1909; *S. pacificus* Bohart, 1936 = *S. polemonii* Pierce, 1909; *S. bisalicidis* Pierce, 1919 = *S. medionitans* Pierce, 1919 = *S. subcandidae* Pierce, 1909. Twelve names were recognized as unjustified emendations and these names are new junior objective synonyms: *S. trimmeranae* Kinzelbach, 1978 = *S. trimmerana* Smith, 1857 (= *S. aterrimus*); *S. dalei* Kinzelbach, 1978 = *S. dalii*; *S. gwynanai* Luna de Carvalho, 1974 = *S. gwynanae*; *S. hammelae* Kinzelbach, 1978 = *S. hammella*; *S. nitidiusculai* Luna de Carvalho, 1974 = *S. nitidiusculae* Poluszyński, 1927 (= *S. hammella*); *S. kirbyi* Kinzelbach, 1978 = *S. kirbii* Leach, 1817 (= *S. melittae*); *S. spencei* Kinzelbach, 1971 = *S. spencei* Luna de Carvalho, 1974 = *S. spencii* Pickering, 1836 (= *S. melittae*); *S. melittai* Luna de Carvalho, 1974 = *S. melittae*; *S. spretae* Ulrich, 1930 = *S. spretus* Luna de Carvalho, 1974 = *S. spreta*; *S. thwattei* Luna de Carvalho, 1969 = *S. thwaitesi*. Nine names are recognized as nomina nuda and therefore unavailable in zoological nomenclature. Years of publications of the species names were corrected based on the original literature. Bee hosts are summarized for each species according to the new synonymies.

Key words. Strepsiptera, Stylopidae, integrative taxonomy, DNA barcode, nomenclature, revised status, new synonym, bee parasite, *Andrena*, Andrenidae

Introduction

Genus *Stylops* Kirby, 1802 belongs to the order Strepsiptera, obligate parasites of various insect orders (Kathirithamby 2009, Kinzelbach 1971). Strepsiptera are well known for complicated life cycles and unusual morphological differences between alate males and wingless neotenic females. The first instars develop inside the female's body and leave her through the front part of body in the case of obligate endoparasitic species. First instars actively find a host and invade its host body. In the species, which parasitise Hymenoptera, the first instar larvae need to be transferred by a vector (Kathirithamby et al. 2012, Linsley & MacSwain 1957). When first instars reach the final host individual, they moult into endoparasitic larvae and grow inside the host body. Mature larvae extrude their cephalothoraxes through the host cuticle, males make puparium and pupate, while females become neotenic imago inside puparium. Basal lineages (Mengenillidia) pupate outside the host body, the others (Stylopidia) pupate inside the host, but with exposed front part of body (Kathirithamby 2009, Kathirithamby et al. 2003). Adult females release sex pheromone and attract short-living males for mating (Cvačka et al. 2012, Lagoutte et al. 2013, Tolasch et al. 2012). Both sexes are known to manipulate their host's morphology and behaviour, which seems to enhance their mating (Straka et al. 2011) and reproductive success (Linsley & MacSwain 1957).

The genus *Stylops* is the most species rich genus of the Strepsiptera in terms of the described species (Kathirithamby 2014, Kinzelbach 1971). There are more than 110 available species names; however, the number of species that are really valid is uncertain. The diversity of *Stylops* depends on the species concept used, and the species concepts were previously very variable among taxonomists. Genus *Stylops* and the first single species was first described by Kirby (1802). At that time, he presented only a single host species, the bee *Andrena nigroaenea* (Kirby, 1802). More than one hundred years later, Pierce (1909, 1911, 1919) and Perkins (1918) described approximately 60 species from North America and Europe based on the principle of single host association. This species classification strategy was followed by Hofeneder (1924a), Noskiewicz & Poluszyński (1927), Pasteels (1949, 1954) in general and for the genus *Stylops*, this strategy was also largely followed by Kifune & Hirashima (1985), Kifune & Maeta (1990), Kifune (1991) and Luna de Carvalho (1969). By contrast, Bohart (1936, 1937, 1941) and also, in part, Luna de Carvalho (1974) took into account existing morphological differences of *Stylops* (males, females and first instars) from different hosts and the similarities of *Stylops* from related hosts that belong to the same host subgenus of *Andrena* (Borchert 1963). Bohart's classification method, that species correspond to the subgeneric rank of the host, was adopted in earlier studies published by Kinzelbach (1971), but he decided that the variability of the West Palaearctic species forms a continuum, so all the European species were synonymized under the single name *S. melittae* Kirby, 1802 (Kinzelbach 1978). Kinzelbach (1978), however, proposed to recognise former species that were allied to hosts as subspecies. Since that time, faunistic lists of species from various European countries have contained a single *Stylops* name for specimens from all the hosts (Bleidorn et al. 2004, Kuhlmann 1998, Pekkarinen 1997, Pohl 2004, Pohl & Oehlke 2003, Smit & Smit 2005, Soon et al. 2012), unlike lists of Nearctic (Kathirithamby & Taylor 2005) or

East Palaearctic species (Kifune et al. 1994). Most authors continue listing all the collected host species, which may help associate them with the correct *Stylops* species in the future, especially when host specialisation seems to be relatively stable in this genus and the number of species is considerably higher than one (Jůzová et al. 2015).

In this study we aim to make clearer the overview of the published species names of the genus *Stylops* which are available for taxonomic studies, make new suggestions concerning synonymy, remove unavailable names (nomina nuda), check correct spelling and specify correct dates of publication of available names. We use available information from DNA barcode sequences and compare genetic distances among and within species. Recent view of taxonomy is changing from a single *Stylops* species recognized in Europe to many species in this genus (there is a different situation in North America or Asia) and for this reason, formally, but still preliminarily, we sort all known *Stylops* names to species reflecting variability in barcode sequences (Jůzová et al. 2015), their host specificity and published morphological variability. It is the first conceptual unification of nomenclature and taxonomy in this species rich genus after a hundred years. We also summarize hosts for each published *Stylops* name and discuss possible taxonomic problems for future re-evaluations. We understand this study as a very first step towards modern taxonomy of *Stylops* and better knowledge and easier work on these bee parasites.

Material and methods

We used primary literature for all of the *Stylops* names. We extracted information about the name of author, correct year of publication, original host, and type locality. We prepared list of species with preliminary synonyms. Results of the phylogenetic study by Jůzová et al. (2015) and the distances between DNA barcode sequences (mitochondrial gene Cytochrome c oxidase subunit I; COI) are used as a leading concept for synonymy here, but we never use it as a rule. Each *Stylops* name was considered a separate case that needs to be examined individually. Names of *Stylops* proposed by authors that used a single host specialisation approach for species definition received the most attention. Species synonymy is always considered, when several *Stylops* names are proposed from host bees of the same subgenus. We follow this affiliation of host subgenus to *Stylops* species as a guideline, except for several cases in which multiple *Stylops* species are likely to be parasites of the hosts belonging to the same subgenus; for example when such *Stylops* species are confirmed by DNA sequence differences or significant morphological differences noted in the literature, or live in distant biogeographic regions. These cases are individually commented on in the notes for each species. No species with unknown host was listed under the synonyms, but as a full species because we cannot decide about synonymy of these species now, except using published evidence. Suggestions of a new name synonymy or a name restoration are presented as ‘supposed new junior subjective synonym’, and ‘status restituted (stat. restit.)’, respectively. All the newly proposed nomenclatural changes are given in bold. Names of host species are listed, but only those directly associated with *Stylops* species name, so the host list is neither complete, nor definitive.

Changes in name spelling of numerous names proposed by Luna de Carvalho (1969, 1974) and Kinzelbach (1978) are considered unjustified emendations, and thus new junior objective synonyms of the original names. Those decisions are based on the Article 33.2.1 (ICZN 1999), because 'two or more names in the same work [were] treated in a similar way' and they used the emended spellings systematically throughout their publications.

Each published name is provided with information about the described sex or the first instar (L1); because of the widely used descriptions of morphology of puparia in the Strepsiptera and complicated definition of female, we use 'F' for female including female puparium, 'M' for male. When holotype was designated and information about it presented in the description, then we present this information for each name.

The names of the *Andrena* host species were obtained according to the updated world bee checklist (Ascher & Pickering 2014), and only the current nomenclature of the hosts is used. The subgeneric names of the hosts are used according to Gusenleitner & Schwarz (2002).

DNA barcode sequences from the published phylogenetic study (Jůzová et al. 2015) are available from GenBank (http://www.ncbi.nlm.nih.gov/). All accession numbers to published sequences are listed in a paragraph under each species in the list. The following list of *Stylops* species is divided for better orientation according to the biogeographic regions – West Palaearctic, East Palaearctic and Nearctic.

List of species of the genus *Stylops*

Stylops Kirby, 1802

Stylops Kirby, 1802: 113. Type species: *Stylops melittae* Kirby, 1802. Designation by monotypy.
= *Katastylops* Pierce, 1919: 454. Synonymized by Bohart (1941: 123).
= *Neostylops* Pierce, 1919: 455. Synonymized by Bohart (1936: 9).
= *Prostylops* Pierce, 1919: 455. Synonymized by Bohart (1941: 123).

Note. Generic name *Stylops* is masculinum (ICZN 1999: 30.1.4.3).

West Palaearctic Region

All the West Palaearctic species of *Stylops* were synonymized with *Stylops melittae* by Kinzelbach (1978: 118), except a few forgotten names, thus the statuses of nearly all species are restituted here in this sense.

Stylops analis Perkins, 1918, stat. restit.

Stylops analis Perkins, 1918: 73, F. Type locality: Great Britain, New Forest.

Host. Not reported in the original description (Perkins 1918). The host of this species is likely *Andrena* (*Larandrena*) *ventralis* Imhoff, 1832, which is supposed to by a junior synonym of *A. analis* Fabricius, 1804. The host is presumed based on the Perkins's practice to use the host name as the name of parasite. The previously proposed host, *A.* (*Tarsandrena*) *tarsata* Nylander, 1848 (Kinzelbach 1971, 1978; Ulrich 1930), is incorrect because its association

with the host name *A. analis* was likely based on an old name association mistake in *Andrena* genus (Gusenleitner & Schwarz 2002).
Note. No DNA sequence is known for *Stylops* from *A. ventralis*. The species status of *S. analis* needs to be confirmed in the future based on newly collected material.

Stylops andrenaphilus Luna de Carvalho, 1974, stat. restit.

Stylops andrenaphilus Luna de Carvalho, 1974: 331, F. Type locality: Spain, Burgos, Estépar.

Host. *Andrena* (*Simandrena*) *propinqua* Schenck, 1853 (Luna de Carvalho 1974).
Note. No DNA sequence is known for *Stylops* from *Andrena propinqua*. The species status of *S. andrenaphilus* needs to be confirmed in the future.

Stylops ater Reichert, 1914, stat. restit.

= *Stylops alterimus*: Anonymus (1898a: 509, with reference to plate). Incorrect subsequent spelling of *Stylops aterrimus* Newport, misidentification.
= *Stylops atterimus*: Anonymus (1898b: plate "Zuchtwahl II", Figs 7a and 7b, not paginated). Incorrect subsequent spelling of *Stylops aterrimus* Newport, misidentification.
Stylops ater Reichert, 1914: 151, M. Type locality: Germany, Merseburg.
? = *Stylops krygeri* Pierce, 1919: 445, F. Type locality: Denmark, Fejo. Status uncertain, see note.
= *Stylops ovinae* Noskiewicz & Poluszyński, 1927: 1098. **Nomen nudum.**
= *Stylops muelleri* Borchert, 1971: 18, M, F. Type locality: Germany, Berlin-Spandau, Weinmeisterhorn. **Supposed new junior subjective synonym.**

Host. *Andrena* (*Melandrena*) *vaga* Panzer, 1799 (Borchert 1971, Reichert 1914).
DNA barcode sequences. KF803437, KF803438, KF803490, JN082812, KF803528, KF803529.
Notes. Two names, *Stylops alterimus* and *S. atterimus* [sic!] appeared in a Lexicon (Anonymus 1898a,b). These are not available names, because they obviously refer to *S. aterrimus* Newport and there is no reason to think that new names were intentionally created in a summarizing publication like lexicon, despite the accompanying figure depicting a different species, not *S. aterrimus* Newport.

Stylops ater was subjectively considered an incorrect subsequent spelling (Hofeneder & Fulmek 1943), or a junior synonym of *S. aterrimus* (Ulrich 1930: 14). However, there is no evidence that the name *S. ater* is merely an incorrect original spelling of *S. aterrimus*, and the name fulfils all the formal conditions for available name proposed before 1931 by method of indication (see ICZN 1999: Articles 12, 12.2.7). Therefore, we consider *Stylops ater* Reichert an available and valid name. The name *S. ater* was used consistently in the Reichert's (1914) publication and his reference that he provided the same material for making the figure used in the Lexicon (Anonymus 1898a,b) does not affect its availability, as no available species name was proposed in that book (Anonymus 1898a,b). The name *S. ater* was not mentioned by Kinzelbach (1978: 118), who listed European species names.

Examination of the type specimen of *S. krygeri* is required. According to Kinzelbach (1978), *S. krygeri* might be the appropriate name for the *Stylops* species from *A. vaga*. However, the

description of the female specimen mentions black basal half of the cephalothorax, which does not match *S. ater*. Such a character is typical for *S. nevinsoni* or *S. thwaitesi*.

Distances between DNA barcode sequences of this species and the other *Stylops* species are 10−20 % and almost invariable within *S. ater* (Jůzová et al. 2015).

Stylops aterrimus Newport, 1851, stat. restit.

Stylops aterrimus Newport, 1851: 340, M. Type locality: Great Britain, Hampstead.
= *Stylops spencii* auct., nec Pickering (1836).
= *Stylops Trimmerana* Smith, 1857: 118, M. Type locality: Great Britain. Synonymized with *S. aterrimus* by Pierce (1908: 77).
? = *Stylops dominiquei* Pierce, 1909: 102, M, F. Type locality: France, Nantes. **Supposed new junior subjective synonym.**
? = *Stylops bimaculatae* Perkins, 1918: 71, M. Type locality: Great Britain, Berkshire, Crowthorne, Wellington College. **Supposed new junior subjective synonym.**
= *Neostylops trimmerana*: Pierce (1919: 456). New generic placement.
= *Stylops aterrima*: Ulrich (1930: 14). Incorrect gender agreement.
= *Stylops perkinsi* Pasteels, 1949: 188, M, F. New substitute name for *S. spencii* Perkins, 1918.
= *Stylops niger* v. Beneden: Kinzelbach (1978: 121). **Nomen nudum.** Van Beneden (1878) used common name 'Stylops noir' and reproduced description from Smith (1857).
= *Stylops trimmeranae* Kinzelbach, 1978: 133. Unjustified emendation. **New junior objective synonym.**

Hosts. *Andrena* (*Plastandrena*) *tibialis* (Kirby, 1802) (Luna de Carvalho 1974, Pasteels 1949, Pickering 1836), *Andrena* (*Hoplandrena*) *trimmerana* (Kirby, 1802) (Newport 1851, Smith 1857), *Andrena* (*Agandrena*) *agilissima* (Scopoli, 1770) (Pierce 1909).
DNA barcode sequences. KF803429, KF803504, KF803521, KF803522, KP213298, KP213299, KP213300.
Notes. The original tractate written by Newport was published in two parts (called memoirs). Name *S. aterrimus* was published in the second part, which appeared in the year 1851 and not as early as the first part in 1847 (Pierce 1908).

For differential diagnosis between *S. aterrimus* and *S. nassonowi* and further details about these closely related species see Straka et al. (in prep).

Distances between DNA barcode sequence of this species and the other *Stylops* species are 4−20 % (Jůzová et al. 2015).

Stylops borcherti Luna de Carvalho, 1974, stat. restit.

Stylops borcherti Luna de Carvalho, 1974: 349, F. Type locality: Spain, Madrid, Alcalá de Henares.

Host. *Andrena* (*Melandrena*) *albopunctata* (Rossi, 1792) (Luna de Carvalho 1974).
Note. This name is possibly a synonym, but it is unclear whether it belongs to *S. melittae* or *S. ater*, both known from the subgenus *Melandrena* in Europe. The latter species was used for comparison to *S. borcherti* by the original author, but we expect rather synonymy with *S. melittae* because *S. melittae* is known to occur in Spain, whereas *S. ater* is not (Luna de Carvalho 1974). No DNA sequence is known for *Stylops* from this host species. The status of this species is restituted for the time being until its status is clarified using integrative taxonomy or from the study of the type material.

Stylops dalii Curtis, 1828, stat. restit.

Stylops dalii Curtis, 1828: plate 226, M, F. Type locality: Great Britain.
= *Stylops dalei* Kinzelbach, 1978: 121. Unjustified emendation. **New junior objective synonym.**

Host. *Andrena* (*Holandrena*) *labialis* (Kirby, 1802) (Curtis 1828).
DNA barcode sequence. KF803473.
Note. Distances between DNA barcode sequence of this species and the other *Stylops* species are 9–18 % (Jůzová et al. 2015).

Stylops deserticola Medvedev, 1970, stat. restit.

Stylops deserticola Medvedev, 1970: 200, M (holotype), F. Type locality: Kazakhstan, Almaty Province, Kerbulak.
= *Stylops desertorum*: Medvedev (1970: 201). Incorrect original spelling.

Host. *Andrena* (*Melanapis*) *fuscosa* Erichson, 1835 (Medvedev 1970).
Note. Medvedev (1970) used two names in his original description. The name *S. desertorum* was used only in a figure and was fixed as incorrect original spelling by Kinzelbach (1978: 120).

This species from south-eastern Kazakhstan is traditionally listed among the West Palaearctic species (Kinzelbach 1978), so we maintain this placement.

No DNA sequence is known for *Stylops* from this host subgenus.

Stylops dinizi Luna de Carvalho, 1974, stat. restit.

Stylops dinizi Luna de Carvalho, 1974: 343, F. Type locality: Spain, Madrid, Vaciamadrid.

Host. *Andrena* (*Campilogaster*) *incisa* Eversmann, 1852 (Luna de Carvalho 1974).
Note. No DNA sequence is known for *Stylops* from this host subgenus.

Stylops gwynanae Günther, 1957, stat. restit.

= *Stylops gwynanae* Noskiewicz & Poluszyński, 1927: 1098. **Nomen nudum.**
Stylops gwynanae Günther, 1957 in Günther & Šedivý (1957): 412, F. Type locality not indicated. Implemented in the key for species identification, representing valid description under paragraph 13.1.1. of ICZN (1999).
= *Stylops gwynanai* Luna de Carvalho, 1974: 340. Unjustified emendation. **New junior objective synonym.**

Host. *Andrena* (*Euandrena*) *bicolor* Fabricius, 1775 (Günther & Šedivý 1957, Luna de Carvalho 1974).
DNA barcode sequences. KF803430, KF803431.
Note. Distances between DNA barcode sequences of *Stylops* from the subgenus *Euandrena* and the other host subgenera are 13–23 % (Jůzová et al. 2015). Variability within *Stylops* from *Euandrena* from distant localities in the Palaearctic Region reach up to 6 % in DNA barcode distance, and thus more valid sibling species can be recognized from this host bee subgenus. We propose to call *Stylops* from *Euandrena* hosts *S. gwynanae sensu lato*. More comprehensive sampling and detailed study are necessary for evaluation of this taxonomic problem.

Stylops hammella Perkins, 1918, stat. restit.

Stylops hammella Perkins, 1918: 71, F. Type locality: Great Britain, near Oxford.
= *Stylops hammelae* Kinzelbach, 1978: 122. Unjustified emendation. **New junior objective synonym.**
?= *Stylops nitidiusculae* Poluszyński, 1927: 95, M, F. Type locality: Ukraine, near Lviv, 'Filipkowce'. **Supposed new junior subjective synonym.**
= *Stylops nitidiusculai* Luna de Carvalho, 1974: 327. Unjustified emendation. **New junior objective synonym.**

Hosts. *Andrena* (*Notandrena*) *chrysosceles* (Kirby, 1802) (Perkins 1918), *Andrena* (*N.*) *nitidiuscula* Schenck, 1853 (Luna de Carvalho 1974, Poluszyński 1927).
DNA barcode sequences. KF803448, KF803449, KP213296, KP213297.
Notes. Perkins (1918) named this species after the collector Mr. Hamm; however, he used an unusual word form that we interpret as a noun in apposition rather than an adjective.

Distances between DNA barcode sequences of *Stylops* from *Andrena* subgenus *Notandrena* and the hosts from other subgenera are 10–18 % (Jůzová et al. 2015).

Stylops ibericus Luna de Carvalho, 1969, stat. restit.

Stylops ibericus Luna de Carvalho, 1969: 7, F. Type locality: Portugal, Sagres, Vila do Bispo.

Host. *Andrena* (*Carandrena*) *nigroviridula* Dours, 1873 (Luna de Carvalho 1969).
Note. No DNA sequence is known for *Stylops* from this host subgenus.

Stylops kinzelbachi Luna de Carvalho, 1974, stat. restit.

Stylops kinzelbachi Luna de Carvalho, 1974: 327, F. Type locality: Spain, Alicante, Elche.

Host. *Andrena* (*Rufandrena*) *orbitalis* Morawitz, 1871 (Luna de Carvalho 1974).
Note. No DNA sequence is known for *Stylops* from this host subgenus.

Stylops liliputanus Luna de Carvalho, 1974, stat. restit.

Stylops liliputanus Luna de Carvalho, 1974: 315, M, F (holotype). Type locality: Spain, Madrid.

Host. *Andrena* (*Aciandrena*) *astrella* Warncke, 1975 (holotype) (Luna de Carvalho 1974).
DNA barcode sequences. KF803426, KF803427.
Notes. Luna de Carvalho (1974) also refers to the possible host *A*. (*Graecandrena*) *montarco* Warncke, 1975; however, other proposed hosts, such as *A*. (*Aenandrena*) *hystrix* Schmiedeknecht, 1883, *Andrena* (*Micrandrena*) *bayona* Warncke, 1975, *A*. (*M.*) *exigua* Erichson, 1835, and *A*. (*M.*) *minutuloides* Perkins, 1914, seem to be unlikely.

Distances between DNA barcode sequences of *Stylops* from the subgenus *Aciandrena* and the other host subgenera are very variable: 1–19 % (Jůzová et al. 2015). DNA distances among individuals from the host subgenus *Aciandrena* are up to 10 %. They are distinctly consisting of several different species lineages, and for this reason, we use only sequences acquired from specimens collected in Spain for the species *S. liliputanus*. We propose to call *Stylops* from *Aciandrena* hosts *S. liliputanus sensu lato*. More comprehensive sampling and detailed study are necessary for evaluation of this taxonomic problem.

Stylops lusohispanicus Luna de Carvalho, 1974, stat. restit.

Stylops lusohispanicus Luna de Carvalho, 1974: 317, F. Type locality: Portugal: Lisboa.

Host. *Andrena* (*incertae sedis*) *verticalis* Pérez, 1895 (Luna de Carvalho 1974). The host of uncertain subgeneric placement.
Note. No DNA sequence is known for *Stylops* from *A. verticalis*.

Stylops madrilensis Luna de Carvalho, 1974, stat. restit.

Stylops madrilensis Luna de Carvalho, 1974: 337, M, F (holotype). Type locality: Spain, Madrid, Arganda.

Host. *Andrena* (*Ptilandrena*) *vetula* Lepeletier, 1841 (Luna de Carvalho 1974).
Note. No DNA sequence is known for *Stylops* from this host subgenus in the West Palaearctic Region.

Stylops maxillaris Pasteels, 1949, stat. restit.

Stylops maxillaris Pasteels, 1949: 194, M, F. Type locality: Belgium, Auderghem.
?= *Stylops esteponensis* Luna de Carvalho, 1974: 334, F. Type locality: Spain, Málaga, Estepona. **Supposed new junior subjective synonym.**

Hosts. *Andrena* (*Chlorandrena*) *humilis* Imhoff, 1832 (Pasteels 1949), *Andrena* (*C.*) *livens* Pérez, 1895 (Luna de Carvalho 1974).
DNA barcode sequences. KF803466, KF803467, KF803516.
Note. Distances between DNA barcode sequences of *Stylops* from the subgenus *Chlorandrena* and the other host subgenera are 3–18 %, but usually more than 7 % (Jůzová et al. 2015).

Stylops melittae Kirby, 1802

Stylops melittae Kirby, 1802: 113, M. Type locality not indicated.
= *Stylops Kirbii* Leach, 1817: 135, M. Type locality not indicated. Synonymized with *S. melittae* by Pierce (1909: 94).
= *Stylops Haworthi* Stephens, 1829: 403. **Nomen nudum.**
= *Stylops spencii* Pickering, 1836: 168, M. Type locality: Great Britain. Synonymized with *S. melittae* by Pasteels (1949:188).
= *Stylops kirbyi* Kinzelbach, 1978: 125. Unjustified emendation. **New junior objective synonym.**
?= *Stylops flavipedis* Hofeneder, 1924a: 132, M, F. Type locality: Austria, Wien, Kalksburg. **Supposed new junior subjective synonym.**
?= *Stylops nitidae* Pasteels, 1954: 352, F, L1. Type locality: Switzerland, Lausanne, Bois de Helmont. **Supposed new junior subjective synonym.**
= *Stylops spencei* Kinzelbach, 1971: 169. Unjustified emendation. **New junior objective synonym.**
= *Stylops spencei* Luna de Carvalho, 1974: 342. Unjustified emendation. Junior homonym of *S. spencei* Kinzelbach, 1971. **New junior objective synonym.**
= *Stylops melittai* Luna de Carvalho, 1974: 341. Unjustified emendation. **New junior objective synonym.**
?= *Stylops giganteus* Luna de Carvalho, 1974: 352, F. Type locality: Spain, Madrid, Ciempozuelos. **Supposed new junior subjective synonym.**

Hosts. *Andrena* (*Melandrena*) *nigroaenea* (Kirby, 1802) (Kirby 1802), *A.* (*M.*) *nitida* (Müller, 1776) (Pasteels 1954), *A.* (*Zonandrena*) *flavipes* Panzer, 1799 (Hofeneder 1924a), *A.* (*M.*) *thoracica* (Fabricius, 1775), *A.* (*Z.*) *soror* Dours, 1872 (Luna de Carvalho 1974).

DNA barcode sequences. KF803450, KF803451, KF803452, KF803453, KF803454, KF803455, KF803456, KF803459, KF803460, KF803461, KF803488, KF803489, KF803491, KF803492, KF803493, KF803517, KP213295.

Notes. Distances between DNA barcode sequences of *Stylops* from the subgenera *Melandrena* and *Zonandrena* and the other host subgenera are 8–18 % (Jůzová et al. 2015). In comparison to other *Stylops* species with known barcode sequences, variability within *Stylops* from *Melandrena* and *Zonandrena* is relatively high, but still at most 2 % in distance (for exception, see below). This fits well with association to a single species from both mentioned subgenera together in the West Palaearctic Region. However, *Melandrena* subgenus hosts also *S. ater* in the West Palearctic Region and rarely the species host pool can overlap in both *Stylops* species. Thus, *A. nigroaenea* can rarely be host of two species of *Stylops* (Jůzová et al. 2015). To fix the name *S. melittae*, revision of the type will be necessary.

Pasteels (1949) suggested that the species name *S. spencii*, originally described by (Pickering 1836) from *A. tibialis*, is a synonym of *S. melittae*, and he proposed a new name for the *Stylops* species that parasitises *A. tibialis*. This problem was first noted by Perkins (1918), who described the possible irrelevance of Pickering's host identification, and thus, also Pickering's *Stylops* determination. Some uncertainty remains. Examination of the type material or the designation of a neotype is required to fix the nomenclature of the name *S. spencii*.

Stylops moniliaphagus Luna de Carvalho, 1974, stat. restit.

Stylops moniliaphagus Luna de Carvalho, 1974: 332, F. Type locality: Spain, Madrid, Vaciamadrid.

Host. *Andrena* (*Orandrena*) *monilia* Warncke, 1967 (Luna de Carvalho 1974).

Note. No DNA sequence is known for *Stylops* from the subgenus *Orandrena*.

Stylops nassonowi Pierce, 1909

Stylops nassonowi Pierce, 1909: 105, F. Type locality: Egypt.

= *Stylops savignyi* Hofeneder, 1924: 254, F. Type locality: Egypt, Aswan, Kitchener's Island. Synonymized with *S. nassonowi* by Straka et al. (in prep.).

= *Stylops nassanowi*: Luna de Carvalho (1974: 345). Incorrect subsequent spelling.

Hosts. *Andrena* (*Plastandrena*) *pilipes* Fabricius, 1781 (Pierce 1909), *Andrena* (*Suandrena*) *savignyi* Spinola, 1838 (Hofeneder 1924b).

DNA barcode sequences. KF803433, KF803434, KF803435, KF803436, KF803463, KF803503, KF803518, KF803519, KF803530, KP213301, KP213302, KP213303, KP213304, KP213305, KP213306.

Notes. Pierce (1909) described *S. nassonowi* from a figure drawing made by Nasonov (1893) from specimens from Germany and Egypt. However, only the specimen from Egypt could be assigned to *S. nassonowi*. Species association of the specimen from Germany is uncertain and may be *S. aterrimus*. For differential diagnosis between *S. aterrimus* and *S. nassonowi*, restitution of status of the latter name and further details about these closely related species see Straka et al. (in prep.).

Distances between DNA barcode sequences of this species and the other *Stylops* species are 4–20 % (Jůzová et al. 2015).

Stylops nevinsoni Perkins, 1918, stat. restit.

Stylops nevinsoni Perkins, 1918: 71, F. Type locality: Great Britain.
?= *Stylops transversa* Pasteels, 1949: 191, M, F. Type locality: Belgium, Uccle. **Supposed new junior subjective synonym.**

Hosts. *Andrena* (*Andrena*) *synadelpha* Perkins, 1914 (Perkins 1918), *Andrena* (*Andrena*) *fulva* (Müller, 1766) (Pasteels 1949).
DNA barcode sequences. KF803457, KF803458, KF803462, KF803533.
Notes. Distances between DNA barcode sequences of *Stylops* from the subgenus *Andrena* and the other host subgenera are 8–17 % in the West Palaearctic Region (Jůzová et al. 2015).

Stylops praecocis, which also parasitizes bees of the subgenus *Andrena*, seems to be very closely related and their DNA barcode sequences differ only 1 % in base composition. However, phylogenetic study suggests two distinct *Stylops* clades for early spring and late spring *Andrena* bee hosts (Jůzová et al. 2015). In addition, significant morphological differences were found in first instars from *A. fulva* (likely *S. nevinsoni*) and *A. praecox* (likely *S. praecocis*) (Borchert 1963). For these reasons, we decided to assign both names to valid species; however, more research is needed in this problem.

Stylops obenbergeri Ogloblin, 1923, stat. restit.

Stylops obenbergeri Ogloblin, 1923: 45, M. Type locality: Czech Republic, Prague, Stromovka.

Host. Unknown.
Note. We restitute status of this species for the time being, until studies of the type material clarify its synonymization or validity.

Stylops obsoletus Luna de Carvalho, 1974, stat. restit.

Stylops obsoletus Luna de Carvalho, 1974: 324, F. Type locality: Spain, Tarifa?, uncertain location.

Host. *Andrena* (*Distandrena*) *distinguenda* Schenck, 1871 (Luna de Carvalho 1974).
DNA barcode sequence. KF803445.
Note. Distances between DNA barcode sequences of *Stylops* from the subgenus *Distandrena* and the other host subgenera are 8–19 % (Jůzová et al. 2015).

Stylops paracuellus Luna de Carvalho, 1974, stat. restit.

Stylops paracuellus Luna de Carvalho, 1974: 339, F. Type locality: Spain, Madrid, Paracuellos.

Host. *Andrena* (*Parandrena*) *tunetana* Schmiedeknecht, 1900 (Luna de Carvalho 1974).
Note. No DNA sequence is known for *Stylops* from the host subgenus.

Stylops pasteelsi Luna de Carvalho, 1974, stat. restit.

Stylops pasteelsi Luna de Carvalho, 1974: 326, F. Type locality: Spain, Málaga, Estepona.

Host. Unclear, maybe *Andrena* (*Melittoides*) *ramlehiana* Pérez, 1903.
Notes. There is most likely a mistake either in the identification or in the locality data of the host. The subgenus *Melittoides* has never been collected in Spain, and the species *A. ramlehiana* is known only from the Near East (Ascher & Pickering 2014).

No DNA sequence is known for *Stylops* from the subgenus *Melittoides*.

Stylops praecocis Luna de Carvalho, 1974, stat. restit.

= *Stylops nycthemerae* Noskiewicz & Poluszyński, 1927: 1098. **Nomen nudum.**
= *Stylops praecocis* Noskiewicz & Poluszyński, 1927: 1098. **Nomen nudum.**
Stylops praecocis Luna de Carvalho, 1974: 329, F. Type locality: Spain, Madrid, Vaciamadrid.

Hosts. *Andrena* (*Andrena*) *praecox* (Scopoli, 1763) (Luna de Carvalho 1974), *A*. (*A.*) *nycthemera* Imhoff, 1868 (Noskiewicz & Poluszyński 1927).
DNA barcode sequences. KF803439, KF803484, KF803495, KF803496.
Notes. The name *S. praecocis* Noskiewicz & Poluszyński, 1927 was correctly mentioned as a nomen nudum by Pasteels (1954); however, he did not provide a description. Pasteels (1954) provided good figures of the female cephalothoraces with references to the host. However, this does not make his note of the name *S. praecocis* in his main text available for nomenclatural use.

Distances between DNA barcode sequences of *Stylops* from the subgenus *Andrena* and the other host subgenera are 8–17 % in the West Palaearctic Region (Jůzová et al. 2015). However, see notes under *S. nevinsoni*.

Stylops risleri Kinzelbach, 1967, stat. restit.

Stylops risleri Kinzelbach, 1967: 37, F. Type locality: Spain, Canary Islands, Teneriffe, Teide, Las Canadas.

Host. *Andrena* (*Micrandrena*) *lineolata* Warncke, 1968 (Kinzelbach 1967).
DNA barcode sequence. KF803502.
Notes. Distances between DNA barcode sequences of *Stylops* from *A*. (*M.*) *lineolata* and the other host species including species from the subgenus *Micrandrena* are 6–17 % (Jůzová et al. 2015).

The host name published by Kinzelbach (1967) was mentioned before the description of *Stylops* species, so Kinzelbach used the host name as a nomen nudum.

Stylops ruthenicus Schkaff, 1925, stat. restit.

Stylops ruthenicus Schkaff, 1925: 139, M. Type locality: Ukraine, Kharkiv Oblast, Zmiiv.
= *Afrostylops ruthenicus* (Schkaff): Fox & Fox (1964: 756). New generic placement.

Host. Unknown.
Notes. We restitute status of this species for the time being until studies of the type material clarify its synonymization or validity.

Fox & Fox (1964) placed *S. ruthenicus* Schkaff, 1925 incorrectly in the genus *Afrostylops* Fox & Fox, 1964; however, the type species of *Afrostylops* belongs to the genus *Myrmecolax* Westwood, 1858, and thus *Afrostylops* is junior synonym of *Myrmecolax* (Kinzelbach 1971).

Stylops salamancanus Luna de Carvalho, 1974, stat. restit.

Stylops salamancanus Luna de Carvalho, 1974: 322, F. Type locality: Spain, Salamanca.

Host. *Andrena* (*Aenandrena*) *hedikae* Jaeger, 1934 (Luna de Carvalho 1974).
DNA barcode sequence. KF803428.
Note. Distances between DNA barcode sequences of *Stylops* from the subgenus *Aenandrena* and the other host subgenera are 9–17 % (Jůzová et al. 2015).

Stylops spreta Perkins, 1918, stat. restit.

Stylops spreta Perkins, 1918: 73, F. Type locality: Great Britain.
= *Stylops parvulae* Noskiewicz & Poluszyński, 1927: 1098. **Nomen nudum.**
= *Stylops spretae* Ulrich, 1930: 15. Unjustified emendation. **New junior objective synonym.**
= *Stylops spretus* Luna de Carvalho, 1974: 322. Unjustified emendation. **New junior objective synonym.**
?= *Stylops duriensis* Luna de Carvalho, 1974: 321, F. Type locality: Portugal, Alto Douro. **Supposed new junior subjective synonym.**

Hosts. *Andrena* (*Micrandrena*) *minutula* (Kirby, 1802) (Noskiewicz & Poluszyński 1927, Perkins 1918), *A.* (*M.*) *tenuistriata* Pérez, 1895 (Luna de Carvalho 1974).
DNA barcode sequences. KF803474, KF803475, KF803476, KF803477, KF803478, KF803479, KF803480, KF803481, KF803497, KF803512, KF803513, KF803514, KF803515, KP213292, KP213293, KP213294.
Notes. Distances between DNA barcode sequences of *Stylops* from the West Palaearctic representatives of the subgenus *Micrandrena* and the other host subgenera are 7–17 % (Jůzová et al. 2015). Distances between DNA sequences of *Stylops* within the subgenus *Micrandrena* from the West Palaearctic and East Palaearctic Regions are 0–9 % (Jůzová et al. 2015), which suggests more than one species of *Stylops* parasitizing the subgenus *Micrandrena*. Variability within *Stylops* from *Micrandrena* hosts from distant localities in European continent is only up to 2 % in DNA distance, which suggests only a single species in continental Europe parasitising the bees of the subgenus *Micrandrena*. See also notes under the closely related *Stylops* species, *S. risleri* and *S. kaguyae*.

Stylops thwaitesi Perkins, 1918, stat. restit.

= *Stylops thwaitei* Saunders, 1872: 23. **Nomen nudum.**
Stylops thwaitesi Perkins, 1918: 70, M, F. Type locality: Great Britain.
= *Stylops wilkellae* Perkins, 1918: 70, M, F. Type locality: Great Britain, Surrey, Woking. Synonymized with *S. thwaitesi* by Pasteels (1954: 349).
?= *Stylops championi* Pierce, 1919: 440, M. Type locality: Great Britain, Woking. **Supposed new junior subjective synonym.**
= *Stylops xanthurae* Noskiewicz & Poluszyński, 1927: 1098. **Nomen nudum.**
?= *Stylops alfkeni* Hofeneder, 1939: 187, M, F. Type locality: Germany, Hannover, Leuchtenberg. **Supposed new junior subjective synonym.**
= *Stylops twaithei*: Pasteels (1954: 349). Incorrect subsequent spelling.
?= *Stylops albofasciatae* Günther, 1957: 412, M, F. Type locality: Not indicated, probably Czech Republic. **Supposed new junior subjective synonym.**
= *Stylops thwattei* Luna de Carvalho, 1969: 8. Unjustified emendation. **New junior objective synonym.**
?= *Stylops borealis* Kifune & Hirashima, 1985: 53, M (holotype), F. Type locality: Japan, Hokkaido, Tokachi, Ashoro. **Supposed new junior subjective synonym.**

Hosts. *Andrena* (*Taeniandrena*) *ovatula* (Kirby, 1802) (Kifune et al. 1994, Perkins 1918), *A.* (*T.*) *similis* Smith, 1849 (Hofeneder 1939), *A.* (*T.*) *albofasciata* Thomson, 1870 (Günther & Šedivý 1957, Pasteels 1954), *A.* (*T.*) *ezoensis* Hirashima, 1965 (Kifune & Hirashima 1985, Kifune & Maeta 1990), *A.* (*T.*) *wilkella* (Kirby, 1802) (Günther & Šedivý 1957, Luna de Carvalho 1974, Pasteels 1954).
DNA barcode sequences. KF803470, KF803494, KF803544.
Notes. Pasteels (1949: 186) correctly noted that the name proposed by Saunders (1872) is unavailable, and the author of the name is Perkins (1918).

We suggest a synonymy of *S. championi* with *S. thwaitesi*, because *S. championi* was described based on the same series of specimens collected by G. C. Champion and described also as *S. wilkellae*. Description of morphological characters presented by Pierce (1919), especially length of antennal segments, is identical to description provided by Perkins (1918).

We also suggest synonymization of *S. borealis* with *S. thwaitesi* because of very similar DNA barcode sequences between the West and East Palaearctic individuals. The sequences from the Japanese population differ from the European population of this species by only 1.7–1.9 %. *Stylops thwaitesi* differs from other species in 9–18 % of the DNA barcode sequence base pairs (Jůzová et al. 2015).

Stylops ventricosae Pierce, 1909, stat. restit.

Stylops ventricosae Pierce, 1909: 109, F. Type locality: Croatia, Fiume [= Rijeka].

Host. *Andrena* (*Cryptandrena*) *ventricosa* Dours, 1873.
Note. No DNA sequence is known for *Stylops* from this host subgenus.

Stylops warnckei Luna de Carvalho, 1974, stat. restit.

Stylops warnckei Luna de Carvalho, 1974: 325, F. Type locality: Spain, Madrid, Arganda.

Host. *Andrena* (*Fumandrena*) *pandosa* Warncke, 1968.
Note. No DNA sequence is known for *Stylops* from this host subgenus.

East Palaearctic Region

Stylops circularis Kifune & Hirashima, 1985

Stylops circularis Kifune & Hirashima, 1985: 50, F. Type locality: Japan, Shikoku, Tokushima, Akui.
?= *Stylops orientis* Kifune & Maeta, 1990: 101, F. Type locality: Japan, Honshu, Tokyo, Nerima-ku, Nakamura-cho. **Supposed new junior subjective synonym.**
?= *Stylops hirashimai* Kifune & Maeta, 1990: 102, F. Type locality: Honshu, Matsue, Nagae. Correct original spelling (fixed here). **Supposed new junior subjective synonym.**
= *Stylops hirashinai*: Kifune & Maeta (1990: 102). Incorrect original spelling.

Hosts. *Andrena* (*Melandrena*) *sasakii* Cockerell, 1913 (Kifune & Hirashima 1985), *A.* (*M.*) *watasei* Cockerell, 1913, *A.* (*M.*) *parathoracica* Hirashima, 1957 (Kifune & Maeta 1990).
Notes. In Kifune & Maeta (1990), this species name occurred in two different spellings; once in the title as *S. hirashinai* [*lapsus calami*], and correctly as *S. hirashimai* throughout the rest of article. Here we fix *S. hirashimai* as the correct original spelling of the name.

No DNA sequence is known for *Stylops* from this host subgenus from the East Palaearctic Region.

Stylops japonicus Kifune & Hirashima, 1985

Stylops japonicus Kifune & Hirashima, 1985: 46, F. Type locality: Japan, Kyushu, Mt. Hikosan.
?= *Stylops truncatus* Kifune & Hirashima, 1985: 46, F. Type locality: Japan, Hokkaido, Tokachi, Nukabira. **Supposed new junior subjective synonym.**

?= *Stylops oblongulus* Kifune & Hirashima, 1985: 47, F. Type locality: Japan, Honshu, Saitama, Hodosan. **Supposed new junior subjective synonym.**
?= *Stylops truncatoides* Kifune & Hirashima, 1985: 50, F. Type locality: Japan, Hokkaido, Tokachi, Nukabira. **Supposed new junior subjective synonym.**
?= *Stylops collinus* Kifune & Maeta, 1990: 98, F. Type locality: Japan, Honshu, Yamanashi, Masutomi. **Supposed new junior subjective synonym.**
?= *Stylops aburanae* Kifune & Maeta, 1990: 98, F. Type locality: Japan, Honshu, Nagano, Ina, Todai. **Supposed new junior subjective synonym.**

Hosts. *Andrena* (*Andrena*) *benefica* Tadauchi & Hirashima, 1987, *A.* (*A.*) *maukensis* Matsumura, 1911, *A.* (*A.*) *longitibialis* Hirashima, 1962, *A.* (*A.*) *lapponica shirozui* Hirashima, 1962 (Kifune & Hirashima 1985), *A.* (*A.*) *nawai* Cockerell, 1913, *A.* (*A.*) *aburana* Hirashima, 1962 (Kifune & Maeta 1990), *A.* (*A.*) *sakagamii* Tadauchi, Hirashima & Matsumura, 1987 (Kifune et al. 1994).
DNA barcode sequence. KF803538.
Note. Distances between DNA barcode sequences of *Stylops* from the subgenus *Andrena* from the East- and West Palaearctic Region are 5 % (Jůzová et al. 2015).

Stylops kaguyae Kifune & Hirashima, 1985

Stylops kaguyae Kifune & Hirashima, 1985: 51, F. Type locality: Japan, Kyushu, Fukuoka.

Hosts. *Andrena* (*Micrandrena*) *kaguya* Hirashima, 1965, *Andrena* (*M.*) *minutula* (Kirby, 1802) (Kifune & Hirashima 1985), *Andrena* (*M.*) *hikosana* Hirashima, 1957, *Andrena* (*M.*) *komachi* Hirashima, 1965 (Kifune & Maeta 1990).
DNA barcode sequences. KF803539, KF803537.
Note. Distances between DNA barcode sequences of *Stylops* from the subgenus *Micrandrena* from the East and West Palaearctic Region are 8–9 % (Jůzová et al. 2015).

Stylops montanus Kifune & Maeta, 1990

Stylops montanus Kifune & Maeta, 1990: 103, F. Type locality: Japan, Honshu, Nagano, Karuizawa.

Host. *Andrena* (*Oreomelissa*) *mitakensis* Hirashima, 1963 (Kifune & Maeta 1990).
Note. No DNA sequence is known for *Stylops* from this host subgenus.

Stylops murotai Kifune, 1991

Stylops murotai Kifune, 1991: 157, F. Type locality: Japan, Honshu, Fukui, Izumi-mura, Kebora.

Host. *Andrena* (*Hoplandrena*) *takachihoi* Hirashima, 1964 (Kifune 1991).
Note. Morphological differences between *Stylops* from *A. takachihoi* and other Japanese *Stylops* from the host subgenus *Hoplandrena* presented in the original description are significant. For this reason, we do not synonymize *S. murotai* with *S. yamatonis* until detailed study of the type material or DNA barcode sequence from *Stylops* from the original host species is performed. No DNA sequence is known for *Stylops* from *A. takachihoi*.

Stylops pilipedis Pierce, 1911

Stylops pilipedis Pierce, 1911: 495, F. Type locality: China, Beijing.

Host. *Andrena* (*Plastandrena*) *pilipes* Fabricius, 1781 (Pierce 1911).
Note. No DNA sequence is known for *Stylops* from this host subgenus in the East Palaearctic Region. Status of this species is uncertain. It might be closely related, or conspecific to *S. nassonowi* or *S. yamatonis*.

Stylops thwaitesi Perkins, 1918

(see above under Western Palaearctic species)

Stylops valerianae Kifune & Hirashima, 1985

Stylops valerianae Kifune & Hirashima, 1985: 55, F. Type locality: Japan, Hokkaido, Tokachi, Ashoromura.

Hosts. *Andrena* (*Holandrena*) *valeriana* Hirashima, 1957 (Kifune & Hirashima 1985).
Note. No DNA sequence is known for *Stylops* from this host subgenus from the East Palaearctic Region.

Stylops yamatonis Kifune & Hirashima, 1985

Stylops yamatonis Kifune & Hirashima, 1985: 51, F. Type locality: Japan, Kyushu, Kagoshima, Miyanojo.
?= *Stylops dentatae* Kifune & Maeta, 1990: 99, F. Type locality: Japan, Honshu, Nagano, Todai. **Supposed new junior subjective synonym.**
?= *Stylops aino* Kifune & Maeta, 1990: 99, F. Type locality: Japan, Hokkaido, Teshio, Piuka. **Supposed new junior subjective synonym.**
?= *Stylops izumoensis* Kifune & Maeta, 1990: 102, F. Type locality: Japan, Honshu, Shimane Pref., Mt. Makuragi. **Supposed new junior subjective synonym.**
?= *Stylops nipponicus* Kifune & Maeta, 1990: 103, F. Type locality: Japan, Honshu, Yamanashi, Shosenkyo. **Supposed new junior subjective synonym.**
?= *Stylops subcircularis* Kifune & Maeta, 1990: 104, F. Type locality: Japan, Honshu, Nagano, Ina, Habiro. **Supposed new junior subjective synonym.**
?= *Stylops fukuiensis* Kifune, 1991: 155, F. Type locality: Japan, Honshu, Fukui, Ohno, Koike. **Supposed new junior subjective synonym.**

Hosts. *Andrena* (*Simandrena*) *yamato* Tadauchi & Hirashima, 1983 (Kifune & Hirashima 1985), *A.* (*Hoplandrena*) *dentata* Smith, 1879, *A.* (*H.*) *rosae* Panzer, 1801, *A.* (*S.*) *opacifovea* Hirashima, 1952, *A.* (*S.*) *nippon* Tadauchi & Hirashima, 1983, *A.* (*Plastandrena*) *japonica* (Smith, 1873), *A.* (*P.*) *fukaii* Cockerell, 1914 (Kifune & Maeta 1990), *A.* (*H.*) *miyamotoi* Hirashima, 1964 (Kifune 1991), *A.* (*S.*) *kerriae* Hirashima, 1965, *A.* (*H.*) *pruniphora* Hirashima, 1964 (Kifune et al. 1994).
DNA barcode sequences. KF803536, KF803540, KF803541, KF803543.
Note. This *Stylops* species is closely related to *S. aterrimus* and *S. nassonowi* and can be understood as a member of this species group. On the other hand, DNA barcode distances between sequences from the West-Palaearctic species and *S. yamatonis* are as high as 8 % (Jůzová et al. 2015).

Nearctic Region

Stylops advarians Pierce, 1909

Stylops advarians Pierce, 1909: 97, F. Type locality: Canada, British Columbia, Vancouver.

?= *Stylops mandibularis* Pierce, 1911: 494, F. Type locality: USA, Illinois, Carlinville. **Supposed new junior subjective synonym.**

?= *Stylops moestae* Pierce, 1919: 443, F. Type locality: USA, Washington, Govan. **Supposed new junior subjective synonym.**

?= *Stylops sinuatus* Pierce, 1919: 450, F. Type locality: USA, Illinois, Carlinville. **Supposed new junior subjective synonym.**

Hosts. *Andrena* (*Andrena*) *vicinoides* Viereck, 1904 (Pierce 1909), *A.* (*A.*) *mandibularis* Robertson, 1892 (Pierce 1911, 1919), *A.* (*A.*) *frigida* Smith, 1853 (Pierce 1919).
DNA barcode sequences. KF803441, KF803485.
Note. Distances between DNA barcode sequences of *Stylops* from the Nearctic representatives of the subgenus *Andrena* and the other host subgenera are 9–18 % (Jůzová et al. 2015).

Stylops apicalis Bohart, 1937

Stylops apicalis Bohart, 1937: 54, F, L1. Type locality: USA, California, Berkeley.

Host. *Andrena* (*Andrena*) *saccata* Viereck, 1904.
Note. This name is not synonymized with the previous species based on the description and the discussion of morphological differences between these species (Bohart 1937, 1941).

No DNA sequence is known for *S. apicalis*.

Stylops bipunctatae Pierce, 1909

Stylops bipunctatae Pierce, 1909: 98, F. Type locality: USA, Indiana.

?= *Stylops oklahomae* Pierce, 1909: 110, F. Type locality: USA, Oklahoma, Ardmore. **Supposed new junior subjective synonym.**

Host. *Andrena* (*Larandrena*) *miserabilis* Cresson, 1872 (Pierce 1909).
Note. No DNA sequence is known for *Stylops* from this host subgenus in the Nearctic Region.

Stylops bruneri Pierce, 1909

Stylops bruneri Pierce, 1909: 98, F. Type locality: USA, Nebrasca, Sioux County.

= *Stylops andrenoides* Pierce, 1911: 493, F. Type locality: USA, Illinois, Carlinville. Synonymized with *S. bruneri* by Bohart (1941: 132).

= *Stylops salictariae* Pierce, 1919: 449, F. Type locality: USA, Illinois, Carlinville. Synonymized with *S. bruneri* by Bohart (1941: 132).

?= *Stylops neonanae* Pierce, 1919: 454, F. Type locality: USA, Georgia. **Supposed new junior subjective synonym.**

?= *Stylops duboisi* Bohart, 1937: 52, M (holotype), F. Type locality: USA, California, Davis. **Supposed new junior subjective synonym.**

Hosts. *Andrena* (*Micrandrena*) *illinoiensis* Robertson, 1891 (Pierce 1909), *A.* (*Parandrena*) *andrenoides* (Cresson, 1878) (Pierce 1911), *A.* (*M.*) *salictaria* Robertson, 1905, *A.* (*M.*) *neonana* Viereck, 1917 (Pierce 1919), *A.* (*M.*) sp. (Bohart 1937).

Notes. The name *S. duboisi* is missing in the list of North American species (Bohart 1941). Considering this, Bohart's name is regarded uncertain and thus presented as a synonym according to the host association.

No DNA sequence is known for *Stylops* from *Micrandrena* host subgenus in the Nearctic Region.

Stylops californicus Pierce, 1909

Stylops californica Pierce, 1909: 99, F, L1. Type locality: USA, Southern California.

Host. *Andrena* (*Tylandrena*) *subtilis* Smith, 1879 (Pierce 1909).
Notes. Pierce (1909) discussed close similarity with *S. subcandidae*. We suggest that these species might be identical. This taxonomic problem should be evaluated using barcode sequences from *Stylops* from *A. subtilis* host.

No DNA sequence is known for *Stylops* from *Tylandrena* host subgenus.

Stylops childreni Gray & Westwood, 1832

Stylops childreni Gray & Westwood, 1832 in Griffith (1832): 684*, plate 59, M. Type locality not indicated.
?= *Stylops vicinae* Pierce, 1909: 110, F. Type locality: USA, New Hampshire; Canada. **Supposed new junior subjective synonym.**
= *Stylops dunningi* Pierce, 1919: 438. Nomen nudum (Bohart 1941).

Host: *Andrena* (*Melandrena*) *vicina* Smith, 1853 (Pierce 1909).
DNA barcode sequence. KF803530.
Notes. There is problematic authorship of the species *S. childreni*. Griffith (1832) is most likely not the only author of the book where the species was described. Species description was prepared based on the work of G. R. Gray, who named the species, and J. O. Westwood, who prepared a figure plate with the name of *Stylops* on the plate and signed the plate, and thus we suggest authorship of the name *S. childreni* to Gray & Westwood equally.

Distances between DNA barcode sequences of *S. childreni* and other related species of *Stylops* from the Nearctic representatives of the subgenus *Melandrena* are 3 % (Jůzová et al. 2015). For this reason *S. cornii* is not considered to be a synonym of *S. childreni*.

Stylops claytoniae Pierce, 1909

Stylops claytoniae Pierce, 1909: 99, F. Type locality: USA, Georgia, Thomasville.
= *Stylops imitatrix* Pierce, 1909: 104, F. Type locality: USA, Texas, Round Mountain. Synonymized with *S. claytoniae* by Pierce (1911: 494).
= *Stylops vierecki* Pierce, 1909: 110, F. Type locality: USA, Texas, Fedor. Synonymized with *S. claytoniae* by Pierce (1911: 494).

Host. *Andrena* (*Scrapteropsis*) *imitatrix* Cresson, 1872 (Pierce 1909).
DNA barcode sequences. KF803471, KF803505.
Notes. The names presented as synonyms were proposed for the hosts *A. claytoniae* Robertson, 1891, *A. imitatrix* and *A. profunda* Viereck, 1917 by Pierce (1909). *Andrena claytoniae* and *A. profunda* are, however, junior synonyms of *A. imitatrix*. Pierce (1911, 1919) later

recognised this relationship between the host names and downgraded the name *S. vierecki* as a variety and later both *S. imitatrix* and *S. vierecki* as subspecies of *S. claytoniae*. The host bee name *A. profunda* was presented before the name description (Pierce 1909), and thus the host name was published as a nomen nudum.

Distances between DNA barcode sequences of *Stylops* from the subgenus *Scrapteropsis* and the other host subgenera are 4–20 % (Jůzová et al. 2015).

Stylops cornii Pierce, 1909

Stylops cornii Pierce, 1909: 100, F, L1. Type locality: USA, Wisconsin, Milwaukee.
= *Stylops graenicheri* Pierce, 1909: 103, F. Type locality: USA, Wisconsin, Milwaukee. Synonymized with *S. cornii* by Bohart (1941: 133).
?= *Stylops solidulae* Pierce, 1909: 107, M, F. Type locality: USA, Washington, Pullman. **Supposed new junior subjective synonym.**
= *Neostylops solidulae*: Pierce (1919: 457). New generic placement.

Hosts. *Andrena* (*Melandrena*) *commoda* Smith, 1879, *A.* (*M.*) *nivalis* Smith, 1853 (Pierce 1909).
DNA barcode sequence. KF803440.
Note. According to the phylogenetic analysis based on DNA sequences, *S. childreni* and *S. cornii* could be different species (distance in DNA barcode sequences between these two related *Stylops* species is 3 %) (Jůzová et al. 2015), and for this reason these names are not considered to by synonyms here.

Stylops crawfordi Pierce, 1909

Stylops crawfordi Pierce, 1909: 100, M, F. Type locality: USA, Texas, Dallas.
?= *Stylops swenki* Pierce, 1909: 108, F, L1. Type locality: USA, Nebrasca, Lincoln. **Supposed new junior subjective synonym.**
= *Stylops asteridis* Pierce, 1911: 494, F. Type locality: USA, Illinois, Carlinville. Synonymized with *S. swenki* by Bohart (1941: 130).
= *Neostylops crawfordi*: Pierce (1919: 456). New generic placement.

Hosts. *Andrena* (*Callandrena*) *crawfordi* Viereck, 1909, *A.* (*C.*) *simplex* Smith, 1853 (Pierce 1909), *A.* (*C.*) *asteris* Robertson, 1891 (Pierce 1911).
DNA barcode sequences. KF803444, KF803472.
Note. Distances between DNA barcode sequences of *Stylops* from *Callandrena* subgenus and the other host subgenera are 13–20% (Jůzová et al. 2015).

Stylops cressoni Pierce, 1909

Stylops cressoni Pierce, 1909: 102, F, L1. Type locality: USA, Maine, Waldoboro.

Host. *Andrena* (*Holandrena*) *cressonii* Robertson, 1891.
DNA barcode sequences. KF803442, KF803443.
Note. Distances between DNA barcode sequences of *Stylops* from the Nearctic subgenus *Holandrena* and the other host subgenera are 9–20 % (Jůzová et al. 2015).

Stylops cuneiformis Bohart, 1936

Stylops cuneiformis Bohart, 1936: 16, M. Type locality: USA, California, Coronado.

Host. Unknown.
Note. Validity of this species needs to be studied using morphological methods using the type material as well as the recently collected material preserved for DNA analyses.

Stylops elongatus Bohart, 1937

Stylops elongatus Bohart, 1937: 53, M (holotype), F. Type locality: USA, California, Riverside, Soboba Hot Springs.
= *Stylops elongata*: Bohart (1941: 132). Incorrect gender agreement.

Host. *Andrena* (*Onagrandrena*) *oenothera* Timberlake, 1937, *A.* (*O.*) *blaisdelli* Cockerell, 1924 (Bohart 1937, 1941).
Note. No DNA sequence is known for *Stylops* from this host subgenus.

Stylops erigeniae Pierce, 1919

Stylops erigeniae Pierce, 1919: 446, F. Type locality: USA, Maryland, Plummers Island.

Hosts. *Andrena* (*Ptilandrena*) *erigeniae* Robertson, 1891.
DNA barcode sequences. KF803446, KF803447.
Note. Distances between DNA barcode sequences of *Stylops* from *Ptilandrena* subgenus and the other host subgenera are 6–19 % (Jůzová et al. 2015).

Stylops hartfordensis Pierce, 1909, stat. restit.

Stylops hartfordensis Pierce, 1909: 103, F. Type locality: USA, Georgia, Thomasville.
= *Stylops nasoni* Pierce, 1909: 104, F. Type locality: USA, Pennsylvania, Ashbourne. Synonymized with *S. bruneri* by Bohart (1941: 132).

Host. *Andrena* (*Simandrena*) *nasonii* Robertson, 1895.
DNA barcode sequences. KF803486, KF803487.
Notes. This species was synonymized with *S. bruneri* by Bohart (1941), which seems to be a parasite of *Andrena* subgenus *Micrandrena*. *Stylops hartfordensis*, as a parasite of the Nearctic *Simandrena* species, is tentatively restored from synonymy here because of significant difference in the host subgenera and the size of the hosts.

Distances between DNA barcode sequences of *Stylops* from the Nearctic subgenus *Simandrena* and the other host subgenera are 4–19 % (Jůzová et al. 2015). However, sequences from the Nearctic *Micrandrena* hosts are not yet known.

Stylops heterocingulatus Bohart, 1937

Stylops heterocingulatus Bohart, 1937: 55, F, L1. Type locality: USA, California, Davis.
= *Stylops heterocingulata*: Bohart (1941: 126). Incorrect gender agreement.

Hosts. *Andrena* (*Simandrena*) *pensilis* Timberlake, 1938, *A.* (*S.*) *angustitarsata* Viereck, 1904 (Bohart 1937, 1941).
Note. No DNA barcode sequence is known from this species.

Stylops hippotes Pierce, 1909

Stylops hippotes Pierce, 1909: 103, F. Type locality: USA, Ohio, Columbus.
?= *Stylops salicifloris* Pierce, 1909: 106, F. Type locality: USA, Washington, Washington and Seattle. **Supposed new junior subjective synonym.**
= *Stylops centroclarus* Bohart, 1937: 50, M (holotype), F, L1. Type locality: USA, California, Berkeley. Synonymized with *S. salicifloris* by Bohart (1941: 124).

Host. *Andrena* (*Trachandrena*) *hippotes* Robertson, 1895, *A.* (*T.*) *salicifloris* Cockerell, 1897 (Pierce 1909), *A.* (*T.*) *quintiliformis* Viereck, 1917 (Bohart 1941).
DNA barcode sequences. KF803464, KF803465, KF803506, KF803511.
Note. Distances between DNA barcode sequences of *Stylops* from *Trachandrena* subgenus and the other host subgenera are 5–19 % (Jůzová et al. 2015). See also note under *S. multiplicatae*.

Stylops leechi Bohart, 1941

Stylops leechi Bohart, 1941: 128, M, F. Type locality: Canada, British Columbia, Vancouver.

Host. *Andrena* (*Andrena*) *vicinoides* Viereck, 1904 (Bohart 1941).
Note. No DNA sequence is known from this *Stylops* species.

Stylops multiplicatae Pierce, 1909

Stylops multiplicatae Pierce, 1909: 104, F. Type locality: USA, Wisconsin, Milwaukee.
?= *Stylops grandior* Pierce, 1919: 451, F. Type locality: USA, Montana, Big Fork. **Supposed new junior subjective synonym.**

Host. *Andrena* (*Trachandrena*) *miranda* Smith, 1879 (Pierce 1909, 1919).
DNA barcode sequences. KF803482, KF803483.
Note. We recognise this species name as valid because the DNA barcode sequences indicate significant differences from the related *S. hippotes*. Distance between these two species in DNA sequences is 4 % (Jůzová et al. 2015). However, both species have closely related hosts placed in the same subgenus and this complicates synonymic list of names described from *Trachandrena* host species. This group should be studied using broader sampling of material from wider distribution range.

Stylops nubeculae Pierce, 1909

Stylops nubeculae Pierce, 1909: 105, F. Type locality: USA, Colorado.

Host. *Andrena* (*Cnemidandrena*) *nubecula* Smith, 1853 (Pierce 1909).
Note. No DNA sequence is known for *Stylops* from this host subgenus.

Stylops nudae Pierce, 1911

Stylops nudae Pierce, 1911: 495, F. Type locality: USA, Illinois, Carlinville.

Host. *Andrena* (*Trachandrena*) *nuda* Robertson, 1891 (Pierce 1911).
Note. There are multiple *Stylops* species within the *Trachandrena* subgenus hosts. Without any DNA barcode sequence of *Stylops* from *A. nuda*, we cannot reliably suggest synonymy of this name.

Stylops packardi Pierce, 1909

Stylops packardi Pierce, 1909: 105, M. Type locality: USA, Massachusetts, Salem.

Host. *Andrena* (*Leucandrena*) *barbilabris* (Kirby, 1802) (Pierce 1909).
Note. No DNA sequence is known for *Stylops* from this host subgenus.

Stylops polemonii Pierce, 1909

Stylops polemonii Pierce, 1909: 106, F, L1. Type locality: USA, Colorado.
?= *Stylops pacificus* Bohart, 1936: 15, M, F, L1. Type locality: USA, California, Berkeley. **Supposed new junior subjective synonym.**
= *Stylops pacifica*: Bohart (1941: 128). Incorrect gender agreement.

Hosts. *Andrena* (*Euandrena*) *polemonii* Robertson, 1891 (Pierce 1909), *Andrena* (*E.*) *caerulea* Smith, 1879 (Bohart 1936, 1941), *Andrena* (*E.*) *suavis* Timberlake, 1938 (Bohart 1941).
Notes. Bohart (1941) omitted the name *S. polemonii* from his North American species review. According to the host association, this name is supposed to be a senior subjective synonym of *S. pacificus*.

No DNA sequence is known for *Stylops* from this host subgenus from the Nearctic Region.

Stylops shannoni (Pierce, 1919)

Neostylops shannoni Pierce, 1919: 457, M. Type locality: USA, Maryland, Plummers Island.
= *Stylops shannoni*: Bohart (1941: 125). New generic placement.

Host. Unknown.
Note. Name of *S. shannoni* was associated with findings of stylopized *A. hippotes* by Kenner (2002). However, he did not provide a description of the male or any other reliable species indication in the publication. If such an association is correct, *S. shannoni* will be a synonym of *S. hippotes*. The species status, which was described from a free living male, remains uncertain.

Stylops sparsipilosae Pierce, 1909

Stylops sparsipilosae Pierce, 1909: 108, F. Type locality: USA, Maine, Waldoboro.

Host. Unknown.
Note. Host name '*A. sparsipilosa* Viereck' presented by Pierce (1909) as host association is a nomen nudum (Krombein et al. 1979). Status of this *Stylops* species should be resolved using its type material.

Stylops subcandidae Pierce, 1909

Stylops subcandidae Pierce, 1909: 108, F, L1. Type locality: USA, Southern California.
?= *Stylops bisalicidis* Pierce, 1919: 446, F. Type locality: USA, Alabama. **Supposed new junior subjective synonym.**
?= *Stylops medionitans* Pierce, 1919: 450, F. Type locality: USA, Colorado, Florissant. **Supposed new junior subjective synonym.**
= *Stylops diabola* Pierce, 1919: 454, F. Type locality: USA, North Dakota, Devils Lake. Synonymized with *S. bisalicidis* by Bohart (1941: 131).

Hosts. *Andrena* (*Thysandrena*) *candida* Smith, 1879 (Pierce 1909), *A.* (*T.*) *bisalicis* Viereck, 1908 (Pierce 1919), *A.* (*T.*) *medionitens* Cockerell, 1902 (Bohart 1936, Pierce 1919), *A.* (*Scaphandrena*) *scurra* Viereck, 1904 (Bohart 1941).
DNA barcode sequences. KF803432, KF803509.
Notes. Pierce (1919) used the name *S. medionitans* for this species because he used incorrect spelling for the host species, *Andrena medionitans*. For this reason the *Stylops* name with 'a' is the correct original spelling of the name.

Distances between DNA barcode sequences of *S. subcandidae* and the other *Stylops* species are 12–21 % (Jůzová et al. 2015).

Stylops timberlakei Bohart, 1936

Stylops timberlakei Bohart, 1936: 14, M (holotype), F. Type locality: USA, California, Riverside.

Host. *Andrena* (*Oligandrena*) *macrocephala* Cockerell, 1906 (Bohart 1936).
Note. No DNA sequence is known for *Stylops* from this host subgenus.

Stylops vandykei Bohart, 1936

Stylops vandykei Bohart, 1936: 11, M (holotype), F, L1. Type locality: USA, California, Berkeley.

Hosts. *Andrena* (*Melandrena*) *perimelas* Cockerell, 1905, *Andrena* (*M.*) *pertristis carliniformis* Viereck & Cockerell, 1914 (Bohart 1936).
Note. No DNA sequence is known from this *Stylops* species.

Discussion

The diversity of the order Strepsiptera is poorly known, not only because of insufficient interest in the alpha taxonomy of the order Strepsiptera, but especially because of different species concepts that have been applied simultaneously to this insect group. In case of the genus *Stylops*, we did not know whether the genus included a single (Kinzelbach 1978, Pohl 2004) or a hundred (Kathirithamby 2014) of species in Europe for a long time. Recent molecular analysis (Jůzová et al. 2015) shows that there are numerous species in Europe, but certainly not hundred. Morphological characters of *Stylops*, which correspond to their host associations (Bohart 1941, Borchert 1963), also correspond to their DNA sequences. For this reason, we prepared a preliminary nomenclatoric list of all *Stylops* species as a new starting point for future taxonomic studies. As a result we found 32 West Palaearctic, 9 East Palaearctic and 27 Nearctic species names, which must be considered valid, in total 67 (one overlaps between the West and East Palaearctic Regions) valid species names of *Stylops* in the world. These numbers include also names of species with uncertain validity, especially those described based on free living males collected without knowledge of their host species. A few other uncertain names concern females from the host lineage (subgenus) known to be a host for multiple *Stylops* species, as well as names associated with rarely collected *Andrena* bees, which might only represent other rare hosts of common species, or rare host switches (Jůzová et al. 2015).

DNA distance analysis of COI barcode sequences of *Stylops* species show that the DNA based approach is applicable for this taxonomic group. However, neither strict species delimitation, nor universally definable distance between species can be postulated. Every species and every lineage need to be considered individually with biological rationality. In most cases, we can recognize 'clusters' of closely related individuals with low variability (<2 %) in DNA distances and relatively high (4–12 %) minimal DNA distance from any other individual. We consider a single 'cluster' to be a population of a single species. In a very few cases, the variability within the tentative species is slightly larger than 2 % (*S. nassonowi*, *S. spreta*). Such species are distributed over large geographic areas and distant populations probably represent original old populations. There are also several cases of possible different species, which differ in only 3 % or even in less than 1 % in DNA barcode sequence (e.g., *S. praecocis* vs. *S. nevinsoni*). These populations occur sympatrically, but differ in morphology (Borchert 1963) and seem to be genetically separated (Jůzová et al. 2015), and for these reasons the species are considered valid for the time being.

All the hypotheses about species delimitation that were proposed in our study should be tested in detail based on broader population genetic tools and/or morphological methods. The comprehensive morphological revision of the genus *Stylops* with definitions of the type material would be especially helpful for the future nomenclatoric stability. We recommend to continue in barcoding and sequencing other genes and using an integrative taxonomic approach (Gibbs 2009) to maintain certainty in the taxonomy of this peculiar group.

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PAPER III

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Rediscovered parasitism of *Andrena savignyi* Spinola (Hymenoptera, Andrenidae) by *Stylops* (Strepsiptera, Stylopidae) and revised taxonomic status of the parasite

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Abstract

Parasitism of *Andrena* (*Suandrena*) *savignyi* Spinola (Hymenoptera: Andrenidae) by *Stylops* Kirby (Strepsiptera: Stylopidae) has been recorded only once, and from an individual collected in Egypt almost a century ago, with the parasite described as *Stylops savignyi* Hofeneder. The recent rediscovery of this *Stylops* from an individual of *A. savignyi* permits a reinterpretation of the species and its affinities among other *Stylops*. The bee was collected at flowers of *Zilla spinosa* (Turra) Prantl. (Brassicaceae) in Amariah, Riyadh, Kingdom of Saudi Arabia. Based on DNA barcode sequences from material sampled across Africa, Asia, and Europe, it is apparent that *S. savignyi* is conspecific with *S. nassonowi* Pierce, and we accordingly synonymize this name (**syn. n.**), with the latter representing the senior and valid name for the species. A differential diagnosis is provided for *S. nassonowi* and the morphology of the female is described, as well as the first instars.

Keywords

Stylopidae, Apoidea, Anthophila, Andrenidae, parasitoid, taxonomy, morphology

Introduction

Strepsiptera (twisted-wing parasites) are an order of minute entomophagous insects that are found throughout the world. Despite the fact that Strepsiptera comprise relatively few species for a lineage of Holometabola (ca. 600 species), the breadth of hosts is considerable and includes at least seven insect orders (Zygentoma, Blattaria, Mantodea, Orthoptera, Hemiptera, Hymenoptera, and Diptera) (Kathirithamby 2009). There remains considerable debate about their relationship to other holometabolan lineages (e.g., Grimaldi and Engel 2005; Pohl and Beutel 2008, 2013), but most evidence tends to suggest they are near the Coleoptera (e.g., McKenna and Farrell 2010; Ishiwata et al. 2011; Niehuis et al. 2012; Boussau et al. 2014) and some authors in the past have even classified the group as a subordinate among the beetles (e.g., Crowson 1960). The internal phylogeny of Strepsiptera is less controversial in terms of the broader patterns of character transition (e.g., Kinzelbach 1971, 1978, 1990; Pohl 2002; Grimaldi et al. 2005; Pohl and Beutel 2005; Pohl et al. 2005; Bravo et al. 2009; McMahon et al. 2011), although more refined aspects among the 'higher' groups and within certain families are in need of revision. There are differences of opinion as to those families recognized, although there are usually 11–13 employed in most summaries of the classification (e.g., Pohl and Beutel 2005; Bravo et al. 2009; Kathirithamby and Engel 2014).

Contributing to the 'mystery' of the order is their complex parasitoid life cycle and conspicuous sexual dimorphism, with pronouncedly neotenic females. The male has an ephemeral, free-living adulthood, whereas adult females are obligatory endoparasites, with the sole exception of the basal family Mengenillidae, and are concomitantly tied to their host throughout their maturity (Kathirithamby 1989, 2009; Kinzelbach 1971). In those families more derived than Mengenillidae, adult females have a dramatically reduced body that is largely larviform and is positioned within the host's body. The more sclerotized cephalothorax of the female extrudes from the host and it is from here that she is able to mate and give birth to her brood. Males seek out parasitized hosts and mate with females who then in turn ultimately produce a large number of free-living first instar larvae, or triungulins (Kathirithamby 2009; O'Connor 1959), that disperse into the surrounding area (Linsley and MacSwain 1957). When the first instars locate a suitable host they attach and eventually invade the body (Kathirithamby 1989, 2009; Kathirithamby et al. 2001). A further complication in the system is found among those first instars of the families Xenidae and Stylopidae which must find a suitable vector that transports them to their new host (Kathirithamby et al. 2012; Linsley and MacSwain 1957). Xenids and stylopids parasitize species of the Euaculeata and they typically position themselves in locations (e.g., among flowers) that will place them into contact with foraging wasps or bees which they can then ride back to the nest and from there invade the brood cells and parasitize the developing immatures (Kinzelbach 1971; Kathirithamby 1989; Pohl and Beutel 2008). Because of this there can at times be disruptions to the developmental process of the host, resulting in noticeable phenotypic alterations (e.g., Smith and Hamm 1914; Salt 1927; Brandenburg

1953; Kathirithamby 1989, 1998; Solulu et al. 1998). Indeed, upon maturity the parasitized host is often sterile or even masculinized such that their ability to collect provisions and provision a new nest is diminished (Smith and Hamm 1914; Salt 1927, 1931), and their behavior altered toward aims other than reproduction (Westwood 1839; Kathirithamby and Hamilton 1995; Hughes et al. 2004; Beani 2006; Linsley and MacSwain 1957; Straka et al. 2011). Accordingly, the newly-emerged first-instar strepsipterans cannot rely on using the host from which they emerged as a vector to a newly established host, and continuing their life cycle requires encounters with new, unparasitized, young individuals (e.g., Kathirithamby et al. 2012). The first instars emerge from their parasitized host on flowers and wait for non-parasitized females of the host species to serve as a vector from the inflorescences to the host's nest. Within the nest the larvae seek fresh offspring as their final host. Understandably, such larvae are quite mobile, as are all strepsipteran triungulins, and well adapted for concealment and affixation to an appropriate host. For example, first-instar larvae of the genus *Stylops* Kirby have a number of morphological adaptations that provide for a stronger attachment to the host, such as structures on the dorsal and ventral surfaces of their body or enlargement of the pro- and mesotarsi (Pohl 2000; Pohl and Beutel 2004, 2008); however, their behavior on flowers is unknown.

The genus *Stylops* is the most diversified lineage of the family Stylopidae. Species are obligate parasites of solitary bees of the genus *Andrena* Fabricius (Kinzelbach 1971; Jůzová et al. 2015). The taxonomy of species in the genus is problematic, plagued by a plethora of ill-defined epithets established by authors but without defined hypotheses of circumscription for the biological units involved (Straka et al. 2015). In the past, host specificity was often used as the principle guide for species determination, sometimes in the absence of characters intrinsic to the parasite. While host association can be a good guideline, it does not apply universally across all species of *Stylops*. While some species are truly specialists, partial generalists do exist within the genus and these complicate matters for identification (Jůzová et al. 2015). In fact, there are useful morphological details in the first-instar larvae that are of considerable importance in identification and which, in combination with DNA sequences, are also known to reveal various cryptic species (Hayward et al. 2011; Nakase and Kato 2013). Some host-parasite associations are found rarely and for these every newly acquired specimen is an aid toward resolving long-standing taxonomic conundrums, and when suitable field observations are made also further information about possible host specializations, behavior, and ecology. Detailed and modern systematic and biological studies are needed across the order, and numerous hypotheses of species circumscription require critical investigation, with many having remained untested for a century or more.

One such taxonomic mystery that has persisted for nearly a century centers on the proper identity of *Stylops savignyi* Hofeneder (1924). Hofeneder (1924) described his species from two stylopized females of *Andrena* (*Suandrena*) *savignyi* Spinola collected in Egypt, each with one female *Stylops*. Since that time the true identity of this species has represented a persistent problem for the taxonomy of *Stylops*. Here we report the first find of stylopized *A. savignyi* from Saudi Arabia, females of which have

been found with their stylopid parasite since 1914 (when Hofeneder's material was collected) and represents a unique opportunity to address the circumscription and identity of *S. savignyi*. The species of *Stylops* collected in Saudi Arabia match those described by Hofeneder (1924) and are further identified using new morphological and DNA barcode sequence data. These data reveal the true identity of the parasite species as a new junior synonym of *S. nassonowi* Pierce (Pierce 1909) and allow for a modern characterization of the taxon.

Material and methods

Individuals of *A. savignyi* were collected mostly from flowers of *Zilla spinosa* (Turra) Prantl. (Brassicaceae) at five localities around Amariah, Riyadh, Saudi Arabia (Al Oyanah, Al Kharj, Rouma, Derab, and Al Amariah, the last of which was where most material was sampled), although the species has also been encountered at various localities throughout Saudi Arabia and the Arabian peninsula (e.g., Dathe 2009; Engel pers. obs.). Details of the collection site are available in Alqarni et al. (2012, 2014a), Engel et al. (2012), and Hannan et al. (2012). At the locality in which the stylopized bee was discovered, general collecting had been underway from September 2010 through September 2012, but all individuals of *A. savignyi* were found between 22 February and 10 March 2011 and with peak bee activities at flowers around 20–25 °C. Although there is a diversity flowers around Al Amariah, *A. savignyi* was only encountered at *Z. spinosa* and to a lesser degree at *Rhaphanus sativus* L. and *Eruca sativa* Mill. (both also of Brassicaceae), indicative of its oligolectic pollen-collecting preferences. The stylopized female was collected from *Z. spinosa*, and she made no attempt to collect pollen from the flowers. The cephalothoraxes of the two female *Stylops* is extruded between the bee's metasomal terga IV and V (Figs 1, 2), with one on either side of the midline (Fig. 4). Such an orientation is typical for a stylopized bee, where even when parasitized by a single female *Stylops*, the cephalothorax always protrudes from a more lateral position and never from the midline. Measurements of the parasite cephalothoraxes are shown in Table 1.

The specimens of *Stylops* examined for the present study (Appendix) were deposited in the King Saud University Museum of Arthropods, Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia (**KSMA**), and the personal collection of Jakub Straka housed at Charles University in Prague, Praha, Czech Republic (**JSPC**). Material in Saudi Arabia, and from which the new material of stylopized *A. savignyi* was sampled, has been collected as part of ongoing bee surveys throughout the country and undertaken by A.S.A., M.A.H., and M.S.E. (e.g., Alqarni et al. 2012a, 2012b, 2013, 2014a, 2014b, 2014c, 2014d; Engel et al. 2012, 2013, 2014; Hannan et al. 2012; Hinojosa-Díaz et al. in press). Those bees with Strepsiptera from other countries were collected into 90–96% ethanol, or with yellow pan-traps and then transferred to ethanol. Individual parasites were removed from dissected bees and subjected to further preparation. Female strep-

Figures 1–3. Female of *Andrena* (*Suandrena*) *savignyi* Spinola from Riyadh, Saudi Arabia parasitized by *Stylops nassonowi* Pierce **1** Dorsal habitus of bee **2** Lateral habitus of bee (image inverted); one female of parasite observable at apex of tergum IV **3** Detail of setae at bee's metasomal apex showing numerous first instars of the parasite.

sipterans studied for morphology were cleared using proteinase – a mixture of lysis buffer and proteinase K (Quiagen) heated to 56 °C. The lysis procedure took several hours or overnight. Cleared specimens were cleaned in water several times and then stored in vials with glycerol. Females were observed using an Olympus BX40 light microscope. Temporary slides were prepared with glycerol. First instar larvae were carefully removed from the body of the females and prepared for scanning electron microscopy (SEM) with a JEOL 6380 LV scanning electron microscope. Specimens

Figure 4. Metasomal apex of female of *Andrena* (*Suandrena*) *savignyi* Spinola from Riyadh, Saudi Arabia parasitized by *Stylops nassonowi* Pierce, and depicting two females of the parasite exposed from under the apex of tergum IV.

were dehydrated using progressively more concentrated (90%, 96%, and then 100%) ethanol, each for 5–10 minutes, and then in acetone for 5 minutes. Subsequently, dehydrated samples were critical point dried and coated with gold.

Morphological terminology of first-instar larvae follows that of Pohl (2000), while terminology for females and female puparia follows that of Kinzelbach (1971) and Straka et al. (2014). The following abbreviations were employed: 1L – first-instar larva, F – female, EMP – empty male puparium. The format for the description generally follows that used elsewhere in studies of stylopid systematics (e.g., Straka et al. 2014). Revised descriptions provide a modern framework for species circumscription and build diverse new character sources for studying bee-parasite evolution and systematics (e.g., Engel 2011; Gonzalez et al. 2013), as well as permit the elaboration of patterns of character variation and distribution, reveal relationships, and contribute to a broader understanding of evolution across a clade (e.g., Grimaldi and Engel 2007).

Table 1. Basic measurements of cephalothoraxes of *Stylops aterrimus* Newport and *S. nassonowi* Pierce (W = width at spiracles; L = length). All measurements in millimeters.

Species	Voucher	W	L	W of head	L of head	Intermandibular diameter
S. aterrimus	SAg1	1.35	1.29	0.77	0.34	0.14
	SBm1a	1.29	1.26	0.67	0.31	0.19
	SBm1b	1.34	1.24	0.69	0.29	0.21
	STig2	1.07	1.10	0.64	0.37	0.19
	Ssp1	1.27	1.20	0.67	0.33	0.21
	SCa7	1.31	1.17	0.70	0.31	0.21
	SCa8	1.20	1.17	0.64	0.31	0.19
S. nassonowi	SCa1	1.41	1.19	0.76	0.3	0.19
	SCa4	1.41	1.27	0.74	0.32	0.19
	SCa5	1.21	1.19	0.70	0.35	0.19
	SCa6	1.09	1.10	0.61	0.31	0.18
	SCa9a	1.19	1.17	0.70	0.32	0.20
	SCa9b	1.33	1.18	0.70	0.30	0.20
	SCa10	1.10	1.11	0.61	0.30	0.18
	SSg1	1.05	1.24	0.60	0.30	0.19
	SHo1	1.36	1.27	0.67	0.34	0.19
	STi2	1.16	1.04	0.70	0.30	0.17
	STi4	1.27	1.19	0.69	0.34	0.19
	STi6	1.26	1.19	0.69	0.31	0.19
	STi5	1.26	1.21	0.73	0.36	0.19

For DNA analysis, the entire body of a female strepsipteran was lysed by Proteinase K (Qiagen). Afterwards, DNA was isolated with a DNA Isolation Kit (Qiagen). Partial DNA sequences were amplified using the primers for Cytochrome oxidase subunit I (COI) (Jůzová et al. in press), and using an annealing temperature of 50 °C. Chromatograms were edited with the program Chromas Lite 2.01 (Technelysium Pty Ltd.) and aligned in BioEdit 7.0.9 (Hall 1999). The online application BLAST was used to reveal any potential contamination in the DNA samples, especially the possibility of amplifying any DNA from the host. Genetic distances were calculated using BioEdit 7.1.3.0 (Hall 1999), under standard computational procedures with the F84 model (Felsenstein 1984).

Distances in DNA base composition were compared pairwise (Table 2). The results show a non-random distribution of genetic distances among individuals in accordance with the published phylogeny of *Stylops* (Jůzová et al. 2015). In the case of material used here, the genetic distance under 2% suggests close relatives. The gap in DNA distance between related individuals within a species and other species is also 2% (1.5–2.5%). Genetic differentiation between the studied populations can be defined according to the present genetic relatedness and the gap.

Table 2. DNA distance matrix among samples of *Stylops nassonowi* Pierce, *S. aterrimus* Newport, and other representative species. Distances below 2% are highlighted yellow, showing closely related individuals; *Stylops* Kirby from *Andrena* (*Suandrena*) *savignyi* Spinola in Saudi Arabia indicated in red.

Stylops		***S. m.***	***S. nev.***	***S. spreta***	***S. m.***	***S. ater***	***S. a.***	***S. a.***	***S. a.***	***S. a.***	***S. a.***	***S. a.***	***S. a.***
	voucher	**SFl1**	**SFu1**	**SMi1**	**SNi1**	**SVa2**	**SAg1**	**SBm1**	**STig1**	**STig2**	**Ssp1**	**SCa7**	**SCa8**
S. nevinsoni	SFu1	0.1075	-	-	-	-	-	-	-	-	-	-	-
S. spreta	SMi1	0.1243	0.1456	-	-	-	-	-	-	-	-	-	-
S. melittae	SNi1	0.0035	0.1081	0.1243	-	-	-	-	-	-	-	-	-
S. ater	SVa2	0.1440	0.1451	0.1746	0.1453	-	-	-	-	-	-	-	-
S. aterrimus	SAg1	0.1300	0.1355	0.1511	0.1276	0.1437	-	-	-	-	-	-	-
S. aterrimus	SBm1	0.1176	0.1353	0.1448	0.1157	0.1337	0.0151	-	-	-	-	-	-
S. aterrimus	STig1	0.1300	0.1355	0.1511	0.1276	0.1437	0.0000	0.0151	-	-	-	-	-
S. aterrimus	STig2	0.1410	0.1465	0.1632	0.1383	0.1533	0.0017	0.0178	0.0017	-	-	-	-
S. aterrimus	Ssp1	0.1300	0.1374	0.1489	0.1276	0.1415	0.0017	0.0134	0.0017	0.0035	-	-	-
S. aterrimus	SCa7	0.1155	0.1331	0.1426	0.1136	0.1315	0.0168	0.0017	0.0168	0.0196	0.0151	-	-
S. aterrimus	SCa8	0.1176	0.1353	0.1448	0.1157	0.1337	0.0151	0.0000	0.0151	0.0178	0.0134	0.0017	-
S. nassonowi	SCa1	0.1286	0.1387	0.1385	0.1245	0.1484	0.0431	0.0414	0.0431	0.0438	0.0412	0.0433	0.0414
S. nassonowi	SCa2	0.1265	0.1337	0.1375	0.1242	0.1406	0.0410	0.0394	0.0410	0.0416	0.0393	0.0412	0.0394
S. nassonowi	SCa4	0.1285	0.1357	0.1394	0.1262	0.1426	0.0428	0.0411	0.0428	0.0434	0.0410	0.0429	0.0411
S. nassonowi	SCa5	0.1345	0.1418	0.1457	0.1321	0.1492	0.0446	0.0429	0.0446	0.0435	0.0427	0.0448	0.0429
S. nassonowi	SCa6	0.1265	0.1337	0.1375	0.1242	0.1406	0.0410	0.0394	0.0410	0.0416	0.0393	0.0412	0.0394
S. nassonowi	SCa9	0.1285	0.1357	0.1394	0.1262	0.1426	0.0428	0.0411	0.0428	0.0434	0.0410	0.0429	0.0411
S. nassonowi	SCa10	0.1330	0.1401	0.1439	0.1305	0.1428	0.0464	0.0447	0.0464	0.0472	0.0446	0.0466	0.0447
S. nassonowi	SHo1	0.1262	0.1335	0.1392	0.1240	0.1404	0.0375	0.0358	0.0375	0.0378	0.0357	0.0376	0.0358
S. nassonowi	SSa1	0.1290	0.1340	0.1397	0.1267	0.1410	0.0411	0.0395	0.0411	0.0417	0.0393	0.0413	0.0395
S. nassonowi	SSg1	0.1285	0.1357	0.1394	0.1262	0.1426	0.0428	0.0411	0.0428	0.0434	0.0410	0.0429	0.0411
S. nassonowi	STi1	0.1285	0.1357	0.1394	0.1262	0.1426	0.0428	0.0411	0.0428	0.0434	0.0410	0.0429	0.0411
S. nassonowi	STi2	0.1285	0.1357	0.1394	0.1262	0.1426	0.0428	0.0411	0.0428	0.0434	0.0410	0.0429	0.0411
S. nassonowi	STi4	0.1285	0.1357	0.1394	0.1262	0.1426	0.0428	0.0411	0.0428	0.0434	0.0410	0.0429	0.0411
S. nassonowi	STi6	0.1265	0.1337	0.1375	0.1242	0.1406	0.0410	0.0394	0.0410	0.0416	0.0393	0.0412	0.0394

Table 2. Continued.

Stylops		*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*	*S. nass.*
	voucher	**SCa1**	**SCa2**	**SCa4**	**SCa5**	**SCa6**	**SCa9**	**SCa10**	**SHo1**	**SSa1**	**SSg1**	**STi1**	**STi2**	**STi4**
S. nevinsoni	SFu1	-	-	-	-	-	-	-	-	-	-	-	-	-
S. spreta	SMi1	-	-	-	-	-	-	-	-	-	-	-	-	-
S. melittae	SNi1	-	-	-	-	-	-	-	-	-	-	-	-	-
S. ater	SVa2	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aterrimus	SAg1	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aterrimus	SBm1	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aterrimus	STig1	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aterrimus	STig2	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aterrimus	Ssp1	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aterrimus	SCa7	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aterrimus	SCa8	-	-	-	-	-	-	-	-	-	-	-	-	-
S. nassonowi	SCa1	-	-	-	-	-	-	-	-	-	-	-	-	-
S. nassonowi	SCa2	0.0035	-	-	-	-	-	-	-	-	-	-	-	-
S. nassonowi	SCa4	0.0017	0.0017	-	-	-	-	-	-	-	-	-	-	-
S. nassonowi	SCa5	0.0018	0.0052	0.0035	-	-	-	-	-	-	-	-	-	-
S. nassonowi	SCa6	0.0000	0.0033	0.0017	0.0017	-	-	-	-	-	-	-	-	-
S. nassonowi	SCa9	0.0017	0.0017	0.0000	0.0035	0.0017	-	-	-	-	-	-	-	-
S. nassonowi	SCa10	0.0053	0.0050	0.0033	0.0070	0.0050	0.0033	-	-	-	-	-	-	-
S. nassonowi	SHo1	0.0141	0.0134	0.0151	0.0157	0.0134	0.0151	0.0185	-	-	-	-	-	-
S. nassonowi	SSa1	0.0106	0.0101	0.0118	0.0123	0.0101	0.0118	0.0152	0.0135	-	-	-	-	-
S. nassonowi	SSg1	0.0017	0.0017	0.0000	0.0035	0.0017	0.0000	0.0033	0.0151	0.0118	-	-	-	-
S. nassonowi	STi1	0.0017	0.0017	0.0000	0.0035	0.0017	0.0000	0.0033	0.0151	0.0118	0.0000	-	-	-
S. nassonowi	STi2	0.0017	0.0017	0.0000	0.0035	0.0017	0.0000	0.0033	0.0151	0.0118	0.0000	0.0000	-	-
S. nassonowi	STi4	0.0017	0.0017	0.0000	0.0035	0.0017	0.0000	0.0033	0.0151	0.0118	0.0000	0.0000	0.0000	-
S. nassonowi	STi6	0.0000	0.0033	0.0017	0.0017	0.0000	0.0017	0.0050	0.0134	0.0101	0.0017	0.0017	0.0017	0.0017

Systematics

Genus *Stylops* Kirby

Stylops nassonowi Pierce
Figs 4–13, 20–26

Stylops nassonowi Pierce 1909: 105 [F]. Resurrected name [previously synonymized with *S. melittae* Kirby by Kinzelbach (1978)].
Stylops savignyi Hofender 1924: 254 [F]. New synonyms.

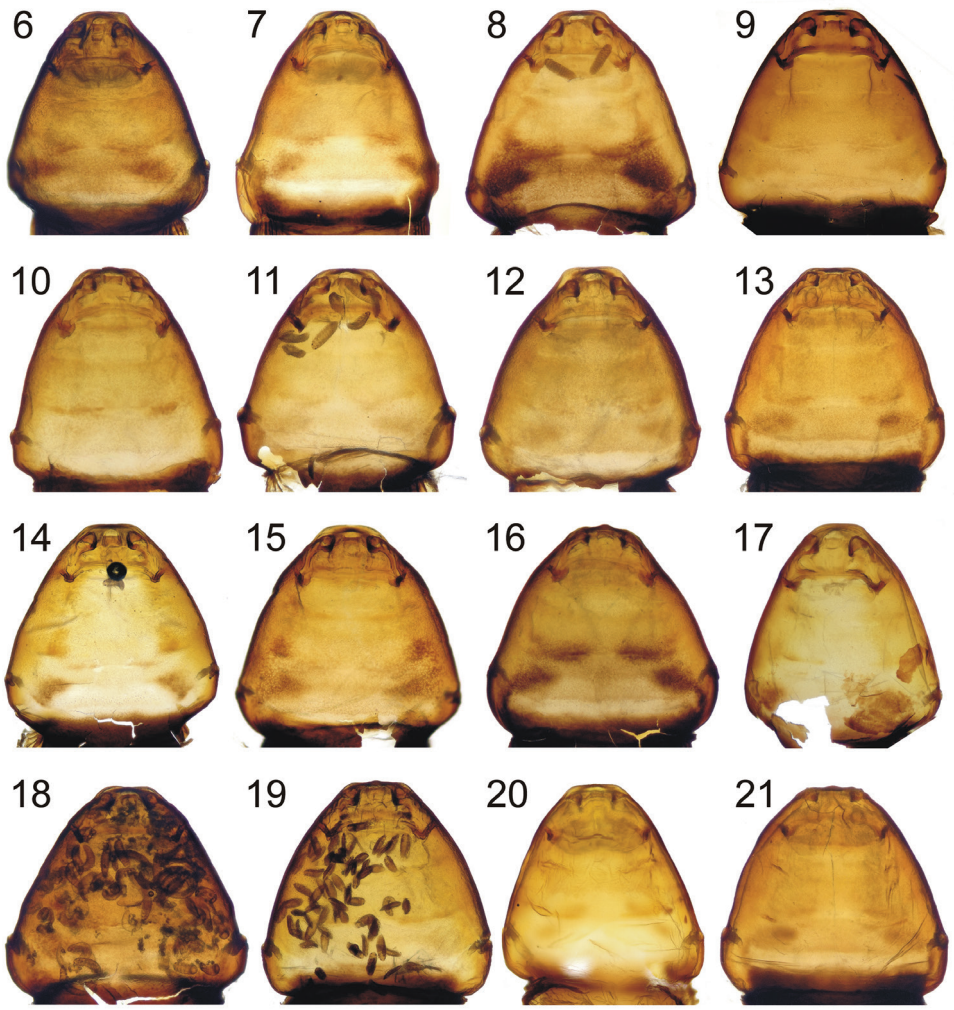
Diagnosis. Female puparium. The female puparium of *S. nassonowi* is almost indistinguishable from its sibling species, *S. aterrimus* Newport (compare Figures 6–13, with Figures 14–19). There is probably no stable character that could differentiate female puparia of both species in terms of their morphology and coloration. However, the following few characters occur in one of the species with a higher probability, or are more pronounced in one of the two species: *Stylops nassonowi* has the prothoracic flange of the brood opening typically more produced forward, less numerous mandibular sensilla (less than 10), and pigmentation of the prothorax more uniform except a pale apical part to the abdominal segment of the cephalothoracic venter (well visible in Figures 12, 13). By contrast, *S. aterrimus* is more complex in pigmentation than *S. nassonowi*, its dark markings on the ventral surfaces of the meso- and metathorax are usually well-developed and the metathorax has a more or less distinct transverse dark band, ultimately giving its apical half a nuanced darker appearance than the basal half (well visible in Figures 14–16). *Stylops nassonowi* differs from other species (such as when compared to *S. ater* Reichert, *S. melittae* Kirby, *S. nevinsoni* Perkins, *S. spreta* Perkins, and *S. thwaitesi* Perkins) mainly in body and head size (larger than *S. nevinsoni*, *S. spreta*, and *S. thwaitesi*), in the short, dark, basal band (large dark basal band in *S. ater*, *S. nevinsoni*, *S. spreta*, and *S. thwaitesi*), described coloration of the cephalothorax, in the shape of the prothoracic flange of the brood opening (strongly curved in *S. thwaitesi*; straight in *S. spreta*; uniformly curved in *S. melittae*, but slightly curved in *S. aterrimus* and *S. nassonowi*), in the shape of the head corners (strongly curved in *S. spreta*, but only slightly curved in the other species), in the shape and sclerotization of the hypostomal and cephalic ridges (strongly sclerotized and dark in *S. melittae*, but less pronounced in the other species), and the length of the clypeal sensilla.

First instar. Body elongate as in other species of *Stylops* except for *S. melittae*, which has wider abdomen. Head dorsally with two olfactory foveae and four pairs of setae in contrast to *S. melittae*, which has seven pairs of setae and no foveae. The frontal margin of the maxillae is not sagging in *S. aterrimus* and *S. melittae*, in contrast to that of *S. nassonowi*. The cervix is indistinct in *S. nassonowi* rather than more defined in *S. melittae*, the latter possessing a narrower head ventrally. The caudal margins of the dorsal segments have spinullae, except for the pro- and mesothoracic segments, which are covered basally (bases are covered by the tergal margin and therefore not visible rather

Figure 5. Detail from Figure 4 showing one female of *Stylops nassonowi* Pierce and numerous emergent first instars.

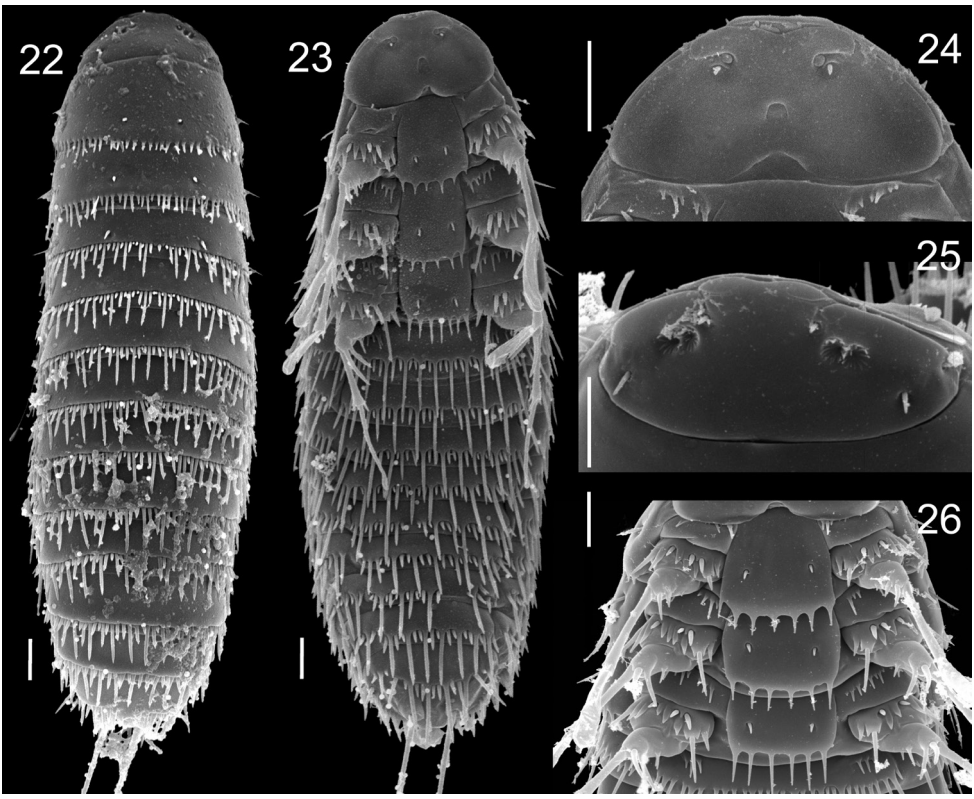
than fully exposed), while in *S. melittae* some spinullae are covered and there is a gap in the center of dorsum where no spinullae are present. The sternal plates are broader than in *S. melittae*.

Redescription. Female and female puparium. Head two times wider than long, width to length 1.97–2.53 (n = 13, $\bar{x}$ = 2.15 mm), width 0.60–0.76 mm ($\bar{x}$ = 0.68 mm), length 0.30–0.36 mm ($\bar{x}$ = 0.32 mm); head posteriorly defined by single incomplete or ill-defined cephalic ridge on dorsal surface, paired cephalic ridge on ventral surface and posterior head thickening (lower margin of brood opening). Head corners short and narrow on ventral surface, slightly diverging posteriorly, head corners shorter than head on dorsal surface laterally, but inner posterior extension of ventral cephalic ridge (joint of ventral cephalic ridge and posterior head thickening) extends as far as head posterior margin on dorsal surface; ventral cephalic ridge posteromedially oriented;



Figures 6–21. Ventral (6–19) and dorsal views (20, 21) of cephalothoraxes of female puparia from *Stylops nassonowi* Pierce (6–13, 20, 21) and *S. aterrimus* Newport (14–19) **6** Voucher SCa5 (Czech Republic) **7** Voucher SCa6 (Czech Republic) **8** Voucher SHo1 (Turkey) **9** Voucher SSa1 (Saudi Arabia) **10** Voucher SSg1 (Czech Republic) **11** Voucher STi2 (Hungary) **12** Voucher STi4 (Czech Republic) **13** Voucher STi6 (Czech Republic) **14** Voucher SAg1 (Tunisia) **15** Voucher SBm1a (Czech Republic) **16** Voucher SBm1b (Czech Republic) **17** Voucher STig2 (Tunisia) **18** Voucher SCa7 (Switzerland) **19** Voucher Ssp1 (Tunisia) **20** Voucher SCa10 (Czech Republic) **21** Voucher STi6 (Czech Republic).

head corners not produced laterally beyond prothorax, head narrower than prothorax and thus cephalothorax continuously diverging posteriorly. Mandibles large, not extending from head contour in ventral view; inner apical tooth well-developed; apex ventrally with 5–8 sensilla, intermandibular distance 0.17–0.20 mm ($\bar{x}$ = 0.19 mm). Labiomaxillary area about 2–2.5× longer than wide; maxillary area distinctly prominent, overlapping mandible at about one third of its width, maxilla with 7–16 sensilla



Figures 22–26. First instar of *Stylops nassonowi* Pierce **22** Dorsal view **23** Ventral view **24** Detail of head, ventral view **25** Detail of head, dorsal view **26** Thoracic segments, ventral view. Scale bars: 10 μm.

laterally; labial area without sensilla, more or less prominent and faintly divided into two parts medially (probably postmentum and prementum). Oral ridge (hypopharynx) well developed, rectangular, apically straight, occupying about half of intermandibular area; epipharynx slightly produced, pale, about as long as oral ridge. Hypostomal ridge (from outer margin of mandible to cephalic ridge and separating maxillary area from head corner) slightly sinuous, about as long as intermandibular distance or slightly longer. Labral area well developed, large, arcuate apically, slightly darker than clypeus in most specimens. Clypeus transverse, exceeding mandibles laterally and apically, apex straight or slightly concave, lateral corners prominent, with about 10–30 short sensilla laterally. Brood opening wide, distinctly wider than distance between mandibles; prothoracic flange (dorsal cover of brood opening) sclerotized, arcuate, laterally curved more than medially, apical margin almost straight, in some specimens more produced forward than in others; posterior head thickening (lower margin of brood opening) more uniformly arcuate than flange; overlap of prothoracic flange and posterior head thickening relatively short, about as long as cephalic ridge thick; joint of posterior head thickening and ventral cephalic ridge small, often serrate, slightly lighter than cephalic ridge. Cephalothorax usually slightly wider than long, but longer than

wide in some specimens, width to length 0.85–1.18 ($\bar{x}$ = 1.05), width 1.05–1.41 mm ($\bar{x}$ = 1.24 mm), length 1.04–1.27 mm ($\bar{x}$ = 1.18 mm); cephalothorax compact, all segments fused, pigmentation denser laterally than medially. Pro- and mesothoracic intersegmental ridges distinct medially on ventral surface; paired pro- and mesothoracic ridges variable in size, usually distinct on dorsal surface. Pro- and mesothorax uniformly light yellowish-brown except pale prothoracic ridge and slightly darker surrounding integument, posterior part of mesothorax with pair of dark brown spots variable in size (absent in some specimens), distinct lighter area in center of mesothoracic ridge; metathorax uniformly pigmented with paired posterolateral dark brown spots (absent in some specimens); abdominal part of cephalothorax dichromatic, apical part lightest of cephalothorax, nearly transparent, and basal band dark brown, basal band short, not extending toward spiracles, division between basal band and remainder of cephalothorax nearly straight in all parts. Spiracles not prominent, positioned at widest part of posterior part of cephalothorax. Canalis prolifer on abdominal segments I–VII; single median tuba prolifera positioned on posterior third of segments II–VI.

First instar. Body length 135–192 μm (without caudal setae); caudal setae approximately one half body length; with minute terminal leaf-like structure ("Haftlappen": *vide* Pohl 2000). Head dorsally with four pairs of setae and two olfactory foveae. Mandibles with short setae. Maxillae distinct; frontal margin of maxillae emarginate; rudimentary maxillary palpi circular; ventral opening of praeoral cavity semicircular and isolated from cervix; labium reduced.

Posterior margin of dorsal tergites with spinullae, all spinullae covered basally by tergal margin except for pro- and mesothoracic segments. Each thoracic tergite with two submedian and lateral rows of setae. Coxae broad; each coxa bearing one coxal bristle and 6–7 cuticular outgrowths distributed among three coxal teeth at anterior part of coxa; coxal bristle on pro- and mesothorax at least two times as long as coxal teeth. Trochanterofemur always with femoral spur and bristle almost as long as coxal bristle, and one cuticular outgrowth. Pro- and mesotarsi elongate and slightly enlarged, metatarsi rod-like. Sternal plates broad, with one pair of setae on each plate, with a few outgrowths (about 6) on their posterior margins. Precoxal pleural membrane with small number of microtrichia (about 3) on prothorax, and with transverse row of microtrichia on meso- and metathorax. Short row of cuticular outgrowths ("Spinulaeplatte" *sensu* Borchert 1963) on sternite I. Posterior margins of abdominal sternites with spinullae, some spinullae covered basally. Abdominal segment X with anus, shortened and fused with segment IX, positioned dorsally; segment XI split in two parts and positioned ventrally, bearing caudal setae.

DNA sequences. *Stylops nassonowi* differs significantly in DNA barcode sequence distance, which is consistently about 4% or more from other species, including *S. aterrimus*. At the same time, the distances within the species are about 1.5% in distance or even less (Table 2). The only exception is an individual collected in eastern Turkey, which differs from all other sequenced individuals of *S. nassonowi* in 1.3–1.9% distance and might represent an isolated population or perhaps different subspecies. Greater sampling is needed across the distribution of the species, particularly the Levant and elsewhere in Arabia.

Discussion

Pierce (1909) described *S. nassonowi* based on a figure provided by Nikolai V. Nasonov (1855–1939) in a comparative morphological study of material the latter ascribed to *S. melittae* and had taken from a female of *A.* (*Plastandrena*) *pilipes* Fabricius (Nasonov 1893a, 1893b). In establishing his new species, Pierce (1909) listed both Germany and Egypt as comprising type localities [referring to the host as *A. carbonaria* (Linnaeus), often considered the senior synonym for *A. pilipes*]; however, no specific locality is mentioned by Nasonov (1893a, 1893b), who could have had material from various places across the Palaearctic. At the time Pierce was publishing, available records of stylopized *A. pilipes* and ascribed by Pierce (1909) to *S. nassonowi* were known from Egypt (Saunders 1872), France (Pérez 1886), and Germany (Friese 1891), and it was from the former and the latter that he likely based his designation. Given this, we consider the type locality to be uncertain and clarification will rely on the eventual designation of a neotype as Nasonov's material is apparently no longer extant. We have hesitated from designating a neotype herein as further investigation into the ultimate disposition and survival of Nasonov's collection is needed.

Phylogenetic analysis of species of *Stylops* sampled from a diversity of hosts (Jůzová et al. 2015) coupled with the new DNA barcode sequences of the present study further demonstrate that the *Stylops* collected in Saudi Arabia belong to the species complex consisting of *S. aterrimus* and *S. nassonowi*. From the results we are able to define an eastern lineage, the oldest available name of which is *S. nassonowi* and a western lineage which accords with *S. aterrimus*. These results further establish the synonymy of *S. savignyi* from *A. savignyi* as a synonym of *S. nassonowi*, and the species appears to be a partial generalist, victimizing multiple species in separate subgenera of *Andrena* (*Plastandrena* Hedicke and *Suandrena* Warncke) (Appendix).

Stylops aterrimus and *S. nassonowi* are close sibling species and are almost indistinguishable morphologically. The two lineages exhibit sequence distances of about 4%, which is quite distinct when compared to many other species. Although we readily admit that there is no definable metric value of percent sequence difference for conferring specific status, 4% is greater than many other closely related species that are easily diagnosed on the basis of additional characters outside of the sequences themselves. Intraspecific variance in the DNA distances of each species is well below 2% and the variability is not overlapping (Table 2), further suggestive of individual evolutionary lineages. Both of these species are more than 10% distant from other common species of *Stylops* in terms of their DNA barcode sequence (Table 2: Jůzová et al. 2015). *Stylops aterrimus* and *S. nassonowi* seem to be largely allopatric across Europe, with their place of contact around the Czech Republic, where both species were recorded although not necessarily from precisely the same locality within that country. The border of contact between the two species is, of course, expected in other countries through Central Europe as well as in northern Africa. This split into a western and eastern species is perhaps a reflection of Pleistocene glaciation across Europe during the Pleistocene, as areas such as western France and Spain were spared from extensive ice coverage, while the same was true for the Italian Peninsula and

Balkans, with some narrow corridors of contact north of the Alps (Ehlers and Gibbard 2004; Ehlers et al. 2011). Naturally, such a pattern of distribution and contact requires further testing through the acquisition of considerably more material, and finer-scale phylogeographic study, ideally coupled with some degree of calibration for purposes of dating. For the moment our limitations largely reflect the infrequent collection of strepsipterans, particularly as many entomologists ignore the presence of such parasites.

The present study demonstrates how a seemingly happenstance and serendipitous encounter with a stylopized female of *A. savignyi* permitted a significant shift in a long-standing taxonomic obstacle. Clarification of the identity of *S. savignyi* provides one further step toward a revised classification of *Stylops* supported by both morphological and molecular data. Given the increased awareness of native pollinators (many of which are wild bees) and their importance for ecosystem health, numerous initiatives are underway to study such species. These endeavors are making available new samples from previously under-collected regions and with this increased effort the probability of acquiring fresh material of their parasites, some unseen for decades. Melittologists and pollination biologists should develop an awareness and maintain alertness for stylopized females, and where possible obtain data on their impact on the host's behavior and development as it not only makes less known the Strepsiptera but simultaneously enhances our knowledge of the hosts.

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Appendix

Material of species of *Stylops* used for taxonomic comparison

Here we provide specimen and collective event details for the various specimens of species of *Stylops* used in our comparative studies, along with the hosts from which they were sampled. In addition, specimens tied to specific sequences deposited in GenBank are identified and their numbers provided.

Stylops ater Reichert

Stylops ater Reichert 1914: 151 [♂]. Type locality: Merseburg, Germany.

Material examined. Czech Republic: Bohemia: Prokopské údolí, Praha-Jinonice, 1F, host: *A.* (*Melandrena*) *vaga* Panzer, 1♂, 13.iii.2007, J. Straka lgt., voucher SVa2, DNA barcode, GenBank: KF803529.

Stylops aterrimus Newport

Figs 14–19
Stylops spencii auctorum (*nec* Pickering 1836).
Stylops aterrimus Newport 1851: 340 [♂]. Type locality: Hampstead, Great Britain.

Material examined. Czech Republic: Bohemia, Velký Luh, sandpit, 2FF+1EMP, host: *A.* (*Plastandrena*) *bimaculata* (Kirby), 1♀, 20.iv.2010, J. Straka lgt., voucher SBm1, DNA barcode, GenBank: KP213298. **Switzerland**: Zürich env., 1F, host: *A.*

(*Hoplandrena*) *carantonica* Pérez, 1F, 25.v.2010, collector unknown, voucher SCa7, DNA barcode, GenBank: KP213300; ditto, 1F+1EMP, voucher SCa8, DNA barcode, GenBank: KP213299. **Tunisia**: Gafsa env., 1F, host: *A.* (*P.*) *bimaculata*, 1F, 1.iv.2006, J. Batelka et J. Straka lgt., voucher STig2, DNA barcode, GenBank: KF803522; Tamerza env., 1F, host: *A.* (*Agandrena*) *agilissima* (Scopoli), 1F, 31.iii.2006, J. Batelka et J. Straka lgt., voucher SAg1, DNA barcode, GenBank: KF803428; ditto, 1F, host: *A.* (*P.*) *bimaculata*, 1F, voucher STig1, DNA barcode, GenBank: KF803521; Wadi Raml, 4.5 km E Douz, 1F, host: *A.* (*H.*) sp., 1F, 4.iv.2006, J. Batelka et J. Straka lgt., voucher Ssp1, DNA barcode, GenBank: KF803504.

Stylops melittae Kirby

Stylops melittae Kirby 1802: 113 [♂]. Type locality: not indicated.

Material examined. Czech Republic: Bohemia, Čelákovice env., 1F, host: *A.* (*Zonandrena*) *flavipes* Panzer, 1♂, 1.v.2006, J. Batelka lgt., voucher SFl1, DNA barcode, GenBank: KF803453; Bohemia, Prokopské údolí, Praha-Jinonice, 1F, host: *A.* (*Melandrena*) *nigroaenea* (Kirby), 1♀, 6.iv.2009, J. Straka lgt., voucher SNi16, DNA barcode, GenBank: KF803488.

Stylops nassonowi Pierce

Figs 4–13, 20–26

Stylops nassonowi Pierce 1909: 105 [F]. Type locality: 'Egypt and Germany' (*vide* Discussion).
Stylops savignyi Hofender 1924: 254 [F]. Type locality: Egypt.

Material examined. Czech Republic: Bohemia, Divoká Šárka, Praha-Liboc, 1F, host: *Andrena* (*Hoplandrena*) *carantonica* Pérez, 1♂, 15.iv.2006, J. Straka lgt., voucher SCa2, DNA barcode, GenBank: KF803434; Bohemia, Chvalské skály, Praha-Horní Počernice, 2FF, host: *A.* (*H.*) *carantonica*, 1♀, 3.vi.2005, J. Straka lgt., voucher SCa9, DNA barcode, GenBank: KF803436; Bohemia, Sušice env., 1F, host: *A.* (*Plastandrena*) *tibialis* (Kirby), 1♀, 9.iv.2007, L. Dvořák lgt., voucher STi1, DNA barcode, GenBank: KF803518; ditto, 2FF, voucher STi6, DNA barcode, GenBank: KP213303; Bohemia, Závišín, Blatná env., 1F, host: *A.* (*P.*) *tibialis*, 1♂, 4.iv.2009, P. Bogusch lgt., voucher STi4, DNA barcode, GenBank: KP213302; Moravia, Dolní Dunajovice env., 1F+1EMP, 16.iv.2007, P. Bogusch lgt., voucher SCa10, DNA barcode, GenBank: KP213304; Moravia, Dolní Věstonice env., 1F, host: *A.* (*H.*) *carantonica*,

1♂, 5.iv.2008, J. Batelka et J. Straka lgt., voucher SCa5, DNA barcode, GenBank: KP213305; Moravia, Dolní Věstonice env., 1F+1EMP, host: *A.* (*H.*) *carantonica*, 1♂, 6.iv.2009, P. Bogusch lgt., voucher SCa6, DNA barcode, GenBank: KF803435; ditto, 3FF, host: *A.* (*H.*) *spinigera* (Kirby), 1♂, voucher SSg1, DNA barcode, GenBank: KF803503; Moravia, Lednice env., 1F, host: *A.* (*H.*) *carantonica*, 1♀, 13.vi.2006, J. Straka lgt., voucher SCa1, DNA barcode, GenBank: KF803433; **Hungary**: Budaörs, Budapest env., 1F, host: *A.* (*H.*) *carantonica*, 1♀, 25.iv.2009, J. Straka et P. Bogusch lgt., voucher SCa4, DNA barcode, GenBank: KP213301; Örkeny (puszta), 1F, host: *A.* (*P.*) *tibialis*, 1♂, 24.iv.2009, J. Straka et P. Bogusch lgt., voucher STi2, DNA barcode, GenBank: KF803519; **Saudi Arabia**: Riyadh, Al Amariah, Majra Al-gasim, 2FF, host: *A.* (*Suandrena*) *savignyi* Spinola, 1♀, 5.iii.2011, M.A. Hannan lgt., voucher SSa1, DNA barcode, GenBank: KP213306; **Turkey**: Hakkari prov., Gözeldere 25 km E, 1F, host: *A.* (*P.*) sp., 1♀, 22.vi.2010, Mi. Halada lgt., voucher SHo1, DNA barcode, GenBank: KF803463.

***Stylops nevinsoni* Perkins**

Stylops nevinsoni Perkins 1918: 71 [F]. Type locality: Great Britain.

Material examined. Czech Republic: Bohemia, Chýnice, 1F, host: *A.* (*A.*) *fulva* (Müller), 1♀, 22.iv.2006, J. Batelka et J. Straka lgt., voucher SFu1, DNA barcode, GenBank: KF803457.

***Stylops spencei* Pickering**

Stylops spencei Pickering 1836: 168 [F]. Type locality: Great Britain.

Material examined. Czech Republic: Bohemia, Chýnice, 1F, host: *A.* (*Micrandrena*) *minutula* (Kirby), 1♀, 22.iv.2006, J. Batelka et J. Straka lgt., voucher SMi1, DNA barcode, GenBank: KF803477.

***Stylops thwaitesi* Perkins**

Stylops thwaitesi Perkins 1918: 70 [♂, F]. Type locality: Great Britain.

Material examined. Spain: Maranchón 3km NW, Castilla-La Mancha prov., 1F, host: *A.* (*Taeniandrena*) *albofasciata* Thomson, 1♀, 10.iv.2012, K. Černá, K. Jůzová et J. Straka lgt., voucher SOv3, DNA barcode, GenBank: KF803494.

PAPER IV

Straka, J., **Jůzová, K.**, Batelka, J., 2014. A new genus of Strepsiptera, *Rozenia* gen. n. (Stylopidae), a parasite of bee genera *Acamptopoeum* and *Calliopsis* (Andrenidae, Panurginae, Calliopsini). Zookeys 31–49.

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A new genus of Strepsiptera, *Rozenia* gen. n. (Stylopidae), a parasite of bee genera *Acamptopoeum* and *Calliopsis* (Andrenidae, Panurginae, Calliopsini)

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Abstract

A new Strepsiptera genus from South America is described, *Rozenia* **gen. n.**, with three new species: *R. calliopsidis* **sp. n.** (type species), *R. peruana* **sp. n.** and *R. platicephala* **sp. n.** These three new species are parasites of bees belonging to the tribe Calliopsini (Andrenidae, Panurginae). *Rozenia calliopsidis* **sp. n.** is a parasite of the bee genus *Calliopsis* Smith, 1853 and *R. peruana* **sp. n.** and *R. platicephala* **sp. n.** are parasites of the bee genus *Acamptopoeum* Cockerell, 1905. Diagnoses and descriptions of female puparia are presented for all three species. Diagnoses and descriptions of first instars (triungulinids) are presented for *R. calliopsidis* **sp. n.** and *R. platicephala* **sp. n.** The first case of increased number of setae on the body of the first instars and augmentation of chaetotaxy of Strepsiptera are discussed.

Keywords

New genus, new species, Apoidea, host-parasite association, morphology, chaetotaxy, description, South America

Introduction

Stylopization of a bee from the tribe Calliopsini (Andrenidae, Panurginae) was recorded for the first time as early as 1931 (Schwarz 1931). Another finding was presented shortly afterwards from Argentina (Hofeneder and Fulmek 1943). Both records were repeated in the literature several times under various combinations of the host names (Hofeneder and Fulmek 1943, Hofeneder 1949, 1952) that recently belong to the genus *Acamptopoeum* Cockerell, 1905. However, no other record of stylopization of a bee from the tribe Calliopsini has been published since that time.

Members of the tribe Calliopsini are not the only known stylopized panurgine bees. Pierce (1904) published a note on a stylopized bee from the tribe Protandrenini and subsequently added data on stylopization of a wider range of species from the tribe Protandrenini from North America (Pierce 1909). All these North American panurgine hosts of Strepsiptera belong to the genus *Pseudopanurgus* Cockerell, 1897; Strepsiptera parasitizing Protandrenini are also known from South America (Holmberg 1921, Ogloblin 1947, Kogan 1989). These hosts belong to the genera *Anthrenoides* Ducke, 1907, *Psaenythia* Gerstaecker, 1868 and *Rhophitulus* Ducke, 1907. There are also two other genera of Panurgini known to be hosts of Strepsiptera in the Palearctic region. The first note about stylopized *Panurgus* Panzer, 1806 (Panurgini) was made by Morice (1913) and later, Ogloblin (1925) recorded Strepsiptera from the genus *Panurginus* Nylander, 1848.

All described Strepsiptera, which parasitize panurgine bees, were placed in the genus *Crawfordia* Pierce, 1908. All bees were from Neotropical, Nearctic, or Palearctic regions. Regarding the Strepsiptera that parasitize bees from the tribe Calliopsini, no taxon has ever been described, even though the host-parasite association has been known for more than eighty years. Here we present a new genus of Strepsiptera associated with the bee tribe Calliopsini, with a description of three new species. We compare the morphology of female puparia and the first instars with other genera of Strepsiptera, and particularly with species parasitizing bees (Stylopidae), especially other members of the bee subfamily Panurginae.

Methods

Collections

Material from the following public and private collections was examined:

AMNH American Museum of Natural History, Jerome G. Rozen Jr., (New York, USA);
JSPC Jakub Straka personal collection, (Praha, Czech Republic);
KUNHM Natural History Museum, Division of Entomology, University of Kansas, Michael S. Engel, (Lawrence, Kansas, USA).

Preparation of material

All host individuals were first relaxed and then dissected. Females and first instar larvae were removed from the host body. Strepsiptera females studied for morphology were cleared using proteinase: a mixture of lysis buffer and proteinase K (Quiagen) was heated to 56 °C. The lysis procedure took several hours or overnight. Cleared specimens were cleaned in water several times and then stored in vials with glycerol. A drawing tube (camera lucida) was attached to an Olympus BX40 light microscope and an Olympus SZX9 binocular microscope and used for morphological studies and drawings. Temporary slides were prepared with glycerol.

First instar larvae were removed from the female's body. Specimens used for morphological studies were prepared using the same method as females, except for scanning electron microscopy (SEM). For SEM, first instars were stored in 96% ethanol and subsequently dehydrated in 100% ethanol for 5–10 minutes and then acetone for 5 minutes. Dehydrated specimens were critical-point dried and coated with gold. For scanning electron microscopy we used a JEOL 6380 LV.

Morphology and terminology

External structures of first instars and female puparia are described. The mature or teneral female is presented inside the external puparium, but these have never been used for species descriptions. The body is weakly sclerotized and, in addition to the number of birth organs (tubae proliferae), lacks any practical characters.

Morphological terminology of female puparium follows Kinzelbach (1971) except:

basal band	pigmented external part of abdominal segment I, usually distinct on ventral side;
cephalic ridge	intersegmental ridge between head and prothorax on ventral side;
cintum	constriction dividing inner and outer part of tergum I;
head corner	lateral extensions of head behind brood opening on ventral side;
oral ridge	mouth sensu Kinzelbach (1971);
prothoracic ridge	intersegmental ridge between prothorax and mesothorax on ventral side.

Terminology of first instar larvae follows Pohl (2000, 2002) except:

interstitial row of setae	additional row of setae between submedian and supralateral row on thoracic tergites.

Specimens of strepsipterans are indicated by the following abbreviations: EMP – empty male puparium; MP – male puparium; FP – female puparium; L1 – first instar larva.

Description style

All newly described species were labeled as follows: "HOLOTYPUS FP, name of taxon sp. nov., Jakub Straka det. 2014" on red card; paratypes analogously on yellow card. Precise label data on locality are cited for the holotypes. Separate lines on a label are indicated by a slash "/" and separate card labels are indicated by a double slash "//".

Information on the distribution and etymology of names are provided in separate paragraphs for each species. An overview of the host-parasite associations with published and updated host names is presented in Table 1. Information concerning host stylopization without classification of the Strepsiptera and other information are within the notes.

Table 1. Summary of host associations for *Rozenia* gen. n. All hosts belong to bees (Apoidea) of the family Andrenidae; (as) host published under the different combination or misidentification; (*) host association corroborated in this study. Valid names are in bold.

Parasite	Host
Strepsiptera	**Hymenoptera**
Stylopidae	**Panurginae** Leach, 1805 (Apoidea: Andrenidae Latreille, 1802)
Rozenia gen. n.	**Calliopsini** Robertson, 1922
R. calliopsidis sp. n.	****Calliopsis* (*Liopoeum*) *mendocina*** (Jörgensen, 1912)
	****Calliopsis* (*Liopoeum*) *trifasciata*** (Spinola, 1851)
R. peruana sp. n.	****Acamptopoeum vagans*** (Cockerell, 1926)
R. platicephala sp. n.	****Acamptopoeum submetallicum*** (Spinola, 1851)
	as *Liopoeum submetallicum* (Spinola, 1851) (Schwarz 1931, Hofeneder and Fulmek 1943)
Rozenia sp.	***Acamptopoeum argentinum*** (Friese, 1906)
	as *Perdita argentina* Friese, 1906 (Hofeneder and Fulmek 1943)
	as *Calliopsis* (*Parafriesea*) *argentina* (Friese, 1906) (Hofeneder 1952)

Genus and species descriptions

Rozenia gen. n.

http://zoobank.org/00957F90-4A0F-4ACB-AAB5-9CFF8B9A303A

Type species. *Rozenia calliopsidis* sp. n.

Diagnosis. Female. *Rozenia* gen. n. differs from other genera of the family Stylopidae in having only four abdominal segments. Similarly to the genus *Crawfordia* Pierce, 1908, canalis prolifer of *Rozenia* gen. n. is with a single median tuba prolifera present on segments II-IV of the abdominal part of female. However, tuba prolifera III of *Rozenia* gen. n. is positioned on the posterior half of abdominal segment IV, but in the middle of segment IV in *Crawfordia*, which possesses also rudimentary segment V. Abdomen of other genera of the family Stylopidae is composed by higher number of segments.

Female puparium. Brood opening of the new genus is very wide, almost from side to side, about four times wider than intermandibular distance, or more in *Rozenia* gen. n. Brood opening is usually much narrower in other genera of the family Stylopidae. Narrow head corners are produced laterally beyond prothorax; this feature causes head to be wider than distal part of prothorax and side of cephalothorax is not continuously diverging posteriorly. This character is developed in *Eurystylops* Bohart, 1943 and some species of the genus *Crawfordia*. Head corners are relatively long, but not as long as in *Crawfordia*, which possess head corners longer than half of cephalothorax. In *Rozenia* gen. n. head corners are as long as head dorsally, but *Crawfordia* has much longer head corners than head dorsally. Mandibles extending from the head contour in ventral view. In contrast to *Crawfordia*, intersegmental ridges are not developed in *Rozenia* gen. n.

First instar. First instars of *Rozenia* gen. n. differ substantially from other genera by having setae of submedian row on thorax as well as on abdominal segments. Caudal setae are distinctly longer than body. Both these characters are unique among all Strepsiptera. *Rozenia* gen. n. does not have spinulae on posterior margin of thoracic tergites as in Xenidae, Halictophagidae, or Elenchidae. These spinulae are developed on posterior margin of thoracic tergites in all other genera of the family Stylopidae. Ventral sublateral bristle is missing on sternum IX in *Rozenia* gen. n., but probably present in all other genera of Strepsiptera. Posterior margin of labiomaxilary area continuous in *Rozenia* gen. n., but emarginated in *Crawfordia*, *Halictoxenos* and *Stylops* (and probably also in other Stylopidae).

Description. Female. Canalis prolifer on abdominal segments I-IV, segment V absent. Single median tuba prolifera on segments II-IV, tuba prolifera on segment IV positioned in posterior half of segment.

Female puparium. Head corners (on ventral side) extending posteriorly as far as head posterior margin on dorsal side; head corners distinct, narrow, forming a lamella on frontal part of cephalothorax, produced laterally beyond prothorax, this feature causes head to be wider than distal part of prothorax and side of cephalothorax is not continuously diverging posteriorly; head corners elevated ventrally over intermandibular part of head, but not over prothorax; brood opening wide, distinctly wider than distance between mandibles; mandibles variable in size, but at least the tip is extending from the head contour in ventral view. Intersegmental ridges not developed; anterior margin of mesothorax ill-defined, but transverse and does not extend forward; spiracles positioned distally above prominent spiracular corners, close to middle of cephalothorax. Prothorax ventrally pigmented, not lighter than head corners.

Male. Unknown.

First instar. Body rounded; thorax approx. half of entire body length (caudal setae not included); caudal setae distinctly longer than body length. Head strongly reduced ventrally; maxilla with single seta; mandibles and labrum overlapping outline of body; labium fused to maxillae forming labiomaxillary area, its posterior margin continuous, not emarginated.

Each segment of thorax bears at least two pairs of setae dorsally and laterally close to posterior margin, forming submedian and lateral rows of setae. Posterior margins

of thoracic tergites smooth. Coxae broad, ovate; three coxal teeth at anterior part of each coxa, all divided into two to four tips; one coxal bristle divided at least into two tips; up to five cuticular outgrowths laterally from coxal teeth and one very short seta anteriorly from cuticular outgrowths; one very short seta at posterior part of coxa. Each trochanterofemur with femoral spur bifid at tip; up to six cuticular outgrowths and one short seta anteriorly and posteriorly on femur; each tibia with five tibial spurs and small projections at distal end of tibia. Tarsi of fore and mid legs enlarged and elongated; tarsi of hind legs rod-like and elongated. Sternal plates broad and smooth on surface (paired setae missing).

Abdomen with rows of setae similar to those present on thorax. Abdominal segment X extremely shortened and fused to segment IX; segment XI split into two parts and restricted to ventral base of caudal setae; segment XI with one particularly long caudal seta and short lateral caudal seta. Posterior margins of abdominal tergites smooth except laterally, spinulae not immersed; posterior margin of abdominal sternites with spinulae, spinulae not immersed; segment IX with only two spinulae, ventral sublateral bristle is missing.

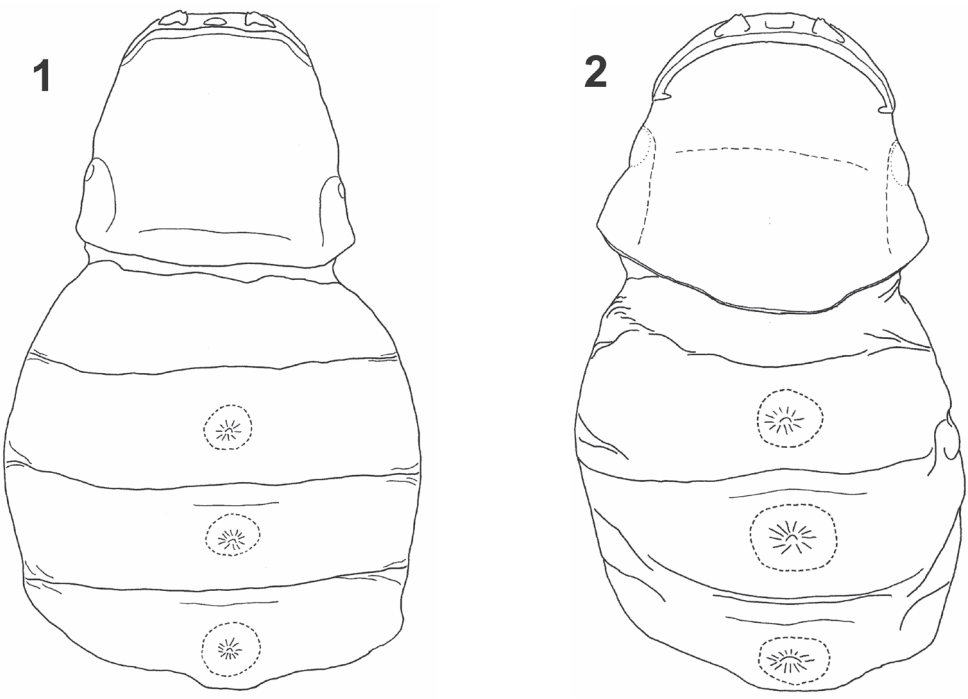
Hosts. Bees of the genera *Acamptopoeum* and *Calliopsis*.

Etymology. Named in honor of the excellent bee expert, teacher of generations of bee students and a friendly and knowledgeable man, Jerome G. Rozen Jr. (American Museum of Natural History, New York, USA). J.G. Rozen Jr., collected most of the specimens of all three new species used for the descriptions.

***Rozenia calliopsidis* sp. n.**
http://zoobank.org/DABEEEE4-1BEF-4764-812D-054FDA6D8FD3
Figures 1, 3, 6, 9, 12, 14, 17, 19, 20, 22

Material examined. Holotype female puparium, in a separate microvial on the same pin as host. Original label: "CHILE: R.M.: Chacabuco / Caleu, nr. Cerro del Robie / 33°00'49"S, 70°58'59"W / 30 Nov 2004, J. S. Ascher, / A. Y. Kawahara, C. Espina". 1 FP, host: *Calliopsis* (*Liopoeum*) *trifasciata* (Spinola, 1851), ♂, AMNH coll. (code: AMNH_BEE 00036534).

Paratypes: ARGENTINA: Salta prov.: Cafayete, 14.xi.1993, 1 FP, host: *Calliopsis* (*Liopoeum*) *mendocina* (Jörgensen, 1912) ♀, JG and BL Rozen leg., AMNH coll. (AMNH_BEE 00036520), ditto, 1 FP (AMNH_BEE 00036521), ditto (AMNH_BEE 00036522); Catamarca prov.: El Desmonte, 7.xi.1989, 1 FP, host: *C. m.* ♂, JG Rozen and A Roig-Alsina leg., AMNH coll. (AMNH_BEE 00036523), ditto 2 FPP (AMNH_BEE 00036524), San Fernando, 3.–6.xi.1989, 1 FP, host: *C. m.* ♀, JG Rozen and A Roig-Alsina leg., AMNH coll. (AMNH_BEE 00036525), ditto, 5.xi.1991, 1 FP, host: *C. m.* ♂, JG Rozen, LE Peña and A Ugarte leg., AMNH coll. (AMNH_BEE 00036529), ditto, 15.xi.1993, 1 FP, host: *C. m.* ♂, JG and BL Rozen leg., AMNH coll. (AMNH_BEE 00036528), Tinogasta 35 km SE, 28.xi.1989, 1 FP, host: *C. m.* ♂, JG Rozen and A Roig-Alsina leg., AMNH coll. (AMNH_BEE 00036526), Co-



Figures 1–2. Female puparium, cephalothorax, with canalis prolifer of female, ventral view. **1** *Rozenia calliopsidis* sp. n. **2** *Rozenia platicephala* sp. n.

pacabana, 30.xi.1993, 1 FP, host: *C. m.* ♂, JG Rozen leg., AMNH coll. (AMNH_BEE 00036527), Punta de Balasto 3–15 km WSW, 25.xi.1993, 1 FP, host: *C. m.* ♀, JG Rozen leg., AMNH coll. (AMNH_BEE 00036530); Tucumán prov.: Amaichá del Valle, 6.iii.1990, 1 MP with pupa, host: *C. m.* ♂, JG Rozen leg., AMNH coll. (AMNH_BEE 00036532); Rio Negro prov.: El Bolson, 17.ii.1960, 1 FP, >50 L1, host: *C. t.* ♂, A Kovacs leg., AMNH coll. (AMNH_BEE 00036533); Neuquén prov.: Junín de los Andes, 21.–23.ii.2004, 2 FPP, host: *C. t.* ♀, J Straka leg. and det., JSPC coll.; CHILE: Apoquindo, Santiago, 1FP, host: *C. t.* ♂, date and collector not indicated, KUNHM coll. (SEMC1008235); Macul, SE Santiago, 5.xi.1974, 2 FPP, host: *C. t.* ♀, LE Peña leg., AMNH coll. (AMNH_BEE 00036536); Petorca prov.: Las Palmas tunnel, 18.x.1994, 2 FPP, host: *C. t.* ♂, JG Rozen, Quinter and JS Ascher leg., AMNH coll. (AMNH_BEE 00036535). Other material examined: Salta prov.: El Carmen, 27 km S Molinos, 1900 m, 6.x.1968, 1 EMP, host: *C. m.* ♀, LE Peña leg., AMNH coll. (AMNH_BEE 00036519). If not indicated otherwise, bee hosts identified by JS Ascher.

Diagnosis. Female puparium. *Rozenia calliopsidis* sp. n. differs from other species of the genus by a narrower head with large mandibles. Brood opening turned backwards laterally, very close to posterior margin of mandible and continued as cephalic ridge. In other species, the brood opening fluently transforms into cephalic ridge and forms an arcuate line. Spiracular corners of this species are weakly prominent, obtuse,

not triangular. Whole cephalothorax is darker than in *R. platicephala* sp. n. and *R. peruana* sp. n.

First instar. Shape of body narrower than in *R. platicephala* sp. n. Ratio of body length and width is on average 2.3. Ratio of body length and length of caudal setae is 0.74–0.96. Caudal setae are shorter than in *R. platicephala* sp. n.

Head dorsally with seven pairs of setae compared to six and usually shorter in *R. platicephala* sp. n. Labrum is not emarginated in the middle in contrary to *R. platicephala* sp. n. Labiomaxillary area more rounded than in *R. platicephala* sp. n., acute posteriorly.

Each segment of thorax bears only two pairs of setae dorsally and laterally, forming submedian and lateral row of setae, both rows continue on abdomen, interstitial and supralateral rows of setae missing. Posterior margin of abdominal tergites with more spinulae laterally than in *R. platicephala* sp. n. These spinulae are visible in dorsal view.

Sternal plates are broad and smooth on surface, posterior margin with fringe of long spinulae in contrast to smooth margins of *R. platicephala* sp. n. Precoxal pleural membrane of prothorax covered with transverse row of microtrichiae and precoxal pleural membrane of meso and metathorax with two cuticular processes laterally and medially.

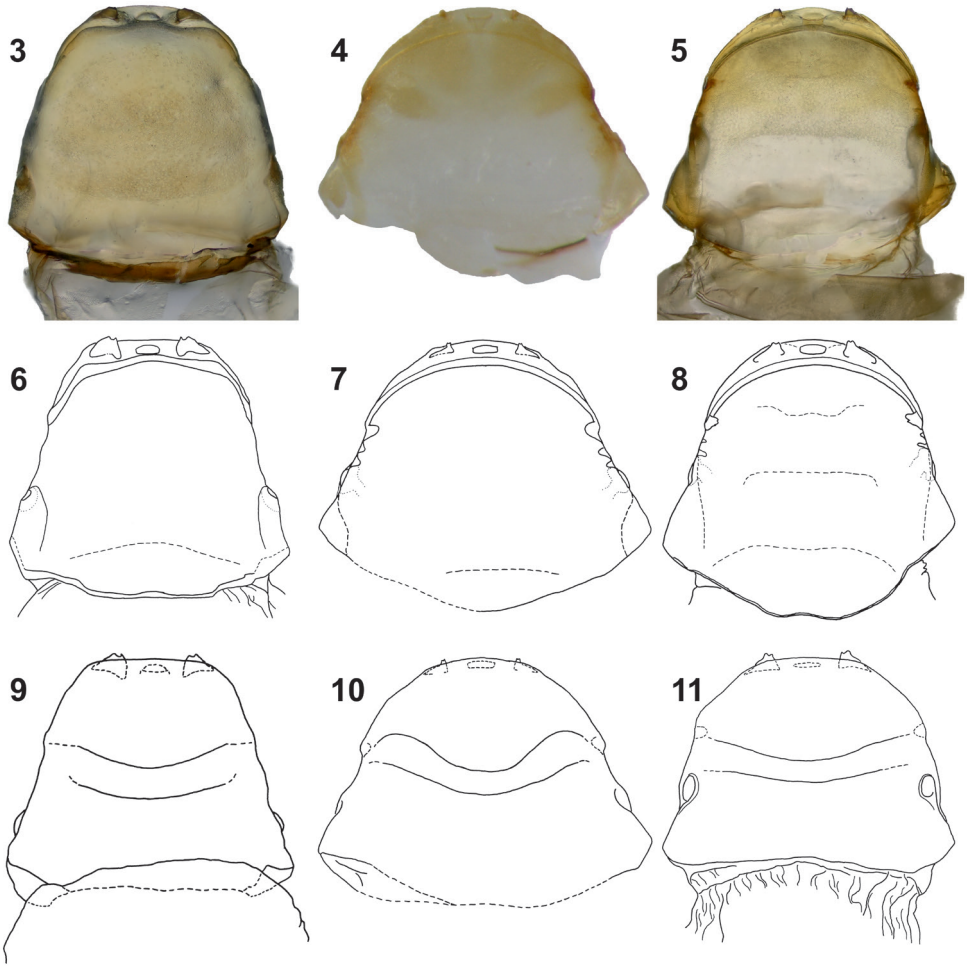
Coxal teeth are usually divided into three to five tips; coxal bristle is divided into four or five tips on foreleg and into two tips on middle and hind legs; this bifurcation is more extensive in comparison to *R. platicephala* sp. n. Coxa and trochanterofemur with more cuticular outgrowths in comparison to *R. platicephala* sp. n.

Description. Female. Canalis prolifer on abdominal segment I–IV. Tuba prolifera on segment IV positioned in posterior half of segment.

Female puparium. Cephalothorax slightly wider than long, approx. 0.7 mm long and approx. 0.8 mm wide between spiracular corners. Head wide, approx. 0.5 mm; mandible large, projecting from head contour, intermandibular distance 0.16–0.17 mm, mandibles approx. two mandibular diameters apart or less; labral apex between mandibles straight; oral ridge well developed; labral area very short; maxilla indistinct, but maxillary area with weak transverse elevation; brood opening wide, nearly from side to side, slightly sinuous, produced forward medially; head corners narrow, laterally turned posteriorly; posterolateral margin of head corner with weak apodeme; cephalic ridge well developed. Thorax without intersegmental ridges; pro-, meso- and metathorax largely fused ventrally as well as dorsally, segments seem to be subequal in length; thoracic stigma not developed; metathoracic ridge distinct, touching cintum and going up spiracle. Spiracular corners weakly prominent, obtuse; spiracula positioned anteriorly to spiracular corners, turned laterally; basal band distinct, arcuate, projecting forward, but anterior end not sharply delimited. Cephalothorax distinctly and uniformly light pigmented, only metathorax pale and translucent ventrally.

First instar. Total length (without caudal setae) 0.160–0.180 mm (n=6) on average; length of caudal setae up to 0.221 mm; ratio of body length and length of caudal seta 0.74–0.96. Ratio of body length and width approx. 2.2–2.3.

Head: Head dorsally with seven pairs of setae; ventrally strongly reduced, with setae on maxillae; mandibles and labrum overlapping outline of body; labrum not



Figures 3–11. Female puparium, cephalothorax. **3, 6, 9** *Rozenia calliopsidis* sp. n., ventral and dorsal view **4, 7, 10** *Rozenia peruana* sp. n., ventral and dorsal view **5, 8, 11** *Rozenia platicephala* sp. n., ventral and dorsal view.

emarginated; labiomaxillary area occupying majority of ventral part of head, rounded, acute posteriorly.

Thorax: Each segment of thorax bears two pairs of setae dorsally and laterally close to posterior margin, forming submedian and lateral rows of setae (Figure 20). Posterior margins of thoracic tergites smooth. Coxae broad and ovate; three coxal teeth at anterior part of each coxa, all variably divided into two to four tips; coxal bristle variably divided into four or five tips on fore leg and extensively bifid on mid and hind legs; single cuticular outgrowth positioned medially from coxal bristle; five cuticular outgrowths laterally from coxal teeth and one very short seta above cuticular outgrowths; one very short seta at the posterior part of coxa. Each trochanterofemur with spur bifid at tip,

five to six cuticular outgrowths and one short seta anteriorly and posteriorly on femur. Each tibia with five tibial spurs and short projections at distal end of tibiae. Tarsi of fore and mid legs enlarged and elongated, tarsus of hind leg rod-like and elongated. Sternal plates broad and smooth on surface and with fringe of long spinulae at its posterior margin. Precoxal pleural membrane with transverse row of microtrichia on prothorax and with two processes laterally and medially on mesothorax and metathorax.

Abdomen: Abdomen with rows of setae dorsally and laterally similar to those present on thorax (Figure 22); submedian row of setae from abdominal tergite I to tergite VIII; lateral row of setae up to tergite IX. Abdominal segment X extremely shortened and fused to segment IX; segment XI split in two parts and restricted on ventral base of caudal setae; segment XI with one particularly long caudal seta and short lateral caudal seta. Posterior margin of abdominal tergites smooth except for a few spinulae (up to six) laterally, few setae present laterally as well as mesally from lateral row of setae; posterior margin of sternites with spinulae, segment IX with only two long spinulae, which extend body outline; no spinulae immersed.

Etymology. Name derived from the generic name of the host bee.

Distribution. Argentina and Chile.

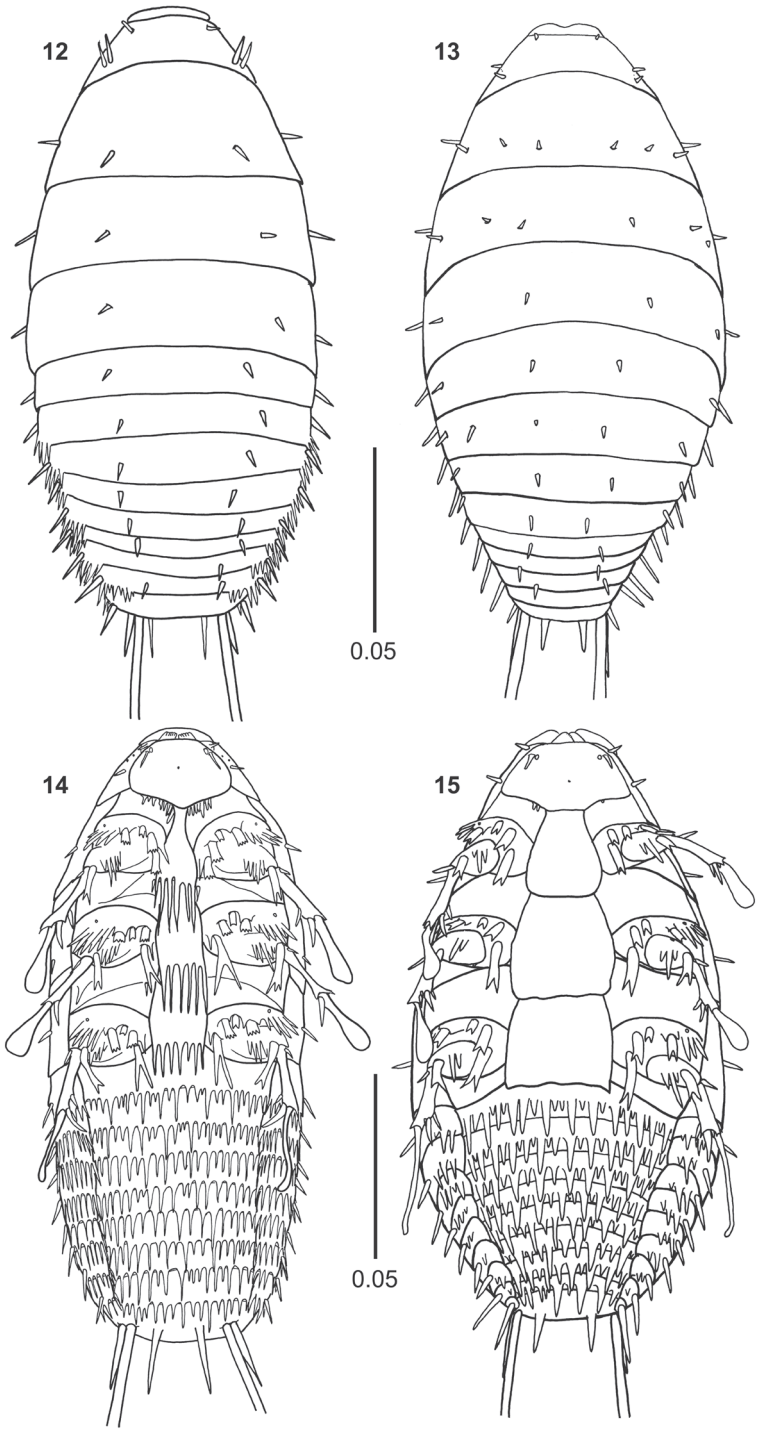
***Rozenia peruana* sp. n.**
http://zoobank.org/84BFDD49-A710-48F1-A304-A4CA92138DCC
Figures 4, 7, 10

Material examined. Holotype female puparium, in a separate microvial on same pin as host. Original label: "PERU: Lima dept. / Ricardo Palma, V-9-96 / J. G. Rozen, A. Ugarte". 1 FP, host: *Acamptopoeum vagans* (Cockerell, 1926), ♀, JS Ascher det., AMNH coll. (code: AMNH_BEE 00026923).

Diferential diagnosis. Female puparium. Cephalothorax of *R. peruana* sp. n. strongly diverging posteriorly behind head (Figure 10). Among all the species of the genus *R. peruana* sp. n. has the smallest mandibles that, as in other species, project from the head contour. A very specific character is the shape of prothorax in dorsal view. Prothorax produced forward on lateral sides to the head margin, thus posterior head margin is sinuous (Figure 10). Prothorax is dorsally pigmented as in *R. platicephala* sp. n.

Description. Female. Canalis prolifer on abdominal segment I–IV, with three large tuba prolifera on segments II-IV, tuba prolifera on segment I distinct, but very small and possibly not functional.

Female puparium. Cephalothorax slightly wider than long, approx. 0.7 mm long and approx. 0.9 mm wide between spiracular corners. Head wide, approx. 0.6 mm; mandible small, projecting from head contour, intermandibular distance 0.16 mm, mandibles nearly three mandibular diameters apart; labral apex between mandibles slightly arcuate; oral ridge well-developed; epipharinx weakly divided from very short labral area; maxilla indistinct; brood opening wide, nearly from side to side, arcuate;



Figures 12–15. First instars, dorsal and ventral view. **12, 14** *Rozenia calliopsidis* sp. n. **13, 15** *Rozenia platicephala* sp. n.

head corners narrow, directed posterolaterally; posterolateral margin of head corner with distinct apodeme; cephalic ridge weak. Thorax without intersegmental ridges; pro-, meso- and metathorax largely fused ventrally, segments seem to be subequal in length; prothorax dorsally slightly shorter than half length of fused meso- and metathorax, prothorax strongly produced forward laterally; metathorax as well as mesothorax laterally with remnant of stigmata; metathoracic ridge ill-defined, but distinct, touching cintum and going anterolaterally to spiracle. Spiracular corners prominent, triangular, well-developed; spiracle positioned anterior to spiracular corners, turned laterally; basal band arcuate, projecting forward, but ill-defined. All cephalothorax pale, head, prothorax dorsally, sides of thorax and spiracular corner light pigmented; rest of cephalothorax pale and translucent.

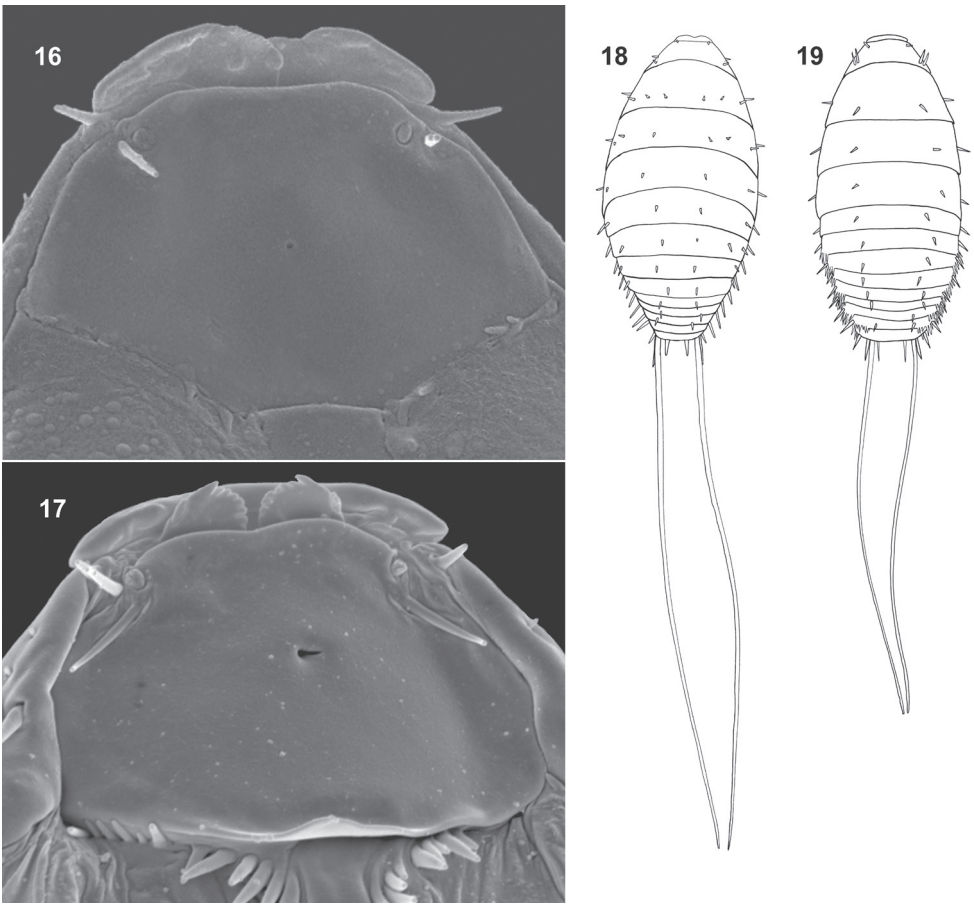
Etymology. Name derived from the country, where the holotype was collected.

Distribution. Peru.

***Rozenia platicephala* sp. n.**
http://zoobank.org/45297119-B4EB-407D-85A1-730D9A514903
Figures 2, 5, 8, 11, 13, 15, 16, 18, 21, 23

Material examined. Holotype female puparium, in a separate microvial on same pin as host. Original label: "CHILE: Cautin Prov. / Cunco, II-1998, / Perez de Arce". 1 FP, host: *Acamptopoeum submetallicum* (Spinola, 1851), ♀, JS Ascher det., AMNH coll. (code: AMNH_BEE 00037984).

Paratypes: ARGENTINA: Córdoba prov.: Parral, Fundo Malcho, xi.1956, 1FP, host: *A. s.* ♂, LE Peña leg., KUNHM coll. (SEMC1006814). CHILE: same as holotype, >500 L1; Limarí prov., 19 km ENE Samo Alto, 10.xi.1992, 1FP, host: *A. s.* ♀, JG Rozen, Sharkov and Snyder leg., AMNH coll. (AMNH_BEE 00037983); Cautin prov.: Cunco, ii.1998, 1 FP, host: *A. s.* ♀, Perez de Arce leg., AMNH coll. (AMNH_BEE 00037985); Bio Bío prov.: Antuco, nr. Hydroeléctrica, 37°23'49"S, 71°27'21"W, 14.xii.2004, 1 FP, host: *A. s.* ♂, JS Ascher leg., AMNH coll. (AMNH_BEE 00037986), ditto, 2 FPP, host: *A. s.* ♀, JS Ascher leg., AMNH coll. (AMNH_BEE 00037391); Dichato, 20.xii.1953, 1 FP, >500 L1, host: *A. s.* ♀, LE Peña leg., KUNHM coll. (SEMC1006914); Coquimbo prov.: Las Breas, 23.–24.x.1989, 1 FP, host: *A. s.* ♀, JG Rozen leg., AMNH coll. (AMNH_BEE 00037393); Santiago prov.: El Manzano, Valle Rio, Maipo, 1000-1500 m, i.1984, 1 FP, host: *A. s.* ♀, LE Peña leg., AMNH coll. (AMNH_BEE 00037394), El Manzano, Quebrada, 900-1500 m, 5.-6.ii.1983, 2 FPP, host: *A. s.* ♀, LE Peña leg., AMNH coll. (AMNH_BEE 00037395); Valdivia prov.: Valdivia, 9.ii.1953, 1 FP, host: *A. s.* ♀, collector not indicated, KUNHM coll. (SEMC1006957); Valparaíso prov.: Viña del Mar, La Quinta Vergara, 18.xii.2004, 1 FP, host: *A. s.* ♀, JS Ascher leg., AMNH coll. (AMNH_BEE 00037397). Other material examined: Coquimbo prov.: Las Breas, 23.–24.x.1989, 1 EMP, host: *A. s.* ♂, JG Rozen leg., AMNH coll. (AMNH_BEE 00037392); Araucanía prov.: Malleco, Victoria, xii.1985, 1 EMP, host: *A. s.* ♀, LE Peña leg., AMNH coll. (AMNH_BEE 00037396). All hosts identified by JS Ascher.



Figures 16–19. First instars, ventral view to head and dorsal view of total body. **16, 18** *Rozenia platicephala* sp. n. **17, 19** *Rozenia calliopsidis* sp. n.

Diagnosis. Female puparium. This species possess relatively and also absolutely the widest head among all species of the genus. Spiracular corners are sharply triangular and distinctly prominent, but not large. Prothorax is more pigmented dorsally than other parts of thorax, like in *R. peruana* sp. n., but anterior and posterior margins are paralel, arcuate, producing forward on sides only slightly. Position of spiracula seems to be characteristic for this species. They are turned more dorsally than in other species, however this character is very variable and may be inconsistent. Pigmentation like in *R. peruana* sp. n.

First instar. Shape of body is more rounded than in *R. calliopsidis* sp. n., width of segments decreases from metathorax more strongly. Ratio of body length and width is on average 2.0. Ratio of body length and length of caudal setae is approx. 0.60–0.65. Caudal setae are relatively longest among all species.

Head dorsally with six pairs of setae compared to seven and usually longer in *R. calliopsidis* sp. n.; labrum is narrow at the middle contrary to *R. calliopsidis* sp. n.; labium is projecting more laterally than in *R. calliopsidis* sp. n.

Each segment of thorax bears four pairs of setae dorsally and laterally, forming submedian, interstitial, supralateral and lateral rows of setae. Sternal plates are broad and smooth on surface, specific are also smooth posterior margins. Precoxal pleural membrane is smooth without any projections except of one or two cuticular outgrowths on prothoracic precoxal pleural membrane. Coxal teeth are always bifid in two tips in contrast to *R. calliopsidis* sp. n. with as many as five tips; coxal bristle is always divided into two tips and bifurcation in middle leg and hind leg is not so extensive, there are no cuticular outgrowth by coxal bristle contrary to *R. calliopsidis* sp. n., also there are not so many cuticular outgrowths on coxa and femur like in *R. calliopsidis* sp. n.

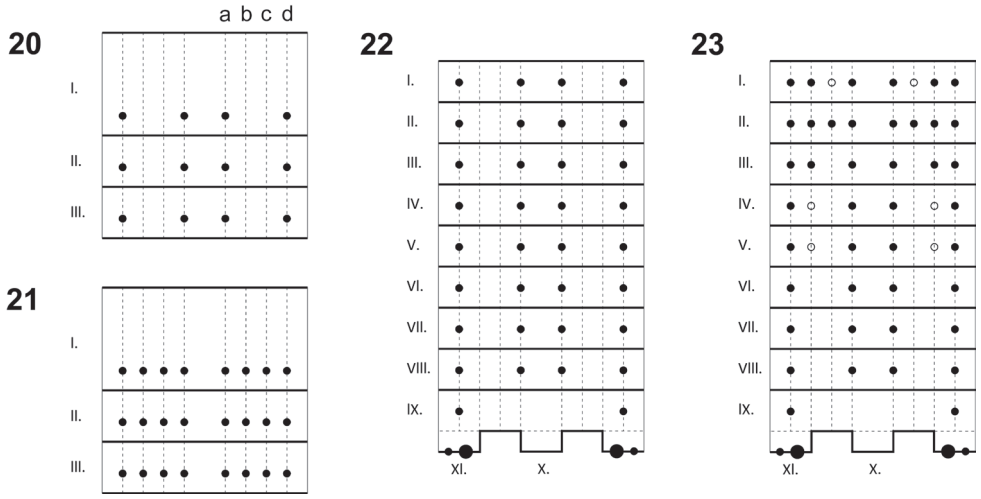
All four pairs of rows of setae continues dorsally on abdomen, submedian row up to tergite XIII, interstitial row is on tergite II or in some specimens also on tergite I, supralateral row is variable and reach up to tergite III, IV or V, and lateral row up to tergite IX. Spinulae on posterial margins of abdominal tergites only beyond lateral row and not visible in dorsal view.

Description. Female. Canalis prolifer on abdominal segment I–IV. Tuba prolifera on segment IV positioned in posterior half of segment.

Female puparium. Cephalothorax slightly wider than long, approx. 0.8 mm long and approx. 0.9–1.0 mm wide between spiracular corners. Head wide, approx. 0.7 mm; mandible projecting from head contour, intermandibular distance 0.17–0.21 mm, approx. two mandibular diameters apart, but variable among different individuals; labral apex between mandibles straight; oral ridge well developed; epipharinx weakly divided from labral area, short; maxilla not developed, but maxillary area with weak transverse elevation; brood opening wide, nearly from side to side, arcuate; head corners narrow, directed posterolaterally; posterolateral margin of head corner with distinct apodeme; cephalic ridge weak. Thorax without intersegmental ridges; pro-, meso- and metathorax largely fused ventrally, segments seem to be subequal in length, prothorax dorsally slightly shorter than half length of fused meso- and metathorax; meso- and metathorax laterally with remnants of stigma, mesothoracic spiraculum very small and hardly visible; metathoracic ridge ill-defined, but distinct, touching cintum and going up spiracle. Spiracular corners prominent, well developed; spiracula positioned anterior to spiracular corners, turned dorsally; basal band distinct but weak, arcuate, projecting forward. All cephalothorax pale, head and prothorax dorsally and head, prothorax and mesothorax light pigmented ventrally; spiracular area and basal band only slightly darker; rest of cephalothorax pale and translucent.

First instar. Total length approx. 0.154–0.175 mm (n=3) without caudal setae; length of caudal setae up to 0.289 mm (on an average 0.276 mm); ratio of body length and length of caudal setae approx. 0.60–0.65. Ratio of body length and width approx. 1.9–2.3.

Head: Head dorsally with six pairs of setae; ventrally strongly reduced; with setae on maxillae; distinctive mandibles and labrum overlapping outline of body; labrum emarginated; labiomaxillary area occupying majority of ventral part of head, rounded, posterior margin straight.



Figures 20–23. Diagram of first instar chaetotaxy. **20, 22** *Rozenia calliopsidis* sp. n., thoracic and abdominal tergites; **21, 23** *Rozenia platicephala* sp. n., thoracic and abdominal tergites; a-submedian row of setae, b-interstitial row of setae, c-supralateral row of setae, d-lateral row of setae; point-stable presence of seta; circle-seta with unstable presence; large point-caudal seta.

Thorax: Each segment of thorax bears four pairs of setae dorsally and laterally close to posterior margin forming submedian, interstitial, supralateral, and lateral rows of setae (Figure 21). Posterior margins of thoracic tergites smooth. Coxae broad and ovate; on each coxa three coxal teeth and one coxal bristle at anterior part of coxa, all bifid at tips; three or four cuticular outgrowths laterally from coxal teeth and one very short seta above cuticular outgrowths and one on posterior margin of coxa. Each trochanterofemur with femoral spur bifid at tip; two or three cuticular outgrowths and one short seta anteriorly and posteriorly on femur. Each tibia with five tibial spurs and little projections at distal end of tibiae. Tarsi of fore and middle legs enlarged and elongated, tarsi of hind legs rod-like and elongated. Sternal plates broad and smooth on surface and on posterior margins. Precoxal pleural membrane smooth without any projections except of one or two cuticular outgrowths on prothoracic precoxal pleural membrane.

Abdomen: Abdomen with rows of setae dorsally and laterally similar to those present on thorax; submedian row from abdominal tergite I to tergite VIII; interstitial row on tergite II or in some specimens also on tergite I; supralateral row variable up to tergite III, IV or V; lateral row up to tergite IX (Figure 23). Abdominal segment X extremely shortened and fused to segment IX; segment XI split in two parts and restricted only on ventral base of caudal setae; segment XI with particularly long caudal seta and short lateral caudal seta. Posterior margin of abdominal tergites smooth except for lateral part with a few spinulae (up to three) more laterally than lateral row of setae; posterior margin of sternites with spinulae, segment IX with only two longer spinulae, which extend body outline; no spinulae immersed.

Etymology. Name of this species refers to characteristic flat head and general flat appearance of all *Rozenia* gen. n. species, when found between tergites of host bees.

Distribution. Argentina and Chile.

Published hosts assigned to *R. platicephala* sp. n. *A. submetallicum*: Schwarz (1931: 78-79), record from Chile (as *Liopoeum submetallicum* (Spinola)), also reported by Hofeneder and Fulmek (1943: 35), but with no original data.

Note: To *R. platicephala* sp. n. could be assigned findings of Strepsiptera in the host bee *Acamptopoeum argentinum* (Friese, 1906): Hofeneder and Fulmek (1943: 42), record from Argentina (as *Perdita argentina* Friese), repeated by Hofeneder (1949: 122) and later by Hofeneder (1952: 489) (as *Calliopsis* (*Parafriesea*) *argentina* (Friese)). The record is impossible to verify as reliable pending a review of the material. The information about material deposition is not known to us.

Key to species of the genus *Rozenia* gen. n.

Female puparia and females

1a More than four abdominal segments developed, with tuba prolifera III (if developed) positioned in the middle part of abdominal segment IV; combination of characters different ..... **other Strepsiptera**

1b Only four abdominal segments developed, with tuba prolifera III positioned on the posterior half of abdominal segment IV (Figures 1–2); brood opening wide, almost from side to side, about four times wider than intermandibular distance, or more; head wider than distal part of prothorax, this character cause that side of cephalothorax is not continuously diverging posteriorly; mandibles extending from the head contour in ventral view; intersegmental ridges not developed (Figures 3–11) ..... **2, *Rozenia* gen. n.**

2a Spiracular corners weakly prominent, obtuse, not triangular; brood opening turned backwards laterally, very close to posterior margin of mandible and continued as cephalic ridge (Figures 3, 6); cephalothorax pigmented in all parts (Figure 3); host bee *Calliopsis* spp. ..... ***Rozenia calliopsidis* sp. n.**

2b Spiracular corners prominent, triangular; brood opening fluently transforms into cephalic ridge and forms an arcuate line (Figures 4, 5, 7, 8); posterior half of cephalothorax nearly transparent (Figures 4, 5); host bee *Acamptopoeum* spp. **3**

3a Prothorax dorsally produced forward on lateral sides to the head margin, thus posterior head margin is sinuous (Figure 10); mandibles very small (Figures 4, 7) ..... ***Rozenia peruana* sp. n.**

3b Anterior and posterior margins of prothorax dorsally parallel, thus posterior head margin arcuate (Figure 11); mandibles of normal size (Figures 5, 8) ..... ***Rozenia platicephala* sp. n.**

First instars

1a Submedian row of setae absent on abdomen; caudal setae shorter or as long as body; posterior margin of labium emarginated; ventral sublateral bristle on sternite IX; posterior margin of thoracic tergites with spinulae..... .....**other Strepsiptera**

1b Submedian row of setae present on abdomen; caudal setae longer then body; posterior margin of labium continuous; ventral sublateral bristle absent; posterior margin of thoracic tergites smooth .....**2, *Rozenia* gen. n.**

2a Sternal plates at posterior margin with spinulae; interstitial and supralateral row of setae on dorsum absent; coxal tooth with two to four tips at apex; coxal bristle in fore leg with multiple tips at apex; coxal bristles in mid and hind leg extensively bifid; numerous cuticular outgrowths on precoxal pleural membrane and coxae; caudal setae slightly longer then body ....***Rozenia calliopsidis* sp. n.**

2b Sternal plates smooth on posterior margin; interstitial and supralateral row of setae on dorsum; coxal tooth bifid at apex; coxal bristles bifid on each leg; few cuticular outgrowths on precoxal pleural membrane and coxae; caudal setae distinctly longer then body .....***Rozenia platicephala* sp. n.**

Discussion

Among all, the newly described genus, *Rozenia* gen. n., is morphologically unusual in having extremely long caudal setae in first instars. No other Strepsiptera species possess such long caudal setae (Pohl 2000). These setae are always longer than the body in *Rozenia* gen. n., and almost two times longer than the body in *R. platicephala* sp. n. (Figures 18–19). This species is also exceptional in having four rows of dorsal thoracic setae, one row more than in the most basal Strepsiptera family Mengenillidae (Pohl 2000). Until now, the chaetotaxy of first instars seemed to be reductive in the evolution of Strepsiptera, because basal lineages possess more abundant setae on dorsal part of the thorax and abdomen than derived lineages. It is, however, clear that *Rozenia* gen. n. is not related to the Mengenillidae, but belongs to the family Stylopidae, which means that at least one row of setae are newly developed in *Rozenia* gen. n. We call the new row of setae the "interstitial row", because at most three rows of setae were known in all other Strepsiptera untill now. This interstitial row continues to abdominal segments I and II in *R. platicephala* sp. n. The second species of *Rozenia* gen. n. with known first instars, *R. calliopsidis* sp. n., has a more standard chaetotaxy, but a submedian row of setae is present on the thorax, as well as on abdominal segments I-VIII, which is a synapomorphy of the genus *Rozenia* gen. n.

Rozenia gen. n. is a genus distinctive from other Strepsiptera genera in numerous characters mentioned in generic diagnosis. According to the host family and a few

shared characters, it seems to be most closely related to the genus *Crawfordia*. In both genera, a single median tuba prolifera on canalis prolifer is present on segments II-IV of the abdominal part of female puparia. In first instars, spinulae are not immersed in any part of the body; two pairs of setae or more are present on each thoracic and abdominal segment dorsally; the sternal plates are completely smooth, no setae are developed; coxal teeth, coxal bristles and femoral spurs are bifid or with multiple tips in both genera. Some of these characters are developed in some other Strepsiptera species, but never in the family Stylopidae (Pohl 2000).

Acknowledgements

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FUTURE PROSPECTS

The elementary characteristic of all of the parasitic organisms is their association with hosts. The study of host-parasite relationship cannot do without knowledge of host specificity and evolutionary history of both parasites and hosts. Without well resolved classification of parasites into hierarchical system it would be hard to do so. We have contributed to the resolution of that part.

At the current stage of knowledge, we proceed deeper into the study of host-parasite specialization in Strepsiptera and we would like to continue in this direction. However, several other issues that need to be resolved stand in our way. The main ones can be investigated by following methods:

- Morphology: It is possible to use the morphology of males, females and also first instars for alpha taxonomy, even though it is not simple due to their parasitic lifestyle. Females lack many important characteristics because of endoparasitism and males are unknown in some groups, as they have extremely short life spans. First instars are great objects for investigation of external morphology, if females are found. The scanning electron microscope has to be used.
- Phylogeny: The comprehensive study of evolutionary relationships between organisms should be established in the family Stylopidae on intraspecific as well as interspecific level. The complex study of interspecific relationships in Strepsiptera is completely missing.

  Our future aim is to create the phylogenetic tree from matrix of morphological characters of first instars of genus the *Stylops*. It is also possible to use combined characters from morphology and molecular markers. Lower price of sequencing resulted in a boom of phylogenetical studies and therefore in a huge increase of genomic data available. We would also like to use more genes and transcriptomes for phylogeny of this family.
- Molecular identification of cryptic species: DNA barcoding should be suitable. It can also reveal matching of males and females (and also hosts) described as separate species.
- Phylogeny and revised taxonomy of hosts.
- Assessment of congruence between host's and parasite's phylogenetical trees to investigate coevolution processes.

Last, but not at least, a good sampling is always crucial. Besides basic information about sampled individual and possibility of comparison with other individuals, it helps us avoid artefacts and misinterpretations.

These methods can help us to get clearer picture about host specialization in Strepsiptera simultaneously with improving of our knowledge of their hosts.