Charles University

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Evolution of Host Specialisation, Phylogeography and Taxonomic Revision of Xenidae (Strepsitera)

Evoluce hostitelské specializace, fylogeografie a taxonomie řasníků čeledi Xenidae (Strepsiptera)

Doctoral Thesis

Supervisor

Mgr. Jakub Straka, Ph.D.

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DECLARATION OF ORIGINALITY

I declare that this thesis or its substantial part has not been submitted to obtain the same or any other academic degree. I have written my thesis independently based on the material cited in the text and in consultation with my supervisor and colleagues.

In Prague on 31 March 2023

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PROHLÁŠENÍ

Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu. Tuto práci jsem zpracoval samostatně a uvedl všechny použité informační zdroje a literaturu.

V Praze dne 31. 3. 2023

Mgr. Daniel Benda

STATEMENT OF CONTRIBUTION

This thesis consists of 5 manuscripts (4 published and 1 submitted). I am the first author of all papers and corresponding author of 4 papers. The Journal Impact Factor Quartile (JIFQ) is from the Web of Science, using the highest quartile from all categories that include a particular journal. Journal metrics are valid for the year of publication. Citation reports are valid on the 1st of April 2023. My contributions to each manuscript comply with the CRediT authorship statement (Brand *et al.* 2015).

PROHLÁŠENÍ O PODÍLU NA PUBLIKACÍCH

Tato práce se skládá z 5 manuskriptů (4 publikovaných a 1 odeslaného k publikaci). Jsem prvním autorem všech publikací a korespondenčním autorem v případě 4 publikací. Pořadí časopisu v kvartilu (Journal Impact Factor Quartile; JIFQ) převzaté z Web of Science odpovídá v případě zařazení časopisu do více oborů jeho nejlepšímu umístění. Metriky časopisů jsou platné pro rok vydání. Můj příspěvek k jednotlivým publikacím je specifikován níže pomocí CRediT statement (Brand *et al.* 2015).

1. **Benda D.**, Nakase Y., Straka J. (2019). Frozen Antarctic path for dispersal initiated parallel host-parasite evolution on different continents. *Molecular Phylogenetics and Evolution*, 135, 67-77. doi: 10.1016/j.ympev.2019.02.023

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2. **Benda D.**, Votýpková K., Nakase Y., Straka J. (2021). Unexpected cryptic species diversity of parasites of the family Xenidae (Strepsiptera) with a constant diversification rate over time. *Systematic Entomology*, 46(1), 252-265. doi: 10.1111/syen.12460

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Mgr. Jakub Straka, Ph.D.

3. **Benda D.**, Pohl H., Nakase Y., Beutel R., Straka J. (2022). A generic classification of Xenidae (Strepsiptera) based on the morphology of female cephalothorax and male cephalotheca with a preliminary checklist of species. *ZooKeys*, 1093, 1-134. doi: 10.3897/zookeys.1093.72339

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4. **Benda D.**, Pohl H., Beutel R., Straka J. (2022). Two new species of Xenos (Strepsiptera: Xenidae), parasites of social wasps of the genus Mischocyttarus (Hymenoptera: Vespidae) in the New World. *Acta Entomologica Musei Nationalis Pragae*, 62(1), 185-195. doi: 10.37520/aemnp.2022.014

Citations (Google Scholar|Web of Knowledge) = 0|0; IF2022 = 0.737; JIFQ = Q4

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Mgr. Jakub Straka, Ph.D.

5. **Benda D.**, Pohl H., Nakase Y., Beutel R., Straka J. (2023): A new species of the genus *Paraxenos* Saunders, 1872 (Strepsiptera: Xenidae) from *Bembix* digger wasps (Hymenoptera: Bembicidae) and a redescription of *Paraxenos hungaricus* (Székessy, 1955). (submitted to *Europaean Journal of Taxonomy*)

Contribution of Daniel Benda: Conceptualization, Methodology, Formal Analysis, Investigation, Resources, Data curation, Writing – Original Draft, Writing – Review & Editing, Visualisation, Project administration, Funding acquisition

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Table of contents

Abstract

Twisted-winged parasites (Strepsiptera: Xenidae) are an excellent group for studying the specialisation of parasites on hosts. Their parasitic lifestyle led to the evolution of numerous morphological, behavioural, physiological, and reproductive adaptations. Moreover, many complex adaptive traits of Strepsiptera have no parallel in other organisms. In contrast, little attention has been paid to the study of strepsipteran molecular phylogeny, phylogeography, species delimitation, and their implications for taxonomic classification.

Using the methods of molecular phylogeny, we created the first dated phylogenetic study of the family Xenidae. By investigating historical biogeography and ancestral host groups, we indicate that multiple lineages were exchanged between the New World and the Old World + Australia until Antarctica became completely frozen over. During the late Paleogene and Neogene periods, several lineages spread from the Afrotropics to other Old World regions and Australia. The original hosts of Xenidae were most likely social wasps, and the subsequent host switch from social to solitary wasps was secondary and probably occurred only once. The parallel host switch from solitary wasps to digger wasps (Sphecidae) occurred independently in the New and Old World. The biogeography and macroevolutionary history of Xenidae can be explained by a combination of dispersal, lineage extinction, and climatic changes during the Cenozoic era. A habitable Antarctica and the presence of now-submerged islands and plateaus that connected the New World and Old World + Australia facilitated the possibility of biotic exchange of Strepsiptera along with their hymenopteran hosts.

To analyse strepsipteran cryptic species diversity, we used three quantitative methods of species delimitation from molecular phylogenetic data – one distance-based (ABGD) and two tree-based (GMYC, bPTP). We found 77–96 putative species in our data and suggested the number of Xenidae species to be more diverse than expected. We identified 67 hosts to species level. Almost half of them were not previously known as hosts of Xenidae. The rate in net diversification is constant, which can be explained by the flexibility of this parasitic group, represented by their ability to colonise new host lineages combined with passive long-range dispersal by hosts.

Based on the results of molecular phylogenetic studies, we provide a taxonomic revision of Xenidae worldwide using morphological characters of female cephalothorax and male cephalotheca. We delimited 13 genera including 3 newly established. Furthermore, we devised a consistent approach of detailed species description, and we demonstrated it on the example of two new species of *Xenos* Rossi and one new species of *Paraxenos* Saunders.

Abstrakt

Řasníci (Strepsiptera: Xenidae) jsou skupinou hmyzích parazitů, která je velmi vhodná pro studium hostitelské specializace. Vyvinula se u nich řada adaptací na parazitický způsob života zahrnující komplexní morfologické, behaviorální, a fyziologické adaptace, které nemají u jiných organismů obdoby. Paradoxně malá pozornost byla naopak věnována studiu molekulární fylogeneze, fylogeografie, vymezení jednotlivých druhů a jejich implikacím pro taxonomickou klasifikaci.

S využitím metod molekulární fylogenetiky jsme vytvořili první datovanou fylogenezi čeledi Xenidae. Pomocí fylogeografických metod a rekonstrukce ancestrálních hostitelských linií jsme zjistili, že mezi Novým světem a Starým světem + Austrálií došlo k výměně některých linií, dokud Antarktida zcela nezamrzla. Během pozdního paleogénu a neogénu se několik linií rozšířilo z Afrotropické oblasti do dalších oblastí Starého světa a Austrálie. Původními hostiteli čeledi Xenidae byly s největší pravděpodobností sociální vosy, přičemž následný přechod od sociálních k samotářským vosám byl sekundární a pravděpodobně k němu došlo pouze jednou. K paralelnímu přeskoku ze samotářských vos na kutilky čeledi Sphecidae došlo nezávisle na sobě v Novém a Starém světě. Evoluční historii Xenidae lze vysvětlit kombinací šíření, vymírání linií a klimatických změn během kenozoika. Nezamrzlá Antarktida a přítomnost komplexu nyní již zatopených ostrovů a částí pevniny, které fungovaly jako spojení mezi Novým světem, Starým světem, a Austrálií, tak usnadnily možnost disperze řasníků spolu s jejich blanokřídlými hostiteli.

K otestování přítomnosti kryptické druhové diverzity jsme použili tři kvantitativní metody vymezení druhů na základě fylogenetických dat – ABGD, GMYC, bPTP. Naše data ukazují, že čeleď Xenidae je mnohem diverzifikovanější, než se předpokládalo. Podařilo se identifikovat 67 hostitelských druhů, přičemž téměř polovina z nich nebyla pro čeleď Xenidae dříve známá. Konstantní diverzifikaci lze vysvětlit velkou flexibilitou těchto řasníků, která spočívá především v dobré schopnosti kolonizovat nové hostitelské linie a také ve schopnosti pasivní disperze spolu s hostiteli na velké vzdálenosti.

Na základě výsledků molekulární fylogeneze jsme provedli taxonomickou revizi čeledi Xenidae. K identifikaci diagnostických znaků pro morfologickou revizi byly využity samičí cephalothorax a samčí cephalotheca. Nově jsme vymezili celkem 13 rodů včetně 3 nově popsaných. Připravili jsme návod na podrobný a konzistentní popis druhů na příkladu dvou nových druhů rodu *Xenos* Rossi a jednoho nového druhu rodu *Paraxenos* Saunders.

List of papers

Phylogeography and host specialisation

I.   **Benda D.**, Nakase Y., Straka J. (2019). Frozen Antarctic path for dispersal initiated parallel host-parasite evolution on different continents. *Molecular Phylogenetics and Evolution*, 135, 67-77. doi: 10.1016/j.ympev.2019.02.023

Cryptic diversity

II.   **Benda D.**, Votýpková K., Nakase Y., Straka J. (2021). Unexpected cryptic species diversity of parasites of the family Xenidae (Strepsiptera) with a constant diversification rate over time. *Systematic Entomology*, 46(1), 252-265. doi: 10.1111/syen.12460

Generic classification

III.   **Benda D.**, Pohl H., Nakase Y., Beutel R., Straka J. (2022). A generic classification of Xenidae (Strepsiptera) based on the morphology of female cephalothorax and male cephalotheca with a preliminary checklist of species. *ZooKeys*, 1093, 1-134. doi: 10.3897/zookeys.1093.72339

Species classification

IV.   **Benda D.**, Pohl H., Beutel R., Straka J. (2022). Two new species of Xenos (Strepsiptera: Xenidae), parasites of social wasps of the genus Mischocyttarus (Hymenoptera: Vespidae) in the New World. *Acta Entomologica Musei Nationalis Pragae*, 62(1), 185-195. doi: 10.37520/aemnp.2022.014

V.   **Benda D.**, Pohl H., Nakase Y., Beutel R., Straka J.: A new species of the genus *Paraxenos* Saunders, 1872 (Strepsiptera: Xenidae) from *Bembix* digger wasps (Hymenoptera: Bembicidae) and a redescription of *Paraxenos hungaricus* (Székessy, 1955) (accepted in *Europaean Journal of Taxonomy*)

Other papers (not included in the thesis)

Talašová A., Straka J., Hadrava J., **Benda D.**, Kocourek F., Kazda J. (2018): High degree of philopatry is required for mobile insects used as local indicators in biodiversity studies. *Ecological indicators*, 94, 99-103.

Mikát M., **Benda D.**, Straka J. (2019): Maternal investment in a bee species with facultative nest guarding and males heavier than females. *Ecological Entomology*, 44(6), 823-832.

Mikát M., **Benda D.**, Korittová C., Mrozková J., Reiterová D., Waldhauserová J., Brož V., Straka J. (2020): Natural history and maternal investment of *Ceratina cucurbitina*, the most common European small carpenter bee, in different European regions. *Journal of Apicultural Research*, 61: 2, 1-12.

Mikát M., **Benda D.**, Straka J. (2021): Unrelated males in societies of a facultatively social bee. *Journal of Apicultural Research*, 1-12.

Hadrava J., Talašová A., Straka J., **Benda D.**, Kazda J., Klečka J. (2022). A comparison of wild bee communities in sown flower strips and semi-natural habitats: A pollination network approach. *Insect Conservation and Diversity*. 1-13.

Leclercq N., Marshall L., Weekers T., Anselmo A., **Benda D.**, Bevk D., Bogusch P., Cejas D., Drepper B., Galloni M., Gérard M., Ghisbain G., Hutchinson L., Martinet B., Michez D., Molenberg J.M., Nikolic P., Roberts S., Smagghe G., Straka J., Vandamme P, Wood T.J., Vereecken N. J. (2022): A comparative analysis of crop pollinator survey methods along a large-scale climatic gradient. *Agriculture, Ecosystems & Environment*, 329, 107871.

Knapp M., González E., Štrobl M., Seidl M., Jakubíková L., Čížek O., Balvín O., **Benda D.**, Teder T., Kadlec T. (2022): Artificial field defects: A low-cost measure to support arthropod diversity in arable fields. *Agriculture, Ecosystems & Environment*, 325, 107748.

Mikát M., Fraňková T., **Benda D.**, Straka, J. (2022): Evidence of sociality in European small Carpenter bees (Ceratina). *Apidologie*, 53 (2), 1-16.

Hlaváček A., Daňková K., **Benda D.**, Bogusch P., Hadrava J. (2022): Batesian-Müllerian mimicry ring around the Oriental hornet (Vespa orientalis). *Journal of Hymenoptera Research*, 92, 211-228.

Bogusch P., Amirmohammedi F., **Benda D.**, Roller L., Sakaki S., Petr L. (2022): Difference in pollen specialisation in spring bees *Andrena vaga* (Andrenidae) and *Colletes cunicularius* (Colletidae) during their nesting season. *Arthropod-Plant Interactions*, 16(5), 459-467.

Straka J., **Benda D.**, Policarová J., Astapenková A., Wood T.J., Bossert S.: Ancestral host reconstruction, biogeography and classification of the West Palearctic *Nomada* (Apidae) using phylogenomics (submitted to *Insect Systematics and Diversity*)

Mikát M., Fraňková T., **Benda D.**, Straka J.: Biparental care occurs despite low paternity in multiple species of small carpenter bee (prepared for submission to *Molecular Ecology*)

"It is for such inquiries the modern naturalist collects his materials; it is for this that he still wants to add to the apparently boundless treasures of our national museums, and will never rest satisfied as long as the native country, the geographical distribution, and the amount of variation of any living thing remains imperfectly known."

Alfred Russel Wallace
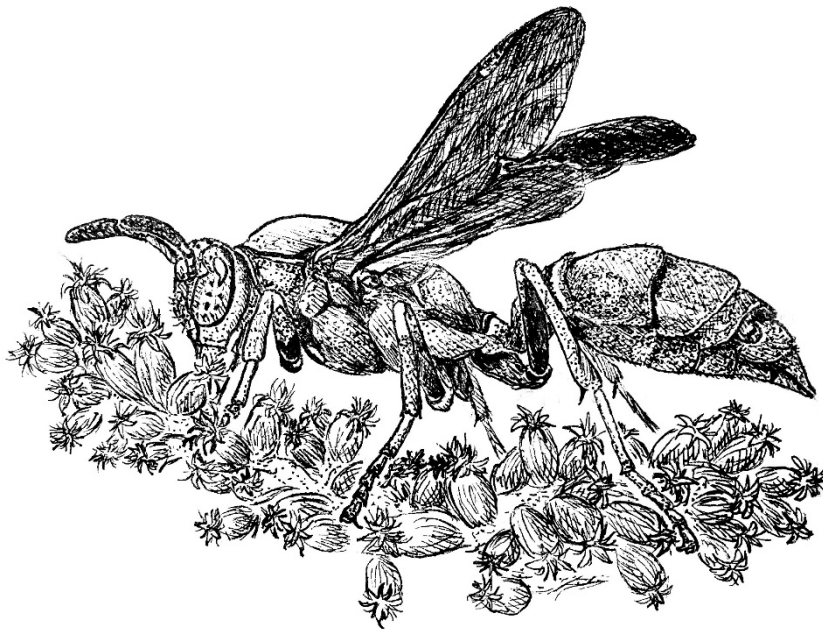
1 Introduction

In this dissertation, I focus on host specialisation and biogeographic history of Xenidae in light of molecular phylogeny. In addition, I examine the cryptic diversity and taxonomy at the genus and species levels. In the introduction, I first provide a brief overview of the phenomenon of parasitism and the definitions of host specialisation and specificity. Then I briefly discuss the topic of phylogeography and finally review the current status of the order Strepsiptera, its characteristics, phylogenetic position, classification, and cryptic diversity.

1.1 Parasitism as an effective life strategy

Parasitism is probably the most common life strategy of organisms (Windsor 1998). Parasites are organisms that spend a significant part of their lives in close physical contact with their host and live at the expense of that host, harming it and reducing its biological fitness. Parasites and their hosts are thus in a constant arms race. While hosts attempt to defend themselves against parasitism through various mechanisms, parasites, in turn, evolve various ways to overcome these defences (Dawkins & Krebs 1979).

During evolution, parasites and their hosts interact through selection pressure in a process called coevolution (Thompson 1994; Townsend *et al.* 2008; Clayton *et al.* 2015). According to the *Red Queen hypothesis*, an evolutionary lineage must keep pace with the rate of evolution of other lineages with which it interacts to avoid extinction (Van Valen 1973).

The parasite's defences against the host include various adaptations: most importantly, resistance to the host's immune system, persistence in the host's body, and the ability to efficiently extract resources from it. Dissemination of infectious stages (in the case of endoparasites) is also an important requirement for successful parasitism. In contrast, the host's most important defence against parasites is an effective immune system and behavioural changes that reduce the risk of contact with the parasite and its infectious stages (Moore 2002). It is therefore advantageous for the parasite to specialise as much as possible on the host and create the effective adaptations in order to successfully resist the host's defence mechanisms (Begon *et al.* 2006).

1.2 Host specialisation and specificity

The specialisation to the host is a coevolutionary process, as parasites are under constant pressure to adapt to the host. This process, driven by natural selection, leads to the emergence of

adaptations (Ridley 1993; Poulin 2007). Better specialisation brings the parasite more efficient use of host resources and less leverage from competition (Begon *et al.* 2006). On the other hand, generalists are forced to maintain a larger repertoire of characters, from which additional variants may arise during evolution, allowing the exploitation of other resources. Therefore, generalists may have a higher evolutionary potential in contrast to specialised species, which have a higher probability of extinction than generalists because they are unable to respond to the disappearance of the resource they use (Futuyma & Moreno 1988). If the parasite eradicates its host, it is likely to simultaneously suffer the same fate (Brooks & McLennan 2002). Narrow specialisation is often accompanied by loss or reduction of a number of characters.

On the other hand, host specificity is a measure of how large a range of hosts a parasite can successfully colonise. It reflects the width of the parasite's ecological niche better than other parameters. In parasitology, the degree of host specificity is a very important characteristic. It provides information on the probability of host switch under current or new conditions (Poulin & Mouillot 2003). Clearly, the incorrect identification of species, unrecognised cryptic species, and synonymisation of species greatly affect the ability to determine host specificity correctly. Therefore, host range is only a rough measure of host specificity (Poulin 2007). Estimation of host specificity may be affected by incorrect synonymisation of species due to the presence of cryptic species (Poulin 2007). Therefore, when considering host specificity, it is necessary to work with a taxonomically well-resolved group.

Host specificity decreases as the number of potential host species increases. However, this is not an accurate measure of parasite specificity in a population because it does not mean that individuals can use all hosts. They can only adapt to locally available host species and are therefore more host-specific than the species as a whole (Poulin 2007). In small populations or in species with limited mobility, the reason for the occurrence of narrow specificity may also stem from the fact that it increases the probability of two individuals meeting, thus increasing the number of mating opportunities (Rohde 1979).
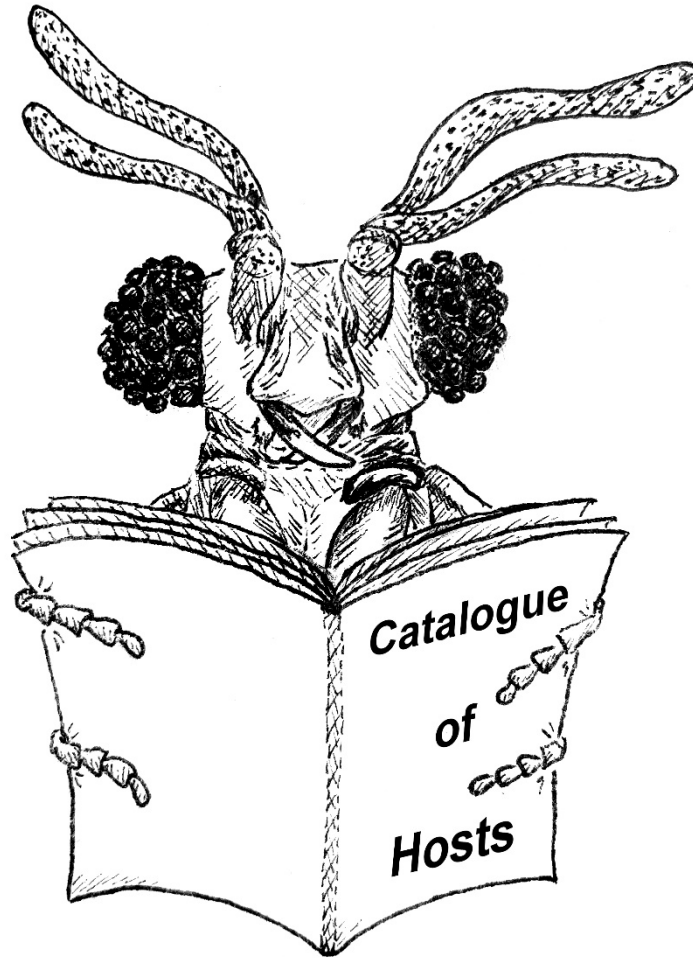
1.3 Phylogeography

To interpret the phenomena of biogeography, we need to understand many different areas of science, for example, evolution, taxonomy, ecology, geology, palaeontology, and climatology (Cox *et al.* 2016). Recently, an increasing range of DNA techniques, combined with new analytical methods and recent palaeoclimatic and geological studies, provides important insights into the distribution of genetic diversity around the globe and how it evolved (Emerson & Hewitt 2005). Vicariance and long-distance dispersion (LDD) are two of the most important mechanisms of global terrestrial biogeography, and they are often considered as competing hypotheses. Disjunct distribution patterns can be explained either by fragmentation of widespread ancestors by vicariant (isolating) events, or by dispersal across a pre-existing barrier (Sanmartín & Ronquist 2004).

The break-up of the supercontinent Gondwana began in the Mesozoic era and lasted until the beginning of the Oligocene about 30 million years ago. This led to its separation into Africa, Antarctica, Australia, India, Madagascar, New Zealand, and South America. An accurate understanding of the sequence and timing of geological events associated with the break-up of the southern continents is crucial for interpreting the biogeographical history of biological taxa (Scotese *et al.* 1988; Jokat *et al.* 2003). The biogeographic history of the Southern Hemisphere was considered a prime example of the vicariance scenario: the disjunct trans-Pacific distribution is the result of the sequential break-up of the southern supercontinent Gondwana, which led to a vicariant partitioning of the ancestral biota (Brundin 1966). Recent molecular estimates suggest that the Cretaceous break-up of Gondwana played an important role in the ordinal diversification of birds and mammals (Eizirik *et al.* 2001; Cracraft 2001).

However, palaeogeographic reconstructions show that the biogeographic history of the Southern Hemisphere cannot be reduced to a simple sequence of vicariation events, at least not entirely. Recent biogeographic studies based on molecular estimates and more accurate palaeogeographic reconstructions suggest that dispersal may have been more important than traditionally assumed (Sanmartín & Ronquist 2004). Although the break-up of Gondwana began in the Cretaceous, the connection between Australia and South America via Antarctica allowed biotic exchange to continue into the late Eocene (McLoughlin 2001). These exchanges were possible because of the habitable Antarctic environment under Eocene climatic conditions (Pross *et al.* 2012). Antarctica was covered with a subtropical rainforest in which southern beech (*Nothofagus*) was the dominant plant (Iglesias *et al.* 2011).

The connection between South America, Antarctica, Australia, and adjacent island archipelagos in the Indian Ocean played a crucial role in the evolution of highly mobile aculeate Hymenoptera (Almeida *et al.* 2012). Antarctica, and now submerged Kerguelen and Crozet Plateaus, probably served as an effective migration bridge for fauna exchange not only between South America and Australia, but also between Australia and Africa (Schwarz *et al.* 2006; Chenoweth & Schwarz 2011; Kayaalp *et al.* 2017).
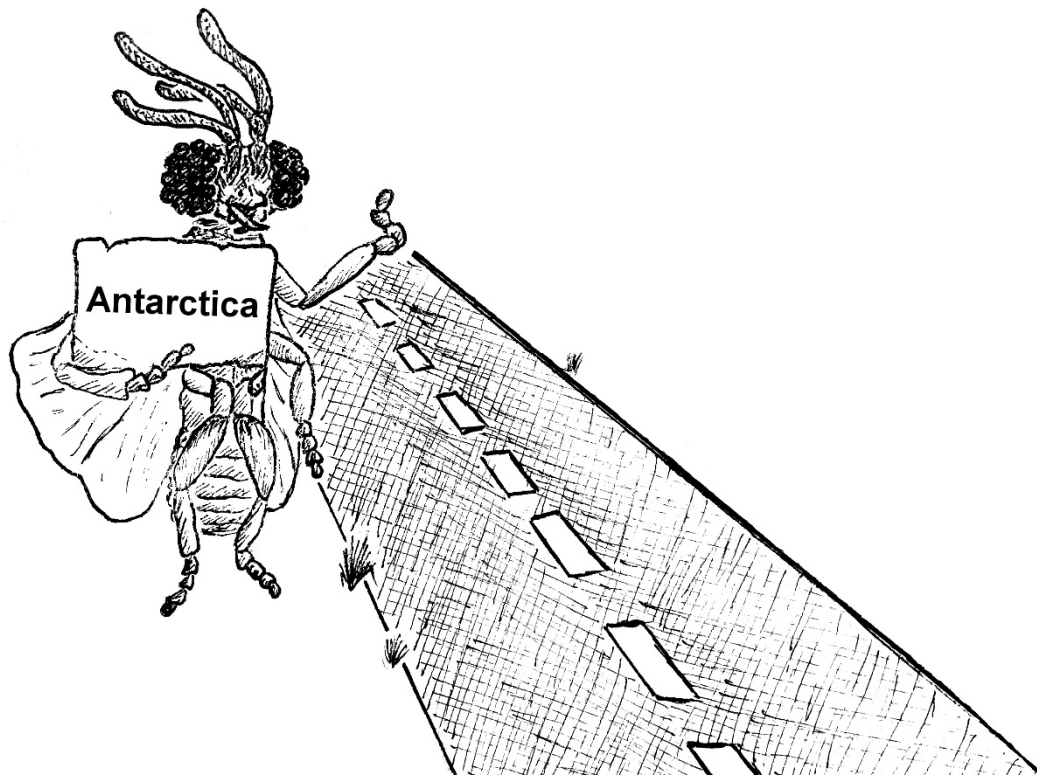
1.4 Characterisation of Strepsiptera

1.4.1 Strepsiptera as specialised parasites

The Strepsiptera are an obligately parasitic order. They parasitise 7 groups of insects (Zygentoma, Blattodea, Mantodea, Orthoptera, Hemiptera, Diptera, Hymenoptera) in more than 30 families. They form more than 600 species in nine extant families (Cook 2019). They are extremely specialised to their hosts and have evolved a number of endoparasitic apomorphies. These include extreme sexual dimorphism, female neoteny, and miniaturisation of the first larval stages (Kinzelbach 1971; Pohl & Beutel 2008).

The males of Strepsiptera are free-living and winged in all families. Their mesothoracic pair of wings is reduced and similar in appearance and function to the halteres of Diptera (Kinzelbach 1978; Kathirithamby 1989). The males are short-lived, living only a few hours, during which a female must be found and fertilised (Linsley & MacSwain 1957; Kifune & Maeta 1975; Kathirithamby 2009). Males of all recent groups do not feed (Pohl & Beutel 2005). The organisation of their compound eyes and the ultrastructure of the ommatidia, which resemble

those of extinct trilobites, are interesting and unusual (Buschbeck *et al.* 1999, 2003; Buschbeck 2005). This is probably an adaptation to an originally nocturnal lifestyle (Buschbeck *et al.* 2003).

Adult females are apterous, neotenic, and viviparous. Representatives of the ancestral family Mengenillidae are free-living; females of other groups remain in the host and are adapted to permanent endoparasitism. They have undergone an extreme reduction of body structures. A sclerotised anterior part, called the cephalothorax, extrudes from the host's body, representing the head, thorax, and a part of the first abdominal segment with a pair of spiracles (Kathirithamby 1989; Pohl & Beutel 2008; Pohl *et al.* 2012). In the family Mengenillidae, copulation occurs by traumatic insemination on any part of the female's body except the head, with the male using his stylet-like copulatory organ (Silvestri 1943). In modern Strepsiptera (Stylopidia), mating occurs through the cephalothorax of the female. However, various speculations have been made about the method of copulation. Traumatic insemination has also been assumed, or the copulatory method has been described as the insertion of the aedeagus into a special organ, the "brood canal opening", through which the first larval stages later emerge (Nassonov 1910; Schrader 1924). The copulatory mechanism was recently elucidated by the work of Peinert *et al.* (2016) in the genus *Stylops*, when it was found that the aedeagus is inserted into a previously unknown paragenital organ formed by an indented cuticle covered by the membranes of earlier larval stages. The paragenital organ and the "brood canal" have a common exit (Peinert *et al.* 2016).

Females release a powerful sex pheromone to attract males (Kirkpatrick 1937; Riek 1970; Tolasch *et al.* 2012; Cvačka *et al.* 2012). Jandausch *et al.* (2022) found that *Stylops ater* females attract sympatrically distributed congeneric males. However, only conspecific males were able to mate. In contrast, the authors did not observe any heterospecific male attraction by *Xenos* females. They hypothesise that the paragenital organ in the genus *Stylops* represents a prezygotic mating barrier that prevents heterospecific matings.

Larvae of Strepsiptera develop in four stages. The first larval instar is invasive, agile, and invades the host's larva or egg. A conspicuous feature of the primary larvae is the extreme degree of miniaturisation. With an average length of ca 230 µm, the larvae are distinctly smaller than many protists. This dramatic size reduction is likely a result of the necessity to produce a large amount of offspring and to enter a relatively small insect host larvae (Pohl & Beutel 2008). Primary larvae are released by endoparasitic females in enormous numbers (up to 750,000) (O'Connor 1959). Larvae of Strepsiptera, which parasitise hemimetabolous insects, can reach their hosts relatively easily, as the adults live in the same environment as the immature stages (Jůzová

2016). Their well-developed larval eyes (stemmata) enable the larvae to identify a suitable host. It was demonstrated that the optical apparatus is even capable of distinguishing colours (Kirkpatrick 1937). To reach the host, the larvae use well-developed bristle-like cerci with strong muscles attached to their base as a jumping apparatus (Pohl & Beutel 2008). Derived body plan features of primary larvae are adhesive pads on the legs and adhesive hairs on ventral body surface that enable them to stick to the host's surface by capillary forces (Pohl & Beutel 2004). The other larval stages are endoparasitic. Their apolysis is not followed by ecdysis (Manfredini *et al.* 2007). In the case of the ancestral family Mengenillidae, both male and female secondary larval stages leave the host completely and pupate within the unusually resistant and hardened larval cuticle which forms the puparium. Only first larval stages and adult males are free-living in Stylopidia (Pohl & Beutel 2008).

In the case of Stylopidae and Xenidae, the most important evolutionary novelty is the phoresy of the primary larvae that get carried by fast-flying aculeate hosts to their nest. This is likely key to the success of these families, which represent the most diversified lineage of Strepsiptera (Pohl & Beutel 2008). Larvae of Xenidae probably attack their vectors directly, clinging to the legs and petiole and attacking the host larvae in the nest (Kathirithamby *et al.* 2012). Xenid larvae have two autapomorphies: the enlarged and rounded adhesive pads on the first and second pair of legs, and filamentous cuticular outgrowths on labium which strongly increase the wettability. These apomorphies are discussed as an adaptation to the smooth body surface of the hosts (Pohl & Beutel 2008). Stylopidae are exclusive parasites of bees. An important evolutionary shift in the primary larvae is the loss of the jumping capacity, as it is made dispensable by the phoretic behaviour (Pohl & Beutel 2008). Moreover, thoracic and abdominal segments are dorsally and ventrally covered by spinulae of two different lengths arranged in distinct patterns, or extremely long caudal setae. These projections anchor larvae in the dense hairs of the phoretic host and may lock with the pollen grains collected by the foraging bee (Straka *et al.* 2014; Balzer & Davis 2019). The larvae are thus transported to the nest on the host's surface (Ulrich 1956; Balzer & Davis 2019), or in a more sophisticated manner in the host crop ingested with nectar and pollen (Linsley & MacSwain 1957).

1.4.2   Phylogenetic position within insects

Perhaps no other order has caused so much trouble to taxonomists with its uncertain position within insects as Strepsiptera. The first species was described by Rossi (1793) as *Xenos vesparum* and was placed by him in the family of parasitic Ichneumonidae. Kirby (1813) then placed Strepsiptera as a separate order of insects. Since then, numerous hypotheses and

speculations about their phylogenetic position arose, based on morphology and developmental biology findings.

Burmeister (1837) hypothesised a relationship between Strepsiptera and beetles from the family Ripiphoridae, and Peters *et al.* (1863) classified them as Trichoptera. The relationship between Strepsiptera and Diptera was also a very popular hypothesis (Newman 1834). Pierce (1936) took this hypothesis further and, based on the morphology of the puparia, established the group Pupariata, which included Coccoidea (Hemiptera) in addition to Diptera and Strepsiptera.

In the second half of the 20th century, the idea of the close position of Strepsiptera and Coleoptera reasserted itself (Hennig 1969; Kinzelbach 1971). The term "the Strepsiptera problem" (Kristensen 1981) is also used as an expression of helplessness in the face of efforts to clearly classify Strepsiptera in the insect system. However, the modern cladistic approach, based on analyses of morphological and ultrastructural data, usually classifies them as a sister group of Coleoptera (Beutel & Gorb 2001; Kukalová-Peck & Lawrence 2004).

Male of *Xenos vesparum* Rossi, 1793, the first described species of Strepsiptera. © Daniel Benda

With the advent of DNA sequencing methods, the first molecular studies showed the sister relationship of Strepsiptera and Diptera, for which the Halteria group was proposed (Chalwatzis *et al.* 1996; Whiting *et al.* 1997). However, these works were based only on ribosomal RNA sequences (18S and 28S). When multigene analyses of many other nuclear genes were performed, Strepsiptera were again supported as a sister group to beetles—the Coleopterida hypothesis (Wiegmann *et al.* 2009; Ishiwata *et al.* 2011). More recent phylogenomic analyses using genomic and transcriptomic data clearly support the Coleopterida hypothesis (Niehuis *et al.* 2012; Misof *et al.* 2014; Boussau *et al.* 2014).

This hypothesis is still accepted today, and the phylogenetic position of Strepsiptera within insects is considered to be resolved. For more information, Pohl & Beutel (2013) provide a detailed and comprehensive overview of the development of hypotheses about the position of Strepsiptera in their work "The Strepsiptera-Odyssey".

1.4.3   Phylogeny and evolution of Strepsiptera

The oldest known fossils of Strepsiptera come from Burmese amber from middle Cretaceous (about 100 million years ago). The species *Cretostylops engeli*, described by Grimaldi *et al.* (2005) based on a single male, was until recently considered the oldest known fossil. However, another male fossil from Burmese amber was recently described—*Kinzelbachilla ellenbergeri* (family Kinzelbachillidae), which, according to morphological analyses, is evolutionarily even older (Pohl & Beutel 2016). Surprisingly, morphological analyses revealed that the evolutionarily oldest known group of Strepsiptera is the family Protoxenidae, represented by a single species *Protoxenos janzeni* that was found in Baltic amber from a relatively later period of 30–50 million years ago. In this group, males were still able to process food as adults (Pohl *et al.* 2005). These findings suggest that Strepsiptera were already a highly derived and specialised group in the Mesozoic, and their origin is probably much older. The most recent phylogenomic study dates the origin of Strepsiptera, according to molecular clock, to the Permian period—almost 300 million years ago (Misof *et al.* 2014).

The most basal recent group of Strepsiptera is the family Bahiaxenidae, of which only one species is known. It occurs in South America, and the host group is unknown, but parasitism on silverfish (Lepismatidae) is likely (Bravo *et al.* 2009). Free-living females are expected in the Bahiaxenidae, as well as in the Mengenillidae, which, in contrast, are widely distributed in all areas except the New World. The significant apomorphy of both families is the absence of specialised attachment structures on the tarsi of males (Pohl & Beutel 2005; Pohl *et al.* 2012).

The most dramatic evolutionary event of Strepsiptera was the transition to parasitising winged insects (Pterygota). It was primarily associated with the permanent endoparasitism of females and the associated formation of attachment structures on the tarsomeres of males, which must cling to the host during copulation. Permanent endoparasitism meant extreme body remodelling and loss of many organs (see above). All strepsipterans parasitising Pterygota belong to the superfamily Stylopidia, which includes the vast majority of all recent Strepsiptera species (Pohl & Beutel 2008; Kathirithamby 2009).

The Corioxenidae are obligate parasites of true bugs (Heteroptera), and their males are primarily nocturnal. The basal position of this family within Stylopidia is supported by both molecular and morphological analyses (Pohl 2002; Pohl & Beutel 2005; McMahon *et al.* 2011). The centre of their distribution lies in tropical areas. This family was reviewed by Tribull & Cook (2013). The other derived families belong to the group Stylopiformia. They include the ancestral group Bohartillidae, represented by the only recent species *Bohartilla megalognatha*, known only from Central America with unknown hosts (Kinzelbach 1969). Of great interest is the finding of a male of the same species from Dominican amber (25–30 million years old), which is morphologically indistinguishable from the recent species (Kathirithamby & Grimaldi 1993).

A very remarkable group is the family Myrmecolacidae, characterised by different host specialisation within the sexes—males parasitise ants (Formicidae) and females Orthoptera and Mantodea (Ogloblin 1939; Kathirithamby & Johnston 2003). Males of this family are relatively common in the fossil record, especially in Baltic amber (Kathirithamby & Grimaldi 1993; Kinzelbach & Pohl 1994; Kathirithamby & Henderickx 2008). However, there is also a known fossil of an endoparasitic female in a host ant, suggesting that both sexes probably parasitised ants in the past (Pohl & Kinzelbach 2001). In a phylogenetic study of Strepsiptera, the peculiar Palaeotropical genus *Lychnocolax* Bohart with unknown hosts was recognised as belonging to the family Lychnocolacidae distinct from the Myrmecolacidae (McMahon *et al.* 2011; Kathirithamby & Engel 2014; Engel 2020). In addition, the family Callipharixenidae was placed in the Halictophagidae (McMahon *et al.* 2011).

According to the mentioned molecular analyses from McMahon *et al.* (2011), the families Halictophagidae and Elenchidae form a monophyletic clade. Both parasitise Auchenorrhyncha, but Halictophagidae have an even broader host range and additionally parasitise representatives of Heteroptera, Orthoptera, Blattodea, and Diptera (Kathirithamby 1989). One of the most derived groups of Strepsiptera is the monophyletic clade consisting of two families: Stylopidae and Xenidae. Its monophyly has been supported by molecular and morphological analyses (Pohl

& Beutel 2005; McMahon *et al.* 2011). They parasitise aculeate Hymenoptera and have strictly diurnal males unlike other strepsipterans (Pohl & Beutel 2008).

Dicranotropis divergens **Kirschbaum, 1868 parasitised by a female of** *Elenchus tenuicornis* **(Kirby, 1815) (Czech Republic, NPR Praděd) © Daniel Benda**

1.4.4 Diversity and cryptic species

Cryptic species are species that are genetically distinct but morphologically indistinguishable, although slight differences are often detected. Extreme environmental conditions might impose stabilising selection on morphology, reducing or eliminating morphological changes that may accompany speciation (Bickford *et al.* 2007). Cryptic species are being revealed in parasites and parasitoids who are usually more diversified, but the species diversity is not obvious due to the reduced morphological structures (León & Poulin 2018). Despite the cryptic species problem, correct identification of species is essential in estimating host specificity of parasites (Poulin 2007). Although phylogenetic relationships within Strepsiptera have been largely elucidated (McMahon *et al.* 2011), only a few studies have dealt with strepsipteran species diversity using molecular phylogenetic methods. Nevertheless, this is an attractive topic, as parasite and

parasitoid diversity has often been frequently discussed in the context of hidden diversity and cryptic species (Smith *et al.* 2008; Santos *et al.* 2018).

For Strepsiptera, the use of molecular phylogenetic data was first tested on the family Myrmecolacidae (Halbert *et al.* 2001). Several myrmecolacid species were detected based on sequencing of COI and 18S genes. Subsequent molecular phylogenetic works studied the host specificity of myrmecolacid species and confirmed that the extremely dimorphic sexes utilise hosts from separate orders (Kathirithamby & Johnston 2003; Kathirithamby *et al.* 2010). Hayward *et al.* (2011) performed a phylogeographic analysis of the species *Caenocholax fenyesi* (Myrmecolacidae) in Central America and discovered at least 10 clades that could be considered separate species. These lineages show some degree of biogeographic separation and host specificity. Host specificity sometimes differs between males and females, with changes in host preference accompanying diversification. Fossil evidence from the *C. fenyesi* complex suggests a very low molecular clock rate and an ancient origin of cryptic lineages, supporting the theory of slow changes in anagenesis. The common ancestor of the entire lineage is estimated to be approximately 30 million years old (Kathirithamby & Henderickx 2008; Hayward *et al.* 2011).

Currently, however, attention is also being paid to other families. Matsumoto *et al.* (2011) focused on phylogeography of the species *Elenchus japonicus* (Elenchidae) in Southeast Asia. The results of this study revealed three species-like lineages with different host specificity. However, no association was found between the three genotypes detected and the host genera or collection site. In Xenidae, the first occurrence of the cryptic species phenomenon was published by Nakase & Kato (2013), who focussed on the giant strepsipteran *Xenos* Rossi parasites in large hornets (Vespidae: *Vespa*). They found two distinct species of *Xenos* with different host specificity and inconspicuous distinguishing characteristics. Jůzová *et al.* (2015) performed a molecular phylogeny of the species-rich genus *Stylops* (Stylopidae), which parasitises *Andrena* Fabricius bees. This study revealed that *Stylops* species are mostly specialised to specific host subgenera, as predicted in previous morphology-based studies by Bohart (1941) and Luna de Carvalho (1974).

1.4.5   Generic and species classification

Although a proper understanding of parasite taxonomy is important, especially for the assessment of host specificity, the genus and species taxonomy of Strepsiptera have been understood differently by various authors. In the history of Strepsiptera research, there have been many different views on their taxonomy, which were closely connected to their host specificity. The *a priori* view of host specificity has largely been a direct guide to the taxonomic approach

due to the lack of easily distinguishable characters associated with the presence of cryptic species and genera. Although several papers have investigated the interspecific relationships of Strepsiptera using molecular methods, there has been no molecular phylogenetic study that has addressed the position of genera within families in detail. Although Pohl & Beutel (2005) indicate intergeneric relationships for individual families based on morphological characters, genera are still considered as predefined taxonomic units.

McMahon *et al.* (2011) primarily deal with relationships between Strepsiptera families, but also provide some interesting information on the phylogenetic placement of genera within families. For example, they indicate the paraphyly of Myrmecolacidae and polyphyly of the genus *Halictophagus*. Specifically, in the genus *Halictophagus*, there are 13 other names that have been synonymised (Kinzelbach 1971). It is therefore very likely that some of them are valid, and a revision of this genus is highly desirable.

The first generic classification of Xenidae was provided by Pierce (1908, 1909, 1911) who described several genera based on a concept that each genus of Xenidae is specialised on one host genus of wasps. This concept was later rejected by Bohart (1941). A more recent classification of Xenidae has proposed four genera, each specialised on one or several families or subfamilies of hosts (Kinzelbach 1971; Cook 2019).

Although the definition of species is problematic in general (Nixon & Wheeler 1990; de Queiroz 2005), the most problematic was the definition of species in the genus *Stylops* Kirby, not only because it is the most numerous genus of Strepsiptera. The species concept mostly depended on the author's opinion for species recognition related to *a priori* defined host specificity. Many of the more than 110 available species of the genus *Stylops* were described on the principle of single host association. This approach has been applied to species in North America and Japan (Perkins 1918; Kifune & Hirashima 1985; Kifune 1991). A different concept was used by Bohart (1936, 1937, 1941) and Luna de Carvalho (1974). They investigated morphological characters and considered similarities of *Stylops* species from related hosts from the same subgenus. By contrast, Kinzelbach (1978) used a generalistic concept, in which all the recognised species of the Western Palaearctic were synonymised and lowered to the subspecies level. It meant that one species, *Stylops mellitae* Kirby parasitised all available species of the bee genus *Andrena*. This conservative hypothesis has been used until recently (Pohl & Oehlke 2003; Bleidorn *et al.* 2004; Smit & Smit 2005). Jůzová *et al.* (2015) provided the first molecular phylogeny of Stylopidae on interspecific level using broad sampling of *Stylops* species. They tested three hypotheses of parasitic strategy: specificity to host species, specificity to host subgenus, and

specificity to host genus. The authors rejected the generalistic concept as well as the concept of superspecialised *Stylops* species and revealed that *Stylops* species are sorted according to host subgenera. This concept can be used as a support tool, but not as a strict one, and it is necessary to approach each lineage individually.

2 Research questions and aims

In my dissertation, I explored four topics concerning the family Xenidae (Strepsiptera) as a model group: (1) phylogeography and host specialisation (**paper I**), (2) cryptic diversity and host specificity on species level (**paper II**), (3) classification of the family Xenidae at the genus level (**paper III**), and (4) species classification (**papers IV–V**). My general research questions for each topic and the specific objectives are as follows:

(1a) Can we detect any patterns in the historical biogeography of Xenidae? Did they disperse well with their hosts, or were there only a few dispersal events in their evolutionary history? Were they able to migrate frequently in a stepping-stone fashion between the Old and New World? Has there been long-distance dispersal in recent history?

- To reconstruct phylogeny of the family Xenidae based on phylogenetic analyses of selected mitochondrial and nuclear markers
- To perform divergence dating
- To reveal the historical biogeography of Xenidae

(1b) Can we identify any patterns of host specificity on a higher taxonomic level in the evolution of Xenidae? What were the ancestral hosts? Are the traditional Xenidae genera specialised, or rather more generalistic? Do they follow host evolution, or can we detect common host switching? Could a switch to the same host group occur in different lineages in parallel?

- To map host groups to the phylogenetic tree (1a), detect host switches on a long-term scale, and reveal the stability of host associations in Xenidae
- To interpret the patterns of host-parasite interactions between Xenidae and host wasps in the context of Xenidae systematics

(2) Does the family Xenidae contain cryptic species that have been overlooked? What is the host specificity of species of the family Xenidae? Could adaptations to parasitism limit the morphological changes associated with speciation? Is there any important pattern in net diversification through Xenidae evolution?

- To apply three quantitative methods of species delimitation for molecular phylogenetic data – one distance-based (ABGD) and two tree-based (GMYC, bPTP)

- To estimate host specificity based on the results of the species delimitation methods
- To analyse net diversification rate through Xenidae evolution

(3) Can we perform a genus-level revision of Xenidae based on previous results of molecular phylogeny? Can we find appropriate diagnostic characters to establish revised generic lineages?

- To document in detail external features of female cephalothorax and male cephalotheca using different techniques
- To provide detailed diagnoses and descriptions for all newly delimited genera
- To provide a practical determination key for the revised genera
- To discuss the new generic classification and compare it with the approach of previous authors

(4) Can we find suitable characters for species identification and for the construction of a determination key?

- To document in detail external features of female cephalothorax and male cephalotheca using different techniques
- To provide detailed diagnoses and descriptions of selected species groups of Xenidae
- To provide recommendations for future species descriptions and evaluate suitable and unsuitable characters for species identification

3 Results and discussion

3.1 Phylogeography and host specialisation

Xenidae are a diverse family that arose in post-Gondwanan time (median age of 54 Ma) when the continents were already distantly separated (McMahon *et al.* 2011). As the females remain permanently in the host throughout their life (Kinzelbach 1971; Pohl & Beutel 2008), their dispersal ability is fully dependent on their hymenopteran hosts. This situation provides a good opportunity to test hypotheses regarding long-distance dispersion or dispersal via stepping stones. In **paper I**, we reconstructed the dated phylogeny of the family Xenidae based on phylogenetic analyses of 6 selected mitochondrial and nuclear markers. Although the ultimate geographical origin of the family remains uncertain based on the biogeographic analyses, there is very good evidence for divergence into two lineages—one of Palaearctic origin and one of New World origin. We have evidence that there were two parallel dispersals of Xenidae from the New World to Australasian and Afrotropical regions during the late Eocene/early Oligocene. There was probably no intercontinental dispersal in the Northern Hemisphere during this time. After glaciation of Antarctica, we have no evidence of transcontinental dispersal through the southern route between the New World and the Old World (and Australia), which is consistent with other studies (McLoughlin 2001; Almeida *et al.* 2012). The results are also consistent with the timing of geological connections between the southern continents which were closely linked until the beginning of the Oligocene (Lawver *et al.* 1992).

Migration events during the more recent evolution of Xenidae are quite complex. Subsequent late Eocene dispersal events from the New World to Africa occurred in parallel during Xenidae evolution. During this period, it is more complicated to explain the dispersion events by southern way. Opening of the Drake and Tasmanian Passages, and the subsequent development of a southern circumpolar current and glaciation of Antarctica was the most likely cause of the rapid cooling of the planet (McLoughlin 2001). In the most recent history of Xenidae, we revealed the parallel dispersal of originally Afrotropical and Palaearctic lineages to Indomalayan and Australasian regions. We revealed this dispersal pattern in the lineage parasitising *Bembecinus* and in the lineage parasitising Eumenini. This supports the hypothesis that Afrotropics were the main source of xenid diversity for the Old World and Australia from the Miocene until the present. The increasing incidence of dispersal events in the last 7 million years could be caused by the emerging climate change at the end of the Miocene. Jansen *et al.* (1990) describe a series of glacial episodes in the area surrounding the Norwegian-Greenland Sea from the late Miocene

(5.45 million years ago) through the Pliocene period. These glacial episodes had a smaller magnitude than those of the episodes postdating the major onset of large-scale northern hemisphere glacial cyclicity. The effects of these climatic fluctuations could have exposed ecological niches for hosts of xenids, leading to expansions in which they brought their parasites with them.

Host-specific parasites are often good models tracking the biogeography of their hosts (Štefka *et al.* 2011). However, host specificity can decrease when a number of suitable hosts increases, especially in new environment (Dawkins & Krebs 1979; Poulin 2007). In **paper I** we also investigated host specificity on a higher taxonomic level and evolution of host specialisation during Xenidae history. According to the mapping analysis, the ancestral host group of Xenidae were social wasps. The host switch from social to solitary wasps was secondary and probably occurred only once. There were two or three independent switches from solitary wasps to digger wasps of the Sphecidae family, which happened in parallel in the New World and Old World + Australia. The parasites may have switched to sphecid wasps once and subsequently returned to solitary wasps, or two independent switches to sphecid wasps may have occurred. This result contradicts the traditional hypothesis that parasites are tied to their hosts and follow host evolution (Eichler 1948; Brooks 1979), and it also denies the traditional classification of Xenidae with four genera, each specialised on one or several families or subfamilies of hosts (Kinzelbach 1971; Cook 2019).

3.2 Cryptic diversity

With the advent of rapid DNA sequencing methods during recent decades, molecular taxonomy has been proposed for quick species diversity assessment. It has helped to resolve species diversity of various little-known taxa, and the enormous diversity in morphologically homogeneous groups has been successfully uncovered thanks to the use of molecular species delimitation methods (Tautz *et al.* 2003; Blaxter 2004). For insect parasites and parasitoids especially, the former species concept has been dramatically changed by molecular taxonomy (Hayward *et al.* 2011; Veijalainen *et al.* 2012). For testing the presence of hidden diversity in Xenidae, we used three quantitative methods of species delimitation based on molecular phylogenetic data (**paper II**). We found 77–96 putative species in our data and suggested the number of Xenidae species to be more diverse. Our findings are consistent with Hayward *et al.* (2011), who suggested that if cryptic species commonly exist in Strepsiptera, current order-wide estimates of diversity may be underestimated by more than an order of magnitude.

We showed evidence of 122 host species sorted out, from which 67 were identified to species level (name assigned), and almost half of them were not previously known as hosts of Xenidae. Our results suggest relatively high host specificity in Xenidae with 70–77% of putative species with only one host. The mean number of host species per putative species varied between 1.39 and 1.55. However, there can be significant differences in host specificity at the species level across Xenidae. We explain this results by the effect of a dominant host, as was suggested by Nakase & Kato (2013) in *Xenos* species parasitising hornets. In contrast, the diversification of Xenidae lineages was found to be nearly constant through time. It could be explained by increased flexibility of this group, represented by the relatively low host fidelity of Xenidae at long time scale, as well as their frequent passive (host-mediated) long-range dispersal.

3.3 Generic classification

Until recently, Xenidae included four genera. Traditionally, *Paragioxenos* Ogloblin is an enigmatic genus specialised on pollen wasps (Masarinae) with an endemic distribution in Australia. *Paraxenos* Saunders is distributed worldwide and specialises on wasps of the families Crabronidae, Sphecidae, and Bembicidae. *Pseudoxenos* Saunders is also cosmopolitan and specialises on solitary potter wasps (Eumeninae). *Xenos* Rossi, which occurs on all continents except for Australia and Antarctica, parasitises social wasps of the subfamilies Polistinae and Vespinae (Cook 2019). In contrast, results from **paper I** and **paper II** suggested the necessity of taxonomic changes in traditional generic classification due to the paraphyly of the genus *Pseudoxenos* and polyphyly of the genera *Xenos* and *Paraxenos*.

In **paper III**, we provided a classification of the genera of Xenidae based on morphology in accordance with results of our molecular phylogenetic studies. External morphological features of female cephalothoraces and male cephalothecae were documented in detail with different techniques. Detailed diagnoses and descriptions were presented for all 13 delimited genera. 5 previously described genera were removed from synonymy, 1 former subgenus was elevated to generic rank, and 3 genera were newly described. An identification key for female cephalothoraces and male cephalothecae was provided for all genera. The earliest diverging genera are usually well-characterised by unique features, whereas deeply nested genera are usually characterised by a combination of characters.

Although the previous classification of the genera of Xenidae implied a specialisation at the level of host genus (e.g., Pierce 1908, 1911) or at the level of host family or subfamily (Kinzelbach 1971), we provided a more complex generic concept combining both approaches. Some representatives of the current genera parasitise only one host genus (e.g., *Paragioxenos*

Ogloblin, *Nipponoxenos* Kifune & Maeta, *Tachytixenos* Pierce), whereas others can utilise hosts from three families (e.g., *Eupathocera* Pierce). Species diversity of a lineage depends probably on the ability to utilise new hosts which would also facilitate the dispersion and increase the distribution range (Benda *et al.* 2019).

3.4 Species classification

Although several species of Xenidae have recently been described or redescribed (e.g., Garza & Cook 2021; Dong *et al.* 2022), the original species descriptions usually lack a consistent concept of detailed morphological description and diagnoses. In **paper IV**, the revision of *Xenos* species parasitising *Mischocyttarus* is provided as a model for detailed species description. Diagnoses and descriptions of female cephalothorax are presented for three species. Diagnoses and descriptions of male cephalothecae are presented for two newly described species. Additionally, a key for *Xenos* species parasitic on *Mischocyttarus* is provided based on characters of the female cephalothorax and male cephalotheca. Identification of *Xenos* species based on external morphology is discussed. Species are easily recognisable by a combination of cephalothoracic colouration, cephalothoracic shape, length proportion of the head versus cephalothorax in females, and by the colouration and shape of the cephalotheca in males. In this paper, the recommendations for future species descriptions are also provided and various morphologic features are evaluated.

In **Paper V**, species of the genus *Paraxenos* from *Bembix* Saunders host were reviewed with a new species description. The first occurrence of *Paraxenos* from *Bembix* in the Afrotropical region is presented with a species determination key based on characters of the female cephalothorax and male cephalotheca. The *Paraxenos* species parasitising *Bembix* hosts can be easily distinguished from other species of the genus by very wide cephalothecae and cephalothoraces. Important characters for species identification are the shape of the mandibles and maxillae, the sculpture and colouration of the cuticle, and the shape of the clypeus. These characters coincide with important diagnostic features of the female cephalothorax and male cephalotheca used for differentiation of *Xenos* species in **paper IV**. The distribution and conservation status of *Paraxenos* spp. on *Bembix* are also discussed.

The findings of **papers IV** and **V** are consistent with the statement of Bickford *et al.* (2007) that cryptic species seem to be morphologically indistinguishable, but differences are often detected once researchers are prompted to look. Reliable identification of the conspecific individuals can be possible without comparing them to the type specimens, but only when descriptions with precise documentation are provided (Pohl *et al.* 2012).

4   General conclusion and future perspectives

Although Strepsiptera have been studied little until recently, nowadays there is a growing number of studies with varying emphasises. Modern studies on Strepsiptera address various topics such as manipulation of host transcriptome expression (Geffre *et al.* 2017), mating behaviour (Jandausch *et al.* 2022), or ultrastructural anatomy (Fischer *et al.* 2021). Some species have even become a model in Strepsiptera research (Weingardt *et al.* 2023). In this case, family Xenidae proved to be an excellent group for studying phylogeography and host specialisation of parasites.

We have shown that Southern Hemisphere provided an important migratory route for Xenidae, which were able to disperse effectively over long distances with their hymenopteran hosts, at least until the early Oligocene. Once they reached a new environment, they were able to switch to a new host lineage. In this way, the parallel switch from social wasps to digger wasps (Sphecidae) occurred independently in the New and Old World. Since the freezing of Antarctica, there have probably been no intercontinental dispersal via the southern route. In the recent history of Xenidae, we have demonstrated the parallel, long-range dispersal of two original Afrotropical and Palaearctic lineages into Indomalayan and Australasian regions. The original hosts of Xenidae were most likely social wasps. Although Xenidae are generally host specialists, they have repeatedly switched to unrelated but ecologically similar hosts during their evolution. As such, there is little or no evidence of co-phylogeny between Strepsiptera and their hymenopteran host lineages. Across Xenidae, we found a large number of putative species, suggesting that the family is more diverse than expected. The number of hosts could also be at least twice as large.

Results of molecular phylogenetic studies have also suggested the need for a major change in traditional taxonomy. The new taxonomic studies presented in this thesis, based on the results of phylogenetic studies, also change the view of the host specificity of the newly delimited genera and species. However, in the era of phylogenomics, a more detailed evaluation of the current hypothesis using hundreds or thousands of protein-coding genes will be required. Whole genome sequencing is becoming cheaper and provides the opportunity to sequence more and more samples. We are currently sequencing dozens of genomes of species from the family Xenidae and Stylopidae which can bring more detailed information about the evolution and biogeographical history of these remarkable parasites of aculeate Hymenoptera. In this regard, the investigation of host phylogeny and taxonomy is also important. We are working on

ultraconserved elements (UCEs) sequencing of selected host genera (e.g., *Andrena*), and other laboratories are in the process of doing so as well.

Finally, we will look for other possibilities for practical morphological evaluation of Strepsiptera. The most promising is the usage of first larval instar morphology, which provides a large number of morphological characters. However, females producing first instar larvae are relatively rare in museums. Therefore, more collections need to be studied to obtain sufficient material for a clearer picture of the evolution of morphological characters.

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Appendix: papers