PALACKÝ UNIVERSITY OLOMOUC

Faculty of Science

Ecology of spider parasitoids: koinobiont ectoparasitoids from *Polysphincta* genus group (Ichneumonidae, Ephialtini)

Habilitation thesis in Ecology

M.Sc. Stanislav Korenko, Ph.D.

Prague 2017

Content

Supplements 1 – 15 Author’s publications included in habilitation thesis

Supplement 16 Corresponding author’s statement in connection to the habilitation thesis

1 Introduction

The presented habilitation thesis deals with ecology of various parasitoid taxons associated with spider hosts (Araneae). My research over the last decade was focused on parasitoid ecology and host-parasitoid interactions including parasitoid life history, phenology, host range, host shift, host behavioural manipulation, and evolution of host utilisation. I studied various parasitoid groups associated with spiders, including small headed flies (Acroceridae) families (Kehlmaier et al. 2012) and wasps from Eupelmidae and Cryptinae (Ichneumonidae) (Korenko et al. 2013), but my main research into spider host-parasitoid interactions was conducted on wasps from the *Polysphincta* genus group (Ichneumonidae, Ephialtini).

Most papers of mine concern wasps from the *Polysphincta* genus group (Ichneumonidae, Ephialtini), whose taxonomy is relatively well documented in Europe (e.g. Fitton et al. 1987; 1988; Zwakhals 2006; Fritzén, Shaw 2014); however, their ecology and interactions with spider hosts has been little studied. Initial work on their ecology and behaviour was done by Nielsen (e.g. 1923, 1928, 1929, 1935, 1937) but essential studies were conducted only in the last decade (e.g. Belgers et al. 2013; Bovee, Leech, 2014; Fritzén 2010, 2014; Fritzén, Fjellberg 2014 and the studies presented here as part of this habilitation thesis). My first encounter with polysphinctine parasitoids was during my Ph.D. project, in which I studied the biological control potential of spiders in apple orchards in Brno. I was surprised to find my first parasitoid larvae on spiders' abdomens and I wondered what these creatures were. I reared them in the laboratory and it was identified as *Zatypota percontatoria* (Müller, 1776). I still use this relatively common species as a model organism for various behavioural studies. From that time, the interactions between polysphinctine parasitoids and spiders became one of my main research interests, and my first results were included in my Ph.D. thesis (Korenko 2010).

Nowadays I study the ecology of polysphinctine parasitoids in several parts of Europe. My collaboration with Dr. Kamil Holý from the Crop Research Institute, prof. Stano Pekár from Masaryk University, and other colleagues from communities of Czech arachnologists and hymenopterologists was fruitful and resulted in several manuscripts which have already been published, have been accepted for publication, or are in preparation. In Slovakia, I have collaborated for several years with M.Sc. Martin Štefánik from Trenčianské Museum on the study of habitat preference and on collecting live parasitoids and their hosts for behavioural studies in the laboratory. The distribution and ecology of rare polysphinctines are investigated in collaboration with M.Sc. Ľudmila Černecká and Dr. Ivan Mihál from the Slovak Academy of Science at Zvolen. Polysphinctines are distributed sparsely and the parasitism rate is mostly low, between 0 – 2 % in Central Europe. Therefore, the collaboration with colleagues in the field and help with the collection of specimens and data on distribution is essential for my behavioural studies. In 2009, I began to investigate spider parasitoids in ecological farms and forest habitats in northern Italy (Piemonte) in collaboration with Dr. Marco Isaia from

Università di Torino. Our fruitful collaboration is still running. Most of my behavioural studies were performed on individuals collected in northern Italy because the parasitism rate there is much higher (often above 10%) in comparison with localities in the Central Europe (Korenko unpubl. data). Other important collaborations on resolving taxonomical and ecological issues have been conducted with Dr. Seraina Klopfstein from the Institute of Ecology and Evolution at Universität Bern in Switzerland, Kees Zwakhals from the Netherlands, Filippo Di Giovanni from Sapienza University of Rome in Italy, Dr. Keizo Takasuka from Keio University in Japan, prof. William G. Eberhard from the Smithsonian Tropical Research Institute in Panama, Dr. Marcelo O. Gonzaga from Universidade Federal de Uberlândia, and Dr. Thiago G. Kloss from Universidade Federal do Espírito Santo in Brazil, as well as several other colleagues from various parts of the world. In 2013, collaboration also began with prof. Gimme Walter from Queensland University, which hosted me in their laboratory during a stay in Australia. Some of our unique findings on the ecology of Australian spider parasitoids are discussed in the presented habilitation thesis; others are being acquired.

Recently, I have been working on an understanding of the evolution of host utilisation among polysphincitine wasps using a combination of molecular, morphological, and behavioural data in collaboration with most of above mentioned colleagues.

The presented habilitation thesis consists of fourteen articles and manuscripts in which I was the corresponding author and to which my contribution was between 40 - 100%. One article has a different corresponding author and my contribution to the work was 25% (see Correspondence author’s statement in Supplement 16).

2 Structure of habilitation thesis

The presented thesis is a review of my published research on the ecology of spider parasitoids from 2011 to the present. One article deals with dipteran endoparasitoids and the other eleven with ichneumonid parasitoids, especially with wasps from the *Polysphincta* genus group (Ephialtini). The first chapter is a brief summarisation of knowledge on the ecology of the most important spider parasitoids. The next three chapters are concentrated on several aspects of the ecology of wasps from the *Polysphincta* genus group: 1) life history and phenology, 2) host association, and 3) the behavioural manipulation of spider hosts. Each chapter is a short summary of knowledge on the particular topic and includes my own results. The final section consists of a list of articles which are included in the thesis, references, and supplements. The full texts of articles are included.

3 Parasitoids of spiders

The most important parasites and parasitoids associated with spider hosts belong to the order Hymenoptera, followed by Diptera and Nematodes (e.g. Godfray 1984; Fitton et al. 1987).

3.1 Nematode and dipteran parasitoids associated with spiders

Nematode parasites associated with spider hosts known from nature are restricted to the family Mermithidae (Poinar 1987). Mermithids were found parasitizing spiders of several families from araneomorph spiders (the families Gnaphosidae, Anyphaenidae, Thomisidae, Salticidae, Linyphiidae, and Araneidae (e.g. Poinar, Benton 1986)) and several families of mygalomorph spiders (the families Ctenizidae, Dipluridae, and Migidae (e.g. Poinar, Early 1990)).

Dipteran parasites and parasitoids associated with spiders are restricted to a few families and vary considerably in the mode of host utilization; for example, some are egg predators, while others are endoparasitoids (e.g. Schlinger 1987), or even ectoparasitoids (Schlinger et al. 2013). Several dipteran families – Drosophilidae, Chloropidae, Phoridae and Sarcophagidae – are known to be predators of spider eggs, while only Acroceridae and Tachinidae are considered to be true parasitoids of spiders. The most detailed host range has been documented for the genus *Ogcodes*, with about 40 host-parasite relationships known, from at least 15 families of araneomorph spiders (e.g. Schlinger 1987; Kehlmaier et al. 2012; Kehlmaier, Almeida 2014).

3.2 Hymenopteran parasitoids of spiders

The ancestors of hymenopteran parasitoids appear to be xyelid sawflies (Xyelidae), a group which first appeared in fossils from the Triassic (Riek 1955). The evolutionary path from the most primitive xyelids to highly specialised parasitoids associated with specific hosts was undoubtedly very long and it is not clear how it evolved. The parasitoid habit caused explosive adaptive radiation in Hymenoptera, forming their host specialisation. Most of them prefer insects as hosts. However, a few parasitoid lineages evolved into spider parasitism. Hymenopteran parasitoids develop singly or in small broods by feeding on spider egg masses hidden in a silken sag (e.g. several Cryptinae, primitive Ephialtini in Ichneumonidae and several lineages in Chalcidoidea); others are so tiny and highly specialise in each egg of spiders where they develop individually (Scelionidae). Araneophagy is also known in hunting wasps (Aculeata), attacking one large spider (Pompilidae) or several smaller spiders (some Sphecidae) per offspring, which are stored inside a shelter or cell constructed by a wasp female and permanently paralysed before being consumed by the wasp's larvae (Fitton et al. 1987).

Interactions between parasitoids and spider hosts differ considerably among species and are mostly very specific. The eupelmid wasp *Calymmochilus dispar* Bouček & Andriescu, 1967 and the ichneumonid wasp *Gelis apterus* (Pontoppidan, 1763) are interesting spider ectoparasitoids which were recorded as parasitoids of the ant-eating spider *Zodarion styliferum* (Simon, 1870) (Araneae, Zodariidae) in Portugal (Korenko et al. 2013). The female of both species attack the resting spider in his igloo-shaped retreat. The female oviposits on the spider ophistosoma and the larva feeds on the still living spider until the larva reaches the final stage, kills the spider, and pupates. There are many other published studies which describe unique host-parasitoid interactions from different parts of the world, but one of the most sophisticated is koinobiont ectoparasitism in the *Polysphincta* genus group within Ephialtini Hellen, 1915 (Ichneumonidae, Pimplinae).

3.3 Parasitoid wasps from the *Polysphincta* genus group (Ephialtini)

Twenty-six genera within the Ephialtini tribe are known to be associated with spiders (Yu et al. 2012). A phylogenetic study based on morphological characteristics performed by Gauld, Dubios (2006) revealed that all groups of pimplines that utilize araneomorph spiders or their egg sacs as larval nourishment form a monophyletic clade within the Ephialtini (Yu et al. 2012). There are two ecological groups within Ephialtini. The first group includes three genera of paraphyletic spider egg mass parasitoids (pseudo-parasitism). The second group includes 23 genera of derived monophyletic true spider parasitoids from the polysphinctines.

It is expected that the association of these wasps with spiders has evolved just once within *Sericopimpla*, *Gregopimpla,* and *Iseropus* of the *Sericopimpla* genus group, one of the five genus groups composing Ephialtini, and parasitizing cocooned Lepidoptera (Gauld et al. 2002; Gauld, Dubois 2006). The ability to search for cocooned hosts, perhaps via chemical cues from the cocoon silk, may have assisted the host shift to silken spider egg sacs performed by egg-mass parasitoids such as *Clistopyga, Tromatobia*, and *Zaglyptus*, these being paraphyletic with respect to the *Polysphincta* genus-group (Gauld, Dubois 2006; Matsumoto 2016). The next step in the host specialisation process was presumably a shift from spider egg-masses to spider body masses. The habit of killing a mother spider guarding her egg sac(s) has evolved in egg-mass parasitoids, as in *Zaglyptus varipes* (Gravenhorst 1829), which was observed to kill and consume only the guarding female and to ignore the spider's egg-mass (Nielsen 1935). This habit is thought to have initiated true spider parasitism typical for the *Polysphincta* genus group (e.g. Takasuka, Korenko, in prep.).

4 Life cycle and phenology of polysphinctine parasitoids

Polysphinctines are narrowly specialized on a specific spider host; therefore, the synchronisation of host availability with parasitoid activity is one of the most important features of a parasitoid’s life. This synchronization of parasitoid and host phenology is important especially in temperate zone. Similarly to other animals, polysphinctines are constrained by a shorter period of reproduction (from spring to autumn) in the temperate zone. Therefore, most polysphinctines overwinter as larvae and the first generation of adults appears in early spring (Fitton et al. 1988). Knowledge on the phenology and life history of polysphinctines is sporadic and mostly estimated on the basis of the presence of adults or larvae during a season (e.g. Fitton et al. 1987, 1988; Eberhard 2001; Matsumoto, Konishi 2007; Barrantes et al. 2008; Korenko et al. 2011; Takasuka et al. 2017). Polysphinctines are mostly expected to be univoltine or bivoltine in the temperate zone, but in some wasps even more generations can be expected in warm years (Fitton et al. 1988). In tropics, where wasps can shift the host according to seasonal changes in the abundance of suitable host species, it could be often multivoltine (e.g. Korenko et al. 2017c).

Detailed studies of the phenology of polysphinctines are scarce; they include studies by Tanaka (2007) and Takasuka, Tanaka (2013) on *Zatypota albicoxa* (Walker, 1874) in Japan, studies by Korenko et al. (2011, 2016) on *Z. percontatoria* in Europe, and studies by Weng, Barrantes (2007) and Barrantes et al. (2008) on *Zatypota petronae* Gauld, 1991 in Costa Rica. Tanaka (2007) and Korenko et al. (2011) observed that both wasps, *Z. albicoxa* and *Z. percontatoria*, continue to grow and reproduce during the whole warm season with an adequate food supply. Takasuka, Tanaka (2013) found four generations per year in *Z. albicoxa* in Japan according to field observations, while Korenko et al. (2016) estimated at least three generations per year for the Czech Republic, on the basis of the estimated sum of effective temperatures (SET) and the lower developmental threshold (LDT) using temperature records from the period 1970 – 2010. In both species, evidence was provided for the considerable effect of geography on phenology. The season for growth and reproduction in *Z. albicoxa* was four months longer in warmer south-western Japan than in colder northern Japan (Takasuka, Tanaka 2013). Further, Korenko et al. (2016) calculated that the phenology of *Z. percontatoria* changes with latitude and altitude and over time.

Details on several traits of the natural history of polysphinctines were provided by Eberhard (2000a, b), who described three larval instars in *Hymenoepimecis argyraphaga* (Gauld, 2000) associated with the spider *Leucauge argyra* (Walckenaer, 1841). A detailed study on polysphinctine life history based on both field and laboratory observations was provided by Korenko et al. (2016), who focused on *Z. percontatoria* in Central Europe. *Zatypota percontatoria* overwinters at each instar attached to the spider abdomen, and the adults of the first generation hatch at the beginning of May. The egg stage was found to last an average of 6.4 days, the larval stage 27.7 days, and the pupal stage 9.6 days. Adult longevity for *Z. percontatoria* was documented to be 14 days on average, but with a maximum of 38 days (Korenko et al. 2016). Similar results were obtained by Tanaka (2007), who noted that adult wasps of *Z. albicoxa* did not live more than 1 month in the laboratory. One month of longevity seems to be typical for small multivoltine wasps, while the longevity of univoltine or bivoltine species, which are often of bigger size, seems to be much higher. Further investigation into the life history of polysphinctines is necessary in order to make a clearer picture of their phenology.

The wasp *Z. percontatoria* is expected to be univoltine in the cold Scottish Highlands, bivoltine in England (Fitton et al. 1988), but multivoltine in the warm parts of Central Europe (Korenko et al. 2016). The temporal model provided by Korenko et al. (2016) estimated 2.5 – 3 generations per year for *Z. percontatoria* in cold years and 4-5 generations per year in extremely warm years, but variables such as low precipitation and other unknown factors could further limit the number of generations especially in extremely warm seasons. The Holarctic wasp *Reclinervellus nielseni* Roman, 1923 seems to be strictly bivoltine; it is expected to have two generations per year and overwinters in the larval stage. The interesting avoidance of competition between two sympatric species, *Reclinervellus tuberculatus* (Uchida, 1932) and *R. masumotoi* Matsumoto & Konishi, 2007, was documented in Japan by Matsumoto, Konishi (2007). These wasps share the same host in Japan, namely *Cyclosa octotuberculata* Karsch, 1879, but the larvae of *R. masumotoi* were found to mature 10–20 days earlier than those of *R. tuberculatus*, and via this difference in phenology *R. masumotoi* has an advantage over its competitor in the case when a single *Cyclosa* host is attacked by larvae of both species (Matsumoto, Konishi 2007). No competitor for *R. nielseni* was documented in Japan, but its host in Europe, *C. conica*, was also attacked by the wasp *Zatypota picticollis* (Thomson, 1888) (Korenko et al. 2015a; Takasuka et al. 2017).

5 Host association

The *Polysphincta* genus group is considered to be monophyletic on the basis of both morphology (Gauld, Dubois 2006) and molecular markers (Matsumoto 2016). Gauld, Dubois (2006) divided it into seven taxonomical groups, A – G. Taxonomical groups A, B and C consist of the *Piogaster* genus complex, the *Zabrachypus sensu stricto* complex, and the *Dreisbachia/Schizopyga* genus complex. Matsumoto (2016), using molecular data, supported the results presented by Gauld, Dubois (2006) and included genus groups A, B and C *Brachyzapus, Piogaster*, *Schizopyga* and *Zabrachypus*) into the presumably more ancestral Clade I within the *Polyshincta* genus group. These genera are known to be egg predators and idiobiont spider ectoparasitoids, utilizing spiders for the construction of egg-laying chambers (Clubionidae, Miturgidae, Salticidae) or koinobionts utilizing funnel sheet web builders (Agelenidae) (Fitton et al. 1988; Nielsen 1935; Matsumoto 2009).

Further, Gauld, Dubois (2006) distinguished four more derived taxonomical groups D - G (the *Sinarachna*/*Reclinervellus* genus complex, the *Oxyrrhexis* complex, the *Polysphincta* genus complex, and the *Eruga*/*Acrodactyla*/*Zatypota* genus complex). Matsumoto (2016) grouped Gauld's groups D – G together into Clade II, but with the genera *Acrodactyla* and *Megaetaira* being considered sister-groups to the other genera within clade II. Wasps of the genera of clade II (Gauld's groups D – G) are associated with aerial web building spiders from the families Araneidae, Dictynidae, Linyphiidae, Tetragnathidae and Theridiidae (e.g. Fitton et al. 1987, 1988; Matsumoto, Takasuka 2010; Takasuka et al. 2017; Eberhard 2000a; Gonzaga et al. 2010; Korenko, Pekár 2011; Korenko 2016, 2017; Korenko et al. 2011, 2014, 2015a, b, 2017a, b, c; Korenko et al., in prep.).

Host range was considered to be strictly specific in all polysphincta wasps (e.g. Fitton et al. 1987; Korenko et al. 2011, 2014; Korenko, Di Giovanni 2017) when one wasp species attacks only one spider species or small group of closely related species, strictly from one family. Except for Australian species *Zatypota kauros* Gauld, 1984 which is oligophagous attacking spiders from three different spider families (Korenko et al. 2017c).

Host range has evolved in each particular wasp specifically in response to conditions in particular zoogeographical regions, host availability, and other more/less important known/unknown factors. In some cases, taxonomically related parasitoid species are associated with taxonomically related spider hosts; in other cases this rule does not apply, presumably because of the greater importance of the host's ecology and behaviour than the host's taxonomical position. For example, wasps of the genus *Polysphincta* from South America prefer orb web weavers from the genus *Cyclosa* and its closely related genus *Allocyclosa* (e.g. Kloss et al. 2016). Spiders of both these genera exhibit very similar ecology and webs are almost always with stabilimenta. In contrast, wasps of the genus *Polysphincta* from Europe never attack spiders of the genus *Cyclosa*, but prefer genera like *Araneus* and *Araniella*, which build webs without a stabilimentum (e.g. Korenko et al. 2014, 2017a).

5.1 Host association of the genus *Zatypota*

The genus *Zatypota* is the largest genus within the *Polysphincta* genus group, comprising at least 50 described species (Gauld, Dubois 2006; Matsumoto, Takasuka 2010; Yu et al. 2012; Fritzén 2014), but many undescribed species exist in museum collections. Gauld, Dubois (2006) predicted that *Zatypota* probably includes as many species as there are in all other genera of the *Polysphincta* genus group combined. *Zatypota* wasps of each different zoogeographical zone have several unique taxonomical characteristics (Gauld, Dubois 2006). All *Zatypota* species are more or less narrowly associated with a specific host spider or a small group of closely related species, except for the Australian taxon *Z. kauros* which was reared from the families Theridiidae, Araneidae included sub families (Araneinae, Cyrtohporinae and Nephilinae), although it is not clear whether this taxon has such a wide host range, or whether it contains several cryptic species associated with a specific host (Korenko et al. 2017c).

Zatypota species are known mainly as parasitoids of theridiid spiders (Nielsen 1923; Fitton et al. 1988; Gauld, Dubois 2006; Korenko, Pekár 2011; Korenko et al. 2011). Only four *Zatypota* species are associated with spiders other than theridiids: *Zatypota sulcata* Matsumoto, 2010 in Japan is associated with sheet web weaving spiders from the family Linyphiidae (Matsumoto, Takasuka 2010), *Zatypota picticollis* in Europe is associated with orb-web building spiders from the family Araneidae (Zwakhals 2006; Korenko et al. 2015a), and *Zatypota anomala* (Holmgren, 1860) in the Holarctic is associated with cribellate tangle-web building spiders from the family Dictynidae (Vincent 1979; Korenko 2017). The fourth is the problematic taxon *Z. kauros*, which has the widest host range within all polysphinctines (Korenko et al. 2017c).

The systematics of the *Zatypota* genus has not been definitively resolved. In particular, *Zatypota* species with different host associations may be treated as a distinct genus, but most of them still remain as members of the genus *Zatypota* at present because of a lack of taxonomical and phylogenetical data (Gauld, Dubois 2006; Matsumoto, Takasuka 2010; Takasuka et al. in prepar.; Korenko et al. in prepar.).

5.1.1 European species of *Zatypota*

The genus *Zatypota* is distributed worldwide. Most knowledge of the biology of *Zatypota* wasps comes from Europe and Japan, where research on polysphinctine wasps has a long history.

Eight wasp species from the genus *Zatypota* occur in Europe (Fitton et al. 1988; Fritzén 2010, 2014); six of them (*Z. percontatoria, Z. bohemani* (Holmgren, 1860), *Z. discolor* (Holmgren, 1860), *Z. kerstinae* Fritzén, 2010*, Z. albicoxa,* and *Z. flamma* Fritzén, 2014) seem to be strictly associated with tangle-web weavers from the family Theridiidae. *Zatypota percontatoria, Z. bohemani, Z. discolor* and *Z. kerstinae* are associated with spiders from the genus *Theridion*, or closely-related genera (*Neottiura, Phylloneta, Platnickina*). All of them have similar habitus and behaviour and are grouped here as the "*Theridion* group". In contrast, *Z. albicoxa* and *Z. flamma* seem to be strictly associated with the genus *Parasteatoda* (e.g. Fitton et al. 1987, 1988; Fritzén 2014). A further two *Zatypota* species are associated with Araneidae and Dictynidae (discussed below).

Some *Zatypota* wasps seem to be associated strictly with one spider species (e.g. Z. *kerstinae*, known only from forests in Finland, attacks *Theridion palmgreni* Marusik & Tsellarius, 1986) (Fritzén 2010). Others have broader host range, see e.g. *Z. percontatoria.*

Zatypota albicoxa is a Palearctic species with relatively well-known biology (Nielsen 1923; Takasuka et al. 2009; Takasuka, Matsumoto 2011a, b; Takasuka, Tanaka 2013). The species seems to be associated exclusively with several species of the genus *Parasteatoda*, which builds a three-dimensional tangle-web and hangs inside the centre of the web, often hidden in a shelter built from dry leaves and/or detritus. The wasp was reared from *Parasteatoda lunata* (Clerck, 1757) and *Parasteatoda simulans* (Thorell, 1875) in Europe (Fitton et al. 1988; Bordoni 2003; Korenko, Di Giovanni 2017), and other species of the genus *Parasteatoda* in different parts of the Palearctic (Matsumoto, Takasuka 2010; Yu et al. 2012).

Zatypota anomala is a Holarctic species associated with cribellate tangle web weaving spiders belonging to the family Dictynidae. The species was excluded from the genus *Sinarachna* and transferred to the genus *Zatypota* by Gauld, Dubois (2006). It was grouped with *Z. kauros*, which is also known to be associated with spiders of the family Theridiidae. Vincent (1979) found this wasp to be associated with the dictynid spider *Mallos pallidus* (Banks, 1904) in North America and the species was repeatedly reared from the dictinid spiders *Dictyna* sp. and *Dictyna pusilla* Thorell, 1856 in Europe (Miller et al. 2013; Korenko 2017; Korenko, Di Giovanni 2017).

Zatypota discolor is an uncommon European species, which is often found together with the more common and abundant *Z. percontatoria*, presumably because their hosts occur sympatrically. *Zatypota discolor* is known to be associated with juvenile spiders of the genus *Phylloneta*, which belongs to the "*Theridion* group", but which differs in its web architecture compared to other members of "*Theridion* group"; for example, the tangle web contains a dome-shaped shelter built from silk, which is used by the female for the protection of its offspring (Roberts 1985). Only two host species are documented for *Zatypota discolor*: *Phylloneta sisyphia* (Clerck, 1757) and *Phylloneta impressa* (Koch, 1881) (Fitton et al. 1988; Korenko, Di Giovanni 2017; S. Korenko unpubl. data).

Zatypota percontatoria is one of the most common polyshinctine wasps in the Holarctic (e.g. Fitton et al. 1988; Zwakhals 2006; S. Korenko unpubl. data). Its biology is one of the most documented. Details on its life history and biology are provided by Nielsen (1923), Korenko et al. (2011, 2016), and Korenko, Pekár (2011). The host range of *Z. percontatoria* is wider in comparison with any other *Zatypota* wasps; it parasitizes several closely-related web-building spiders belonging to the *Theridion* group of the family Theridiidae (listed below). These hosts have a similar habitus and behaviour (tangle web), but take on different levels of abundance and different sizes over the season, so the wasp seems to select proper host spiders opportunistically. Laboratory experiments showed that this wasp species ignores linyphiid, araneid, and dictynid spiders and accepts only theridiid spiders from the *Theridion* group, as was observed in the field (Korenko et al. 2011). The host range of *Z. percontatoria* includes *Theridion varians* Hahn, 1831; *Neottiura bimaculata* (Linnaeus, 1767); *Phylloneta impressa* (Korenko et al. 2011; S. Korenko, unpubl. data); *Theridion pinastri* Koch, 1872 (Matsumoto, Takasuka 2010; S. Korenko unpubl. data); *Theridion melanurum* Hahn, 1831; *Theridion simile* Koch, 1836, and *Theridion tinctum* Walckenaer, 1802 (Fitton et al. 1988; Yu et al. 2012; Korenko, Di Giovanni 2017; S. Korenko unpubl. data).

Zatypota picticollis is a species known from Central and Western Europe (Zwakhals 2006; Korenko et al. 2015a; Takasuka et al. 2017) which attacks orb web weaving spiders from the family Araneidae (Korenko et al. 2015a). The Araneid spiders *Cyclosa conica*, *Mangora acalypha* (Walckenaer, 1802) and *Zilla diodia* (Walckenaer, 1802) are known to be its hosts (Zwakhals 2006; Korenko et al. 2015a).

5.1.2 Oligophagy in Australian *Zatypota kauros*

Australian taxon *Z. kauros*, which consist from three divergent mitochondrial lineages, attacks hosts from two different families (three foraging guilds), each differing widely in their web-building behaviours. The first lineage is associated exclusively with spiders of the genus *Anelosimus* (Theridiidae). Second one is associated with spiders of the genus *Cyrtophora* (Araneidae), all of which weave tent webs. The third one has a broader host range, including spiders of the both families, Araneidae and Theridiidae. It was also observed that the last one shifted host preferences according to seasonal changes in the abundance of hosts of suitable body length (Korenko et al. 2017c).

5.2. Host association of *Sinarachna pallipes* and *Polysphincta* spp.

Sinarachna pallipes (Holmgren, 1860) belongs to a genus whose members seem to be associated exclusively with the family Araneidae. The wasp is known to be associated with *Araneus diadematus* Clerck, 1757, *Araneus quadratus* Clerck, 1757, and *Larinioides cornutus* (Clerck, 1757) (Araneidae) (Aubert 1969; Fitton et al. 1988; Korenko et al. 2014). Several spiders from family Linyphiidae were also recorded as hosts of *S. pallipes* under the name of *Polysphincta pallipes* in Nielsen (1923) but the species in this paper was later identified as *Acrodactyla degener* (Haliday, 1838) (Nielsen 1928). A recent study on the parasitoids of arboreal spiders in the Czech Republic, Slovakia and northern Italy additionally found strong host associations between *S. pallipes* and spiders of the genus *Araniella* (Araneidae); *A. cucurbitina* (Clerck 1757), *A. displicata* (Hentz, 1847), *A. opisthographa* (Kulczyński, 1905) (Korenko et al. 2014)***.***

The genus *Polysphincta* is represented by five valid taxa in Europe [*P. boops* Tschek, 1869, *P. longa* Kasparyan, 1976, *Polysphincta rufipes* Gravenhorst, 1829, *P. tuberosa* Gravenhorst, 1829, and *P. vexator* Fitton, Shaw & Gauld, 1988] (Yu et al. 2012). All of them are known to be strictly associated with orb-web building spiders from the family Araneidae, but their host spectrum seems to be specific (Schmitt et al. 2012; Fritzén, Shaw 2014; Korenko et al. 2014, 2017a).

Polysphincta tuberosa is widely distributed throughout the Holarctic region (Yu et al. 2012) and is common at least in Europe (Fitton et al. 1988; S. Korenko unpubl. data). *P. tuberosa* exceptionally utilizes a wide array of spider species but seems to be associated exclusively with the family Araneidae: *Araneus diadematus*, *A. quadratus* Clerck, 1757, *Araniella cucurbitina* and *A. opisthographa* (Fitton et al. 1988; Korenko et al. 2014), *Agalenatea redi* (Scopoli, 1763) (Nielson 1923), *Zygiella atrica* Koch, 1845 (Fitton et al. 1988), and *Araneus sturmi* (Hahn, 1831) (Korenko et al. 2017a). *P. tuberosa* in Denmark is thought to shift hosts between *A. redi* in autumn, winter and spring and *A. quadratus* in summer (Nielsen 1923).

Polysphincta boops is a relatively rare species distributed in the Western (mainly Europe) and Eastern edges (partly the Russian Far East) of the Palaearctic (Yu et al. 2012), and is presumed to be associated exclusively with spiders of the genus *Araniella*: *A. cucurbitina* (Fitton et al. 1988) and *A. opisthographa* (Fitton et al. 1988; Korenko et al. 2014).

Polysphincta longa is probably widely distributed across Europe but presumably often misidentified as the related and morphologically similar species *P. boops*. Recent revision of European material provided by Fritzén, Shaw (2014) documented *P. longa* from several parts of Europe. *Polysphincta longa* is exclusively associated with the orb web building spider *Araneus angulatus* Clerck, 1758 (Fritzén, Shaw 2014; Korenko et al. 2017a).

5.3 Host association of the genera *Acrodactyla* and *Megaetaira* in Europe

This moderately large genus currently includes 32 described species with a subspecies in the Holarctic and the Indo-Australian regions (Yu et al. 2012). The genus had been divided into two genera, *Acrodactyla* and *Colpomeria*, but the latter was synonymized by Townes, Townes (1960) and, at present, is generally treated as a junior synonym of *Acrodactyla*.

Townes (1969) divided the genus *Acrodactyla* into three species-complexes on the basis of several morphological distinctions; the *degener* species-complex, the *quadrisculpta* species-complex (formerly *Colpomeria*), and the *madida* species-complex. Later, Gauld, Dubois (2006) excluded the *madida* species-complex from *Acrodactyla* on the basis of phylogenetic analysis and proposed a new genus *Megaetaira* for only one species of the complex, *A. madida*. Recently, two *Acrodactyla* species were transferred to the genus *Megaetaira* by Matsumoto (2016), namely *M. varicarinata* (Uchida & Momoi, 1958) from Japan-Russia-Sakhalin territory, and *M. inoperta* Kusigemati 1958 from Taiwan and Japan. Because Gauld, Dubois (2006) did not obtain strong evidence for the monophyly of the *degener* species-complex, they did not recognize *Colpomeria* as a distinct genus and retained it as a species-group within *Acrodactyla*. The two current species-complexes within *Acrodactyla* (and also the already separated genus *Megaetaira*) each seem to be associated with specific spider taxa as described below. All the above indicates the likely validity of the genus *Colpomeria*, but detailed revision is required to establish it.

Acrodactyla degener from the *degener* species-complex (*Acrodactyla* s. str.) is a common species widely distributed in most of the Holarctic region and associated exclusively with sheet web building spiders from the family Lyniphiidae with a very wide host range: *Bolyphantes alticeps* (Sundevall, 1833), *Kaestneria dorsalis* (Wider, 1834), *Tenuiphantes mengei* (Kulczyński, 1887), *Tenuiphantes tenuis* (Blackwall, 1852), *Tenuiphantes zimmermanni* (Bertkau, 1890), *Lepthyphantes minutus* (Blackwall, 1833), *Linyphia triangularis* (Clerck, 1757), *Neriene montana* (Clerck, 1757), *Neriene peltata* (Wider, 1834), *Bathyphantes* sp., *Linyphia* sp. (Fitton et al. 1987, 1988; S. Korenko unpubl. data), *Obscuriphantes obscurus* (Blackwall, 1841), *Pityohyphantes phrygianus* (Koch, 1836) (Aubert 1969), *Improphantes decolor* (Westring, 1861), *Microneta viaria* (Blackwall, 1841), *Erigone* sp. (Nielsen 1923, 1928), *Tenuiphantes tenebricola* (Wider, 1834) (O. Macháč unpubl. data) and *Tenuiphantes flavipes* (Blackwall, 1854) (A. Šestáková unpubl. data).

Acrodactyla quadrisculpta (Gravenhorst, 1820) from the *quadrisculpta* species-complex (the former potential genus *Colpomeria*) is widely distributed in the Holarctic and partly Oriental and Australian regions (Fitton et al. 1988; Gauld 1984; Townes, Townes 1960; Yu et al. 2012), though the Australian population is thought to have been introduced (Gauld 1984). The species is associated exclusively with spiders from the genus *Tetragnatha* (Tetragnathidae): *T. extensa* (Linnaeus, 1758), *T. obtusa* Koch, 1837, *T. montana* Simon, 1874 in Europe (Fitton et al. 1988), and *T. laboriosa* Hentz, 1850 in North America (Townes, Townes 1960). Another common species from the *quadrisculpta* species-complex is Palearctic *A.*

carinator (Aubert, 1965), which is associated with *T. montana*, but presumably also attacks other species of this genus. *Acrodactyla carinator* was misidentified as Holarctic *A. quadrisculpta* in studies by Belgers et al. (2013) and Korenko et al. (2015b) (material revised by K. Holý and K. Zwakhals). Additional investigation revealed that the cocoon web of *T. montana* induced by the larva of *A. quadrisculpta* has the same architecture as that induced by *A. carinator* (S. Korenko unpubl. data).

Megaetaira madida (Haliday, 1838) is widely distributed in Europe but relatively uncommon (Fitton et al. 1988, Korenko 2016). The wasp is distributed in several parts of the Palaearctic and is associated exclusively with spiders of the genus *Metellina*, which build a typical orb-web with a small hole in the hub (e.g. Roberts 1985). The spiders *Metellina mengei* (Blackwall, 1869), *M. merianae* (Scopoli, 1863), and *M. segmentata* (Clerck, 1757) were already reported as hosts (Fitton et al. 1987, 1988; Korenko 2016). The wasp *M. varicarinata*, recently transferred to this genus, is known to parasitize the spider *Meta reticuloides* Yaginuma, 1958, which is closely related to the genus *Metellina*.

5.4 Host association of *Reclinervellus nielseni* across the Palearctic

Reclinervellus nielseni is distributed across the Palaearctic. Records are from various parts of Europe on the western side of the Paleartic, and Primorye Kray (Russia) and the Japanese Archipelago on the eastern side. The wasp is associated with *Cyclosa conica* (Pallas, 1772) (Araneidae) in Europe and with *C. argenteoalba* and *C. laticauda* in Japan (Matsumoto, Konishi 2007; Takasuka et al. 2015, 2017).

5.5 Divergence in host association within genus *Eriostethus*

Studies Gauld (1984b); Masumoto et al. (2016) recorded only orb-web building spiders from family Araneidae as hosts for genus *Eriostethus*. Therefore, it was expected that species of this genus are associated exclusively with araneid orb-web weavers. Recent study Korenko et al. (2017b) confirmed araneids as hosts of *Eriostethus perkinsi* (Baltazar, 1964), but also found, that tangle web building spiders from family Theridiidae as host of *Eriostethus minimus* Gauld, 1984. Taxonomy of genus *Eriostethus* is evidently not definitely resolved. Findings of Korenko et al. (2017b) on differences in biology between two groups within genus *Eriostethus*, supported by differences in morphology (Baltazar 1969) and by molecular data (Korenko, unpubl. data) opened discussion on resurrection of genus *Millironia* (Baltazar, 1969), which was synonymised by Gauld (1984a, b). More data are required to definitely solve its taxonomy.

6 Behavioural manipulation of spider hosts

Larvae of polysphinctine wasps are attached to the dorsal side of the spider's prosoma/opisthosoma, where they develop while the spider continues in web construction and foraging. The most serious problem confronting later instar larvae and pupae after the host spider has been killed is the fragility (i.e. the vulnerability) of the original spider's web. To overcome this problem, a fascinating adaptation making use of the spider's web building behaviour has evolved in penultimate instar larvae.

Shortly before killing the spider and pupation, the penultimate instar larvae of several polysphinctines manipulate the spider’s web building behaviour. The spider is forced to spin a special web structure called a "cocoon web" in order to establish a safe place for larval pupation. This structure presumably serves to protect the immobile final instar larva and then pupa against enemies and natural elements (e.g. Eberhard 2000a). These effects of the parasitoid larva on the spider host are apparently due to chemical products that are introduced into the spider body (Eberhard 2000a, b, 2010; Kloss et al. 2017). The mechanism responsible for host manipulation in parasitoid wasps was recently suggested by Kloss et al. (2017), who studied manipulation in *Polysphincta janzeni* Gauld, 1991 and *P*. sp. nr. *purcelli*. Such manipulation probably involves the inoculation of psychotropic chemicals by the parasitoid larva. They suggested that the substance responsible for induced behavioural changes might be a moulting hormone or a precursor chemical of this hormone, which is produced during ecdysis. Spiders under parasitoid larval control presented higher 20-OH-ecdysone levels than parasitised spiders acting normally or unparasitised individuals.

Several studies have recently been devoted to the behavioural manipulation of orb web weaving spiders. For example, Eberhard (2000a, b, 2013), Sobczak et al. (2009), Belgers et al. (2013), Korenko (2016) and Korenko et al. (2015a) studied parasitoids associated with orb web building spiders from the family Tetragnathidae. Further, Eberhard (2010, 2013), Gonzaga, Sobczak (2011), Gonzaga et al. (2010), Kloss et al. (2016, 2017), Korenko et al. (2014, 2015a, 2017a, b, c) Matsumoto, Konishi (2007), Schmitt et al. (2012), and Takasuka et al. (2015, 2017) studied the manipulation of spiders from the family Araneidae.

Not all polysphinctines manipulate the host spider's web-building behaviour (e.g. Fritzén 2010; Korenko 2017) and in other cases it is not easy to distinguish between a normal web and a modified "cocoon web" (e.g. in 3D web weavers, which produce a dense 3D structure) (S. Korenko, unpubl. data). In this case, wasp larvae use the spider's normal 3D web or its shelter to protect themselves without any manipulation of spider behaviour. A normal 3D web or spider's shelter seems often to be sufficient protection for larval pupation and it is not necessary to invest more resources into surplus manipulation of the spider host.

6.1 Host utilisation by *Zatypota* wasps

The genus *Zatypota* includes both species which evidently modify spider host behaviour (e.g. *Z. percontatoria*) and those in which no alteration in web architecture has been identified (e.g. *Z. anomala*). The most important species are discussed below.

6.1.1 *Zatypota* wasps associated with theridiid spiders in Europe

Zatypota albicoxa associated with tangle web building spiders from the genus *Parasteatoda* utilises a three-dimensional tangle web without any retreat [e.g. juvenile *P. tepidariorum* (C. L. Koch, 1841) and *P. oculiprominens* (Saito, 1939)] or with a retreat in the centre of the web built from dry leaves (e.g. some adults of *P. tepidariorum*) or soil [e.g. *P. tabulate* (Levi, 1980)]. The modification of web architecture has not so far been detected and the parasitoid larva pupates inside the 3D tangle web or in the spider retreat if present. A reduction in gumfoot lines was observed in some cases by K. Takasuka (personal com.).

Zatypota percontatoria exhibited species-specific manipulation of the web architecture of two theridiid spiders, *N. bimaculata* and *T. varians* (Korenko, Pekár 2011). The alterations to the web architecture induced by larvae differed, but the resulting architecture was functionally similar. Final stage larva of *Z. percontatoria* induced the production of a dense web in *N. bimaculata*, whereas a cupola-like structure was induced in *T. varians*. Similar spinning activity was also observed in unparasitised spiders. *Neottiura bimaculata* produced an analogous dense web around its egg sac and for itself during winter, while *T. varians* constructed an analogous 'cupola' only for overwintering. The parasitoid larvae were thus thought to evoke protective behaviours that occur in unparasitised hosts only during specific life-history periods (Korenko, Pekár 2011). In the host *P. impressa*, the larva of *Z. percontatoria* induces an increase in thread density in the surroundings of the spider's retreat, in which the parasitoid larva pupates, similarly to *Z. discolor* (see above).

Zatypota discolor is a closely-related species to the more common *Z. percontatoria*. These two wasps occur sympatrically at least in Europe and share some hosts (S. Korenko, unpubl. data). *Zatypota discolor* is known to be associated with spiders of the genus *Phylloneta*, which build three-dimensional tangle webs consisting of horizontal and vertical threads placed at random and of a upward cone retreat made of in the centre of the tangle (the retreat is not present in all juvenile spiders). Small pieces of plants or prey remnants can be incorporated into the wall of the retreat. In the host *P. impressa*, the parasitoid cocoon is constructed inside the 3D tangle web. The thread density seems to be higher in the cocoon web than in the normal web in the surroundings of spider/cocoon position. The wasp's cocoon is also protected by a cone-shaped silk platform, if this is present in the spider's normal web (S. Korenko, unpubl. data).

6.1.2 *Zatypota anomala* associated with dictynid spiders

Zatypota anomala is a Holarctic species associated with cribellate tangle web weaving spiders from the family Dictynidae. The parasitoid larva pupates in the spider's position at the centre of a dense tangle of cribellate (sticky) threads. This sticky silk structure seems to be an effective protection against potential enemies and the modification of the spider's innate web is not necessary. Therefore, no modification of web architecture was observed in the interaction of *Z. anomala* with arboreal spider species of the genus *Dictyna* in Europe (Korenko 2017).

6.1.3 *Zatypota picticollis* associated with araneid spiders

Zatypota picticollis attacks several spider species from the family Araneidae. The final stage larva of *Z. picticolis* induces changes in the web architecture of all three documented host spiders (Korenko et al. 2015a). Under the influence of the final stage larva, the spiders built a specific web architecture which differed considerably from the capturing orb web. The modified web lacked any sticky spiral and also debris decoration which is usually formed in unparasitised *C. cyclosa* (Korenko et al. 2015a; Takasuka et al. 2017). The number of radii was reduced and multiple threads were accumulatively laid along each remaining radius strengthening the web. The cocoon was attached in a perpendicular direction to the plane of the cocoon web. In one out of ten observations of the manipulation of *Z. diodia*, the web was reduced to a single strong horizontal thread consisting of several lines woven together and the cocoon was suspended from the thread (Korenko et al. 2015a).

6.1.4 Australian oligophagous *Zatypota kauros*

Taxon *Z. kauros*, which includes three mitochondrial lineages, exhibited high plasticity in host utilisation when induces different modifications in the web architecture of spiders from different families. This specificity seems to be more host-specific than lineage-specific (Korenko et al. 2017c). Lineage associated exclusively with tangle web building spiders of the genus *Anelosimus* (Theridiidae) did not induce any changes in the structure of its host's tangle webs. In contrast, lineage associated with spiders of the genus *Cyrtophora* (Araneidae, subfamily Cyrtophorinae) and lineage, which has broader host range, including spiders of both families, Araneidae (including subfamilies Araneinae, Cyrtophorinae, Nephilinae) and Theridiidae, modified web-building behaviours specifically in the different host groups.

6.2 The specificity of host utilisation in the genera *Acrodactyla* and *Megaetaira*

Two current species-complexes in the recently accepted genus *Acrodactyla* (the *degener* and *quadrisculpta* species-complexes sensu Townes 1969) and the genus *Megaetaira* (the former *madida* species-complex within *Acrodactyla* sensu Townes (1969) show different manipulations.

Acrodactyla degener from the *degener* species-complexes *sensu* Townes 1969, associated exclusively with sheet web building spiders from the family Linyphiidae, does not seem to manipulate web architecture. It pupates in a horizontally-oriented cocoon lying parallel on the sheet of the unmodified web of *Neriene* sp. (S. Korenko unpubl. data), or inside a sparse web tangle of *T. tenebricola* (O. Machač unpubl. data).

Acrodactyla quadrisculpta and *A. carinator* from the *quadrisculpta* species-complex sensu Townes 1969 are associated exclusively with spiders from the genus *Tetragnatha*. The host spider *T. montana*, which normally constructs a horizontal or vertical orb web, constructs, under manipulation of the penultimate instar larva of *A. carinator* (misidentified as *quadrisculpta* in Belgers et al. 2013 and in Korenko et al. 2015b), a horizontal "I" or "T" shaped and reinforced cocoon web composed of a highly reinforced main thread, mostly tensioned by a reinforced side thread (Korenko et al. 2015b). The larva was observed to kill the spider at the centre of the main thread or the cross point of two threads and weave a cocoon horizontally (Belgers et al. 2013; Korenko et al. 2015b). A modified cocoon web of a similar architecture was also observed in the interaction between *A. quadrisculpta* and *Tetragnatha* spp. (S. Korenko unpubl. data).

Megaetaira madida from the former *madida* species-complex within *Acrodactyla* sensu Townes (1969) is associated with spiders of the genus *Metellina* (Tetragnathidae). The host *M. merianae* builds an orb web with an open hub, very similar to the web of *M. mengei* and *M. segmentata*. A juvenile female of *M. merianae* under manipulation of the final stage larva of *M. madida* was observed to build a 3D silk structure. The larva pupates in the position of the highest thread concentration (Korenko 2016).

6.3 The *Reclinervellus nielseni* parasitoid of *Cyclosa* spiders in the Palearctic

The wasp is associated exclusively with *Cyclosa* spiders from the family Araneidae. The normal web of the European host *C. conica* is a 2D orb with a linear web decoration made from silk, prey remnants, and detritus. In contrast, the Japanese host *C. argenteoalba* seldom forms debris decoration but frequently linear decorations above and below the hub (Nakata 2009). The cocoon web of the host *C. conica* is a reduced orb consisting of reinforced radii and remaining debris decoration; the parasitoid larva pupates on the hub along the debris decoration, which seemingly camouflages the cocoon (Nielsen, 1923; Takasuka et al. 2017). The cocoon web of *C. argenteoalba* is a simple orb-web with reduced numbers of radii like that of *C. conica* but with numerous conspicuous fluffy thread decorations laid on the radii. The modified web is almost the same as the resting web built before the moulting of the unparasitised spider, not only in terms of its shape but also in terms of its specific decorations (Takasuka et al. 2015, 2017).

6.4 Three-dimensional cocoon webs of *Polysphincta* spp. and *Sinarachna pallipes* in Europe

The genus *Polysphincta* in Europe is associated exclusively with orb-web building spiders from the family Araneidae. The penultimate instar larva of *P. tuberosa* manipulates *A. opisthographa* to build a three-dimensional structure in which the wasp's cocoon is nested in a horizontal position. The spider under manipulation is forced to find a safe place away from the original orb-web (if presented), protected from all sides by spider silk or habitat structures, such as the base of tree twigs or the corner of an experimental arena (Korenko et al. 2014). When no safe shelter is provided (e.g. in laboratory tubes), the spider builds a dense three-dimensional structure with the highest density in the centre, where the larva pupates (S. Korenko, unpubl. data). Nielsen (1923) called this three-dimensional structure a 'loose-meshed sheet' enclosing a cocoon, but he was mistaken that this was spun by the wasp larva itself. The alteration of the web architecture in *A. opisthographa* induced by *P. boops* is similar to that found in *P. tuberosa*. The wasp cocoon is located horizontally inside the three-dimensional structure, which is significantly denser around the pupa than in the outer parts of the cocoon web, as in the case of *P. tuberosa* (Korenko et al. 2014, 2017a).

Polysphincta longa is probably widely distributed across Europe, but the species has been presumably often misidentified as the related and morphologically similar species *P. boops*. The wasp is exclusively associated with the araneid spider *A. angulatus*. The revision of European material provided by Fritzén, Shaw (2014) documented *P. longa* from several parts of Europe. Recently, *P. longa* was recorded in Slovakia for the first time (Korenko et al. 2017a). Fritzén, Shaw (2014) did not observe the modification of spider web building behaviour by the parasitoid final stage larva; however, in the laboratory, Korenko et al. (2017a) observed that the host *A. angulatus* parasitised by the final stage larva of *P. longa* built a unique structure, called the death chamber, (a cocoon web consisting of a 3D tangle) which protected the parasitoid during the pupal stage. Differences between the observations of Korenko et al. (2017a) and those of Fritzén, Shaw (2014) can be explained by the fact that experimental arenas of different sizes were used. Fritzén, Shaw (2014) used a rearing arena of small size, whereas Korenko et al. (2017a) employed a large arena, where the spider had sufficient space to build both a normal web and a cocoon web.

Sinarachna pallipes shares hosts with European wasps of the genus *Polysphincta*. The penultimate stage larva of *S. pallipes* induces the production of a more or less symmetrical three-dimensional structure in *Araniella* spp., in which the pupa hangs vertically from its centre (Korenko et al. 2014).

The production of a 3D tangle by non-parasitised *Araniella* spiders is known in adult females and is used to protect the spider’s egg sac. This behaviour seems to be evident only in adult females, but parasitised juvenile spiders are always killed before reaching adulthood. The production of a 3D tangle has never been observed in unparasitised juvenile spiders.

Parasitoid penultimate stage larvae thus might evoke this innate (adult) behaviour in manipulated juvenile spider hosts (Korenko et al. 2014).

6.5 Divergence in host utilisation within the genus *Eriostethus*

Recently valid genus *Eriostethus* Morley, 1914 went through several taxonomical treatments and its taxonomy is still unresolved (Korenko et al. 2017b). The genus was designated based on morphological characteristics as a sister-group to *Flacopimpla*, and these are a sister-group to *Zatypota* and *Longitibia* (Gauld, Dubois 2006). A recent phylogenetic study (Matsumoto, 2016), based on several molecular markers, showed weak relatedness of *Eriostethus rufus* (Uchida, 1932) to *Zatypota*. The most related genus is *Sinarachna*, which was considered a distant group in analyses by Gauld and Dubois (2006). This mismatch on *Eriostethus* classification between Gauld, Duboid (2006) and Matsumoto (2016) opens a new taxonomical questions on validity of this genus. Korenko et al. (2017b) suggest that *Eriostethus* could consist of two genera as designed Baltazar (1964), which separated a group of species from *Eriostethus* and designated a new genus, *Millironia*. Baltazar's considered several important morphological characters of *Millironia*, but several of them intergraded to genus *Eriostethus*, therefore Gauld (1984a) considered wasps of these two genera, compared with other polysphinctines, as very closely related species and combined them to a single genus, *Eriostethus*. Recently accepted genus *Eriostethus* is a moderately large genus with eighteen species distributed mainly in the Indo-Australia and in Japan (Yu et al., 2012). The last study on their ecology (Korenko et al. 2017b) suggested to re-consider the resurrection of genus *Millironia* sensu Baltazar (1969). This is supported by morphological data (e.g. Morley 1914; Baltazar 1964; Gauld 1984a, b; Momoi 1966), behaviour and DNA data (Matsumoto 2016; Korenko unpubl. data). However, the resurrection of genus *Millironia* requires next intensive investigation of all aspects on classification of these wasps.

7 Acknowledgements

I would like to express my gratitude to all my above mentioned colleagues, my family and friends for collaboration and help on my research in last decade.

8 References

Aubert J-F. 1969. Les Ichneumonides Ouest-Paléarctiques et leurs Hôtes. 1. Pimplinae, Xoridinae, Acaenitinae. Editeur Quatre-Feuilles: Alfortville. 302 pp.

Baltazar CR. 1964. *Eriostethus* Morley and a new polysphinctine genus (Ichneumonidae, Hymenoptera). Pacific Insects 6, 388-401.

Barrantes G, Eberhard WG, Weng JL. 2008. Seasonal patterns of parasitism of the tropical spiders *Theridion evexum* (Araneae, Theridiidae) and *Allocyclosa bifurca* (Araneae, Araneidae) by the wasps *Zatypota petronae* and *Polysphincta gutfreundi* (Hymenoptera, Ichneumonidae). Revista de Biología Tropical 56, 749-754.

Belgers D, Zwakhals K, van Helsdingen P. 2013. De bijzondere levensloop van de sluipwesp *Acrodactyla quadrisculpta* op de schaduwstrekspin *Tetragnatha montana* (Hymenoptera: Ichneumonidae, Araneae: Tetragnathidae). Nederlandse Faunistische Mededelingen 39, 1-6.

Bordoni A. 2003. Osservazioni su *Zatypota albicoxa* (Walker) (Hymenoptera, Ichneumonidae) e sul suo ospite *Achaearanea lunata* (Clerck) (Araneae, Theridiidae). Doriana 8, 1-4.

Bovee J, Leech R. 2014. *Araneus gemmoides* (Araneae: Araneidae) death by a parasitoid (Hymenoptera: Ichneumonidae). Bulletin of the Entomological Society of Canada 46, 86-90.

Eberhard WG. 2000a. Spider manipulation by a wasp larva. Nature 406, 255-256.

Eberhard WG. 2000b. The natural history and behavior of *Hymenoepimecis argyraphaga* (Hymenoptera: Ichneumonidae) a parasitoid of *Plesiometa argyra* (Araneae: Tetragnathidae). Journal of Hymenoptera Research 9, 220-240.

Eberhard WG. 2001. Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). Journal of Arachnology 29, 354-366.

Eberhard WG. 2010. Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. Animal Behaviour 79, 375-383.

Eberhard WG. 2013. The Polysphinctine wasps *Acrotaphus tibialis*, *Eruga* ca. *gutfreundi*, and *Hymenoepimecis tedfordi* (Hymenoptera, Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. Annals of the Entomological Society of America 106, 652-660.

Fritzén NR. 2010. Natural history and description of *Zatypota kerstinae* sp. nov. (Hymenoptera: Ichneumonidae) reared from *Theridion palmgreni* Marusik et Tsellarius (Araneae: Theridiidae) in Finland. Zootaxa 2487, 52-60.

Fritzén NR. 2014. Two new species of the *Polysphincta* genus-group (Hymenoptera: Ichneumonidae: Pimplinae) reared from their spider (Araneae) hosts in Europe. Zootaxa 3894, 117-130.

Fritzén NR, Fjellberg A. 2014. Natural history of *Oxyrrhexis zephyrus* sp. n. (Hymenoptera: Ichneumonidae), a parasitoid of *Enoplognatha serratosignata* (Araneae: Theridiidae), with notes on taxonomy and other host species of *Oxyrrhexis*. Arthropoda Selecta 23, 135-144.

Fritzén NR, Shaw MR. 2014. On the spider parasitoids *Polysphincta longa* Kasparyan and *P. boops* Tschek (Hymenoptera, Ichneumonidae, Pimplinae), with the first host records of *P. longa*. Journal of Hymenoptera Research 39, 71-82.

Fitton MG, Shaw MR, Austin AD. 1987. The Hymenoptera associated with spiders in Europe. Zoological Journal of the Linnean Society 90, 65-93.

Fitton MG, Shaw MR, Gauld ID. 1988. Pimpline ichneumon-flies. Handbooks for the Identification British Insects 7, 1-110.

Gauld ID. 1984a. The Pimplinae, Xoridinae, Acaenitinae and Lycorininae (Hymenoptera: Ichneumonidae) of Australia. Bulletin of the British Museum (Natural History) Entomology 49, 235-339.

Gauld, I.D. 1984b. An introduction to the Ichneumonidae of Australia. British Museum (Natural History), London, UK.

Gauld ID, Dubois J. 2006. Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae), a taxonomic revision of spider ectoparasitoids. Systematic Entomology 31, 529-564.

Gauld, I, Wahl DB, Broad GR. 2002. The suprageneric groups of the Pimplinae (Hymenoptera: Ichneumonidae): a cladistic re-evaluation and evolutionary biological study. Zoological Journal of the Linnean Society 136, 421-485.

Godfray HCJ. 1994. Parasitoids, behavioural and evolutionary ecology Princeton University Press, Princeton.

Gonzaga MO, Sobczak JF. 2011. Behavioral manipulation of the orb-weaver spider *Argiope vargentata* (Araneae: Araneidae) by *Acrotaphus chedelae* (Hymenoptera: Ichneumonidae). Entomological Science 14, 220-223.

Gonzaga MO, Sobczak JF, Penteado-Dias AM, Eberhard WG. 2010. Modification of *Nephila clavipes* (Araneae Nephilidae) webs induced by the parasitoids *Hymenoepimecis bicolor* and *H. robertsae* (Hymenoptera Ichneumonidae). Ethology Ecology & Evolution 22, 151-165.

Hashimoto R. 1963. [Six species of wasps parasitizing spiders]. Acta Arachnologica 18, 27-29 [In Japanese].

Kehlmaier Ch, Michalko R, Korenko S. 2012. *Ogcodes fumatus* (Diptera: Acroceridae) reared from *Philodromus cespitum* (Araneae: Philodromidae), and first evidence of *Wolbachia* in Acroceridae. Annales Zoologici 62, 281-286.

Kehlmaier Ch, Almeida JM. 2014. New host records for European Acroceridae (Diptera), with discussion of species limits of *Acrocera orbiculus* (Fabricius) based on DNA-barcoding. Zootaxa 3780, 135-152.

Kloss TG, Gonzaga MO, Roxinol JAM, Sperber CF. 2016. Host behavioural manipulation of two orb-weaver spiders by parasitoid wasps. Animal Behaviour 111, 289-296.

Kloss TG, Gonzaga MO, de Oliveira LL, Sperber CF. 2017. Proximate mechanism of behavioral manipulation of an orb-weaver spider host by a parasitoid wasp. PLoS ONE 12, e0171336.

Korenko S. 2010. Tritrophic relationship of arboreal spiders. Disertation Thesis, Masaryk University, Brno, Czech Republic.

Korenko S. 2016. Web architecture alteration of the orb web weaving spider *Metellina merianae* (Araneae, Tetragnathidae) induced by the parasitoid *Megaetaira madida* (Ichneumonidae, *Polysphincta* group). Arachnologische Mitteilungen 52, 35-37.

Korenko S. 2017. First record from Italy of *Zatypota anomala* (Ichneumonidae, Ephialtini), a parasitoid of the cribellate spider *Dictyna pussila* (Araneae, Dictynidae). Arachnologische Mitteilungen 54, 1-4.

Korenko S, Di Giovanni F. 2017. Spider parasitoids of tribe Ephialtini (Ichneumonidae, Pimplinae) in Italy and their host association. Submited in North-Western Journal of Zoology.

Korenko S, Pekár S. 2011. A parasitoid wasp induces overwintering behaviour in its spider host. PLoS ONE 6, e24628.

Korenko S, Michalková V, Zwakhals K, Pekár S. 2011. Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid (Hymenoptera: Ichneumonidae). Journal of Insect Science 11, 101.

Korenko S, Schmidt S, Schwarz M, Gibson GAP, Pekár S. 2013. Hymenopteran parasitoids of the ant-eating spider *Zodarion styliferum* (Simon) (Araneae: Zodariidae). Zookeys 262, 1-15.

Korenko S, Isaia M, Satrapová J, Pekár S. 2014. Parazitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecological Entomology 39, 30-38.

Korenko S, Satrapova J, Zwakhals K. 2015a. Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimlinae). Entomological Science 18, 383-388.

Korenko S, Korenková B, Satrapova J, Hamouzová K. Belgers D. 2015b. Modification of *Tetragnatha montana* (Araneae, Tetragnathidae) web architecture induced by larva of the parasitoid *Acrodactyla quadrisculpta* (Hymenoptera, Ichneumonidae, *Polysphincta* genus-group). Zoological Studies 54, 40.

Korenko S, Potopová V, Satrapová J, Pekár S. 2016. Life history of the spider parasitoid *Zatypota percontatoria* (Hymenoptera: Ichneumonidae). Entomological Science 19, 104-111.

Korenko S, Kysilková K, Černecká Ľ. 2017a. New records of two spider parasitoids of the genus *Polysphincta* (Ichneumonidae, Ephialtini) from Central Europe, with notes on their host interactions. Arachnologische Mitteilungen 54, 28-3.

Korenko S, Hamouzová K, Kysilková K, Kolářová M, Kloss TG, Takasuka K, Pekár S. 2017b. Divergence in host utilisation in two spider parasitoids of the genus *Eriostethus* (Ichneumonidae, Ephialtini). In submission, Zoologischer Anzeiger.

Korenko S, Pekár S, Walter GH, Korenková V, Hamouzová K, Kolářová M, Kysilková K, Spasojevic T, Klopfstein S. 2017c. One generalist or several specialist species? Wide host range and diverse manipulations of the hosts' web building behaviour in the true spider parasitoid *Zatypota kauros* (Hymenoptera: Ichneumonidae). In submission, Frontiers in Zoology.

Korenko S, Kloss TG, Gonzaga MO, Takasuka K, Pekár S. (in preparation) Evolution of host utilisation by true spider parasitoids (Ichneumonidae, Ephialtini, *Polysphincta* genus group).

Matsumoto R. 2009. ''Veils'' against predators: Modified web structure of a host spider induced by an ichneumonid parasitiod, *Brachyzapus nikkoensis* (Uchida) (Hymenoptera). Journal of Insect Behavior 22, 39-48.

Matsumoto R. 2016. Molecular phylogeny and systematics of the *Polysphincta* group of genera (Hymenoptera, Ichneumonidae, Pimplinae). Systematic Entomology 41, 854-864.

Matsumoto R, Konishi K. 2007. Life histories of two ichneumonid parasitoids of *Cyclosa octotuberculata* (Araneae), *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomological Science 10, 267-278.

Matsumoto R, Takasuka K. 2010. A revision of the genus *Zatypota* Förster of Japan, with descriptions of nine new species and notes on their hosts (Hymenoptera: Ichneumonidae: Pimplinae). Zootaxa 2522, 1-43.

Miller JA, Belgers JDM, Beentjes KK, Zwakhals K, van Helsdingen P. 2013. Spider hosts (Arachnida, Araneae) and wasp parasitoids (Insecta, Hymenoptera, Ichneumonidae, Ephialtini) matched using DNA barcodes. Biodivers Data Journal 1, e992.

Morley C. 1914. A revision of the Ichneumonidae based on the collection in the British Museum, part III. Tribes Pimplines and Bassides.British Museum, London, England.

Momoi S. 1966. Some new ichneumonidae (Hymenoptera) from New Guinea and adjacent areas. Pacific Insects 8 (1): 152-164.Nakata K. 2009. To be or not to be conspicuous: the effects of prey availability and predator risk on spider's web decoration building. Animal Behaviour 78, 1255-1260.

Nielsen E. 1923. Contribution to the life history of the Pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*). Entomologiske Meddelelser 14, 137-205.

Nielsen E. 1928. A supplementary note upon the life histories of the Polysphinctas (Hym. Ichneum.). Entomologiske Meddelelser 16, 152-155.

Nielsen E. 1929. A second supplementary note upon the life histories of polysphinctas. Entomologiske Meddelelser 16, 366-368.

Nielsen E. 1935. A third supplementary note upon the life histories of polysphinctas. Entomologiske Meddelelser 19, 191-215.

Nielsen E. 1937. A fourth supplementary note upon the life histories of polysphinctas. Entomologiske Meddelelser 20, 25-28.

Poinar GO. 1987. Nematode parasites on spiders. In: W Nentwig (ed) Ecophysiology of spiders. Berlin, Springer Verlag, pp. 299-308.

Poinar GO Jr, Benton CLB. 1986. *Aranimermis aptispicula* new genus new species (Mermithidae: Nematoda), a parasite of spiders (Arachnida: Araneida). Systematic Parasitology 8, 33-38.

Poinar GO Jr, Early JW. 1990. *Aranimermis giganteus* n. sp. (Mermithidae: Nematoda), a parasite of New Zealand mygalomorph spiders (Araneae: Arachnida). Revue de Nématologie 13, 403-410.

Riek EF. 1955. Fossil insects from the Triassic beds at Mt. Crosby, Queensland. Australian Journal of Zoology 3, 654-691.

Roberts MJ. 1985. Spiders of Britain & Northern Europe. Harper Collins Publishers London, England.

Schlinger EI. 1987. The biology of Acroceridae (Diptera): True endoparasitoids of spiders. In: W Nentwig (ed) Ecophysiology of spiders. Berlin, Springer Verlag, 319-327.

Schlinger EI, Gillung JP, Borkent ChJ. 2013. New spider flies from the neotropical region (Diptera, Acroceridae) with a key to New World genera. ZooKeys 270, 59-93.

Schmitt M, Richter D, Göbel D, Zwakhals K. 2012. Beobachtungen zur Parasitierung von Radnetzspinnen (Araneidae) durch *Polysphincta rufipes* (Hymenoptera: Ichneumonidae). Arachnologische Mitteilungen 44, 1-6.

Sobczak JF, Loffredo APS, Penteado-Dias AM, Gonzaga MO. 2009. Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. Journal of Natural History 43, 2691-2699.

Tanaka K. 2007. Seasonal life cycle of *Zatypota albicoxa* (Hymenoptera: Ichneumonidae), an ectoparasitoid of *Achaearanea tepidariorum* (Araneae: Theridiidae), in northern Japan. Entomological Science 10, 421-423.

Takasuka K, Matsumoto R. 2011a. Infanticide by a solitary koinobiont ichneumonid ectoparasitoid of spiders. Naturwissenschaften 98, 529-536.

Takasuka K, Matsumoto R. 2011b. Lying on the dorsum: unique host-attacking behaviour of *Zatypota albicoxa* (Hymenoptera, Ichneumonidae). Journal of Ethology 29, 203-207.

Takasuka K, Tanaka K. 2013. Seasonal life cycle of *Zatypota albicoxa* (Hymenoptera: Ichneumonidae), an ectoparasitoid of *Parasteatoda tepidariorum* (Araneae: Theridiidae), in Southwestern Japan. Pacific Science 67, 105-111.

Takasuka K, Matsumoto R, Ohbayashi N. 2009. Oviposition behavior of *Zatypota albicoxa* (Hymenoptera, Ichneumonidae), an ectoparasitoid of *Achaearanea tepidariorum* (Araneae, Theridiidae). Entomological Science 12, 232-237.

Takasuka K, Yasui T, Ishigami T, Nakata K, Matsumoto R, Ikeda K, Maeto K. 2015. Host manipulation by an ichneumonid spider-ectoparasitoid that takes advantage of preprogrammed web-building behaviours for its cocoon protection. Journal of Experimental Biology 218, 2326-2332.

Takasuka K, Korenko S, Štefánik M, Černecká Ľ, Mihál I, Dolejš P, Holý K. 2017. Host utilization of koinobiont spider-ectoparasitoids (Ichneumonidae, Ephialtini, *Polysphincta* genus-group) associated with *Cyclosa* spp. (Araneae, Araneidae) across Palaearctic. Zoologischer Anzeiger 267, 8-14.

Takasuka K, Korenko S. (in preparation) Systematics and biology of spider-associated parasitoids in Ephialtini Hellen 1915 (Hymenoptera, Ichneumonidae, Pimplinae) – Review.

Townes H. 1969. The genera of Ichneumonidae, part 1. Memoirs of the American Entomological Institute 11, 1-300.

Townes H, Townes M. 1960. Ichneumon-flies of America North of Mexico: 2. Subfamilies Ephialtinae, Xoridinae, Acaenitinae. United States National Museum Bulletin, 216, 1-676.

Vincent LS. 1979. A new record for *Sinarachna anomala* (Hymenoptera: Ichneumonidae), an external parasitoid of *Mallos pallidus* (Araneae: Dictynidae). Pan-Pacific Entomologist 553, 192-194.

Weng JL, Barrantes G. 2007. Natural history and larval behavior of the parasitoid *Zatypota petronae* (Hymenoptera: Ichneumonidae). Journal of Hymenoptera Research 16, 326-335.

Yu DS, van Achterberg C, Horstmann K. 2012. Taxapad 2012. Ichneumonoidea 2011. Taxonomy, biology, morphology and distribution. Ottawa, Ontario. online at http://www.taxapad.com (accessed – 1st August 2017)

Zwakhals K. 2006. The European species of the genera *Zatypota* and *Sinarachna* (Hymenoptera: Ichneumonidae, Pimplinae, Polysphinctini). Entomologische Berichten 66, 34-37.

9 List of supplements

Supplement 1: Korenko S, Pekár S. 2011. A parasitoid wasp induces overwintering behaviour in its spider host. PLoS ONE 6(9), e24628. doi:10.1371/journal.pone.0024628.

Supplement 2: Korenko S, Michalková V, Zwakhals K, Pekár S. 2011. Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid (Hymenoptera: Ichneumonidae). Journal of Insect Science 11, 101.

Supplement 3: Kehlmaier Ch, Michalko R, Korenko S. 2012. *Ogcodes fumatus* (Diptera: Acroceridae) reared from *Philodromus cespitum* (Araneae: Philodromidae), and first evidence of *Wolbachia* in Acroceridae. Annales Zoologici 62(2), 281-286.

Supplement 4: Korenko S, Schmidt S, Schwarz M, Gibson GAP, Pekár S. 2013. Hymenopteran parasitoids of the ant-eating spider *Zodarion styliferum* (Simon) (Araneae: Zodariidae). Zookeys 262, 1-15.

Supplement 5: Korenko S, Isaia M, Satrapová J, Pekár S. 2014. Parazitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecological Entomology 39, 30-38.

Supplement 6: Korenko S, Korenková B, Satrapova J, Hamouzová K, Belgers D. 2015. Modification of *Tetragnatha montana* (Araneae, Tetragnathidae) web architecture induced by larva of the parasitoid *Acrodactyla quadrisculpta* (Hymenoptera, Ichneumonidae, *Polysphincta* genus-group). Zoological Studies 54, 40.

Supplement 7: Korenko S, Satrapova J, Zwakhals K. 2015. Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimlinae). Entomological Science 18, 383-388.

Supplement 8: Korenko S. 2016. Web architecture alteration of the orb web weaving spider *Metellina merianae* (Araneae, Tetragnathidae) induced by the parasitoid *Megaetaira madida* (Ichneumonidae, *Polysphincta* group). Arachnologische Mitteilungen 52, 35-37.

Supplement 9: Korenko S, Potopová V, Satrapová J, Pekár S. 2016. Life history of the spider parasitoid *Zatypota percontatoria* (Hymenoptera: Ichneumonidae). Entomological Science 19, 104-111.

Supplement 10: Takasuka K, Korenko S, Kysilková K, Štefánik M, Černecká Ľ, Mihál I, Dolejš P, Holý K. 2017. Host utilization of koinobiont spider-ectoparasitoids (Ichneumonidae, Ephialtini, *Polysphincta* genus-group) associated with *Cyclosa* spp. (Araneae, Araneidae) across the Palaearctic. Zoologischer Anzeiger 267, 8-14.

Supplement 11: Korenko S. 2017. First record from Italy of *Zatypota anomala* (Ichneumonidae, Ephialtini), a parasitoid of the cribellate spider *Dictyna pussila* (Araneae, Dictynidae). Arachnologische Mitteilungen 54, 1-4.

Supplement 12: Korenko S, Kysilková K, Černecká Ľ. 2017. Further records of two spider parasitoids of the genus *Polysphincta* (Ichneumonidae, Ephialtini) from Central Europe, with notes on their host interactions. Arachnologische Mitteilungen54: 28-32.

Supplement 13: Korenko S, Hamouzová K, Kysilková K, Kolářová M, Kloss TG, Takasuka K, Pekár S. Divergence in host utilisation in two spider parasitoids of the genus *Eriostethus* (Ichneumonidae, Ephialtini). In submission, Zoologischer Anzeiger.

Supplement 14: Korenko S, Di Giovanni F. Spider parasitoids of tribe Ephialtini (Ichneumonidae, Pimplinae) in Italy and their host association. In submission, North-Western Journal of Zoology.

Supplement 15: Korenko S, Pekár S, Walter GH, Korenková V, Hamouzová K, Kolářová M, Kysilková K, Spasojevic T, Klopfstein S. One generalist or several specialist species? Wide host range and diverse manipulations of the hosts' web building behaviour in the true spider parasitoid *Zatypota kauros* (Hymenoptera: Ichneumonidae). In submission, Frontiers in Zoology.

Supplement 16: Corresponding author's statement in connection to the habilitation thesis.

Supplement 1

Korenko S, Pekár S. 2011. A parasitoid wasp induces overwintering behaviour in its spider host. PLoS ONE 6(9), e24628. doi:10.1371/journal.pone.0024628.

Citation report:
Web of Science^{TM}: 10
Scopus/Google Scholar: 19

ISI WOS:
PLoS ONE
IF$_{(2011)}$ = IF = 4.092
Quartile $_{(2011)}$: Biology – Q1

A Parasitoid Wasp Induces Overwintering Behaviour in Its Spider Host

Stanislav Korenko[1,2]*, **Stano Pekár**[2]

1 Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Suchdol, Czech Republic, **2** Department of Botany and Zoology, Faculty of Sciences, Masaryk University, Brno, Czech Republic

Abstract

Parasites and parasitoids control behaviors of their hosts. However, the origin of the behavior evoked by the parasitic organism has been rarely identified. It is also not known whether the manipulation is universal or host-specific. Polysphinctine wasps, koinobiont ectoparasitoids of several spider species that manipulate host web-spinning activity for their own protection during pupation, provide an ideal system to reveal the origin of the evoked behavior. Larva of *Zatypota percontatoria* performed species-specific manipulation of theridiid spiders, *Neottiura bimaculata* and *Theridion varians*, shortly before pupation. Parasitized *N. bimaculata* produced a dense web, whereas parasitized *T. varians* built a cupola-like structure. The larva pupated inside of either the dense web or the cupola-like structure. We discovered that unparasitized *N. bimaculata* produce an analogous dense web around their eggsacs and for themselves during winter, while *T. varians* construct an analogous 'cupola' only for overwintering. We induced analogous manipulation in unparasitized hosts by altering ambient conditions. We discovered that the behavior evoked by larvae in two hosts was functionally similar. The larva evoked protective behaviors that occur in unparasitized hosts only during specific life-history periods.

Citation: Korenko S, Pekár S (2011) A Parasitoid Wasp Induces Overwintering Behaviour in Its Spider Host. PLoS ONE 6(9): e24628. doi:10.1371/journal.pone.0024628

Editor: Markus M. Heimesaat, Charité, Campus Benjamin Franklin, Germany

Received January 26, 2011; **Accepted** August 16, 2011; **Published** September 8, 2011

Funding: The study was supported by grant no. MSM0021622416 provided by the Ministry of Education, Youth and Sports of the Czech Republic. SK was also supported by project no. MSM6046070901. URL: http://www.isvav.cz/researchPlanDetail.do?rowId = MSM0021622416 and http://www.isvav.cz/researchPlanDetail.do?rowId = MSM6046070901. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: korenko.stanislav@yahoo.com

Introduction

Many parasites and parasitoids have evolved remarkable strategies to manipulate the behavior of their hosts in order to promote their own survival and reproduction [1,2]. The behavioral manipulations described include altered phototaxis, changes in locomotion, and the alteration of foraging and defensive behaviors [2–19]. The most fascinating manipulations are those that lead to unnatural host behaviors. The parasitic trematode, *Dicrocoelium dendriticum* Rudolphi, forces its intermediate ant-host to move up onto blades of grass during the night and early morning. This action increases the ingestion of infected ants by grazing sheep, the final host [3]. Mermithid nematodes induce their terrestrial arthropod hosts to commit suicide by jumping into water, after which the hairworms desert the host to spend their adult stage in their natural habitat [8].

Behavioral manipulations often result in the induction of innate behaviors. Acanthocephalan, *Polymorphus paradoxus* (Connell & Corner), evokes evasive behavior in the amphipod intermediate host, *Gammarus lacustris* Sars, which is then eaten by ducks [4]. The braconid parasitoid, *Glyptapanteles* spp., makes their caterpillar host behave as a bodyguard of the parasitoid pupae [15]. The caterpillar stands bent over the parasitoid pupae and violently lashes out at approaching predators, resulting in reduced predation of parasitoid pupae.

Evidence for benefits of the host manipulations for the parasitoid has been gained from several host-parasitoid systems [9–12]. But there might be also costs involved. This has been rarely studied. Maure et al. [13] investigated bodyguarding of the braconid pupae, *Dinocampus coccinellae* (Schrank), by ladybird *Coleomegilla maculate* Timberlake. Laboratory experiments revealed that duration of bodyguarding suppressed predation by lacewings but also decreased the parasitoid fecundity.

Within ichneumonid wasps a large group of ectoparasitoids (Polysphinctini) are specialized on spiders [20]. The larva of this koinobiont parasitoids is attached to the dorsal side of the abdomen, where it develops (from egg via three larval instars), while the spider continues foraging. Shortly before pupation the parasitoids manipulate the web-spinning activity of the host in order to establish effective protection against enemies and the environment, though there are a few cases of absent manipulation [21–23]. The larva of *Hymenoepimecis* sp. (Ichneumonidae) makes its spider host build a highly modified web that is smaller than the capture web [6,7,24]. The protective effect of the web for the parasitoid larva was recently documented by Matsumoto [16], who found that the web structure safely guarded parasitoid larva against predators and scavengers.

The mechanism of manipulation used by parasites or koinobiont parasitoids, i.e. the origin of the induced behavior has not been revealed yet. This is because the host response to manipulation is often very complex; it is composed of a series of behaviors. In this respect, the scenario of wasp parasitoids attacking spiders offers an ideal system for identifying the origin of such induced behavior, because the evoked behavior is simple, i.e. leading to an unique product. Furthermore, koinobiont parasitoids are often not so strictly specialized as idiobionts [25], so the behavioral response can be compared among several hosts.

Here, we chose the wasp *Zatypota percontatoria* Müller (Ichneumonidae), which is specialized on several theridiid spiders. Most frequently it attacks two species, *Neottiura bimaculata* (Linnaeus) and *Theridion varians* Hahn [26]. We performed a comparative analysis of evoked behaviors in two hosts in conditions when these behaviors occur naturally. The obtained results enabled us to identify the origin of the evoked behaviors.

Results

Species-specific response

Shortly (24–48 hours) before the larva's pupation the behavior of the host spider changed from typical foraging and web-building activity to the production of a specific structure on the web as detailed below. Once the spider finished the structure, the larva killed the spider, consumed it and built a pupal cocoon inside of the structure.

The result of host manipulation induced by the *Z. percontatoria* larva differed dramatically between *N. bimaculata* and *T. varians*. Nearly all parasitized *N. bimaculata* (93%, N = 31) constructed a structure made of dense silk threads surrounding the spider's resting position (Figure 1A). This web was significantly denser (Figure 2) in parasitized *N. bimaculata* than the web of unparasitized individuals (Welch test, $t_{15.1} = 4.3$, $P = 0.0025$, Figure 1B). Most parasitized *T. varians* (82%, N = 56) constructed a closed, spherical cupola-like structure around the spider's resting site in the web (Figure 1C), whereas none of unparasitized spiders constructed such a structure (0%, N = 20) ($X^2_1 = 38.3$, $P < 0.0001$). The interior of the 'cupola' contained only sparse silk threads. The cupola-like structure was completely closed in 80% of cases (N = 56), and open at the bottom in 20% of cases. The mean horizontal and vertical diameters of the 'cupola' were 5.4 mm (SD = 0.06, N = 42) and 6.7 mm (SD = 0.09). The larva pupated either inside the dense web (*N. bimaculata*) or the 'copula'-like structure (*T. varians*).

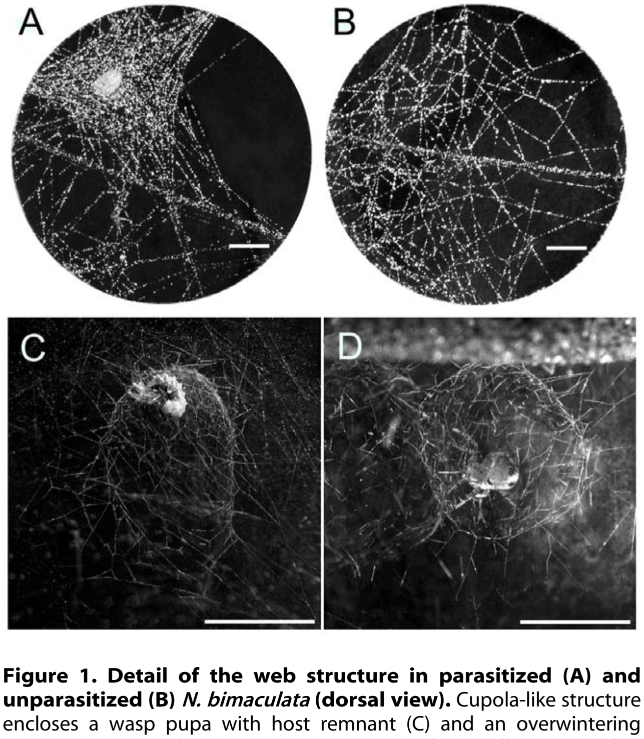


Figure 1. Detail of the web structure in parasitized (A) and unparasitized (B) *N. bimaculata* (dorsal view). Cupola-like structure encloses a wasp pupa with host remnant (C) and an overwintering *T. varians* (D) (lateral view). The wasp larva is in the middle (A, C). Scales: 5 mm.
doi:10.1371/journal.pone.0024628.g001

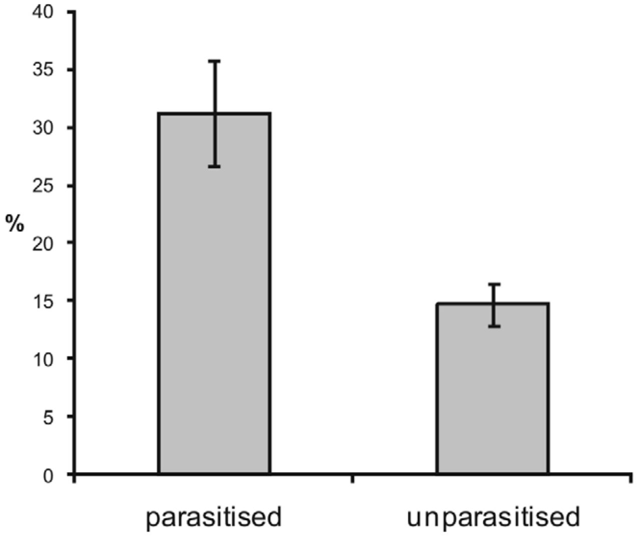


Figure 2. Comparison of the mean density of web in parasitised and unparasitised *N. bimaculata* spiders. Whiskers are standard errors of the mean.
doi:10.1371/journal.pone.0024628.g002

Origin of the evoked behavior

In the field we observed that during winter, unparasitized immature individuals of *T. varians* were hidden in the cupola-like structures built among leaf debris (N = 14) and under tree bark (N = 5). Unparasitized immature individuals of *N. bimaculata* were hidden in a 'web' consisting of several threads spread among leaf debris (N = 10).

In the laboratory, study of the life history of unparasitized *N. bimaculata* revealed that denser webs were not produced before molting, but during overwintering and around the egg sac (Figure 3). Study of the life history of unparasitized *T. varians* revealed that the spider did not construct the cupola-like structure either before molting or around the egg sac (Figure 3). However, majority of *T. varians* juveniles constructed the cupola-like structure

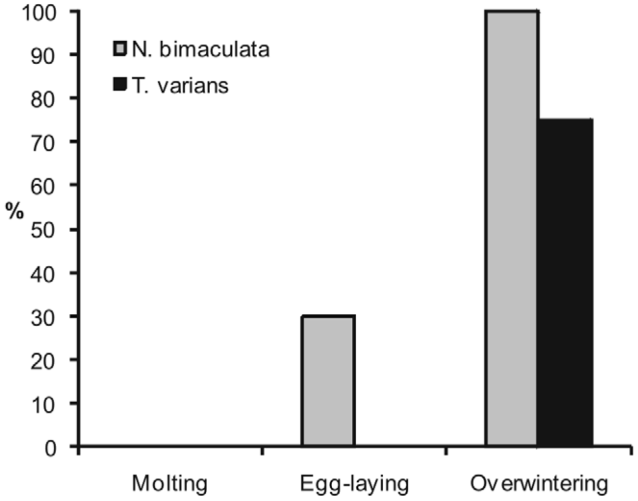


Figure 3. Comparison of the realtive frequency of occurence of dense webs (in case of *N. bimaculata*) or copula-like structure (in case of *T. varians*) at three life-history stages. N varies between 12 and 28 (see Methods for details).
doi:10.1371/journal.pone.0024628.g003

around themselves when exposed to low temperature imitating overwintering (Figure 1D).

Discussion

We found here that the larva of *Z. percontatoria* manipulated web-spinning activity in two spider hosts. The induced activities were innate to both host species but occurred only during specific life-history periods of unparasitized hosts. In *T. varians* induced activity occurred only before overwintering, while in *N. bimaculata* it occurred both at oviposition and before overwintering. Thus, we identified the origin of the behavior evoked by the parasitic organism. At the same time we revealed that the response of hosts to the parasitoid manipulation was species-specific, though similar in function.

The investigated parasitoid wasp, *Z. percontatoria*, is an example of a parasitoid that attacks a few closely related web-building spiders of the family Theridiidae. All species belong to the '*Theridion*' group, which was recently split into several genera, namely *Theridion*, *Neottiura*, *Phylloneta*, *Platnickina*, *Paidiscura* [27]. These spiders are similar with respect to body size, phenology pattern, and capture web-building [28,29] but may differ with regard to the construction of defensive structures. We expect that spiders of all these species parasitized by *Z. percontatoria* can produce a variety of silk structures but of similar protective function.

Host manipulation by the polysphinctine parasitic wasp can lead to an unique product that is apparently not produced by unparasitized spiders [23,24] or it can be a more or less homologous product of a web produced by unparasitized spiders [16,18,30]. Manipulated *T. varians* created a cupola-like structure, which was very different from the capture web. The capture web of *T. varians* was an irregular 3-dimensional web about 10 cm across, while the 'cupola' was considerably smaller regular 3-dimensional structure. The parasitized spider constructed the 'cupola' in the capture web. In nature, the 'cupola' is built in the debris among leaves in October and November when the ambient temperature is low. It is used as a shelter by unparasitized spiders during winter. But parasitized spiders construct such a structure in summer on vegetation when temperatures are considerably higher. On the other hand, manipulated *N. bimaculata* produced a protective structure in the capture web by adding more silk around the position for pupation. Such denser silk is used by unparasitized *N. bimaculata* during summer at the time of eggsac production or before overwintering. Again, the parasitized spider constructed such a structure in summer.

Induced changes in host behavior are most likely adaptive as they lead to increased fitness of the parasitoid [13,31]. In our study system, the behavioral manipulation led to the construction of specific structures that were innate to the spider. We did not study the effect of the structures on the fitness of the parasitoid pupa, but we expect that it is used to increase its survival. The denser web of *N. bimaculata* as well as the cupola-like structure of *T. varians* are assumed to have a similar function for the parasitoid as for the unparasitized spider. The cupola-like structure most likely protects *T. varians* against winter-active predators, and the dense webbing seems to protect eggs of *N. bimaculata* from rain or wind and predators.

Although there are several studies that describe host manipulation by koinobiont parasitoid wasps in detail [1,5–7,9–13,15–19], an understanding of the proximate mechanisms of manipulation is largely lacking. This is in contrast to our knowledge of the mechanism in idiobiont wasp parasitoids, where the wasp injects its venom directly into the host's head ganglia and induces long-term hypokinesia [32,33]. In koinobionts, the influence of the parasite/parasitoid on host behavior can be either direct by manipulating the host's nervous system, or indirect by manipulating the host's immune or endocrine system, or its metabolism [2,34]. If the effect is direct, then the behavior is controlled by neuromodulators, such as taurine [35]. Parasites are able to use the same or similar neuromodulators as those of the hosts to usurp control of the host's behavior [2]. However, the production of neuromodulators may be energetically expensive. Less expensive methods may be seen in indirect ways, when parasites induce the host's immune system to produce the appropriate neuromodulators. In such cases the hosts, not the parasites, produce the neuromodulators that alter their own behavior [2,36].

At present we do not know whether the host behavioural changes observed in our study system was a direct or an indirect influence. The fact that the evoked behavior occurred naturally only during reproduction or overwintering suggests control via the endocrine system. This is also supported by our own induction of the manipulation in unparasitized spiders. By placing unparasitized *T. varians* into cold chambers we induced the production of the cupola-like structure. However, the fact that only a specific short-lived behavioral process was induced rather suggests manipulation via use of neuromodulators. We hypothesize that the larva produced a signal molecule responsible for the onset of such behavior. The molecule should be homologous to a molecule that the spider is naturally producing in conditions requiring protection. Differences in the response of *T. varians* and *N. bimaculata* could be a result of different substances being introduced into different host-species. We are inclined to assume they are a result of species-specific host responses to the same substance (possibly signal molecule) because, in both species, functionally similar behavior was induced.

Parasitism as a life-history strategy among polysphinctine wasps is known for several species. All such species are specialized on spiders from different taxonomical groups and foraging guilds [37]. The more basal genera of the polysphinctine wasps, such as *Clistopyga*, *Dreisbachia*, and *Schizopyga* attack wandering hunters (e.g. Clubionidae, Lycosidae), while more derived genera exploit spiders that construct aerial webs [38]. For example, wasps of the genera *Polysphincta*, *Reclinervellus*, and *Sinarachna* attack orb-web builders from the families Araneidae [37,39], Nephilidae [19] and Tetragnathidae [6,7,17,24]. Representatives of the genera *Acrodactyla* and *Brachyzapus* attack sheet-web builders from the family Linyphiidae [37] and Agelenidae [16]. Species of the genus *Zatypota* attack space-web builders of the family Theridiidae [18, 26,30,37] and Dictynidae [37]. Parasitoids specialized on orb-web builders induce production of a unique simplified web that provides support for the wasp pupa [6,7,24,39]. Parasitoids specialized on sheet-web builders either do not modify host-spinning activity [21] or induce production of webbing for protecting the parasitoid [16]. Parasitoids that attack space-web builders induce production of dense sheet webbing [18]. Thus, the manipulation of web-spinning activity has arisen independently in several lineages within hymenopterans as well as other insect orders. An alteration in spider spinning behavior induced by parasitoid larva has also been observed in acrocerid flies, where the manipulated spider produced a silk cell similar to that made prior to molting [40].

Here, we showed that the parasitoid wasp *Zatypota percontatoria* induced web-spinning activity in both spider hosts. Their response to manipulation was species-specific as parasitized *N. bimaculata* constructed a structure made of dense silk threads and parasitized *T. varians* constructed a cupola-like structure in the web. We revealed the origin of the evoked behavior that the parasitoid used in manipulation. The behaviors were innate to both hosts and occurred only during specific life-history periods of unparasitized hosts, i.e. when spiders needed protection. In *T. varians* such behaviour occurred only before overwintering, while in *N. bimaculata* it occurred both at oviposition and before overwintering. The products of manipulation most likely provided effective protection of the wasp pupa against enemies and the environment.

Materials and Methods

Spiders and parasitoids

Parasitized and unparasitized spiders of both species, *Neottiura bimaculata* and *Theridion varians*, were collected in a commercial apple orchard in the Czech Republic, Brno (49° 09′ 37″N, 16° 33′ 35″E) in 2007 and 2008, and in an ecologically managed apple and hazel-nut orchard in Italy, Caraglio, Cascina Rosa (44° 24′ 47.85″N, 7° 24′ 43.39″E) in autumn 2009. Spiders were collected by beating tree branches in spring and in late autumn, when the incidence of parasitism was highest (26). A square shaped beating tray (1 m^2 area) was placed beneath the tree-crown and all tree branches above the net were beaten. Spiders were kept at a room temperature of 22±3.5°C, natural L:D regime and fed with a surplus of *Drosophila melanogaster* Meigen flies. Unparasitized spiders were observed until adulthood and parasitized spiders until they were killed and consumed by the parasitoid larva. Once adult parasitoids emerged, they were preserved in ethanol and identified by Kees Zwakhals (Arkel, The Netherlands).

Host response

We investigated differences in the web structures between parasitized (N = 31) and unparasitized *N. bimaculata* spiders (N = 24) and parasitized (N = 56) and unparasitized (N = 20) *T. varians* spiders. Juvenile spiders collected in the field were placed singly into plastic containers (diameter 34 mm, height 40 mm). When parasitoid larva achieved the last larval instar (2–4 days before the spider was killed) all spiders, including the unparasitized ones, were moved to new plastic containers (of the same size), where spider web construction was observed until the larva had consumed the spider. The web structures of both parasitized and unparasitized spiders were then photographed using a digital camera (Konica Minolta DZ2). The pictures of web structures were analyzed using SigmaScan Pro, v. 5.0. In pictures of *N. bimaculata* webs, the silk density (% of white colour) per selected area that included the center (resting-place) of the web was estimated. In pictures of *T. varians* webs, the size and shape of the cupola-like structures were evaluated.

Behavior of unparasitized hosts

To reveal whether the manipulated behavior is innate or unique to the two spider species and when it is used, we collected unparasitized specimens of both species and reared them under controlled laboratory conditions with a surplus of *D. melanogaster* flies. Twenty immature *N. bimaculata* individuals and 28 immature *T. varians* individuals were collected in September, placed in plastic containers (as above) and observed during molting. In May we collected 14 adult females of *N. bimaculata* and 12 adult females of *T. varians*, placed them into containers, and observed the web structures until eggsac production. In the orchard we investigated the leaf litter where both spider species overwintered in winter between October 2007 and January 2008 when the average day temperature was 2°C. We recorded the conditions, i.e. microhabitat and type of webbing, in which they were overwintering. The next season, in November 2008, we collected 20 juvenile specimens of *N. bimaculata* and 24 specimens of *T. varians* and placed them into tubes (diameter 34 mm, height 40 mm) with a piece of moist gauze. The tubes were placed into a thermostat at 5°C, L:D = 10:14. After 10 days we recorded the frequency and shapes of the constructed web structures.

Acknowledgments

We would like to thank M. Isaia, A. Chiarle and M. Paschetta for their help in collecting spiders and wasps in Italy, K. Zwakhals for the identification of wasp specimens and W. G. Eberhard for fruitful comments.

Author Contributions

Conceived and designed the experiments: SK SP. Performed the experiments: SK. Analyzed the data: SP. Contributed reagents/materials/analysis tools: SK SP. Wrote the paper: SK SP.

References

1. Brodeur J, McNeil JN (1989) Seasonal microhabitat selection by an endoparasitoid through adaptative modification of host behavior. Science 244: 226–228.
2. Moore J (2002) Parasites and the behavior of animals. Oxford: Oxford University Press. 338 p.
3. Carney WP (1969) Behavioral and morphological changes in carpenter ants harboring *Dicrocoelium metacercariae*. Am Midl Nat 82: 605–611.
4. Bethel WM, Holmes JC (1977) Increased vulnerability of amphipods to predation owing to altered behaviour induced by larval acanthocephalans. Can J Zool 55: 110–115.
5. Brodeur J, Vet LEM (1994) Usurpation of host behaviour by a parasitic wasp. Anim Behav 48: 187–192.
6. Eberhard WG (2000) Spider manipulation by a wasp larva. Nature 406: 255–256.
7. Eberhard WG (2000) The natural history and behavior of *Hymenoepimecis argyraphaga* (Hymenoptera: Ichneumonidae) a parasitoid of *Plesiometa argyra* (Araneae: Tetragnathidae). J Hymenopt Res 9: 220–240.
8. Thomas F, Schmidt-Rhaesa A, Martin G, Manu C, Durand P, et al. (2002) Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? J Evolution Biol 15: 356–361.
9. Tanaka S, Ohsaki N (2006) Behavioral manipulation of host caterpillars by the primary parasitoid wasp *Cotesia glomerata* (L.) to construct defensive webs against hyperparasitism. Ecol Res 21: 570–577.
10. Tanaka S, Ohsaki N (2009) Does manipulation by the parasitoid wasp *Cotesia glomerata* (L.) cause attachment behaviour of host caterpillars on cocoon clusters? Ethology 115: 781–789.
11. Harvey JA, Kos M, Nakamatsu Y, Tanaka T, Dicke M, et al. (2008) Do parasitized caterpillars protect their parasitoids from hyperparasitoids? A test of the 'usurpation hypothesis'. Anim Behav 76: 701–708.
12. Harvey JA, Tanaka T, Kruidhof M, Vet LEM, Gols R (2011) The usurpation hypothesis revisited: dying caterpillar repels attack from a hyperparasitoid wasp. Anim Behav 81: 1281–1287.
13. Maure J, Brodeur J, Ponlet N, Doyon J, Firlej A, et al. (2011) The cost of a bodyguard. Biol Lett Published online, doi:10.1098/rsbl.2011.0415.
14. Vyas A, Kim SK, Giacomini N, Boothroyd JC, Sapolsky RM (2007) Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. Proc Natl Acad Sci USA 104: 6442–6447.
15. Grosman AH, Janssen A, de Brito EF, Cordeiro EG, Colares F, et al. (2008) Parasitoid increases survival of its pupae by inducing hosts to fight predators. PLoS ONE 3(6): e2276. doi:10.1371/journal.pone.0002276.
16. Matsumoto R (2009) "Veils" against predators: Modified web structure of a host spider induced by an ichneumonid parasitiod, *Brachyzapus nikkoensis* (Uchida) (Hymenoptera). J Insect Behav 22: 39–48.
17. Sobczak JF, Loffredo APS, Penteado-Dias AM, Gonzaga MO (2009) Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. J Nat Hist 43: 2691–2699.
18. Eberhard WG (2010) New types of behavioral manipulation of host spiders by a parasitoid wasp. Psyche ID 950614 doi:10.1155/2010/950614.
19. Gonzaga MO, Sobczak JF, Penteado-Dias AM, Eberhard WG (2010) Modification of *Nephila clavipes* (Araneae Nephilidae) webs induced by the parasitoids *Hymenoepimecis bicolor* and *H. robertsae* (Hymenoptera Ichneumonidae). Ethol Ecol Evol 22: 151–165.

20. Gauld ID, Dubois J (2006) Phylogeny of the Polysphincta group of genera (Hymenoptera: Ichneumonidae; Pimplinae), a taxonomic revision of spider ectoparasitoids. Syst Entomol 31: 529–564.
21. Nielsen E (1923) Contributions to the life history of the Pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*) (Hym. Ichneum.). Entomol Medd 14: 137–205.
22. Fincke OM, Higgins L, Rojas E (1990) Parasitism of *Nephila clavipes* (Araneae, Tetragnathidae) by an ichneumonid (Hymenoptera, Polysphinctini) in Panama. J Arachnol 18: 321–329.
23. Gonzaga MO, Sobczak JF (2006) Parasitoid-induced mortality of *Araneus omnicolor* (Araneae, Araneidae) by *Hymenoepimecis* sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. Naturwissenschaften 94: 223–227.
24. Eberhard WG (2001) Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). J Arachnol 29: 354–366.
25. Althoff DM (2003) Does parasitoid attack strategy influence host specificity? A test with New World braconids. Ecol Entomol 28: 500–502.
26. Korenko S, Michalková V, Zwakhals K, Pekár S (2011) Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid (Hymenoptera: Ichneumonidae). J Insect Sci. In press.
27. Arnedo AM, Agnarsson I, Gillespie RG (2007) Molecular insights into the phylogenetic structure of the spider genus *Theridion* (Araneae, Theridiidae) and the origin of the Hawaiian Theridion-like fauna. Zoologica Skripta 4: 337–352.
28. Heimer S, Nentwig W (1991) Spinnen Mitteleuropas. Berlin: Paul Parey. 543 p.
29. Eberhard WG, Agnarsson I, Levi HW (2008) Web forms and the phylogeny of theridiid spiders (Araneae: Theridiidae): chaos from order. Syst Biodivers 6: 415–475.
30. Weng J-L, Barrantes G (2007) Natural history and larval behavior of the parasitoid *Zatypota petronae* (Hymenoptera: Ichneumonidae). J Hymenopt Res 16: 326–335.
31. Poulin R (2000) Manipulation of host behaviour by parasites: a weakening paradigm? Proc R Soc B 267: 787–792.
32. Libersat F, Delago A, Gal R (2009) Manipulation of host behavior by parasitic insects and insect parasites. Annu Rev Entomol 54: 189–207.
33. Gal R, Libersat F (2010) A wasp manipulates neuronal activity in the sub-esophageal ganglion to decrease the drive for walking in its cockroach prey. PLoS ONE 5(4): e10019. doi:10.1371/journal.pone.0010019.
34. Thomas F, Adamo S, Moore J (2005) Parasitic manipulation: where are we and where should we go? Behav Process 68: 185–199.
35. Harris-Warwick RM, Marder E (1991) Modulation of neural networks for behavior. Annu Rev Neurosci 14: 39–57.
36. Beckage NE (1985) Effects on host hormones and behavior. In: Beckage NE, ed. Parasites and pathogens. New York: Chapman and Hall. pp 3–36.
37. Fitton MG, Shaw MR, Austin AD (1987) The Hymenoptera associated with spiders in Europe. Zool J Linn Soc-Lond 90(1): 65–93.
38. Gauld ID, Wahl DB, Broad GR (2002) The suprageneric groups of the Pimplinae (Hymenoptera: Ichneumonidae): a cladistic re-evaluation and evolutionary biological study. Zool J Linn Soc-Lond 136: 421–485.
39. Matsumoto R, Konishi K (2007) Life histories of two ichneumonid parasitoids of *Cyclosa octotuberculata* (Araneae), *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomol Sci 10: 267–278.
40. Schlinger EI (1987) The biology of Acroceridae (Diptera): true endoparasitoids of spiders. In: Nentwig W, ed. Ecophysiology of spiders. New York: Springer-Verlag. pp 319–327.

Supplement 2

Korenko S, Michalková V, Zwakhals K, Pekár S. 2011. Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid (Hymenoptera: Ichneumonidae). Journal of Insect Science 11, 101.

Citation report:
Web of Science^{TM}: 12
Scopus/Google Scholar: 17

ISI WOS:
Journal of Insect Science
IF$_{(2011)}$ = IF = 0,947
Quartile $_{(2011)}$: Entomology – Q2

Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid *Zatypota percontatoria*

Stanislav Korenko[1,2,a*], Veronika Michalková[3,b], Kees Zwakhals[4,c], Stano Pekár[2,d]

[1]Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic
[2]Department of Botany and Zoology, Masaryk University, Faculty of Science, Kotlářská 2, 611 37 Brno, Czech Republic
[3]Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 06 Bratislava, Slovakia
[4]Dr. Dreeslaan 204, NL-4241 CM Arkel, Netherlands

Abstract

Current knowledge about polysphinctine parasite wasps' interactions with their spider hosts is very fragmented and incomplete. This study presents the host specificity of *Zatypota percontatoria* (Müller) (Hymenoptera: Ichneumonidae) and its adaptation to varying host availability. Two years of field observations show that *Z. percontatoria* is a stenophagous parasitoid that parasitizes only five closely related web-building spiders of the family Theridiidae (Araneae). Within the Theridiidae it attacks only species belonging to a small group of species, here called the "*Theridion*" group. These hosts have a similar biology, but are available at different levels of abundance and at different sizes over the season. Laboratory experiments showed that this wasp species ignores linyphiid, araneid or dictynid spiders and accepts only theridiid spiders of the "*Theridion*" group. In the field study, wasp females preferred older juvenile and sub-adult female spider instars with intermediate body size. Only 5% of the parasitized spiders were males. Parasitism in the natural population of theridiid spiders was on average 1.3%. Parasitism was most frequent on two species, *Theridion varians* Hahn in 2007 and *Neottiura bimaculata* Linnaeus in 2008. The parasitization rate was positively correlated with spider abundance. The wasp responded adaptively to seasonal changes in host abundance and host body size and shifted host preference according to the availability of suitable hosts during, as well as between, seasons. In spring and summer the highest percentage of parasitism was on *T. varians* and in autumn it was on *N. bimaculata*.

Keywords: foraging strategy, host-parasitoid interaction, *Neottiura bimaculata*, *Theridion varians*
Correspondence: [a*] korenko.stanislav@yahoo.com, [b] vmichalkova@yahoo.com, [c] keeszwakhals@yahoo.com, [d] pekar@sci.muni.cz, *Corresponding author
Editor: Jay Rosenheim was Editor of this paper.
Received: 17 December 2010, **Accepted:** 31 December 2010

ISSN: 1536-2442 | Vol. 11, Number 101

Cite this paper as:
Korenko S, Michalková V, Zwakhals K, Pekár S. 2011. Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid *Zatypota percontatoria. Journal of Insect Science* 11:101 available online: insectscience.org/11.101

Introduction

Foraging is one of the central issues in ecology as it shapes the interactions between predators and their prey. Foraging of a parasitoid includes attack on a single free-living host and oviposition of an egg that develops only on the host tissue leading to complete host consumption. Due to this restriction, parasitoids have evolved adaptive foraging and host-selection strategies that enable them to survive in situations when host sources are restricted (Godfray and Shimada 1999).

Two different parasitoid developmental strategies exist: idiobiosis and koinobiosis. Idiobiont species develop on a paralyzed and non-growing host (Askew and Shaw 1986), hence their host resource is static in terms of size during their development. Parasitoid size is thus a function of host species size at the time of attack. By contrast, koinobiont species attack hosts that continue to feed and grow (Askew and Shaw 1986). The amount of resources exploited by such parasitoid larvae for growth and development is not fixed; their development depends upon host feeding rate and growth (Godfray 1994).

Hymenopteran parasitoids show a great variety of host specializations. The host range of specialist parasitoids, assessed at the taxonomic level, is often very narrow, whereas generalists exploit a broad range of hosts (e.g., Shaw 1994; Shaw and Aeschlimann 1994). They tend to attack a group of host species that share a similar set of characteristics, either ecological or phylogenetic. When encounters with one host species become infrequent, generalist parasitoids expand their host selection criterion to include other (even less profitable) host species (Henry et al. 2009). As a result, generalist parasitoids can have multiple generations per season (Pyke et al. 1977).

Koinobionts are generally more host-specific than idiobionts (Althoff 2003). In strictly specialized parasitoids, their life history is tightly coupled with that of their host species. Such parasitoids occur only in the habitat of their host and synchronize their development with them (Barrantes et al. 2008). Examples of rather specialized parasitoids include polysphinctine ichneumonids that attack spiders (e.g. Fitton et al. 1987).

Host size or developmental stage vary during the season. Therefore, choosing suitable hosts is fundamental to the parasitoid–host relationship. Some parasitoids, for example, attack hosts only at a certain developmental stage or size (Henry et al. 2006). Henry et al. (2009) demonstrated that smaller parasitoids receive the greatest benefits from selecting small, but less defended and abundant hosts. Hosts, such as web spiders, are habile hunters that can easily overcome the handling capabilities of smaller parasitoids (Gentry and Dyer 2002; Henry et al. 2006). The host's ability to physically defend itself typically increases with host age and size. Female parasitoids must make a choice between increased handling time and risking injury in attempting to oviposit on larger dangerous hosts (Gerling et al. 1990).

The koinobiont ectoparasitoid wasp, *Zatypota percontatoria* (Müller) (Hymenoptera: Ichneumonidae), belongs to polysphinctine pimplines, which exhibit a unique trait within the Ichneumonidae in terms of development (Fitton et al. 1987). A wasp larva develops externally on an active spider and the spider host is killed and consumed shortly before the ichneumonid's pupation. Biologically and morphologically, polysphinctines belong to the most specialized Pimplinae (Fitton et al. 1987). Overall, the trophic relationship between polysphinctines and spiders is clearly highly specialized, and *a priori* it is expected that the host ranges of these species are narrow (Shaw 1994). Our knowledge about this host – parasitoid system still remains very fragmented and incomplete.

The few available records about the *Zatypota* species show that it attacks spiders from several different families (Aubert 1969; Shaw 1994; Gauld and Dubois 2006). Shaw (1994) summarized host records of *Z. percontatoria* and concluded that this species attacks spiders from several web-building families, namely Araneidae, Dictynidae, Tetragnathidae and Theridiidae. However, Gauld and Dubois (2006) presumed a misidentification of the reared wasp in several cases and questioned all hosts other than theridiid spiders. Thus, verified records of hosts of *Z. percontatoria* include only a few spider species from the family Theridiidae: *Phylloneta impressa* (L. Koch), *Platnickina tincta* (Walckenaer), *Theridion varians* Hahn, *T. simile* Koch and *T. melanurum* Hahn (Aubert 1969; Fitton et al. 1987; Gauld and Dubois 2006).

The aim of this study was to investigate the foraging strategy of *Z. percontatoria* in terms of host specificity at a taxonomic, developmental and sexual level.

Materials and Methods

Field study

A population of *Z. percontatoria* was investigated in an apple orchard situated in Brno, Czech Republic (49° 09′ 37″ N, 16° 33′ 35″ E). The orchard consists of two tree varieties, Golden Delicious and Champion, both between 27 and 30 years old. The orchard was under an integrated pest management regime during our study.

The percentage of parasitism (defined as the total number of cases of parasitized spiders in the population at a given time, divided by the number of individuals in the population) was investigated during 2007 and 2008, by collecting potential and parasitized hosts. The hosts, arboreal spiders, were collected by beating apple tree branches. This was done from spring (April) to late autumn (October) each year. Spiders were collected in a square shaped beating net (1 m^2 area) placed beneath the tree crown. A single sampling consisted of beating the branches of 25 trees. The sampling was performed on 12 days in April, May, August, September, and October in 2007 and on 10 days in April, May, June, July, August, and September in 2008. On each investigated day three samples were taken. All spiders were fixed in 70% alcohol and identified to species/genus level using Heimer and Nentwig (1991), Roberts (1995) and Pekár (1999). The spider nomenclature is according to Platnick (2009).

During both years, 11 theridiid spider species were identified to species level based upon adult specimens. The majority of these belonged to the "*Theridion*" group. In the terms of this study the "*Theridion*" group includes the following closely related species with a similar biology and web architecture (see, e.g. Heimer and Nentwig 1991): *Theridion varians* Hahn, *T. pinastri* L. Koch, *Phylloneta impressa* Linnaeus, *Neottiura bimaculata* (Linnaeus), *Platnickina tincta* (Walckenaer), *Heterotheridion nigrovariegatum* (Simon) and *Paidiscura pallens* (Blackwall). The remaining species are *Dipoena melanogaster* C. L. Koch, *Enoplognatha latimana* Hippa and Oksala, *E.*

ovata (Clerck) and *Parasteatoda lunata* (Clerck). The body size (prosoma length), developmental stage (juvenile or adult), and sex of each parasitized spider were determined with an Olympus stereomicroscope SZ 40.

The sex ratio of both unparasitized (N = 204) and parasitized (N = 75) "*Theridion*" spiders was estimated from individuals of sub-adult and adult growth stage. The sex of spiders could be recognized only at later juvenile and sub-adult stages, i.e. when their prosoma was larger than 0.7 mm. At these stages, males were recognized by swollen pedipalps and females by a swollen and dark area of the epigyne. The length of the prosoma is a commonly used parameter for body size because unlike the length of the abdomen it changes only during molting (Schaefer 1987).

Wasp rearing

Living parasitized spiders were collected in the same periods but in a different part of the orchard. Parasitized spiders (N = 138) were collected by means of branch beating. Hosts with parasitoids were placed singly in cylindrical containers (diameter 35 mm, height 40 mm) with a layer of plaster of Paris at the bottom. The plaster was moistened at three-day intervals. Spiders were kept at room temperature 22 ± 3.5° C under a natural L:D regime and fed with a surplus of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). The spiders were reared until the wasps' emergence. Spider hosts were identified and the results were included into host-specificity frequencies. Hatched wasps were identified using Fitton et al. (1988) and Zwakhals (2006). The nomenclature of the polysphinctines follows Fitton et al. (1988) and Yu and Horstmann (1997). Some of these wasps were used in the experiments described below.

Laboratory experiments

The host preference of *Z. percontatoria* was investigated under laboratory conditions in two experiments. In the first one, the preference for hosts of four spider genera belonging to four families was tested; in the second one, the preference for spider species of one family was tested. A similar experimental set-up was used in both experiments. After hatching, the virgin female wasps were placed singly in glass vials (height 85 mm, diameter 55 mm), with a cotton ball soaked in 20% aqueous honey solution at the bottom. Three days later three spiders (potential hosts) were released into each vial simultaneously and acceptance of a particular spider host was recorded within 48 hours. Arrhenotokous parthenogenesis of virgin females seems to be common in *Z. percontatoria* (Korenko, unpublished).

In the first experiment with 18 wasp females, web-building spiders, specifically *Araniella* sp. (Araneidae, N = 12), *Meioneta* sp. (Linyphiidae, N = 11), *Dictyna* sp. (Dictynidae, N = 13), and *Theridion* spp. (Theridiidae, N = 18) collected in the orchard, were offered as potential hosts. The prosoma length of these spiders was between 0.6 and 1 mm. All spiders were juvenile and did not build a complete web in such a short time. If the spider built a dense web, it was destroyed with a stick in order to prevent the female wasp from becoming caught in the web.

In the second experiment with 30 wasp females, six species of the family Theridiidae were offered. The following species were used: *N. bimaculata* (N = 17), *P. impressa* (N = 11), *T. varians* (N = 30), "*Theridion*" (N = 15), *Dipoena melanogaster* (N = 9) and *Enoplogntha* sp. (N = 8). All spiders were juvenile with a prosoma length between 0.6 and 1 mm.

Data analysis of the laboratory experiments

Data were analyzed using various methods within the R environment (R Development Core Team 2009). The Chi-square "goodness of fit" test was used to compare frequencies of abundance of potential hosts with the rate of parasitized hosts. The Proportion test was used to compare proportions of each sex among available and parasitized hosts. ANOVA was used to compare host sizes during each season, as the response variable was continuous and residuals were homoscedastic. The results of laboratory experiments were analyzed using two different methods. As in both experiments, observations of the frequency of parasitization were not independent due to blocked design, therefore methods that can handle correlated data were used. The first experiment included only 18 vials (blocks); thus, the Generalized Linear Model with a quasibinomial setting (GLM) was used to correct for over-dispersion due to the correlated response within vials (Pekár and Brabec 2009). The second experiment included 30 vials (blocks); therefore, Generalized Estimating Equations with binomial error structure (GEE) were used (Hardin and Hilbe 2003). Within this model an exchangeable association structure was used.

Results

Host specificity from the collected spiders

Of 9,314 spider specimens collected in the field, the potential spider hosts, i.e. the web-building spiders, were represented by five families amounting to 85.7% of all collected spiders. These were Theridiidae (92%, N = 7,353), Araneidae (6.2%), Dictynidae (0.5%), Tetragnathidae (0.6%) and Linyphiidae (0.6%). The parasitoid, however, attacked only Theridiidae (100%, N = 105).

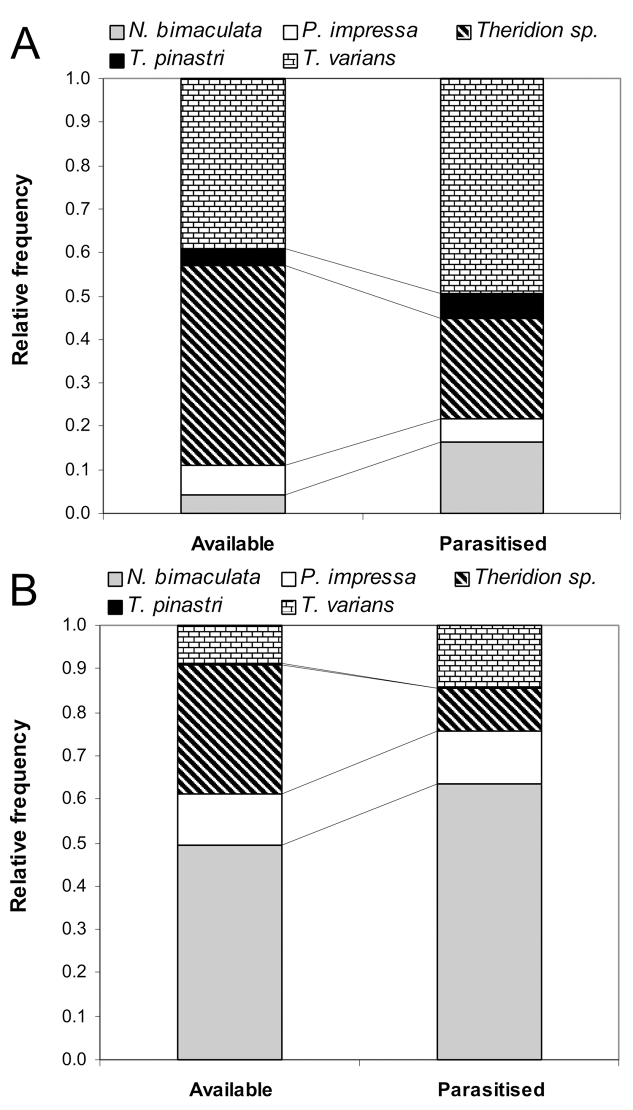


Figure 1. Comparison of relative frequency of available and parasitized "*Theridion*" spider hosts in the field in 2007 (A) and 2008 (B). High quality figures are available online.

Using all data from both the samples from the parasitism rate study (N = 105) and the rearing of wasps (N = 138), it was shown that parasitized theridiid spiders belonged mainly to *Theridion varians* (43%, N = 104), *Neottiura bimaculata* (24.4%), *Phylloneta impressa* (6.6%), *T. pinastri* (4.5%) and unidentified juveniles of the "*Theridion*" group (21.5%). The frequency of attacked theridiid hosts did not correspond to their

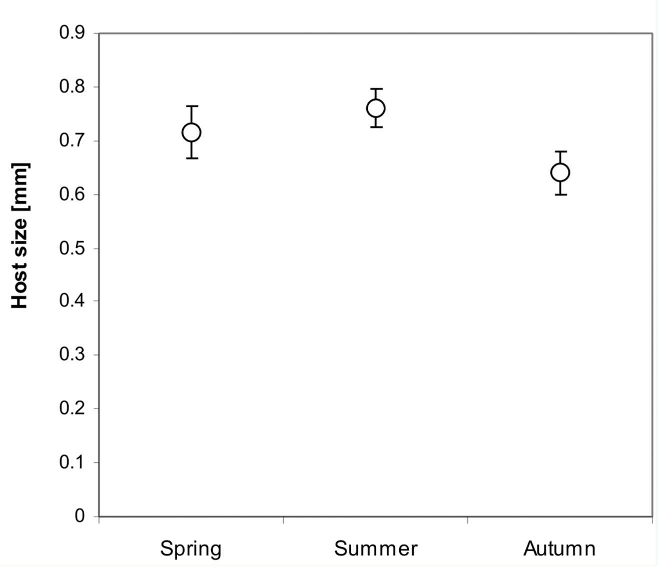


Figure 2. Comparison of the size (prosoma length) of available juvenile "*Theridion*" hosts during season. Points are means; uncertainty bars represent 95% confidence intervals. High quality figures are available online.

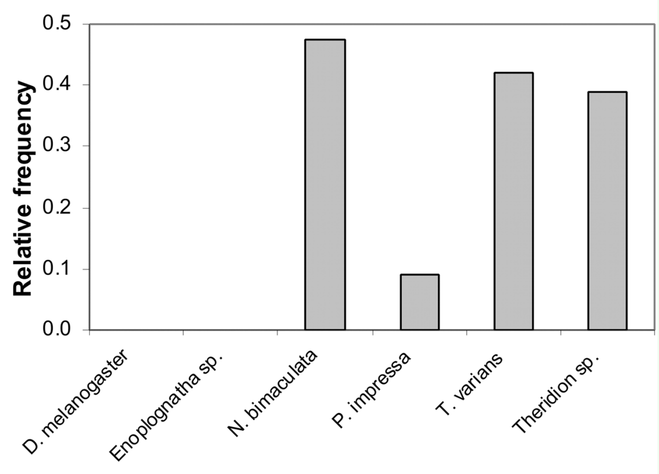


Figure 3. Relative frequencies of six taxa parasitized by wasps in laboratory experiments. High quality figures are available online.

availability both in 2007 (Goodness of fit, $\chi^2_4 = 1674$, $p < 0.0001$) and in 2008 (Goodness of fit, $\chi^2_4 = 1137$, $p < 0.0001$), (Figure 1).

The vast majority of the parasitized spiders (89.5%) were at the juvenile stage (from N = 105). Adults, which comprised 10.5% of all parasitized hosts, were parasitized merely in August 2007, and these were exclusively females of *T. varians*. The mean size of the prosoma of the parasitized host was 0.71 mm (N = 105, SE = 0.013) and changed somewhat during the season (Figure 2).

The natural sex ratio of subadult and adult "*Theridion*" spiders was biased in favor of females, as only 25% (from N = 204) of spiders were males. The sex ratio of parasitized "*Theridion*" spiders was significantly skewed in favor of females, as only 5% (from N = 75) of spiders were males (Proportion test, $\chi^2_1 = 12.2$, $p = 0.0005$).

Ovopositioning results in the laboratory

Under laboratory conditions, *Z. percontatoria* attacked exclusively spiders of the family Theridiidae and ignored linyphiid, araneid or dictynid spiders (GLM, $F_{3,32} = 19.1$, $p = 0.004$). When six taxa of the family Theridiidae (N = 30) were offered, parasitism differed significantly among them (GEE, $\chi^2_5 = 87.2$, $p < 0.0001$). *N. bimaculata*, *T. varians* and "*Theridion*" were parasitized with similarly high frequency, *P. impressa* was parasitized with a low frequency, and *D. melanogaster* and *Enoplognatha* sp. were not parasitized at all (Figure 3).

Host shifts

In 2007 the rate of parasitism by *Z. percontatoria* wasps on theridiid spiders was 1.74% (from N = 4,814). In 2008 the rate halved to 0.83% (from N = 2,539). Comparison of the host spectrum between the two seasons, 2007 and 2008, revealed a shift in host preference. While in 2007 the highest portion of parasitized hosts was represented by *T. varians*, in 2008 it was represented by *N. bimaculata* (Figures 1A, B). In 2007 (April – August) *T. varians* was the most abundant species with a mean abundance of 40.1 specimens (N = 7, SD = 23.5). This species was also the most parasitized (49.5%) spider host (Figure 1A). In 2008, the abundance of *T. varians* decreased to 12.9 (N = 8, SD = 13.8), and its parasitized portion decreased to 14.6%. *Neottiura bimaculata* had a lower abundance in 2007, specifically 4.3 (N = 7, SD = 4.4) spiders and a parasitized portion 16.3%. In 2008, it was the most abundant species with 20.9 specimens (N = 8, SD = 6.5) and a higher portion of parasitism (63.4%) (Figure 1B). The abundance of *P. impressa* was similar in both years, i.e. 7 specimens per day (N = 7, SD = 9.8) in 2007 and 5.4 specimens (N = 8, SD = 4.84) in 2008. Its portion of parasitism increased from 5.4 to 12.2% between years.

The body size of parasitized hosts changed significantly according to the seasons (ANOVA, $F_{2,102} = 10.4$, $P < 0.0001$): it was smallest in autumn and largest in summer (Figure 2). The frequency of occurrence of the three main hosts, *T. varians*, *N. bimaculata* and *P. impressa*, at a size preferred by the parasitoid, also changed with the seasons. In spring, all three host species were juvenile and thus occurred at high frequencies at the preferred body size (Figure 4A). In spring (of both years) the highest parasitized portion was in *T. varians* (43.5%, N = 10), followed by *N. bimaculata* (26%), the "*Theridion*" group (15.5%), *P. impressa* (13%) and *T. pinastri* (2%). In summer, spiders with a suitable body size were rare, as the majority of them had reached adulthood. The prosoma size of adult *T. varians*, however, falls within the preferred host range and adult females were thus accepted as host (Figure 4B). This was not the case in *P. impressa,* whose adult prosoma length was far larger than 1.1 mm. Neither the first instar spiderlings (prosoma size < 0.5 mm) of *T. varians* or *N. bimaculata* that hatched from egg sacs were accepted as hosts (field data). In summer, the highest parasitized portion was observed on *T. varians* (57%, N = 26), followed by indeterminable spiders at early instars (prosoma > 0.6 mm) of the "*Theridion*" group (26%), *N. bimaculata* (8.7%), and *P. impressa* (6%) and *T. pinastri* (2.3%). In autumn, spiderlings (prosoma size > 0.6 mm) of all three species were accepted by the wasp (Figure 4C). The parasitized

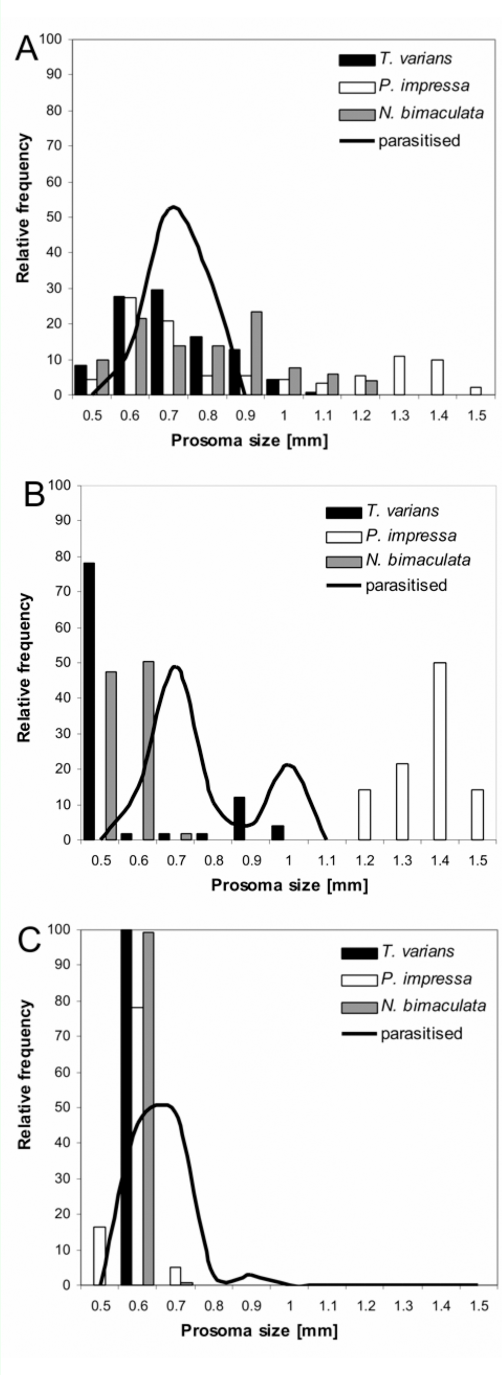


Figure 4. Relative frequencies of prosoma sizes of three major available host species (bars) and parasitized individuals (line) in spring (A), in summer (B) and in autumn (C). Bars were generated from spider relative abundance in the particular prosoma size (for each species separately). Lines were generated from all parasitized spiders in the particular prosoma size collected in the field in the particular season. High quality figures are available online.

portion was highest on *N. bimaculata* (37%, N = 13), followed by *T. varians* (28.6%), indeterminable spiders at early instars of "*Theridion*" group (25.7%), *P. impressa* (5.7%) and *T. pinastri* (3%).

Discussion

Both field and laboratory data show a strong preference of *Z. percontatoria* for web-building spiders of the family Theridiidae. It is known that host identification may be based on visual and chemical cues and the attacking behavior may be dependent on the invasion of the host web (Fellowes et al. 2005). The limitations of the performed laboratory experiments (i.e. with only virgin females enclosed in a small container and spiders deprived of their webs) could have influenced the searching and attacking behavior of the wasp. We assume that wasp females, which naturally mate in the field, may express different host acceptance behaviors. If female wasps allocate male offspring to smaller hosts and female offspring to larger hosts (e.g. Takasuka et al. 2009), then patterns of host preference for virgin females might differ from those of mated females.

Although laboratory experiments have limitations, they clearly support field data. The same spider species were accepted by wasps in the laboratory as in the field. Thus, as predicted by Gauld and Dubois (2006), previous records of hosts from other families, namely Agelenidae, Araneidae, Dictynidae and Tetragnathidae, cannot be supported. *Zatypota percontatoria* clearly attacks spiders building 3-dimensional webs with similar biology and web architecture. This study confirmed three hosts published previously (Aubert 1969; Fitton et al. 1987; Gauld and Dubois 2006) and revealed two new hosts: *N. bimaculata* and *T. pinastri*. The results of this study provide data only for a single population. To identify the entire host spectrum of *Z. percontatoria*, studies in other places of its distribution area should be undertaken. This species has a Holartic distribution, but most of the records come from Europe, where about 30 spider species of the family Theridiidae belong to the potential pool of hosts.

Host size/stage preference

Within the "*Theridion*" group we found *Z. percontatoria* to select only hosts of a certain size range. Populations of spider hosts are generally characterized by a high degree of body size variation (Henry et al. 2009). Large hosts provide more resources and, therefore, produce larger parasitoids, which is a proxy mechanism positively correlated with parasitoid fitness (Sequeira and Mackauer 1994; Takasuka et al. 2009). Parasitoids can distinguish between high- and low-quality hosts and preferentially oviposit on higher quality ones (Godfray 1994). Fincke et al. (1990) observed an intermediate-size preference for juvenile female spiders by *Hymenoepimecis* sp. in Panama. This is because the developmental stage of a host becomes far more important for larva survival than the actual size of the host at the moment of parasitization (Henry et al. 2006).

Zatypota percontatoria is a medium-sized parasitoid and it attacks medium-sized spiders corresponding to older juvenile and sub-adult spider instars. It does not accept hosts that are either too small (early instars) or too large (adult specimens). Tiny spiders are probably not accepted because the larva would be too great a burden for the spider, likely decreasing its foraging efficiency, which would negatively affect the fitness of the parasitoid. *Zatypota percontatoria* females significantly preferred female juvenile spiders as host. Generally, female spiders are larger, can be more abundant and have higher longevity (e.g. Schaefer 1987); therefore, they are a more profitable source of food for larvae than males. The selection of female sex in spider hosts is profitable due to divergent ontogenetic developmental trajectories. As females are larger than males at the adult stage, they have a higher foraging rate (Givens 1978), pass through more instars and attain a larger body size (Vollrath 1987). We can assume that adult males are avoided due to their short life expectancy, while large adult females are presumably avoided because of their stronger defenses against an ovipositing female wasp. It is, however, not known how wasps recognize the sex of immature spiders.

Host shifts

Zatypota percontatoria adaptively changed hosts according to their size, which was changing during the season. The two most preferred hosts, *T. varians* and *N. bimaculata*, attain an acceptable body size even at maturity, but the latter species was not parasitized at the adult stage. This is probably because adult female specimens of *N. bimaculata* are hidden while guarding their eggsac underneath the leaves of an herb layer (Korenko, unpublished). Thus adult females of *T. varians* were the only acceptable hosts of the preferred size in the middle of a season, presumably because they build webs at a higher vegetation stratum.

Species of the "*Theridion*" group are among the most abundant spiders in crowns of fruit trees (Pekár and Kocourek 2004). In central Europe, three species, *T. varians*, *N. bimaculata* and *P. impressa* are the most abundant 3-D web weavers, but they differ in phenology and body size during the season. *Zatypota percontatoria* responds adaptively to changes in abundance, body size and age of the three hosts. Spider hosts may become unavailable, for example due to reproduction when males die soon after mating, or due to large body size, which is not accepted by the wasp. This adaptive behavior is an effective means of maintaining a stable population dynamic throughout the season.

We also observed shifts in host preference between years. In the first year, 2007, *T. varians* was the dominant host, while in the second year, 2008, the parasitoid shifted to *N. bimaculata*. Parasitoids may influence parasitoid–host dynamics (Mangel and Roitberg 1992), which can further produce cascading effects on populations that shape the structure of the entire arthropod community (Petchey et al. 2008). Theridiid spiders are polyphagous predators, very abundant in agrobiocenoses and thus important for the natural control of pests (Bogya 1999). The rate of parasitism on theridiid hosts by *Z. percontatoria* was relatively low in both years. This complements observations on other related species: 1.4% in *Zatypota petronae* Gauld parasitized on *Theridion evexum* Keyserling (Barrantes et al. 2008) and 5% in *Zatypota albicoxa* (Walker) parasitized on *Parasteatoda tepidariorum* (C.L.Koch) (Tanaka 2007). In the study orchard, the frequency of parasitism did not exceed 6% throughout the season, except for the summer of 2007, when parasitism rate was 33% (sample in August). This high increase in parasitism rate was caused by a low abundance of acceptable theridiid hosts. Adult females of *T. varians* were frequently parasitized in the summer of the first year of the investigation (50% of all *T. varians* specimens). In general, such a situation could lead to a dramatic decline in the population of this species in the following year. The impact of parasitism on the host population seems to be most disastrous when adult females are attacked. The high rate of parasitism on adult female hosts was observed by Eberhard (2000) and Gonzaga and Sobezak (2007), but they did not investigate how parasitism rate influences host population dynamics.

This study confirms that *Z. percontatoria* is a stenophagous parasitoid attacking a group of ecologically similar and taxonomically related spider hosts. Such narrow host specialization is advantageous because it requires similar attack and handling behavior. Not being strictly monophagous allows shifts between hosts when their availability changes during and between seasons.

Acknowledgements

We would like to thank J. Viktorín of Fruit-growing Company Brno – Starý Lískovec for permitting us to collect spiders at their commercial orchard. We also thank two anonymous referees for their constructive comments. Stanislav Korenko was supported by grant no. 526/09/H025 of the Czech Science Foundation and project MSM 6046070901 of the Ministry of Education, Youth and Sports of the Czech Republic. Stano Pekár was supported by project MSM 0021622416 of the Ministry of Education, Youth and Sports of the Czech Republic.

References

Althoff DM. 2003. Does parasitoid attack strategy influence host specificity? A test with New World braconids. *Ecological Entomology* l28: 500-502.

Askew RR, Shaw MR. 1986. Parasitoid communities: their size, structure and development. In: J Waage J, Greathead D, Editors. *Insect parasitoids*. pp. 22-234. Academic Press.

Aubert JF. 1969. Les Ichneumonides Ouest-Paléarctiques et leurs Hôtes. 1. Pimplinae, Xoridinae, Acaenitinae. *Laboratoire d'Evolution des Etres Organises,*, Paris.

Barrantes G, Eberhard WG, Weng JL. 2008. Seasonal patterns of parasitism of the tropical spiders *Theridion evexum* (Araneae, Theridiidae) and *Allocyclosa bifurca* (Araneae, Araneidae) by the wasps *Zatypota petronae* and *Polysphincta gutfreundi* (Hymenoptera, Ichneumonidae). *Revista de Biologia Tropical* 56: 749-754.

Bogya S. 1999. Spiders (Araneae) as polyphagous natural enemies in orchards. Ph.D. Thesis, Landbouwuniversiteit Wageningen.

Eberhard WG. 2000. The natural history and behaviour of *Hymenoepimecis argyraphaga* (Hymenoptera: Ichneumonidae) a parasitoid of *Plesiometa argyra* (Araneae: Tetraganthidae). *Journal of Hymenoptera Research* 9: 220-240.

Fellowes MDE, van Alphen JJM, Jervis MA. 2005. Foraging behaviour. In: Jervis MA, Editors. *Insects as natural enemies: a practical perspective.* pp. 1-71. Kluwer Academic Publishers.

Fincke O, Higgins L, Rojas E. 1990. Parasitism of *Nephila clavipes* (Araneae, Tetragnathidae) by an ichneumonid (Hymenoptera, Polysphinctini) in Panama. *Journal of Arachnology* 18: 321-329.

Fitton MG, Shaw MR, Austin AD. 1987. The Hymenoptera associated with spiders in Europe. *Zoological Journal of the Linnean Society* 90: 65-93.

Fitton MG, Shaw MR, Gauld ID. 1988. Pimpline ichneumon-flies. *Handbooks for the Identification of British Insects* 7: 1-110.

Gauld ID, Dubois J. 2006. Phylogeny of the Polysphincta group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider ectoparasitoids. *Systematic Entomology* 31: 529-564.

Gentry GL, Dyer LA. 2002. On the conditional nature of neotropical caterpillar defenses against their enemies. *Ecology* 83: 3108-3119.

Gerling D, Roitberg BD, Mackauer M. 1990. Intraspecific defense of the pea aphid, *Acyrthosiphon pisum* – influence on oviposition success of the parasite *Aphelinus asychis* (Hymenoptera, Aphelinidae). *Journal of Insect Behavior* 3: 501-514.

Givens RP. 1978. Dimorphic foraging strategies of a salticid spider (*Phidippus audax*). *Ecology* 59**:** 309-321.

Godfray HCJ. 1994. *Parasitoids: Behavioral and evolutionary ecology*. Princeton University Press.

Godfray HCJ, Shimada M. 1999. Parasitoids as model organisms for ecologists. *Population Ecology* 41: 3-10.

Gonzaga MO, Sobezak JF. 2007. Parasitoid-induced mortality of *Araneaus omnicolor* (Araneae, Araneidae) by *Hymenoepimecis* sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. *Naturwissenschaften* 94: 223-227.

Hardin JW, Hilbe JM. 2003. *Generalized Estimating Equations*. Chapman and Hall/CRC.

Heimer S, Nentwig W. 1991. *Spinnen Mitteleuropas*. Paul Parey.

Henry LM, Ma BO, Roitberg BD. 2009. Size-mediated adaptive foraging: a host-selection strategy for insect parasitoids. *Oecologia* 161: 433-445.

Henry LM, Roitberg BD, Gillespie DR. 2006. Covariance of phenotypically plastic traits induces an adaptive shift in host selection behaviour. *Proceedings of the Royal Society B* 273: 2893-2899.

Mangel M, Roitberg B. 1992. Behavioral stabilization of parasite–host dynamics. *Theoretical Population Biology* 42: 308-320.

Nielsen E. 1923. Contributions to the life history of the pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*) (Hym. Ichneumon.). *Entomologiske Meddelelser* 14: 137-205.

Pekár S. 1999. Life-histories and the description of developmental stages of *Theridion bimaculatum*, *Theridion impressum* and *Theridion varians* (Araneae: Theridiidae*)*. *Acta Societatis Zoologicae Bohemicae* 63: 301-309.

Pekár S, Brabec M. 2009. *Modern Analysis of Biological Data*. 1. Generalised Linear Models in R. Scientia [in Czech].

Pekár S, Kocourek F. 2004. Spiders (Araneae) in the biological and integrated pest management of apple in the Czech Republic. *Journal of Applied Entomology* 128: 561-566.

Petchey OL, Beckerman AP, Riede JO, Warren PH. 2008. Size, foraging, and food web structure. *Proceedings of the National Academy of Science of the United States of America* 105: 4191-4196.

Platnick NI. 2009. The world spider catalogue, version 10.0. American Museum of Natural History. Available online, http://research.amnh.org/entomology/spiders/catalog/index.html.

Pyke GH, Pulliam HR, Charnov EL. 1977. Optimal foraging: a selective review of theories and tests. *Quarterly Review of Biology* 52: 137-154.

Roberts MJ. 1995. *Spiders of Britain and Northern Europe*. Harper Collins Publishers.

R Development Core Team 2009 R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Availble online, http://www.R-project.org.

Schaefer M. 1987. Life cycles and diapause. In: Nentwig W, Editor. *Ecophysiology of Spiders*. pp. 331-347. Springer.

Sequeira R, Mackauer M. 1994. Variation in selected life-history parameters of the parasitoid wasp, *Aphidius ervi* – influence of host developmental stage. *Entomologia Experimentalis et Applicata* 71: 15-22.

Shaw MR. 1994. Parasitoid host ranges. In: Hawkins BA, Sheehan W, Editors. *Parasitoid community ecology*. pp. 111-144. Oxford University Press.

Shaw MR, Aeschlimann JP. 1994. Host ranges of parasitoids (Hymenoptera: Braconidae and Ichneumonidae) reared from *Epermenia chaerophyllella* (Goeze) (Lepidoptera: Epermeniidae) in Britain, with description of a new species of *Triclistus* (Ichneumonidae). *Journal of Natural History* 28: 619–629.

Takasuka K, Matsumoto R, Ohbayashi N. 2009. Oviposition behavior of *Zatypota albicoxa* (Hymenoptera, Ichneumonidae), an ectoparasitoid of *Achaearanea tepidariorum* (Araneae, Theridiidae). *Entomological Science* 12: 232–237.

Tanaka K. 2007. Seasonal life cycle of *Zatypota albicoxa* (Hymenoptera: Ichneumonidae), an ectoparasitoid of *Achaearanea tepidariorum* (Araneae: Theridiidae), in north Japan. *Entomological Science* 10: 421–423.

Vollrath F. 1987. Growth, foraging and reproductive success. In: Nentwig W, Editor. *Ecophysiology of Spiders*. pp. 357-370. Springer-Verlag.

Yu D, Horstmann K. 1997. Catalogue of world Ichneumonidae (Hymenoptera). *Memoirs of the American Entomological Institute* 58: 1–1558.

Zwakhals K. 2006. The European species of the genera *Zatypota* and *Sinarachna* (Hymenoptera: Ichneumonidae, Pimplinae, Polysphinctini). *Entomologische Berichten* 66: 34–37.

Supplement 3

Kehlmaier Ch, Michalko R, Korenko S. 2012. *Ogcodes fumatus* (Diptera: Acroceridae) reared from *Philodromus cespitum* (Araneae: Philodromidae), and first evidence of *Wolbachia* in Acroceridae. Annales Zoologici 62(2), 281-286.

Citation report:
Web of Science^{TM}: 6
Scopus/Google Scholar: 8

ISI WOS:
Annales Zoologici
IF$_{(2012)}$ = IF = 0,660
Quartile $_{(2012)}$: Zoology – Q4

ANNALES ZOOLOGICI (Warszawa), 2012, 62(2): 281-286

OGCODES FUMATUS (DIPTERA: ACROCERIDAE) REARED FROM *PHILODROMUS CESPITUM* (ARANEAE: PHILODROMIDAE), AND FIRST EVIDENCE OF *WOLBACHIA* ALPHAPROTEOBACTERIA IN ACROCERIDAE

Christian Kehlmaier[1], Radek Michalko[2] and
Stanislav Korenko[3]

[1]*c/o Senckenberg Natural History Collections Dresden, Museum of Zoology,*
Königsbrücker Landstrasse 159, 01109 Dresden, Germany;
e-mail: kehlmaier@web.de
[2]*Department of Botany and Zoology, Faculty of Sciences, Masaryk University,*
Kotlářská 2, 611 37 Brno, Czech Republic
[3]*Department of Agroecology and Biometeorology, Faculty of Agrobiology,*
Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129,
165 21 Prague 6, Suchdol, Czech Republic

Abstract.— A first rearing record of the small-headed fly *Ogcodes fumatus* (Erichson, 1846) from the running crab spider *Philodromus cespitum* (Walckenaer, 1802) is reported. Aberrant web spinning activity of the host spider is documented. Molecular work comprises DNA barcoding (COI) for the host and parasitoid as well as PCR assays (16S rRNA, wsp) detecting the presence of at least two strains of *Wolbachia* bacteria in *O. fumatus*. A Neighbour-joining search of the 16S rRNA clusters these strains within supergroup A of *Wolbachia*.

⁂

Key words.— Acroceridae, *Ogcodes*, Philodromidae, *Philodromus*, *Wolbachia*, 16S rRNA.

INTRODUCTION

This paper presents a novel host association between *Ogcodes* Latreille, 1796 and *Philodromus* Walckenaer, 1826, plus data on aberrant spider spinning activity, DNA-barcodes, and the occurrence of *Wolbachia* Hertig, 1936 bacteria in Acroceridae.

Most known Acroceridae larvae develop as endoparasitoids in the opisthosoma of true spiders (Araneae). Known exceptions comprise the Neotropical *Sphaerops appendiculata* Philippi, 1865, developing in an ectoparasitic mode on *Ariadna maxima* (Nicolet, 1849) (Schlinger 1987), and recent findings by Sferra (1986) and Kerr and Winterton (2008) suggesting a relationship between Acroceridae and Acari. A synopsis of the family's life history is given by Schlinger (2003). So far, host data for more than 60 Acroceridae taxa (about 10% of the world fauna), recorded from 23 spider families, have been gathered (Schlinger 1987, 2003). For *Ogcodes* about 40 host-parasite relationships have been documented which mostly belong to the Lycosidae (de Jong *et al.* 2000, Eason *et al.* 1967, Larrivée and Borkent 2009).

Wolbachia is a maternally inherited, endosymbiontic intracellular alphaproteobacteria widespread among arthropods and nematodes. Eleven supergroups (A–K) have been proposed based on molecular evidence — note that the status of supergroup G is a matter of dispute as it has been considered a natural recombinant between supergroups A and B recently —

PL ISSN 0003-4541 © Fundacja *Natura optima dux*
doi: 10.3161/000345412X652819

of which A, B and F occur in Diptera (Ros *et al.* 2009). Recent estimates suggest that more than 20% of arthropod species (Ros *et al.* 2009) and more than 65% of insect species are infected with *Wolbachia* (Hilgenboecker *et al.* 2008). The endosymbiont is well known for manipulating the reproduction of its hosts leading to feminization, parthenogenesis, male killing and sperm-egg incompatibility (Werren *et al.* 2008). Next to vertical transmission through infected host eggs (from mother to offspring), evidence of horizontal transfer between predator and prey (e.g. Ahmed *et al.* 2010) as well as host and parasitoid (e.g. Cordaux *et al.* 2001) is known. Lateral gene transfer into the host nuclear genome has also been documented on several occasions recently (e.g. Dunning Hotopp *et al.* 2007, Nikoh *et al.* 2008). Inserted fragments range from <500 bp to >1 mb and remain partly functional (Dunning Hotopp *et al.* 2007).

MATERIALS AND METHODS

Living *Philodromus aureolus* (Clerck, 1757) (n=40), *P. cespitum* (Walckenaer, 1802) (n=40) and *P. albidus* Kulczyński, 1911 (n=35) were collected with a beating-net in the canopy and understorey of an abandoned fruit orchard (old peach and plum trees, *Rosa* sp. and *Crataegus* sp.) and in the surrounding young forest (*Tilia cordata* Miller, *Acer platanoides* L., *A. pseudoplatanus* L. and *Crataegus* sp.), both within the city boundary of Brno, Czech Republic (49°09'18"N, 16°33'40"E). Each spider was placed in a glass vial (diameter 15 mm, height 55 mm), kept at room temperature at 22 ± 3.5°C under a natural light/dark regime and fed with *Drosophila melanogaster* (Meigen, 1830). The vials were moistened with drops of water at three-day intervals. Spiders were reared to maturity to guarantee an unambiguous identification using Heimer and Nentwig (1991). The identification of the acrocerid parasitoid is based on the works of Chvála (1980a, 1980b).

Genomic DNA was extracted using the innuPREP DNA Mini Kit of Analytik Jena AG (Jena, Germany). The amplified genetic loci are listed in table 1 including primer pairs and PCR-conditions. Each PCR was performed with 1–5 μl of DNA extraction in a 20 μl volume (1 μl of each primer at 10 pmol, 1 μl of dNTP-mix at 10 pmol, and 1 unit of Taq polymerase (Bioron DFS Taq, Ludwigshafen, Germany), 2 μl PCR buffer 10× incl. $MgCl_2$, ultra pure H_2O). Cleaning of PCR and sequencing-PCR products were performed by salt-ethanol precipitation using 4M NH_4Ac (PCR) or 3M NaAc (PCR-sequencing). For sequencing-PCR the same forward and reverse primers were used in a total reaction volume of 10 μl (2 μl sequencing buffer, 1 μl premix, 1 μl primer at 5pmol concentration, 0.5–6 μl of DNA template, ultra pure H_2O) with 25–30 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min using the ABI PRISM Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing was done on an ABI 3130XL Genetic Analyser. PCR products were visualisation of a 1% agarose gel. If necessary, excision of distinct DNA bands was performed from a 2% agarose gel and purified using the peqGold Gel Extraction Kit of peqlab Biotechnologie GmbH (Erlangen, Germany).

Table 1. Gene regions amplified for the different organisms, primer pairs used in PCR and sequencing, including their literature reference, and PCR-conditions.

Gene	Primer pair	PCR
cox1 amplified for *Ogcodes*, *Philodromus* and *Wolbachia*	Forward: LCO1490 GGTCAACAAATCATAAAGATATTGG Reverse: HCO2198 TAAACTTCAGGGTGACCAAAAAATCA (Folmer *et al*. 1994)	Initial 94°C for 4 min, 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 45 s, final elongation of 72°C for 10 min
16S *Wolbachia*	Forward: 16Sf TTGTAGCCTGCTATGGTATAACT Reverse: 16Sr GAATAGGTATGATTTTCATGT (O'Neill *et al*. 1992)	Initial 95°C for 5 min, 35 cycles of 95°C for 1 min, 52°C for 1 min, 72°C for 1 min, final elongation of 72°C for 10 min
wsp *Wolbachia*	Forward : *wsp*-81F TGGTCCAATAAGTGATGAAGAAAC Reverse: *wsp*-691R AAAAATTAAACGCTACTCCA (Zhou *et al*. 1998)	Initial 95°C for 5 min, 35 cycles of 95°C for 1 min, 55°C for 1min, 72°C for 1 min, final elongation of 72°C for 10 min

DNA barcoding of the 5'-half of the COI gene was conducted to generate molecular evidence that acts as a surrogate voucher, ensuring that future nomenclatural changes to host or parasitoid taxonomy will be easy to track. The resulting spider barcodes were also used to corroborate the morphological identifications by comparing them to a set of reference barcodes obtained from GenBank and BOLD (www.barcodinglife.org), partly published in Barrett and Hebert (2005) and Muster *et al.* (2007). A Neighbour-Joining (NJ) tree of uncorrected pairwise genetic distance was computed using PAUP* 4.0b10 (Swofford 2001). For testing for a possible *Wolbachia* infection, specific primer pairs targeting the 16S rRNA and the *wsp* gene (*Wolbachia* surface protein) were used. DNA of three *Wolbachia* infected specimens — two *Altica* spp. (Coleoptera: Chrysomelidae), one *Nasonia* sp. (Hymenoptera: Pteromalidae) — acted as positive control. Determination of supergroup affiliation of *Wolbachia* was conducted by a NJ search based on the 16S rRNA data, using MEGA5 (Tamura *et al.* 2011). The alignment for this reconstruction was adopted from Ros *et al.* (2009, see their supplementary table S1), representing all currently recognised supergroups. Alignment length was cropped to 804 bp (originally 1,255 bp) due to the restricted length of the obtained *Wolbachia* sequences. Software settings: Kimura 2-parameter model, all codon positions and substitutions included partial deletion of missing data (site coverage cutoff 95%). Branch support was assessed with 1000 bootstrap replicates.

GenBank sequence accession numbers are listed in table 2.

RESULTS AND DISCUSSION

A single female *Ogcodes (Ogcodes) fumatus* (Erichson, 1846) was reared from a subadult female *Philodromus cespitum* (Walckenaer, 1802), representing a new host and family record for *O. fumatus*. Previous rearing records for *O. fumatus* comprise *Zygiella x-notata* (Clerck, 1757) (Araneidae), *Oxyopes lineatus* (Latreille, 1806) (Oxyopidae), *Misumena vatia* (Clerck, 1757) (Thomisidae), *Thomisus onustus* (Walckenaer, 1806) (Thomisidae) and *Pardosa lugubris* s. str. (Walckenaer, 1802) (Lycosidae) (de Jong *et al.* 2000). Both host and parasitoid have extensive distributions, *P. cespitum* being widely Holarctic and frequently collected (Heimer and Nentwig 1991), and *O. fumatus* widely Palaearctic (Czech Republic, Germany, Kazakhstan, Mongolia, The Netherlands, Poland, Russia, Ukraine) but rarely encountered (Nartshuk 1988 & 2004).

Collection and rearing details:

Parasitized *P. cespitum*: collected 7.VI.2010, Czech Republic, Brno, Starý Lískovec, old fruit orchard, 49°09'18"N, 16°33'40"E, leg. R. Michalko. DNA-voucher CK515.

Ogcodes fumatus: Pupation of larva: no data. Eclosion of adult *O. fumatus*: 18.VI.2010. DNA-voucher CK459.

Philodromus cespitum: 1.VI.2011, same locality, leg. R. Michalko, DNA-voucher CK516.

Philodromus aureolus: 11.V.2011, same locality, leg. S. Korenko, DNA-voucher CK517.

The host spider is kept as dry material in its glass vial. The other two spiders used for barcoding and the parasitoid are ethanol preserved. All specimens are currently in the collection of the senior author but will eventually be deposited in a public collection to ensure their long term accessibility.

It has been repeatedly highlighted in the literature that parasitized spiders show an abnormal web-building behaviour previous to the emergence of their parasitoids, and that the larvae attach themselves to the web when leaving their hosts, a process that can last 90 to 150 minutes in the case of *O. pallidipennis* Loew,

Table 2. GenBank accession numbers.

	cox1	*CtaG* putative pseudogene	16S rRNA
Ogcodes fumatus (Erichson, 1846)	HE575183	HE576770	—
Philodromus cespitum (Walckenaer, 1802) host specimen	HE575184	—	—
Philodromus cespitum (Walckenaer, 1802) additional specimen	HE575185	—	—
Philodromus aureolus (Clerck, 1757) additional specimen	HE575186	—	—
Wolbachia Hertig, 1936 long sequence	—	—	HE575187
Wolbachia Hertig, 1936 short sequence	—	—	HE575188

1865 (Eason *et al.* 1967). This could also be observed partly in the case of *P. cespitum*, where the spider produced a more or less horizontally directed web near the bottom of the glass vial before the final instar larva emerged (Fig. 1) — none of the other 114 unparasitized spiders produced any silk aggregation as philodromids are non-web building, using their silk in certain occasions like egg sac production for example (Uetz *et al.* 1999). The actual emergence of the larva from its host could not be observed, but the pupa was found hanging from below the main silk aggregation.

Considering all philodromid spiders (n=115), the observed rate of parasitism is 0.87%. If only *P. cespitum* is regarded (n=40), the rate of parasitism rises to 2.5%. A similar rate of parasitism (0.88%) was observed by Larrivée and Borkent (2009) who collected app. 455 foliage spiders from tree canopies and understorey saplings.

The DNA barcodes proved valuable in corroborating the identity of the host spider and the use of this technique is strongly advocated in any future work focusing on host parasitoid relationships. Whereas for *Ogcodes* only two DNA barcodes are currently available in public databases, the computed NJ-tree for *Philodromus* clusters the acrocerid host spider with other *P. cespitum*, showing a maximum intraspecific genetic distance of 3.8% compared to North American specimens (Fig. 2).

Figure 1. Lower part of the rearing vial showing the dead host spider *Philodromus cespitum*, its abnormal web and the translucent pupal skin of the parasitoid *Ogcodes fumatus*.

All specimens involved in this study were tested for *Wolbachia* using 16S rRNA and *wsp*, but only *O. fumatus* proved to harbour the endosymbiont. The obtained forward and reverse sequences of the 16S rRNA were of good quality but proved to be a mixture of at least two closely allied strains of *Wolbachia*, differing at five positions (mixed-bases) plus by a single nucleotide deletion/insertion. We abstained from cloning the PCR product as the obtained sequences were sufficient for determining the supergroup affiliation. The Neighbour-joining analysis clustered these sequences within supergroup A of *Wolbachia* (Fig. 3). The *wsp* sequences could not be analysed as the trace files show two overlapping sequences of equal length that considerably diverge after 60 bp. However, this first unambiguous fragment was positively attributed to *wsp* when entered into BLASTN 2.2.25 (Zhang *et al.* 2000).

The infestation of *O. fumatus* was first detected when amplifying the COI barcode. Several PCRs resulted in two distinct bands when visualised. Eventually, both amplicons were excised and sequenced, resulting in the *O. fumatus* barcode (658 bp) and a somewhat shorter fragment (readable sequence length 458 bp). When searching the gene database using the heuristic

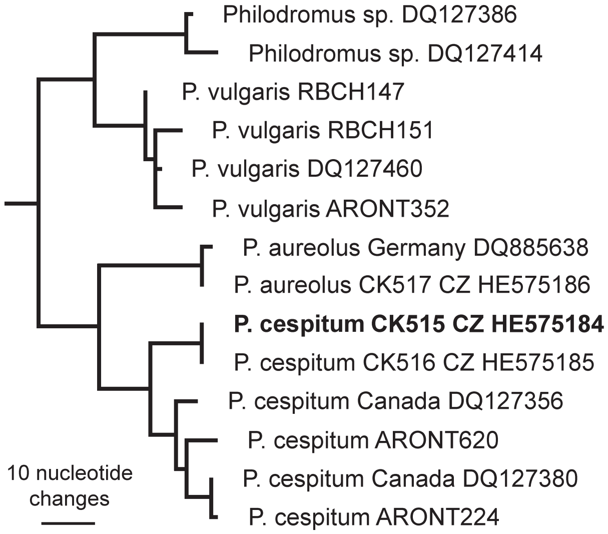


Figure 2. Excerpt of rooted Neighbour-joining phenogram of genetic uncorrected pairwise distance for the COI dataset comprising 42 specimens of *Philodromus* spp. – the tree is rooted on *Tibellus oblongus* (Walckenaer, 1802). The parasitized *P. cespitum* is in bold lettering. The maximum intraspecific genetic distance for *P. cespitum* is 3.8%. The scale bar represents ten nucleotide changes.

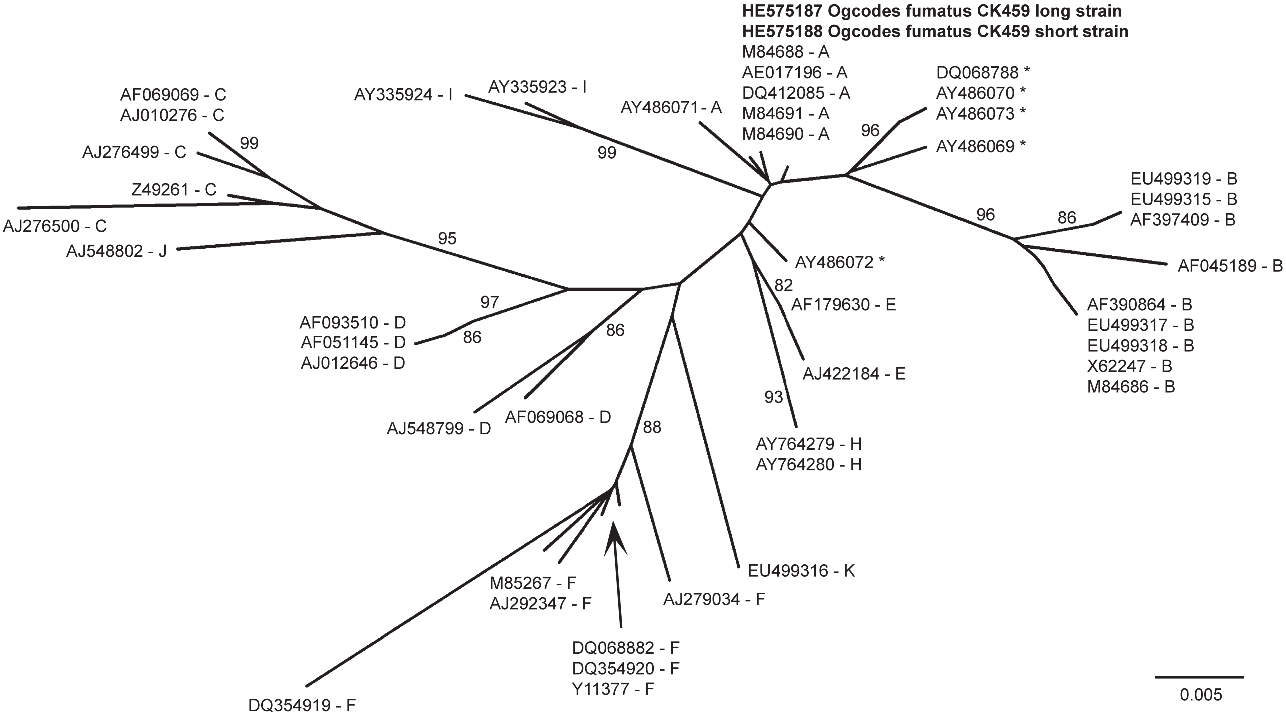


Figure 3. Unrooted Neighbour-joining network of *Wolbachia* 16S rRNA sequences (804 bp) for 47 strains (characterized by their GenBank accession numbers) calculated with MEGA5. Software settings: Kimura 2-parameter model, all codon positions and substitutions included partial deletion of missing data (site coverage cutoff 95%). Bootstrap support is based on 1000 replicates. Values below 80% are not depicted. Supergroup affiliation is indicated in capital letters after GenBank numbers. Specimens characterized by an asterisk exhibit an unclear supergroup affiliation (supergroup G or natural A/B recombinants). The *Wolbachia* strains of *Ogcodes fumatus* (bold lettering) fall within supergroup A. The scale bar indicates 0.005 substitutions per amino acid position.

BLAST algorithm, the query was specified as *'cytochrome c oxidase assembly protein CtaG, putative'* of AE017196 complete genome of *Wolbachia* (strain *wMel*) from *D. melanogaster* with a 97% sequence identity over 84% of the read length (388 bp). The first appox. 100 bp of the query sequence can be assigned to various regions of the mentioned *Wolbachia* genome with a sequence identity above 80% and differs strongly from the flanking gene fragment preceding *CtaG*. At present, it cannot be specified whether this sequence is inherent to the *Wolbachia* endosymbiont. Due to its divergence from the known *Wolbachia* genome structure and the presence of internal stop codons, it more likely represents a pseudogene remnant of a lateral gene transfer of *Wolbachia* into its host (Nikoh *et al.* 2008), and is thus deposited at GenBank under this definition.

ACKNOWLEDGEMENTS

We would like to thank Dr. Regina Jäckel (Hamburg) for providing DNA of *Wolbachia* infected insects used as positive control in this study. SK was supported by the Ministry of Education and Youth of the Czech Republic by grant MSM 6046070901.

REFERENCES

Ahmed, M. Z., Ren, S.-X., Mandour, N. S., Greeff, J. M. and B.-L. Qiu. 2010. Prevalence of *Wolbachia* Supergroups A and B in *Bemisia tabaci* (Hemiptera: Aleyrodidae) and some of its natural enemies. Journal of Economic Entomology, 103: 1848–1859.

Barrett, R. D. H. and P. D. N. Hebert. 2005. Identifying spiders through DNA barcodes. Canadian Journal of Zoology, 83: 481–491.

Chvála, M. 1980a. Acroceridae (Diptera) of Czechoslavakia. Acta Universitatis Carolinae, Biologica, 1977: 253–267.

Chvála, M. 1980b. Two new European *Ogcodes* species (Diptera, Acroceridae). Acta entomologica bohemoslovaca, 77: 131–137.

Cordaux, R., Michel-Salzat, A. and D. Bouchon. 2001. *Wolbachia* infection in crustaceans: novel hosts and potential routes for horizontal transmission. Journal of Evolutionary Biology, 14, 237–243.

de Jong, H., Noordam, A. P. and T. Zeegers. 2000. The Acroceridae (Diptera) of The Netherlands. Entomologische Berichten, 60: 171–179.

Dunning Hotopp, J. C., Clark, M. E., Oliveira, D. C. S. G., Foster, J. M., Fischer, P., Muñoz Torres, M. C., Giebel, J. D., Kumar, N., Ishmael, N., Wang, S., Ingram, J., Nene, R. V., Shepard, J., Tomkins, J., Richards, S., Spiro, D. J., Ghedin, E., Slatko, B. E., Tettelin, H. and J. H. Werren. 2007. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Science, 317: 1753–1756.

Eason, F. R., Peck, W. B. and W. H. Whitcomb. 1967. Notes on spider parasites, including a reference list. Journal of the Kansas Entomological Society, 40: 422–434.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3: 294–299.

Heimer, S. and W. Nentwig. 1991. Spinnen Mitteleuropas. Paul Parey, Berlin & Hamburg. 543 pp.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A. and J. H. Werren. 2008. How many species are infected with *Wolbachia*? — a statistical analysis of current data. FEMS Microbiology Letters, 281: 215–220.

Kerr, P. H. and S. L. Winterton. 2008. Do parasitic flies attack mites? Evidence in Baltic amber. Biological Journal of the Linnean Society, 93: 9–13.

Larrivée, M. and C. J. Borkent. 2009. New spider host associations for three acrocerid fly species (Diptera, Acroceridae). Journal of Arachnology, 37: 241–242.

Muster, C., Bosmans, R. and K. Thaler. 2007. The *Philodromus pulchellus*-group in the Mediterranean: taxonomic revision, phylogenetic analysis and biogeography (Araneae: Philodromidae). Invertebrate Systematics, 21: 39–72.

Nartshuk, E. P. 1988. Acroceridae, pp. 186–196. *In*: Á. Soós and L. Papp (eds). Catalogue of Palaearctic Diptera, Volume 5, Akadémiai Kiadó/Elsevier, Budapest & Amsterdam.

Nartshuk, E. P. 2004. Fauna Europaea: Acroceridae. *In*: T. Pape (ed.). Fauna Europaea: Diptera: Brachycera, Version 1.0. Available online: http://www.faunaeur.org [last updated: 7.ix.2004, accessed: 3.vii.2011].

Nikoh, N., Tanaka, K., Shibata, F., Kondo, N., Hizume, M., Shimada, M. and T. Fukatsu. 2008. *Wolbachia* genome integrated in an insect chromosome: Evolution and fate of laterally transferred endosymbiont genes. Genome Research, 18: 272–280.

O'Neill, S. L., Giordano, R., Colbert, A. M., Karr, T. L. and H. M. Robertson. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proceedings of the National Academy of Sciences of the United States of America, 89: 2699–2702.

Ros, V. I. D., Fleming, V. M., Feil, E. J. and J. A. J. Breeuwer. 2009. How Diverse Is the Genus *Wolbachia*? Multiple-Gene Sequencing Reveals a Putatively New *Wolbachia* Supergroup Recovered from Spider Mites (Acari: Tetranychidae). Applied and Environmental Microbiology, 75: 1036–1043.

Schlinger, E. I. 1987. The biology of Acroceridae (Diptera): true endoparasitoids of spiders, pp. 319–327. *In*: W. Nentwig (ed.). Ecophysiology of Spiders, Springer-Verlag, Berlin.

Schlinger, E. I. 2003. Acroceridae, spider-fly endoparasitoids, pp. 734–740. In: M. Goodman and J. P. Benstead (eds). The Natural History of Madagascar, University of Chicago Press, Chicago.

Sferra, N. J. 1986. The first record of *Pterodontia flavipes* Gray (Diptera: Acroceridae) larvae in the mites *Podothrombium* (Acari: Trombidiidae) and *Abrolophus* (Acari: Erythraeidae). Entomological News, 97: 121–123.

Swofford, D. L. 2001. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods), Version 4.0b10. [Computer software and manual]. Sinauer Associates.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and S. Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution. doi: 10.1093/molbev/msr121.

Uetz, G. W., Halaj, J. and A. B. Cady. 1999. Guild structure of spiders in major crops. Journal of Arachnology, 27: 270–280.

Werren, J. H., Baldo, L. and M. E. Clark. 2008. Wolbachia: master manipulators of invertebrate biology. Nature Reviews Microbiology, 6: 741–751.

Zhang, Z., Schwartz, S., Wagner, L. and W. Miller. 2000. A greedy algorithm for aligning DNA sequences. Journal of Computational Biology, 7: 203–214.

Zhou, W., Rousset, F. and S. O'Neil. 1998. Phylogeny and PCR-based classification of Wolbachia strains using wsp gene sequences. Proceedings of the Royal Society of London. Series B, Biological sciences, 265(1395): 509–515.

Received: August 26, 2011
Accepted: December 13, 2011

Supplement 4

Korenko S, Schmidt S, Schwarz M, Gibson GAP, Pekár S. 2013. Hymenopteran parasitoids of the ant-eating spider *Zodarion styliferum* (Simon) (Araneae: Zodariidae). Zookeys 262, 1-15.

Citation report:
Web of Science^{TM}: 2
Scopus/Google Scholar: 4

ISI WOS:
Zookeys
IF$_{(2013)}$ = IF = 0,917
Quartile $_{(2013)}$: Zoology – Q3

ZooKeys 262: 1–15 (2013)
doi: 10.3897/zookeys.262.3857
www.zookeys.org

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Hymenopteran parasitoids of the ant-eating spider *Zodarion styliferum* (Simon) (Araneae, Zodariidae)

Stanislav Korenko[1], Stefan Schmidt[2], Martin Schwarz[3],
Gary A.P. Gibson[4], Stano Pekár[5]

1 *Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic* **2** *Zoologische Staatssammlung, Münchhausenstr. 21, 81247 Munich, Germany* **3** *Eben 21, 4202 Kirchschlag, Austria* **4** *Agriculture and Agri-Food Canada, Canadian National Collection of Insects, K. W. Neatby Bldg., 960 Carling Avenue, Ottawa, Ontario, Canada, K1A 0C6* **5** *Department of Botany and Zoology, Faculty of Sciences, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*

Corresponding author: *Stanislav Korenko* (korenko.stanislav@yahoo.com)

Academic editor: *Gavin Broad* | Received 15 August 2012 | Accepted 27 December 2012 | Published 1 February 2013

Citation: Korenko S, Schmidt S, Schwarz M, Gibson GAP, Pekár S (2013) Hymenopteran parasitoids of the ant-eating spider *Zodarion styliferum* (Simon) (Araneae, Zodariidae). ZooKeys 262: 1–15. doi: 10.3897/zookeys.262.3857

Abstract

Calymmochilus dispar Bouček & Andriescu (Hymenoptera, Eupelmidae) and *Gelis apterus* (Pontoppidan) (Hymenoptera, Ichneumonidae) are newly recorded as parasitoids of the ant-eating spider *Zodarion styliferum* (Simon) (Araneae, Zodariidae). The larvae of both parasitoid species fed on juvenile spiders. The final instar larva and pupa of *C. dispar* and the male of *G. apterus* are described for the first time. Both species represent new distribution records for Portugal. The biology and host associations of the parasitoids are discussed.

Keywords

Ectoparasitoid, host, *Calymmochilus*, *Gelis*, larva, pupa, male description

Introduction

Several groups of Hymenoptera develop on spider hosts, their larvae either feeding on the spider or on its eggs (Fitton et al. 1987). Species known to use spiders as hosts include those of Ichneumonidae (Ichneumonoidea), Eulophidae, Eupelmidae, Eurytomidae and Pteromalidae (Chalcidoidea), Diapriidae (Diaprioidea), Scelioninae (Platygastroidea, Platygastridae), Pompilidae (Vespoidea), and Sphecidae (Sphecoidea) (Aubert 1969; Fitton et al. 1987; Noyes 2012). The parasitoids attack a number of spiders ranging from ground dwelling and fast moving hunters like wolf spiders of the family Lycosidae (Kessler and Fokkinga 1973) to web spiders such as orb-web weavers of the family Araneidae (Gonzaga and Sobczak 2011) that stay on webs during most of their life. Some parasitoids parasitize a wide range of spider species whereas others are narrow specialists of a single species (Fitton et al. 1987). The parasitoids and predators include solitary species or those that develop in small broods feeding in cocooned spider egg masses to endoparasitoids that develop individually within eggs, and from external koinobiont parasitoids of mobile spiders to idiobionts that paralyse one (Pompilidae) or more (Sphecidae) spiders as prey (Austin 1985; Eberhard 1970; Gauld and Dubois 2006).

Zodarion Walckenaer is the most species-rich genus of ant-eating spiders in the family Zodariidae Pickard-Cambridge (Araneae) (Platnick 2012). They are restricted almost exclusively to the Palearctic region with at least 35 species reported for the Iberian Peninsula (Platnick 2012). Available data on their biology show that all species are compulsory ant eaters (Wiehle 1928; Cushing and Santangelo 2002; Pekár 2004; Pekár et al. 2005a,b, 2011). Some *Zodarion* spiders (Fig. 1A) are Batesian mimics with various colour patterns and morphological resemblance to ants. They are crepuscular and often nocturnal wanderers. During the day they remain hidden in igloo-shaped retreats (Fig. 1B) that are attached to the underside of rocks or dead wood. The igloos provide protection against unfavourable environmental conditions and enemies such as ants.

The only previous record of a predator or a parasitoid of a *Zodarion* spider is that of a larva tentatively identified as a parasitoid feeding on *Z. cyrenaicum* Denis, 1935 in Israel (Pekár et al. 2005a). Here we newly present information about two parasitoids associated with *Z. styliferum* (Simon, 1870) in Portugal, *Calymmochilus dispar* Bouček & Andriescu (Chalcidoidea, Eupelmidae) and *Gelis apterus* (Pontoppidan) (Ichneumonoidea, Ichneumonidae). Notes on their biology are provided and the final instar larva and pupa of *C. dispar* and the male of *G. apterus* are described for the first time.

Methods

During 2008–2011 we conducted 31 field excursions in 18 localities in Central and Southern Portugal, in early spring between the last week of March and the first week of April in 2008–2010, and in late spring in the last week of May in 2011. Different habi-

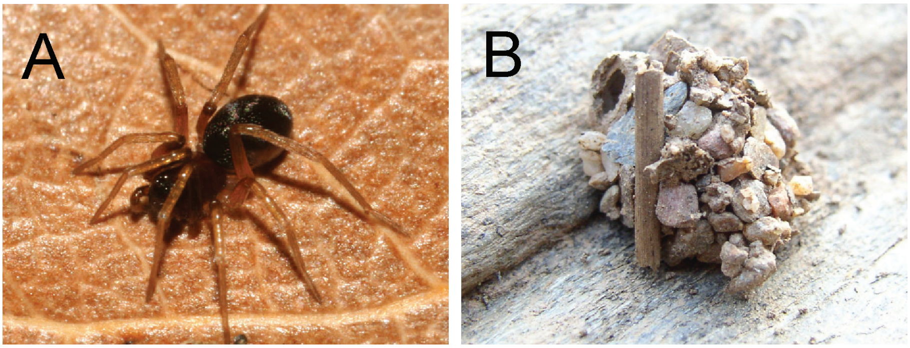


Figure 1. Spider host, juvenile *Zodarion styliferum* (**A**), igloo-shaped retreat (**B**).

tats were investigated, including arid meadows with sparse vegetation, meadows surrounding a castle, sand beaches with sparse vegetation, slopes of river banks, olive groves, and wooded habitats. Larvae and pupae of hymenopteran parasitoids were collected from the igloos of *Z. styliferum* in seven localities (Fig. 2). Entomological pincers were used to open the spider igloos and parasitized spiders were transferred to plastic containers using an aspirator. The whole igloo was collected if there was a pupa inside an igloo.

The diversity of *Zodarion* spiders, their abundance, and the rate of the parasitism was recorded for each of the localities where *G. apterus* and *C. dispar* parasitized spiders. The rate of parasitism per excursion was calculated as the number of parasitized spiders compared to all observed spiders during the excursion. Parasitoid larvae and pupae were reared until emergence of the adult wasps (1 of the 11 specimens was lost). Duration of the pupal stage, size of the pupa, and sex of the adult wasp were recorded. Emerged wasps were preserved in pure ethanol and identified using Bouček and Andriescu (1967), Gibson (1995), and Schwarz (1995, 1998 and 2002). Spiders were identified using Pekár and Cardoso (2005). The juvenile *Zodarion* hosts were identified to species-level based on knowledge of the species diversity in the investigated localities and using distinct differences in body proportions and coloration among occurring species. Wasp specimens are deposited in the private collection of M. Schwarz, Eben, Austria, the Canadian National Collection of Insects (CNCI), Ottawa, Canada, and the collection of Zoologische Staatssammlung, Münchhausenstr (ZSM), Munich, Germany. Morphological terms largely follow Gibson (1997) for Chalcidoidea and Schwarz (1998, 2002) for Ichneumonidae. The mature female and male larva and pupa of *C. dispar* were described based on photographs taken once they were detached from the spider at two day intervals and reared to adults.

Microphotographs of adult wasps (Figs 3, 5) were obtained using a Nikon D300s DSLR camera with a Leitz Photar 1:2/25 mm lens connected via a Novoflex Universal Bellows (total views) and a ProgRes SpeedXT core 5 (Jenoptik AG) camera attached to Leitz M205 stereo microscope (images of heads). Images were captured in raw format, developed using Adobe Lightroom 3, and extended depth-of-field images obtained using Zerene Stacker 1.04 (Zerene Systems LLC). Stacked images were enhanced using

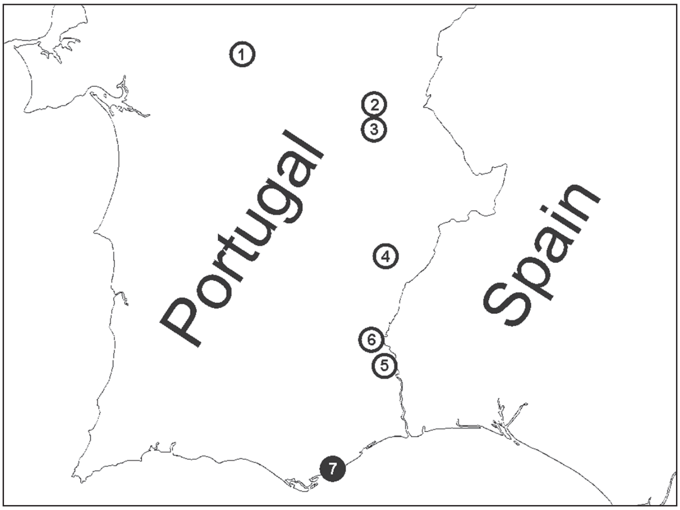


Figure 2. Localities where parasitoids were recovered from *Z. styliferum*. White circle = spiders parasitized by *Gelis apterus*, black circle = spiders parasitized by *Calymmochilus dispar*. Localities, in order from north to south: **1** Montemor o Novo – surrounding of castle ruins **2** Monsaraz – old olive grove **3** Alqueva – bank of water reservoir close to Moura **4** Ribeira de Limas – arid habitat with sparse vegetation **5** Alcoutim – arid habitat with sparse vegetation **6** Mesquita - arid habitat with sparse vegetation **7** Moncarapacho – arid slope in road surrounding.

Adobe Photoshop CS5 (Adobe Systems Inc.). The microphotographs of the cocoons, larvae and pupae (Figs 4, 6) were obtained using an Olympus U-TV 0.5 XC-3 camera with ColorView Soft Imaging System III-U software attached to an Olympus SZX-ILLK200 microscope.

Results

Host and parasitism rate

Three species of *Zodarion* were recorded from seven localities where parasitoids were found (Fig. 2). *Zodarion styliferum* (Simon, 1870) was dominant in all localities, whereas *Z. alacre* (Simon, 1870) and *Z. atlanticum* Pekár & Cardoso, 2005 were collected rarely. Parasitoids were found only in the igloos of *Z. styliferum*, which were parasitized, by the larvae of one species of Eupelmidae, *C. dispar*, and one species of Ichneumonidae, *G. apterus*. This represents the first host records for these two species and new distribution records for Portugal. *Calymmochilus dispar* was recovered from only 1 of the 18 surveyed localities (Fig. 2, locality 7) where 2 of 10 examined igloos of *Z. styliferum* were parasitized. *Gelis apterus* was recovered from 6 of the 18 localities surveyed (Fig. 2, locality 1 – 6), with an average parasitism rate of 7% (min – max = 3% – 13%, SD = 0.03).

Species survey

Hymenoptera: Eupelmidae (Eupelminae)

Calymmochilus dispar Bouček & Andriescu, 1967

http://species-id.net/wiki/Calymmochilus_dispar
Figures 3A–D,4A–J

Calymmochilus dispar Bouček & Andriescu (1967): 233–238. Holotype female, Romania, Agigea, 28.vii.1964, A. Andriescu (not examined).

Recognition. *Calymmochilus dispar* is the only one of four European species of *Calymmochilus* (Noyes 2012) with brachypterous females. Bouček and Andriescu (1967) provided a detailed description of both sexes of *C. dispar* in German, which are summarized below. The descriptions of the larval and pupal stages are new.

Description. Female (Fig. 3A, C). Length 3.0–4.6 mm. Body mostly brown to black, but partly with greenish or bluish metallic lustre, particularly frontovertex; antenna brown, clava yellowish-brown; legs brown with apices of tibiae and tarsi except for apices yellowish-brown. Head (Fig. 3C) slightly broader than mesosoma. Supraclypeal area (Fig. 3C: Sa) with about five transverse carinae and glabrous interspaces, strongly inclined from clypeus, hence clypeus below face level. Clypeus (Fig. 3C: Cl) protruding over the mandibles, with a strongly elevated carina and a serrate margin. Lower face with a blunt crest extending from clypeus almost to ventral margin of eye (Fig. 3C: Cr). Mandible (Fig. 3C: Md) very slender, sickle-shaped. Antenna long and slender, all funicular segments longer than broad, anellus about 1.3× as long as broad, clava as long as 3.5 apical funicular segments. Mesonotum dorsoventrally compressed, with alutaceous surface sculpture. Mesoscutum flat, on same level as scutellar-axillar complex. Axillae distinguished from scutellum by only slightly finer surface sculpture. Propodeum transverse, anteriorly with a distinct transverse carina connecting propodeal spiracles, with indistinct plicae and median carina; callar region strongly declining posteriorly. Prepectus slightly larger than tegula. Wings reduced, infuscate fore wing barely extending to base of metasoma (Fig. 3A). Metasoma evenly tapered posteriorly, syntergum tapered with rounded apex, laterally curved over to conceal very slightly exserted ovipositor sheaths.

Male (Fig. 3B, D). Length 1.4–2.3 mm. Head and body very dark brown to black with blue metallic lustre, metasoma brown (Fig. 3B). Antenna brown. Legs brown with knees and tarsi except for apices yellowish-brown. Head (Fig. 3D) slightly broader than thorax, nearly triangular in frontal view. Structure of lower face, clypeus (Fig. 3: Cl), and mandibles (Fig. 3: Md) similar to female except crest extending from clypeus to compound eye indicated only by slight elevation. Antenna (Fig. 3B) long, each funicular segment at least twice as long as broad, anellus indistinct and hardly discernible, claval segments fused. Mesoscutum convex with distinct notauli. Wings fully developed. Scutellum strongly convex, almost parallel-sided, with strongly inclined

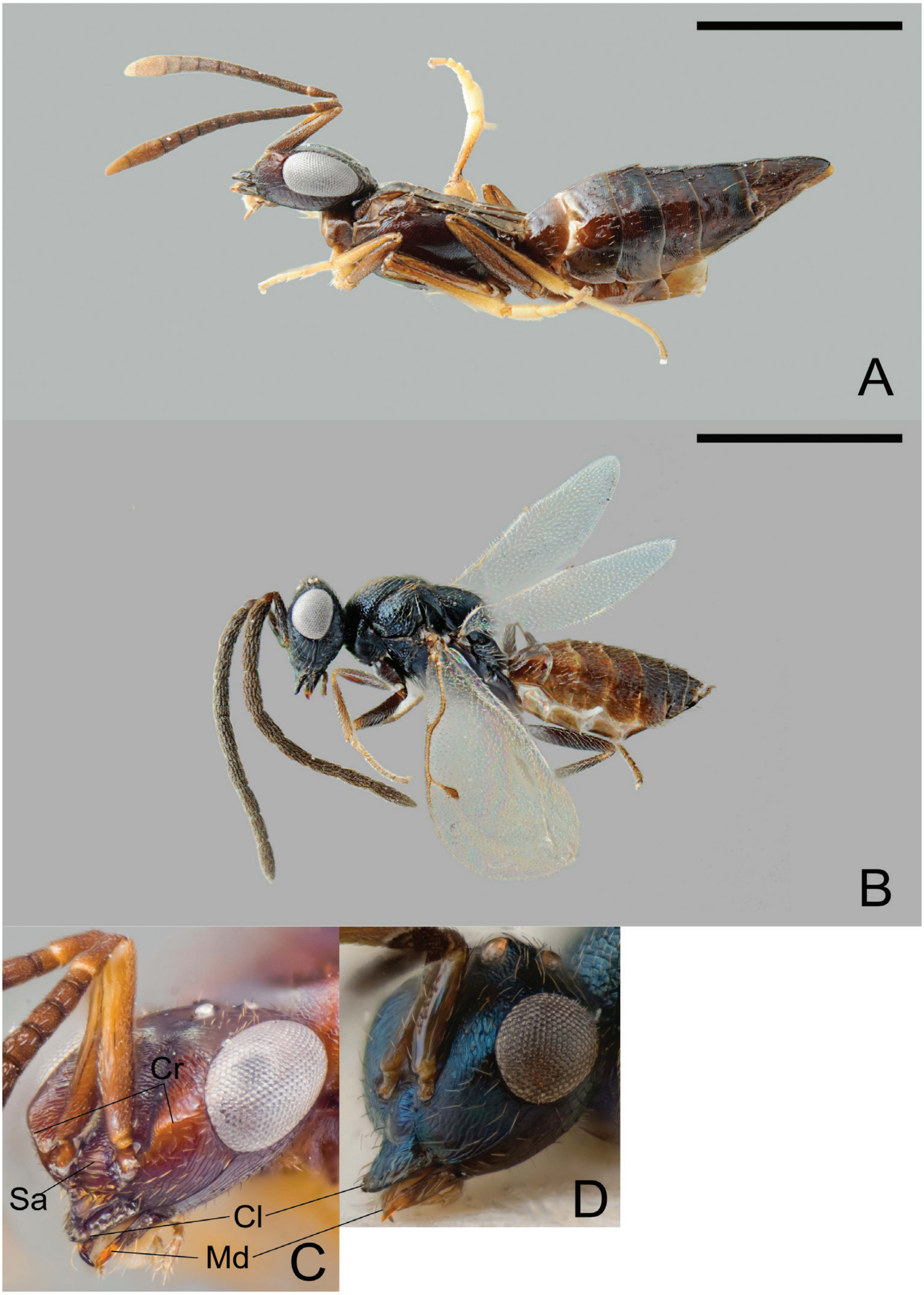


Figure 3. *Calymmochilus dispar*, female in lateral view (**A**), head (**C**); male in lateral view (**B**), head (**D**). Abbreviations: **Cl**: clypeus; **Cr**: crest; **Sa**: supraclypeal area; **Md**: mandible. Scale = 1 mm.

sides. Metanotum almost vertical, dorsellum almost triangular with surrounding furrow, dorsally with sharp carina. Propodeum with distinct median carina.

Larva (Fig. 4A, B, F). Brownish-yellow, female length about 2.5 mm (N = 1) and male length = 1.6 mm (N = 1). Mature larva with one pair of long, strong, dorsal setae (0.25–0.3× maximal diameter of larva) on each body segment plus two pairs of smaller dorsal setae (0.8× length of longer setae), one pair between long dorsal setae of first and second segment and second pair between those of second and third segment; laterally with one pair of smaller lateral setae (0.5–0.6× length of dorsal setae) on each body segment and irregularly placed short setae. Larval head very weakly sclerotized (not easily discernible in photographs).

Pupa (Fig. 4C–E, G–J). Pupa brown, about 3 mm length for female (Fig. 4C) and 2.2 mm for male (Fig. 4G). Eyes and mandibles becoming dark brown (Fig. 4C, H) as part of sclerotization process after 3 days. Eyes and mandibles dark brownish-black and first dark spots appearing inside pupa (Fig. 4D, I) seven days after pupation; pupa completely dark brown (Fig. 4E, J) after nine days.

Material. PORTUGAL, Faro district: 1 ♂ and 1 ♀ Moncarapacho; rocky slope near road, in spider igloos under rocks (37°05'N, 7°47'W, Fig. 2, locality 7), penultimate larvae attached to spider abdomen, 31.iii.2009, S. Korenko leg., larvae pupated 7.iv.2009 (male) and 8.iv.2009 (female), adults emerged 22.iv.2009, (1 ♂, CNCI; 1 ♀, ZSM).

Distribution. Armenia, Bulgaria, Croatia, France, Germany, Italy, Serbia, Spain, Yugoslavia (Noyes 2012) and Portugal (new record).

Host. Juvenile *Z. styliferum* with prosoma length of 0.4–0.5 mm (N = 2) (new host record).

Biology. The two *Z. styliferum* igloos from which *C. dispar* were reared were collected in an open, rocky habitat with sparse vegetation. When collected, a larva was attached to the abdomen of an immobilised juvenile inside the igloo. Exuviae of the previous moults were attached to the apex of the abdomen of the last instar. The larvae did not build any cocoon inside the igloo, being protected only by their long setae. The final instar and prepupal stage combined lasted 7 days for the male and 8 days for the female, after which the larvae pupated. The female emerged 15 days and the male 16 days after pupation at 23°C (±1.5°C).

Remarks. Little is known about the biology and host associations of *Calymmochilus* wasps. Previously, *C. russoi* Gibson, 1995 was reared from olive branches infested with *Pheloeotribus scarabaeoides* (Bernard, 1788) (Coleoptera, Scolytidae) (Russo 1938) and *C. longbottomi* Gibson, 1998 was reared from *Synsphyronus lathrius* Harvey, 1987 (Pseudoscorpionidae, Garypidae) (Austin et al. 1998). The *Z. styliferum* host was of a similar body size and created structurally similar igloos as the pseudoscorpion documented by Austin et al. (1998). Larvae of *C. dispar* do not create a cocoon for pupation; rather they use the already built spider igloo to help protect the bare larvae, which is isolated from the inner surface of the igloo by their long dorsal setae. The larvae we reared from the two *Z. styliferum* igloos were on the underside of a rock approximately

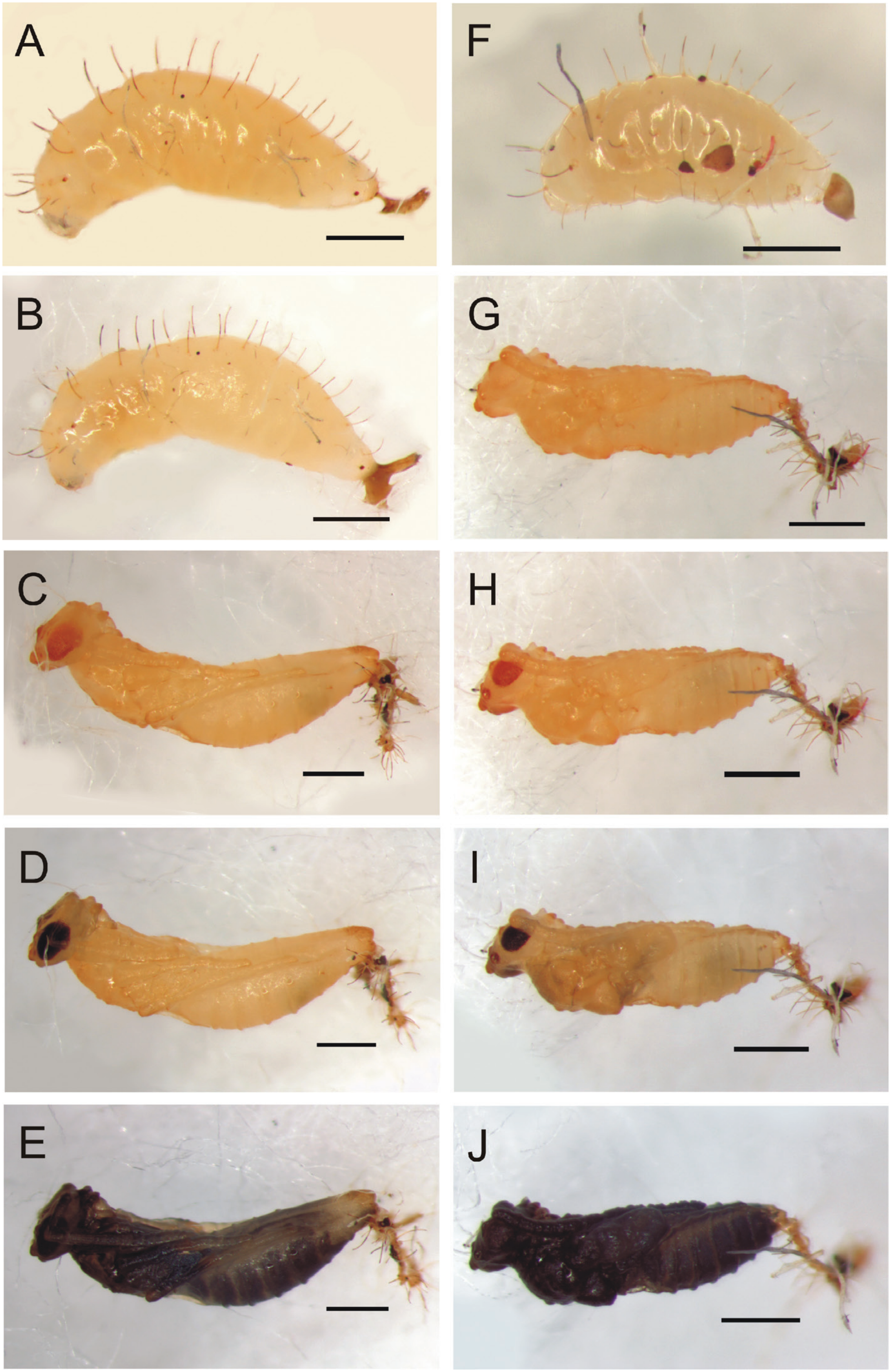


Figure 4. *Calymmochilus dispar*, mature larva (**A, B, F**) and pupa (**C–E, G–J**). Female final instar larva (**A, B**); female pupa after two days (**C**), six days (**D**), nine days (**E**). Male final instar larva (**F**). Male pupa after one day (**G**), four days (**H**), six days (**I**), nine days (**J**). Scale = 1 mm.

5 cm apart from each other. The parasitized pseudoscorpions reared by Austin et al. (1998) were also located under rocks, whereas the beetle larvae associated with *C. russoi* were under tree bark (Russo 1938). These concealed habitats presumably provide additional shelter for the *Calymmochilus* larvae and support Bouček (1988), who suggested that *Calymmochilus* species are primarily associated with hosts in sheltered places, e.g. under bark or rocks. The unusual, protuberant clypeus that characterizes adults may be a structural adaptation to help the adults emerge and the female to access restricted spaces to parasitize new hosts. However, it remains to be shown whether *C. dispar* is narrowly associated with *Zodarion* species or parasitizes taxonomically more diverse hosts in similar niches.

Hymenoptera: Ichneumonidae (Cryptinae)

Gelis apterus (Pontoppidan, 1763)

http://species-id.net/wiki/Gelis_apterus
Figures 5A–C, 6A–B

Ichneumon apterus Pontoppidan (1763): 692–693. Holotype female, missing. Comments about description in Schwarz (1995).

Recognition. Schwarz (2002) gave a key to the western Palaearctic species of *Gelis* with apterous females, and Schwarz (1995, 1998) provided a diagnosis and description of the female of *G. apterus.* The main diagnostic features of the female are summarised below; the descriptions of the male and pupa are new.

Description. Female (Fig. 5A). Length 3.2–5.5 mm. Apterous. Body mostly black but base of antenna orange and thorax, propodeum and first segment of gaster varying from entirely black to nearly entirely orange; legs mainly blackish or dark brown with yellowish brown parts; tibiae white basally. Antenna with 21–25 segments; third segment (without anellus) 3.7–4.4× as long as wide. Malar space 1.2–1.3× as long as wide. Mesoscutum in lateral view not or only weakly sloping anteriorly, with a strong or weak median longitudinal furrow. Mesopleuron with fine striation anteriorly or more rarely almost entirely striate. Metapleuron entirely granulate or more rarely partly smooth and lustrous. Hind femur 4.0–4.9× as long as wide. Ovipositor sheath 1.9–2.5× as long as hind tibia. Ovipositor curved upwards, its tip with only very weak teeth ventrally.

Male (Fig 5B, C). Length 3.0–4.1 mm. Macropterous. Body mostly black but mandible with teeth reddish, palpi brown, and tegula yellowish brown or brown. Legs with coxae, trochanters, trochantelli and femora except for following black or blackish brown; fore and mid femora yellowish-brown apically; tibiae basally whitish (most distinct on hind tibia), fore tibia except basally, and mid tibia except basally and apically yellowish-brown, hind tibia except basally blackish brown; tarsi brown or blackish brown. Fore wing with pterostigma brown except white basally. Body mostly distinctly granulate and matt, without distinct punctures. Antenna with 21–23 segments; third

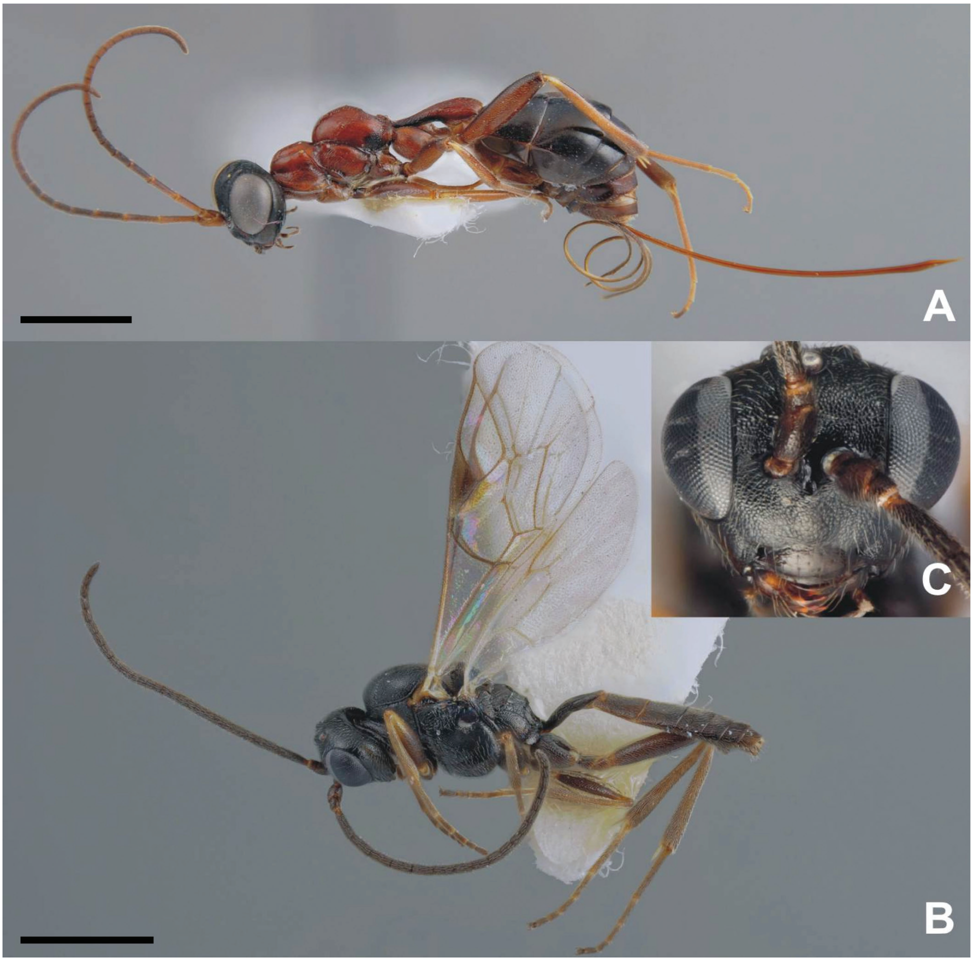


Figure 5. Adult of *Gelis apterus*, female in lateral view (**A**), male in lateral view (**B**), and male head in front view (**C**). Scale = 1 mm.

segment (without anellus) 2.9–3.3× as long as wide; segments 11–13 with linear tyloids. Clypeus in profile evenly and rather weakly convex, smooth or weakly granulate dorsally in addition to some scattered punctures; lower margin convex and region just above lower margin depressed. Mandible rather long, its teeth of equal length, outer surface with a distinct swelling subbasally. Malar space 1.2× as long as basal width of mandible, and without a furrow. Genal carina joining oral carina behind base of mandible. Ocelli small. Head behind eyes in dorsal view moderately narrowed and distinctly convex. Pronotum without dorsomedian longitudinal ridge. Mesopleuron with fine granulation and weak rugosity, speculum and hind margin below speculum smooth. Prepectal carina present but rather weak. Propodeum of moderate length and with both transverse carinae complete and distinct; longitudinal carinae anterior to posterior transverse carina rather weak and absent basally except for lateral longitudinal

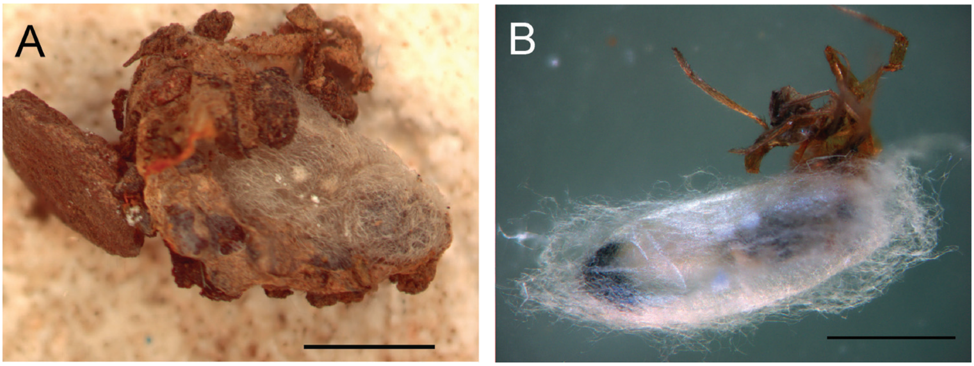


Figure 6. Pupa of *Gelis apterus*, inside the spider igloo (**A**) during pupation when parasitized spider was removed from igloo (**B**). Scale = 2 mm.

carina; propodeum lustrous between transverse carinae and with longitudinal striation about as distinct as longitudinal carinae; lustrous anterior to posterior transverse carina, nearly smooth and with distinctly separated area petiolaris. Legs slender with hind femur 5.0–5.1× as long as wide. Fore wing with areolet rather small. Gaster with first segment slender, without median dorsal carinae, and with dorsolateral and ventrolateral carinae rather weak.

Pupa (Fig. 6A, B). Pupa brownish (becoming dark brown as part of sclerotization process), about 5.5–7 mm.

Material. PORTUGAL, Beja district: 2 ♀ Ribeira de Limas, direction to Guadiana River, slope close to unpaved road (37°51'N, 7°31'W, Fig. 2, locality 4), 24.v.2011, S. Korenko leg. (1 pupa and 1 larva), larva on *Z. styliferum*, pupated 25.v.2011, adults emerged 5.vi.2011 and 7.iv.2011. Specimens deposited in collection of M. Schwarz.

Évora district: 1 ♂ Montemor o Novo, surrounding of castle ruins (38°38' N, 8°13'W, Fig. 2, locality 1), 10.iv.2010 (pupal stage), S. Korenko leg., adult emerged 12.iv.2010. 1 ♀ Monsaraz, olive grove (38°26' N, 7°32' W, Fig. 2, locality 2), 11.iv.2010, E. Líznarová and S. Korenko leg., larva on *Z. styliferum*, pupated 21.iv.2010, adult emerged 4.v.2010 (one empty cocoon and one larva that died in the laboratory were also collected from same locality). 1 ♀ and 1 ♂ Alqueva close to Moura, bank of water reservoir (38°12'N, 7°32'W, Fig. 2, locality 3), 11.iv.2010, S. Korenko leg., larvae on *Z. styliferum*, pupated 14.iv.2010, adults emerged 27.iv.2010 (female) and 28.iv.2010 (male), female escaped.

Faro district: 1 ♀ Casa do Canavial close to Mesquita in Guadiana Valley Natural Park (37°32'N, 7°31'W, Fig. 2, locality 6), 4.iv.2008, S. Korenko leg., larva on *Z. styliferum*, pupated 21.iv. 2008, emergence date not recorded. 1 ♀ and 1 ♂ Alcoutim, view terrace close to town (37°27'N, 7°28'W, Fig. 2, locality 5), 29.iii.2009, S. Korenko and S. Pekár leg., larvae on *Z. styliferum*, pupated 2.iv.2009 (female) and 1.iv.2009 (male), adults emerged 9.iv.2009.

Distribution. South and Central Europe (Schwarz 1998) including Portugal (new record), Azerbaijan, Tajikistan (Schwarz 1998).

Hosts. Juvenile spiders of *Z. styliferum* with prosoma length averaging 2.47 mm (N = 9, min/max = 1.6/3.2 mm) (new host record).

Biology *Gelis apterus* and its spider hosts appear to be associated with open arid habitats with sparse vegetation. Females attack the host spiders in the igloo, penetrating the igloo wall with their long ovipositors (laboratory observation). Unfortunately nothing more is known about oviposition behaviour. The larva makes a cocoon inside the spider igloo before pupation (Figs. 6). Details about the sclerotization process of the pupa were not recorded because of its location in the cocoon. The cocoon consists of white to brownish weaved threads which fill space inside the spider igloo. Adults emerged 9–14 days after pupation.

Remarks. Females of *G. apterus* reared from *Z. styliferum* in Portugal differ from those collected in other parts of Europe by somewhat longer ovipositor sheaths and the thorax laterally having smooth patches. In these two features they resemble the closely related species *G. atratus* (de Stefani, 1884), but females of *G. apterus* reared from *Z. styliferum* do not have the mesoscutum sloping downwards caudally.

The previously unknown macropterous males of *G. apterus* are very distinct from their apterous females. Males of *Gelis* are often unknown or unassociated with females because of the difference in aptery and because they are more difficult to distinguish in many species. The two sexes of *G. apterus* possess very few similar features that indicate they are conspecific and we consider the males we reared as *G. apterus* primarily because they were reared with females of *G. apterus*, and because only females of *G. apterus* were reared from *Z. styliferum*.

Diagnostic features of *G. apterus* males include an evenly and weakly convex, smooth or mainly smooth clypeus, long mandible and malar space, moderately narrowed and distinctly convex head, and the pattern of sculpture and carinae of the propodeum. These features enable separation of males from those of most other *Gelis* species. However, it is expected that the unknown males of *G. atratus* will be very similar to *G. apterus* and the two may not be easily distinguishable.

We found *G. apterus* associated with *Z. styliferum* in several parts of Central and South Portugal, and it seems to be widespread but in low abundance throughout the Iberian Peninsula based on the observation of three empty pupae inside *Zodarion* igloos in the Spanish provinces of Málaga and Granada (Korenko unpub.). *Gelis* is a large genus of parasitoid wasps that are worldwide in distribution but with most species in the Holarctic region. Some species are fully-winged but many are ant-like micropterous or wingless. Hosts of different species of *Gelis* include eggs or larvae or cocoons or cocoon-like structures of a wide range of holometabolous insects as well as egg sacs of spiders. Fitton et al. (1987) listed ten *Gelis* species reared from spider egg sacs of both wandering and web-building spiders. The larvae of *Gelis* species known to attack spiders have been regarded as exclusively feeding on spider eggs (Fitton et al. 1987). We did not observe this for *G. apterus*, which fed on juvenile spiders. Diurnally active female *G. apterus* presumably attack nocturnal *Zodarion* that are resting in their igloos during the day.

Zodarion styliferum is common in Portugal and has two overlapping generations (Pekár unpub.). Juvenile spider hosts are therefore available to *G. apterus* during the whole year and likely provide a highly available resource. *Gelis apterus* is documented from South – Central Europe to 70° eastern longitude. Several *Zodarion* species have overlapping distributions within this area. Their life history, behaviour, body size and igloo architecture are similar to *Z. styliferum* and could be potential hosts for *G. apterus*. Although we did not rear *G. apterus* from either *Z. alacre* or *Z. atlanticum*, this may simply reflect their relative rarity and low parasitism rates. Whether *G. apterus* is also associated with *Zodarion* species outside of the Iberian Peninsula is not known. Knowledge of the host range of *G. apterus* in other parts of its range is essential to confirm whether *Zodarion* spiders are their only hosts and, if not, understand how an association with spider hosts evolved.

Acknowledgements

We would like to thank Rudolf Macek for permitting us to use his photography in figure 1A. SK was supported by the project of European Science Foundation and Ministry for Education and Youth of the Czech Republic CZ.1.07/2.3.00/30.0040.

References

Aubert JF (1969) Les Ichneumonides Ouest-Palearctiques et leurs hôtes. Tome I. Pimplinae, Xoridinae et Acaenitinae. Quatre Feuilles Editeur France 74: 49–63.

Austin AD (1985) The function of spider egg sacs in relation to parasitoids and predators, with special reference to the Australian fauna. Journal of Natural History 19(2): 359–376. doi: 10.1080/00222938500770261

Austin AD, Gibson GAP, Harvey MS (1998) Synopsis of Australian *Calymmochilus* Masi (Hymenoptera: Eupelmidae), description of a new Western Australian species associated with a pseudoscorpion, and review of pseudoscorpion parasites. Journal of Natural History 32: 329–350. doi: 10.1080/00222939800770171

Bouček Z (1988) Australasian Chalcidoidea (Hymenoptera). A Biosystematic Revision of Genera of Fourteen Families, with a Reclassification of Species. CAB International, Wallingford, 832 pp.

Bouček Z, Andriescu I (1967) Notizen über die Gattung *Calymmochilus* Masi, mit Beschreibung einer neuen Art aus Südeuropa (Hymenoptera, Eupelmidae). Acta Entomologica Musei Nationalis Pragae 37: 233–238.

Cushing PE, Santangelo RG (2002) Notes on the natural history and hunting behavior of an ant-eating zodariid spider (Arachnida, Araneae) in Colorado. Journal of Arachnology 30(3): 618–621. doi: 10.1636/0161-8202(2002)030[0618:NOTNHA]2.0.CO;2

Eberhard W (1970) The predatory behavior of two wasps, *Agenioideus humilis* (Pompilidae) and *Sceliphron caementarium* (Sphecidae), on the orb weaving spider, *Araneus cornutus* (Araneidae). Psyche 77(2): 243–251.

Fitton MG, Shaw MR, Austin AD (1987) The Hymenoptera associated with spiders in Europe. Zoological Journal of the Linnean Society 90(1): 65–93. doi: 10.1111/j.1096-3642.1987.tb01348.x

Gauld ID, Dubois J (2006) Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae), a taxonomic revision of spider ectoparasitoids. Systematic Entomology 31(3): 529–564. doi: 10.1111/j.1365-3113.2006.00334.x

Gibson GAP (1995) Parasitic wasps of the subfamily Eupelminae: classification and revision of world genera (Hymenoptera: Chalcidoidea, Eupelmidae). Memoirs on Entomology International 5: 1–421.

Gibson GAP (1997) Morphology and Terminology. In: Gibson GAP, Huber JT, Woolley JB (Eds.) Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). NRC Research Press, Ottawa, 16–44.

Gonzaga MO, Sobczak JF (2011) Behavioral manipulation of the orb-weaver spider *Argiope argentata* (Araneae: Araneidae) by *Acrotaphus chedelae* (Hymenoptera: Ichneumonidae). Entomological Science 14(2): 220–223. doi: 10.1111/j.1479-8298.2010.00436.x

Kessler A, Fokkinga A (1973) Hymenopterous parasites in egg sacs of spiders of the genus *Pardosa* (Araneida, Lycosidae). Tijdschrift voor Entomologie 116(3): 43–61.

Noyes JS (2003) Universal Chalcidoidea database. Available from http://www.nhm.ac.uk/research-curation/research/projects/chalcidoids/ [accessed 17 November 2012]

Pekár S (2004) Predatory behavior of two European ant-eating spiders (Araneae, Zodariidae). Journal of Arachnology 32(1): 31–41. doi: 10.1636/S02-15

Pekár S, Cardoso P (2005) Ant-eating spiders (Araneae: Zodariidae) of Portugal: additions to the current knowledge. Zootaxa 1009: 51–60.

Pekár S, Bilde T, Martišová M (2011) Intersexual trophic niche partitioning in an ant-eating spider (Araneae: Zodariidae). PLoS ONE 6(1): e14603. doi: 10.1371/journal.pone.0014603

Pekár S, Král J, Lubin Y (2005a) Natural history and karyotype of some ant-eating zodariid spiders (Araneae, Zodariidae) from Israel. Journal of Arachnology 33(1): 50–62. doi: 10.1636/S03-2

Pekár S, Král J, Malten A, Komposch C (2005b) Comparison of natural histories and karyotypes of two closely related ant-eating spiders, *Zodarion hamatum* and *Z. italicum* (Araneae, Zodariidae). Journal of Natural History 39(19): 1583–1596. doi: 10.1080/00222930400016762

Platnick NI (2012) The world spider catalog, version 13.0. American Museum of Natural History. doi: 10.5531/db.iz.0001

Pontoppidan E (1763) Den Danske Atlas eller Konige-Riget Dannemark. Tomus I, Kiobenhavn (Godicke), 723 pp.

Russo G (1938) Contributo alla conoscenza dei Coleotteri Scolitidi. Fleotribo: *Phloeotribus scarabaeoides* (Bern.) Fauv. Parte seconda. Biografia, simbionti, danni e lotta. Laboratorio di Entomologia Agraria di Portici, Bollettino 2: 1–420.

Schwarz M (1995) Revision der westpaläarktischen Arten der Gattungen *Gelis* Thunberg mit apteren Weibchen und *Thaumatogelis* Schmiedeknecht (Hymenoptera, Ichneumonidae). Teil 1. Linzer Biologische Beiträge 27: 5–105.

Schwarz M (1998) Revision der westpaläarktischen Arten der Gattungen *Gelis* Thunberg mit apteren Weibchen und *Thaumatogelis* Schmiedeknecht (Hymenoptera, Ichneumonidae). Teil 2. Linzer Biologische Beiträge 30: 629–704.

Schwarz M (2002) Revision der westpaläarktischen Arten der Gattungen *Gelis* Thunberg mit apteren Weibchen und *Thaumatogelis* Schwarz (Hymenoptera, Ichneumonidae) – Teil 3. Linzer Biologische Beiträge 34(2): 1293–1392.

Wiehle H (1928) Beiträge zur Biologie der Araneen, insbesondere zur Kenntnis des Radnetzbaues. Zeitschrift für Morphologie und Ökologie der Tiere 11: 115–151.

Supplement 5

Korenko S, Isaia M, Satrapová J, Pekár S. 2014. Parazitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecological Entomology 39, 30-38.

Citation report:
Web of Science^{TM}: 14
Scopus/Google Scholar: 16

ISI WOS:
Ecological Entomology
IF$_{(2014)}$ = IF = 1,699
Quartile $_{(2014)}$: Entomology – Q1

Ecological Entomology

Ecological Entomology (2014), **39**, 30–38 DOI: 10.1111/een.12067

Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae)

STANISLAV KORENKO,[1] MARCO ISAIA,[2] JITKA SATRAPOVÁ[1] and STANO PEKÁR[3] [1]Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Suchdol, Czech Republic, [2]Laboratorio Ecosistemi Terrestri - Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Torino, Italy and [3]Department of Botany and Zoology, Faculty of Sciences, Masaryk University, Brno, Czech Republic

Abstract. 1. Araneid spiders of genus *Araniella* are attacked by three polysphinctine parsitoid wasps *Polysphincta boops* Tschek, *P. tuberose* (Gravenhorst), and *Sinarachna pallipes* (Holmgren). In the present study, the trophic niche of sympatrically occurring parasitoids and the host manipulation they induced were studied. The aim was to identify whether the variation in host response to manipulation is as a result of differences among parasitoids or among host species.

2. It was found that final instar larva forced the spider host to build a three-dimensional (3D) ‘cocoon web’ to protect the parasitoid during pupation. The behaviour of parasitoid larva and the induced modification of the web architecture differed between wasps of genus *Polysphincta* and *Sinarachna* but not among three spider species. The larvae of genus *Polysphincta* forced the spider host to build the ‘cocoon web’ with a high thread density within which the pupa was positioned horizontally. The larvae of *Sinarachna* forced the spider host to build web with sparse threads and the pupa was positioned vertically in the middle of the ‘cocoon web’.

3. There seems to be an investment trade-off in parasitoid wasps: some species manipulate the host to build a dense protective web, while pupating in a sparse cocoon, whereas others make the spider produce a sparse web but build a dense pupa wall.

Key words. Behavioural manipulation, parasitoid–host interaction, Polysphinctini, web architecture.

Introduction

Parasitic organisms, parasites, and parasitoids often develope the ability to manipulate the host phenotype including its morphology, physiology, and behaviour for their own benefit (Moore, 2002). The function of behavioural manipulation has been described in many parasite–host systems (e.g. Moore, 2002) and their evolutionary significance and adaptation significance were discussed by Poulin (1995, 2010), Moore (2013), and Maure *et al.* (2013).

The response to manipulation varies (Thomas *et al.*, 2005; Poulin, 2010). It can result either from differences among hosts or among parasitoids or from the interaction between both. For example, variability of the host response to the same parasitoid has been found in amphipods hosts. The freshwater amphipod *Gammarus pulex* (Linnaeus) is regularly infected with an acanthocephalan parasite *Pomphorynchus leavis* Müller that use either fish or birds as final hosts. *Pomphorynchus leavis* induce a photophilic reaction in *G. pulex*, but not in *Gammarus roeseli* Gervais (Tain *et al.*, 2007). The inability to manipulate the behaviour of the invasive species *G. roeseli* might be explained by tight adaptation of the parasite to the local host species. The host response in this system varied even among populations as some parasitoid individuals induced a strong manipulation whereas others failed to do so (Franceschi *et al.*, 2010). Eberhard (2010) observed variability in the behavioural response of the spider host *Anelosimus octavius* Agnarsson after manipulation by the polysphinctine wasp *Zatypota solanoi* Gauld. All parasitised spiders have been manipulated to build the safe retreat for larva pupation; however, some manipulated

Correspondence: Stanislav Korenko, Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic. E-mail: korenko.stanislav@yahoo.com

spiders build a retreat for parasitoid larva using only silk threads and other incorporated leaves to improve the retreat. Eberhard (2010) suggested that this variability is at higher rather than lower levels in the hierarchy of behavioural manipulation mechanisms because the larva's effect is fine tuned to details of the host's natural history. Korenko and Pekár (2011) described a host-specific response in the theridiid spider *Neottiura bimaculata* (Linnaeus) and *Theridion varians* Hahn attacked by *Zatypota percontatoria* (Müller). The first host species responded by building a dense web, whereas the other one responded by building a specific spherical web.

A few previous studies have been devoted to behavioural manipulation of orb-web building spiders (Eberhard, 2000a,b 2001; Gonzaga & Sobczak, 2007, 2011; Sobczak *et al.*, 2009; Gonzaga *et al.*, 2010). Several polysphinctine wasps of the genus *Hymenoepimecis* associate with tetragnathid spiders and induce the production of a 'cocoon web' that has changed design in the number of radii and the spiral, but two-dimensionality of the web is retained (Eberhard, 2000a,b, 2001). A completely different manipulation, when the spider has been forced to change a two-dimensional (2D) to a 3D structure, has been found in araneid and nephilid spiders (Gonzaga *et al.*, 2010; Gonzaga & Sobczak, 2011).

Here we focus on Polysphinctine wasps (Polysphincitini *sensu* Townes, 1969) that are koinobiont ecto-parasitoids of spiders and they are narrowly associated with the biology of their spider hosts (Fitton *et al.*, 1987). The larva, attached to the dorsal side of the abdomen, develops while the spider continues foraging. Shortly before pupation, the parasitoid final instar larva manipulates the web-building activity of the host in order to construct a safe shelter against natural elements and predators during the parasitoid pupation. The architecture of the innate orb web is changed and becomes stronger providing more durable support for the wasp's pupa.

We selected a host–parasitoid system in which few parasitoid species have overlapping trophic niches, i.e. attack hosts of the same or closely related species. The parasitoid wasps, *Polysphincta boops* Tschek, *Polysphincta tuberosa* (Gravenhorst), and *Sinarachna pallipes* (Holmgren), are exclusive parasitoids of orb-web spiders of the family Araneidae. *Polysphincta boops* was documented to be associated with *Araniella cucurbitina* (Clerck) and *A. opisthographa* (Kulczyński). *Polysphincta tuberosa* and *S. pallipes* were recorded from several araneid species, namely *Araniella* sp., *Araneus diadematus* Clerck, *Araneus quadratus* Clerck, *Agalenatea redii* (Scopoli), and *Zygiella* sp. (Aubert, 1969; Fitton *et al.*, 1988). The spider genus *Araniella* includes several species with a sympatric distribution in Palearctic. These are common orb-web weaving spiders in the canopies, inhabiting similar microhabitats (tree-crowns, shrubs), building a planar orb-web of similar architecture, and having similar life histories (Levi, 1974; Roberts, 1985).

We studied the trophic niche and the niche overlap of the three parasitoid species, and compared several traits of the manipulation induced by larva. Next, we investigated whether the variation in host response to manipulation is because of differences among parasitoids or among host species.

Methods

The frequency of parasitised spiders within communities of orb-web weaving spiders (Araneidae) were investigated in four sites: (i) in a commercial apple orchard in Starý Lískovec (district Brno, Czech Republic, 49°09′38″N, 16°33′38″E) in 2007 and 2008; (ii) at a forest edge of Veľké Lúky (Motešice, district Trenčín, Slovakia, 48°51′34″N, 18°13′45″E) during May and October 2012; (iii) in an organic apple orchard in Bottonasco (Province of Cuneo, Italy, 44°24′47″N, 7°24′43″E) in November 2009–2011; and (iv) at a forest edge in Monterosso Grana (Province of Cuneo, Italy, 44°24′20″N, 7°19′17″E) in November 2011. At each site the spiders were collected by beating tree branches with a square-shaped beating net (1 m^2 area) placed beneath the tree crown. At least three samples (each sample was made of beating 30 trees) were taken per site. Collected spiders were identified to the generic level using Nentwig *et al.* (2013) and Roberts (1985) and matched to the species based on formerly occurring species in investigated localities (Isaia *et al.*, 2010; Korenko *et al.*, 2011, 2012; S. Korenko, unpublished data) and on rearing-collected specimens to adulthood. The host range of the parasitoids was estimated as niche breadth using Smith's formula, FT, which unlike other commonly used measures is less sensitive to selectivity of rare resources and has good statistical properties (Smith, 1982). Estimated host ranges were compared among parasitoids based on the z statistics.

Live parasitised and unparasitised spiders collected in the four sites above plus in two additional ones: (i) in forest in Aisone (Province of Cuneo, Italy, 44°17′29″N, 7°12′26″E) in November 2010; and (ii) private garden in Hradečno (district Kladno, Czech Republic, 50°11′15″N, 13°59′16″E) in June 2012, were reared and observed in the laboratory. The web architecture of parasitised or non-parasitised spiders was studied by placing spiders individually in to experimental arenas (100 × 100 mm base, 130 mm height) with an installed twig providing the space for a spider web. Spiders were kept at a room temperature of $22 \pm 3\,^\circ$C, a natural LD regime and fed with a surplus of fruit flies (*Drosophila melanogaster* Meigen). The web building activity of parasitised spiders ($N = 29$) was recorded until the larva killed and consumed the spider, and built the cocoon. In the case of non-parasitised *Araniella* spiders ($N = 20$), these were observed for 20 days. The web-building activity has been recorded in 1–2 day periods.

Hatched wasps were identified using Fitton *et al.* (1988) and Zwakhals (2006). The nomenclature of the polysphinctines follows Fitton *et al.* (1988) and Yu and Horstmann (1997). Voucher specimens were deposited in the collection of Kees Zwakhals (Netherlands), of Kamil Holý (Crop Research Institute, Prague, Czech Republic), and of the first author.

The web architecture of both parasitised and unparasitised spiders were recorded: the presence/absence of an orb web in the experimental arena was recorded in a 1- to 2-day interval. Spider spinning activity was recorded using a digital camera Canon EOS 500 with an objective EFS 18–55 mm DC III and a macro objective EF 100 mm f/2,8L IS USM.

The body size of live spiders was measured using scale paper placed under a transparent Petri dish with the precision of 0.5 mm. The angle of pupa orientation was measured by transparent goniometer from photographs. The density of threads in the spider web of each parasitised individual and the density of the threads in the wall of wasp cocoon was measured using SigmaScan Pro, Image Analysis V. 5.0.0 (SPSS Inc., Chicago, Illinois). The standardised photographs of the 'cocoon web' and the wasp cocoons (4752 × 3168 pix) were transformed to 1 bit black and white mode (art line style) by Corel Photo Paint software v. 11 (Corel Corporation, Ottawa, Canada). Square-shaped sectors (200 × 200 mm) were cut out from the several sectors of the pictures. We estimated the density of the threads from two different sectors of the web: (i) from the pupa surrounding and (ii) from a distant part of web (sectors far away from pupa). A sector from the pupa surrounding was the place where one side of the square was touching the edge of pupa cocoon. A sector from a distant part of the web was at least 200 mm away from the pupa cocoon edge, but only in places with webbing. A white area of silk threads in a picture was measured as a proportion (of the whole area). The density of silk threads of the web was compared between two sectors for each individual and among particular individuals. The cocoon was halved longitudinally using a blade. Images of the cocoon were taken, standardised and analysed similarly as mentioned above. The density of threads in the cocoon wall was compared among wasp species.

Statistical analysis

All analyses were performed within R environment (R Core Team, 2012). A Linear Model (ANOVA) or a Generalised Linear Model with binomial (GLM-b) or a gamma (GLM-g) error structure was used to study the effect of the spider species and the wasp species on the selected traits of manipulation. ANOVA was used when the errors showed normal distribution and homoscedastic variance (spider body size). GLM-b was used when the response was binary and GLM-g was used when the response was continuous but showed heteroscedastic variance. The sum of squares of the type II were used to assess the effects of the two factors as the design was not orthogonal because of missing factor combinations (Pekár & Brabec, 2009). The density of cocoon wall was near to 1, therefore the data were angularly transformed in order to homogenise variance and stay within 0 and 1 bounds. As the measurements performed on the cocoon were not statistically independent, for example they were repeated measurements per cocoon, the data were compared with Generalised Least Squares (GLS) from the nlme package (Pinheiro & Bates, 2000). GLS models the population average (or marginal) relationship between study variables and takes into account any correlation among measurements by correcting the variances. The correlation structure among measurements was specified as exchangeable owing to only two repeated measurements on each cocoon

Results

Parasitism frequency and host specificity

In total 417 araneid orb-web spiders were collected in the four investigated sites (Table 1). Of these three genera, *Araniella*, *Mangora* followed by *Araneus*, were dominant. Only two genera *Araniella* and *Araneus* were parasitised. Juvenile spiders of the genus *Araniella* were parasitised with a frequency between 4% and 14%, whereas juvenile spiders of the genus *Araneus* were parasitised only with a frequency of 3%. Three species of the genus *Araniella* were parasitised with a varying frequency: *A. cucurbitina* –4%, *A. displicata* –14%, and *A. opisthographa* –7.3% (pooled for two sites).

Three polysphinctine wasp species *P. boops*, *P. tuberose*, and *S. pallipes* were reared from *Araniella* and *Araneus* spider hosts (Table 2). The host range of the three species differed: *P. boops* parasitised only *A. opisthographa* (100%); *P. tuberosa* parasitised *A. cucurbitina* (13.3%), *A. opisthographa* (80%), and *A. diadematus* (6.7%); and *S. pallipes* parasitised *A. cucurbitina* (17%), *A. displicata* (50%), and *A. opisthographa* (33%). Thus the host range was narrower in *P. boops* ($FT = 0.66$) and wider in *P. tuberosa*

Table 1. List of spider host species on four sites with their relative frequency of abundance. The number of parasitised hosts is given after a slash.

	Site			
Species	Bottonasco (I)	Monterosso Grana (I)	Starý Lískovec (CZ)	Veľké Lúky (SK)
Aculepeira ceropegia (Walckenaer)	0	0	0.01	0
Agalenate redii (Scopoli)	0.01	0	0.02	0
Araneus alsine (Walckenaer)	0	0	0	0.03
A. diadematus Clerck	0.02	0.02	0	0.2
Araneus sp.	0.03	0.04/1	0.11	0
Araniella cucurbitina (Clerck)	0	0	0.24/2	0
A. displicata (Hentz)	0	0.86/6	0	0
A. opisthographa (Kulczyński)	0.67/5	0	0	0.74/2
Cyclosa conica (Pallas)	0.15	0.02	0.01	0
Gibaranea bituberculata (Walckenaer)	0.01	0.02	0.01	0.03
Mangora acalypha (Walckenaer)	0.1	0.02	0.58	0
Singa hamata (Clerck)	0	0	0.02	0
Zilla diodia (Walckenaer)	0.01	0.02	0	0
Total individuals	110	50	227	30

Table 2. The number of cases of parasitised araneid spider species by three wasp species and the sites, where these were collected. The wasp individuals were reared to adulthood in order to record details of manipulation and to identify them to species.

	Parasitoid		
Spider host	*P. boops*	*P. tuberose*	*S. pallipes*
Araneus diadematus	–	Hradečno ($N = 1$)	–
Araniella cucurbitina	–	Starý Lískovec ($N = 2$)	Aisone ($N = 2$)
A. displicata	–	–	Monterosso ($N = 6$)
A. ophistographa	Bottonasco ($N = 2$)	Veľké Lúky ($N = 12$)	Bottonasco ($N = 4$)

(0.87) and *S. pallipes* (0.90), but not significantly different among species ($z < 0.9$, $P > 0.39$, Fig. 4a).

The body sizes of parasitised spider hosts were as follows: *A. diadematus* (mean = 3 mm), *A. cucurbitina* (3.3), *A. displicata* (3.4), and *A. opisthographa* (3.4). The difference in body size between spider hosts was not significant (ANOVA, $F_{3,18} = 0.18$, $P = 0.91$): it was on average 3.3 (SE = 0.11).

Web architecture manipulation

Unparasitised spiders of *Araniella ophistographa* ($N = 8$), *A. displicata* ($N = 12$), and *A. cucurbitina* ($N = 5$) constructed only 2D webs during 20 days of observation. The web architecture was similar among species (Fig. 1). *Araniella* spiders were observed in the field to build a 3D web structure only when a female guarded its cocoon ($N = 4$).

Fig. 1. Innate 2-dimensional web of *Araniella displicata* spiders built in the laboratory. Scale = 1 cm.

Final instar larva of all three wasp species induced production of a unique 3D 'cocoon web' in its spider host, but considerable differences were observed in the architecture (Fig. 2). The difference between web density surrounding pupa and the outer part of the web varied significantly among wasp species (GLS, $F_{2,16} = 7.0$, $P = 0.007$, Fig. 3a), but not among spider species (GLS, $F_{2,16} = 1.7$, $P = 0.22$). The density of threads in a web induced by *S. pallipes* did not differ between the pupa surrounding and the outer parts of the web (contrasts, $P > 0.38$). However, pupas of *P. boops* and *P. tuberosa* were surrounded by significantly more dense silk around the pupa than at the outer parts of the web (contrasts, $P < 0.01$). The differences between *A. ophistographa* and *A. displicata* and between the two *Polysphincta* species were not significant (contrasts, $P > 0.1$).

The density of threads surrounding the pupa differed significantly among wasp species (GLM-g, $X^2{}_2 = 16.5$, $P = 0.0003$, Fig. 3b), but not among spider species (GLM-g, $X^2{}_2 = 1.1$, $P = 0.57$). The density of threads around *Sinarachna* pupa was on average 17.6% (SD = 4.6), whereas that of *Polysphincta* was on average 39.4% (SD = 18.0). There was no significant difference between *P. boops* and *P. tuberosa* (contrast, $P = 0.43$).

Pupa

After manipulation, the final instar larva pupated at the same place where she killed the spider. The angle at which pupa was oriented differed significantly among wasp species (ANOVA, $F_{2,16} = 137.9$, $P < 0.0001$, Fig. 4b), but not among spider species (ANOVA, $F_{2,16} = 0.5$, $P = 0.65$): pupa of *S. pallipes* was vertically oriented at an average angle of 81° (SD = 10.5), whereas pupa of *P. boops* and *P. tuberosa* was horizontally oriented at an average angle of 172.5° (SD = 11.5). There was a marginally significant difference between *P. boops* and *P. tuberosa* (contrast, $P = 0.047$). The place where pupa was produced differed significantly among wasp species (GLM-b, $X^2{}_2 = 11.3$, $P = 0.003$), but not among spider species (GLM-b, $X^2{}_2 = 4.5$, $P = 0.1$). The cocoon of *Polysphincta* pupas were built inside the 3D web surrounded by a dense tangle (55%, $N = 11$) or outside the main web. These pupae were built at the base of tree twigs (27%) or in a corner of the experimental arena (18%). The pupa of *Sinarachna* always took a position at the middle of web (100%, $N = 11$). There was no significant difference between *P. boops* and *P. tuberosa* (contrast, $P = 1$).

The cocoons enclosing pupa were fusiform, diaphanous white or yellowish, with an open and sparse construction in *Polysphincta* (Fig. 5a,b) and narrowly fusiform, light brownish–yellowish, and densely woven with a tight outer cover of coarse fibres in *Sinarachna* (Fig. 5c). There was a significant difference among the thread density of the cocoon wall of the three wasp species (GLS, $F_{2,13} = 173$, $P < 0.0001$): the cocoon wall of *S. pallipes* was significantly more dense than those of both *Polysphincta* species, and the cocoon wall of *P. tuberosa* was significantly more dense than that of *P. boops* (contrast, $P < 0.0001$, Fig. 4c).

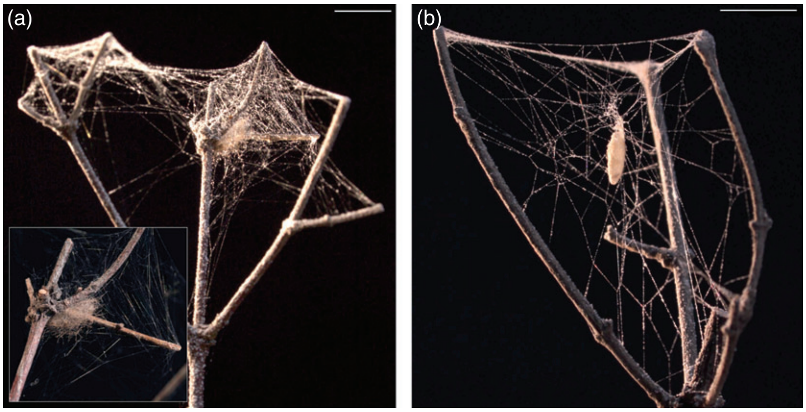


Fig. 2. (a) Cocoon web of *Araniella ophistographa* induced by *Polysphincta tuberosa*. Insert: *P. boops*. (b) Cocoon web built by the spider host *Araniella displicata* under manipulation by *S. pallipes*. Scale = 1 cm.

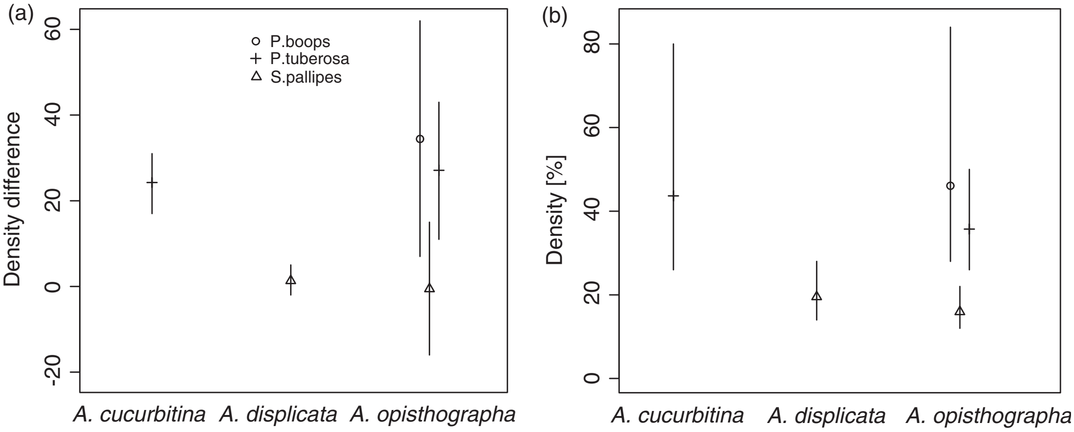


Fig. 3. Comparison of the cocoon web architecture. (a) Difference between thread density surrounding pupa and the web margin. (b) Thread density surrounding pupa. Points are means and whiskers are 95% confidence intervals.

Discussion

We found that investigated *Polysphincta* and *Sinarachna* wasps are associated with spiders of the genus *Araniella*, in particular. The wasps have been reported to be associated with other areneid spider hosts (e.g. Aubert, 1969; Fitton *et al.*, 1987, 1988; Gauld & Dubois, 2006), but records are sporadic and some of them should be revised. *Araniella* spiders were most frequently attacked by parasitoids probably because they were the most dominant species of orb-web weaving spiders in the study spider communities. In all cases, the most abundant species from the spectrum of available hosts was the most parasitised one. This is in contrast with a previous study (Korenko *et al.*, 2011), where the *Zatypota percontatoria* (Müller) wasp preferred the most abundant host species from a spectrum of several suitable hosts, and was able to shift the host based on its quality and abundance. Although there are old notes on *P. boops* and *S. pallipes* to attack spiders of the family Theridiidae (3D web weavers) (Brischke, 1877; Nielsen, 1928), it is likely misidentification owing to pronounced differences in life history and web architecture (2D versus 3D web). This is supported by our field observations (S. Korenko, unpublished data) as no case out of 108 theridiid spiders found in Bottonasco was parasitised by the genus *Polysphincta* and *Sinarachna*. Theridiid spiders are parasitised by wasps of the genus *Zatypota*, instead (Korenko & Pekár, 2011; S. Korenko, unpublished data). Polysphinctines must have evolved a narrow association fine-tuned to host biology (foraging, web building, and antipredatory behaviour) to effectively use specific host biological traits (e.g. behavioural and physiological) for their benefit. They evolved adaptations to attack the 2D web. Their attack strategy is so specific (Takasuka & Matsumoto, 2011) that a parallel association with 3D web weavers (Theridiidae) is unlikely.

The study of the web-building activity of three *Araniella* species (*A. displicata*, *A. cucurbitina*, and *A. ophistographa*) revealed that non-parasitised spiders build a 3D web only as adult females to guard its cocoon. Parasitoids thus might be evoking an innate behaviour in the manipulated host. The behaviour might be naturally used only by adult females, not by juveniles which are parasitised and killed before reaching adulthood.

We studied several traits of web-manipulation. A few architectural characteristics of the induced ‘cocoon web’ were similar between manipulated spider hosts: 2D web was changed to a 3D structure and the absence of the sticky threads. In other traits, such as thread density, or pupa orientation, the hosts differed. The variability was explained by the wasp

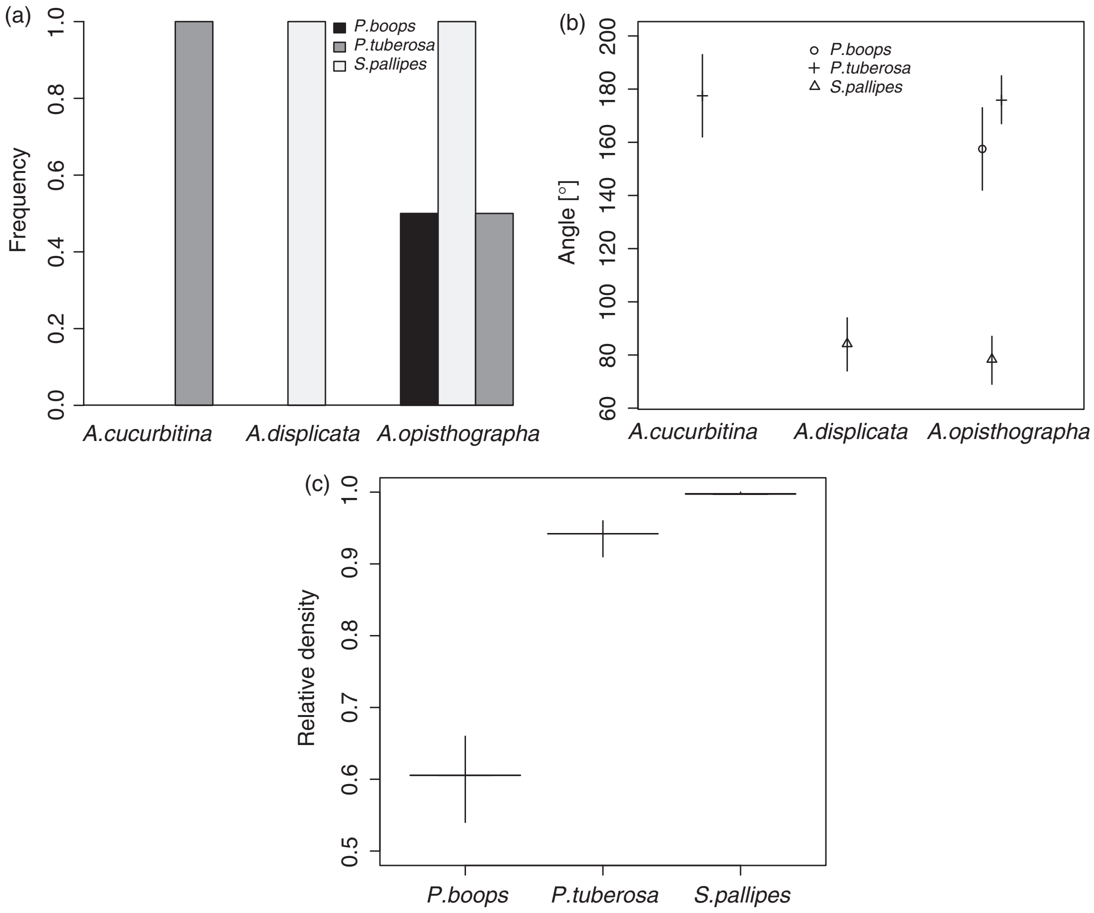


Fig. 4. Comparison of the pupa traits. (a) Relative host frequency of the three wasp species. (b) Angle of orientation. (c) Silk density of the cocoon wall. Points (lines or bars) are means and whiskers are 95% confidence intervals.

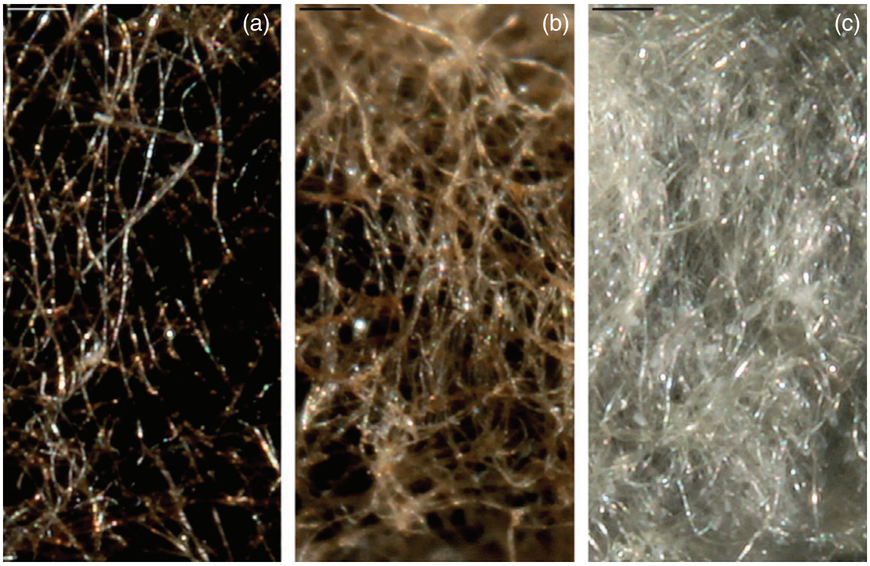


Fig. 5. Cocoon wall of *Polysphincta boops* (a), *P. tuberosa* (b), and *Sinarachna pallipes* (c). Scale = 0.1 mm.

species, not by the spider species. More specifically, wasps of the genus *Polysphincta* manipulated spider host in a different way than *S. pallipes* wasps. For example, all manipulated spiders have been forced to find some 'safe' place, a place protected from all sides by spider silk or a habitat structure, such as tree twig, in the provided space, but position of this safe place differed. Spiders manipulated by *Sinarachna* chose the safest place in the centre of web, whereas spiders manipulated by *Polysphincta* chose a place beneath the base of tree twigs or in the corner of the experimental arena. How could such a difference be explained? We presume that the two parasitoid genera use different chemicals (e.g. neuromodulators), which evoke specific changes in *Araniella* host behaviour. The lack of a difference between two *Polysphincta* species is probably because of their phylogenetic relatedness as compared with *Sinarachna*. According to phylogenetic analysis (Gauld & Dubois, 2006), wasps of the genus *Polysphincta* belong to the taxonomical clade 'F', which also includes species of the genera *Acrotaphus* and *Hymenoepimecis*. The manipulation behaviour observed in these wasps has similar characteristics to that by *Polysphincta* (Gonzaga *et al.*, 2010; Gonzaga & Sobczak, 2011; this study), but differs considerably from manipulations induced by wasps of other taxonomic clades (S. Korenko, unpublished data).

Overall the three wasp species use a different strategy for protection of themselves during pupation. The pupa of *Polysphincta* is protected by dense tangled silk threads produced by the manipulated spider. The wall of the pupa cocoon is thinner and sparser. In contrast, the pupa of *S. pallipes* is surrounded by fewer threads produced by the spider, but is enclosed in a cocoon with a strong wall. So there seems to be a negative correlation between the web and cocoon density. Such a correlation is achieved via trade-off in investment to host manipulation versus the cocoon production. It seems that both strategies are similarly effective in protection. A comparative study is needed to explain whether the lack of host manipulation as shown by *S. pallipes* led to evolution of stronger protection by the wasp larva, or advanced host manipulation as shown by *Polysphincta* has led to evolution of weaker pupa protection.

Poulin (1995) set up four criteria for consideration of behaviour manipulation to be adaptive in parasites: (i) they must be complex, (ii) they must show signs of a purposive designs, (iii) they arose independently in several lineages of hosts or parasites and (iv) they must be shown to increase the fitness of the parasite. All these criteria seem to satisfy that web architecture manipulation induces polysphinctine parasitoids, although it has not been demonstrated yet if this trait is adaptive. Evidence for increasing the survival of parasitoid offspring induced by the host's behaviour was reported by Matsumoto (2009). He observed that the induced webbing had been protecting parasitoid larva against ant predators. Similar induction of the 3D web in the pupa surrounding, as reported here, was also reported for acrocerid parasitoids (Kehlmaier *et al.*, 2012). So far it is not known whether modification of the spider web architecture is effective against enemies of polysphinctines, including hyperparasitoids. We plan to address this topic in the future. The *Sinarachna* genus belongs to clade 'D' which includes wasps of the *Sinarachna/Reclinervellus* genus complex (Gauld & Dubois, 2006). *Sinarachna* induced considerably different manipulations of spider architecture when compared with those of the genus *Reclinervellus*. *Sinarachna* induced a change in the 2D web to a 3D symmetric structure where the pupa hangs in its centre; *Reclinervellus* only rebuilds and reinforces the innate 2D web and pupa is nested in this 2D structure (e.g. Matsumoto & Konishi, 2007). This marked difference in the web architecture change induced by parasitoid larva inside one clade might be explained as follows: *Sinarachna* is associated with hosts with a different life history (including web architecture and antipredatory behaviour) than *Reclinervellus*. *Sinarachna* attacks Palearctic 2D web weavers which do not use stabilimentum (a conspicuous silk structure in the spider web having a variety of shapes (Foelix, 1996)). In contrast, the *Reclinervellus* is mostly a tropical genus associated with 2D web weavers of genera *Cyclosa* and *Allocyclosa*, which use the stabilimentum as a camouflage protecting spiders against potential predators (Neet, 1990). Incorporation of the parasitoid pupa onto the web with stabilimentum provided protection for wasp pupa as well (Matsumoto & Konishi, 2007). The trophic niche of the three investigated wasp species overlapped both in terms of taxonomy and body size of hosts. The overlap in size is given by similar body size of the parasitoids (Fitton *et al.*, 1988). The host spectrum is also restricted taxonomically in other related wasp species. The small genus *Acrotaphus* is associated with *Argiope* (Araneidae) (Gonzaga & Sobczak, 2011; Eberhard, unpublished data). The species-rich genus *Hymenoepimecis* is associated with a wider spectrum of hosts from the families Nephilidae, Tetragnathidae and Araneidae, but their association is strictly species specific. *Nephila clavipes* Linnaeus is attacked by *H. robertsae* Gauld and *H. bicolour* (Brulle) (Gonzaga *et al.*, 2010). Tetragnathid genus *Leucauge* is attacked by *H. japi* Sobczak *et al*. [*L. roseosignata* (Mello-Leitão)], *H. argyraphaga* Gauld [*L. argyra* (Walckenaer)], and by *H. tedfordi* Gauld [*L. mariana* (Taczanowski)] (Eberhard, 2000a,b, 2001; Sobczak *et al.*, 2009; pers. comm.) and *Araneus omnicolor* (Keyserling) is attacked by *H. veranii* Loffredo & Penteado-Dias (Gonzaga & Sobczak, 2007). However, spiders of all three families are orb-web weavers and the general architecture of their innate web is similar, the changes in web architecture induced by in particular *Hymenoepimecis* differ considerable. Nephilids are forced to build 3D tangle web, tetragnathids are forced to build cocoon web in 2D and araneids are forced to add lines to the innate 2D web and transform it to a 3D-tangled structure (Eberhard and Gonzaga, pers. comm.). Manipulation of the web architecture induced by investigated wasps seems to be the most similar to manipulation induced by nephilids. We hypothesise that this discrepancy between taxonomy and behaviour could be a consequence of higher importance of the host's natural history than taxonomical relatedness.

The manipulations induced in web-building spiders reported so far appear to have in common the adaptive value: silk threads in different shapes provide shelter for parasitoid pupa against environment and enemies. We need more examples on behavioural manipulation in spiders by polysphinctine species in order to understand the evolution of the host-parasitoid system in polysphinctines.

Acknowledgements

We would like to thank Sanne Beikes from Ecological Fruit growing Company Cascina Rosso in Bottonasco for permitting us to study spiders and their parasitoids at their ecological orchards; and Mauro Paschetta from Università di Torino, Martin Štefánik from Museum of Trenčín and Barborka Korenková for help with collecting of spiders. S.K. was supported by the project of European Science Foundation and Ministry for Education and Youth of the Czech Republic CZ.1.07/2.3.00/30.0040.

References

Aubert, J.F. (1969) *Les ichneumonides ouest-paléarctiques et leurs Hôtes, 1*. Centre National de la Recherche Scientifique, Paris, France.

Brischke, C.G. (1877) Hymenopterologische notizen. *Deutsche Entomologische Zeitschrift*, **21**, 285–287.

Eberhard, W.G. (2000a) Spider manipulation by a wasp larva. *Nature*, **406**, 255–256.

Eberhard, W.G. (2000b) The natural history and behavior of *Hymenoepimecis argyraphaga* (Hymenoptera: Ichneumonidae) a parasitoid of *Plesiometa argyra* (Araneae: Tetragnathidae). *Journal of Hymenoptera Research*, **9**, 220–240.

Eberhard, W.G. (2001) Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). *Journal of Arachnology*, **29**, 354–366.

Eberhard, W.G. (2010) New types of behavioral manipulation of host spiders by a parasitoid wasp. *Psyche*, **2010**, Article ID 950614.

Fitton, M.G., Shaw, M.R. & Austin, A.D. (1987) The Hymenoptera associated with spiders in Europe. *Zoological Journal of the Linnean Society*, **90**, 65–93.

Fitton, M.G., Shaw, M.R. & Gauld, I.D. (1988) Pimpline ichneumonflies. *Handbooks for the Identification of British Insects*, **7**, 1–110.

Foelix, R. (1996) *Biology of Spiders*. Oxford University Press, New York, New York.

Franceschi, N., Bollache, L., Dechaume-Moncharmont, F., Cornet, S., Bauer, A., Motreuil, S. *et al.* (2010) Variation between populations and local adaptation in acanthocephalan-induced parasite manipulation. *Evolution*, **64**, 2417–2430.

Gauld, I.D. & Dubois, J. (2006) Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae), a taxonomic revision of spider ectoparasitoids. *Systematic Entomology*, **31**, 529–564.

Gonzaga, M.O. & Sobczak, J.F. (2007) Parasitoid-induced mortality of *Araneus omnicolor* (Araneae, Araneidae) by *Hymenoepimecis* sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. *Naturwissenschaften*, **94**, 223–227.

Gonzaga, M.O. & Sobczak, J.F. (2011) Behavioral manipulation of the orb-weaver spider *Argiope argentata* (Araneae: Araniedae) by *Acrotaphus chedelae* (Hymenoptera: Ichneumonidae). *Entomological Science*, **14**, 220–223.

Gonzaga, M.O., Sobczak, J.F., Penteado-Dias, A.M. & Eberhard, W.G. (2010) Modification of *Nephila clavipes* (Araneae Nephilidae) webs induced by the parasitoids *Hymenoepimecis bicolor* and *H. robertsae* (Hymenoptera Ichneumonidae). *Ethology Ecology and Evolution*, **22**, 151–165.

Isaia, M., Beikes, S., Paschetta, M., Sarvajayakesavalu, S. & Badin, G. (2010) Spiders as potential biological controllers in apple orchards infested by *Cydia* spp. (Lepidoptera: Tortricidae). *Proceedings of the 24th European Congress of Arachnology, 25–29 August 2008, Bern*, pp. 79–88.

Kehlmaier, C., Michalko, R. & Korenko, S. (2012) *Ogcodes fumatus* (Diptera: Acroceridae) reared from *Philodromus cespitum* (Araneae: Philodromidae), and first evidence of *Wolbachia* alphaproteobacteria in Acroceridae. *Annales Zoologici*, **62**, 281–286.

Korenko, S. & Pekár, S. (2011) A parasitoid wasp induces overwintering behaviour in its spider host. *PLoS One*, **6**, e24628.

Korenko, S., Michalková, V., Zwakhals, K. & Pekár, S. (2011) Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid (Hymenoptera: Ichneumonidae). *Journal of Insect Science*, **11**, 101.

Korenko, S., Štefánik, M., Trnik, M. & Gajdoš, P. (2012) Spiders (Araneae) in the territory of Žihľavník–Baske (Strážovské vrchy Mts., Slovakia). *Folia faunistica Slovaca*, **17**, 309–315 (in Slovak, with English abstract).

Levi, H.W. (1974) The orb-weaver genera *Araniella* and *Nuctenea* (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology*, **146**, 291–316.

Matsumoto, R. (2009) "Veils" against predators: modified web structure of a host spider induced by an ichneumonid parasitoid, *Brachyzapus nikkoensis* (Uchida) (Hymenoptera). *Journal of Insect Behavior*, **22**, 39–48.

Matsumoto, R. & Konishi, K. (2007) Life histories of two ichneumonid parasitoids of *Cyclosa octotuberculata* (Araneae), *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). *Entomological Science*, **10**, 267–278.

Maure, F., Daoust, S.P., Brodeur, J., Mitta, G. & Thomas, F. (2013) Diversity and evolution of bodyguard manipulation. *Journal of Experimental Biology*, **216**, 36–42.

Moore, J. (2002) *Parasites and the Behavior of Animals*. Oxford University Press, Oxford, New York.

Moore, J. (2013) An overview of parasite-induced behavioral alterations–and some lessons from bats. *Journal of Experimental Biology*, **216**, 11–17.

Neet, C.R. (1990) Function and structural variability of the stabilimenta of Cyclosa insulana (Costa) (Araneae, Araneidae). *Bulletin of the British Arachnological Society*, **8**, 161–164.

Nentwig, W., Blick, T., Gloor, D., Hänggi, A. & Kropf, C. (2013) *Spiders of Europe, version 1.2013* [WWW document]. URL www.araneae.unibe.ch [accessed on 1 April 2013].

Nielsen, E. (1928) A supplementary note upon the life histories of the Polysphinctas (Hym. Ichneum.). *Entomologiske Meddelelser*, **16**, 152–155.

Pekár, S. & Brabec, M. (2009) *Modern Analysis of Biological Data. 1. Generalised Linear Models in R*. Scientia, Praha, Czech Republic.

Pinheiro, J.C. & Bates, D.M. (2000) *Mixed-Effects Models in S and S-PLUS*. Springer, Berlin, Germany.

Poulin, R. (1995) "Adaptive" changes in the behaviour of parasitized animals: a critical review. *International Journal for Parasitology*, **25**, 1371–1383.

Poulin, R. (2010) Parasite manipulation of host behavior: an update and frequently asked questions. *Advances in the Study of Behaviour*, **41**, 151–186.

R Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria [WWW document]. URL http://www.R-project.org/ [accessed on 1 February 2013].

Roberts, M.J. (1985) *The Spiders of Great Britain and Ireland*, Vol. 1. Harley Books, Colchester, U.K.

Smith, E.P. (1982) Niche breadth, resource availability, and inference. *Ecology*, **63**, 1675–1681.

Sobczak, J.F., Loffredo, A.P.S., Penteado-Dias, A.M. & Gonzaga, M.O. (2009) Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. *Journal of Natural History*, **43**, 2691–2699.

Tain, L., Perrot-Minnot, M. & Cézilly, F. (2007) Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on brain serotonergic activity in two congeneric hostspecies. *Biology Letters*, **3**, 68–71.

Takasuka, K. & Matsumoto, R. (2011) Lying on the dorsum: unique host-attacking behaviour of *Zatypota albicoxa* (Hymenoptera, Ichneumonidae). *Journal of Ethology*, **26**, 203–207.

Thomas, F., Adamo, S. & Moore, J. (2005) Parasitic manipulation: where are we and where should we go? *Behavioural Processes*, **68**, 185–199.

Townes, H. (1969) The genera of Ichneumonidae, part 1. *Memoirs of the American Entomological Institute*, **11**, 1–300.

Yu, D.S. & Horstmann, K. (1997) A catalogue of world Ichneumonidae (Hymenoptera). *Memoirs of the American Entomological Institute*, **58**, 1–1558.

Zwakhals, K. (2006) The European species of the genera *Zatypota* and *Sinarachna* (Hymenoptera: Ichneumonidae, Pimplinae, Polysphinctini). *Entomologische Berichten*, **66**, 34–37.

Accepted 22 July 2013

Supplement 6

Korenko S, Korenková B, Satrapova J, Hamouzová K, Belgers D. 2015. Modification of *Tetragnatha montana* (Araneae, Tetragnathidae) web architecture induced by larva of the parasitoid *Acrodactyla quadrisculpta* (Hymenoptera, Ichneumonidae, *Polysphincta* genus-group). Zoological Studies 54, 40.

Citation report:
Web of Science^{TM}: 3
Scopus/Google Scholar: 4

ISI WOS:
Zoological Studies
IF$_{(2015)}$ = IF = 0,885
Quartile $_{(2015)}$: Zoology – Q3

Korenko *et al. Zoological Studies* (2015) 54:40
DOI 10.1186/s40555-015-0119-6

Zoological Studies
a SpringerOpen Journal

RESEARCH Open Access

Modification of *Tetragnatha montana* (Araneae, Tetragnathidae) web architecture induced by larva of the parasitoid *Acrodactyla quadrisculpta* (Hymenoptera, Ichneumonidae, *Polysphincta* genus-group)

Stanislav Korenko[1*], Barbora Korenková[1], Jitka Satrapová[1], Katerina Hamouzová[1] and Dick Belgers[2]

Abstract

Background: The polysphinctine wasp, *Acrodactyla quadrisculpta*, is a koinobiont ecto-parasitoid of spiders and is narrowly associated with the biology of its spider hosts. The larva, attached to the dorsal side of the abdomen, develops while the spider continues foraging. Shortly before pupation, the parasitoid larva manipulates the web-building activity of the host in order to construct a safe shelter against natural elements and predators during parasitoid pupation.

Results: *A. quadrisculpta* was associated exclusively with the orb web weaving spiders *Tetragnatha montana*, with a parasitism incidence of 19%. The manipulated spider constructed a unique cocoon web that provided strong mechanical support for the parasitoid's pupal cocoon. The cocoon web consisted of one highly reinforced main thread, tensioned in 60% of cases by a reinforced side thread. The wasp cocoon, square in cross-section, was fastened along its length to the main cocoon thread.

Conclusions: The wasp *A. quadrisculpta* was exclusively associated with an orb-weaving spider *T. montana* in the family Tetragnathidae. The alteration of the web architecture of *T. montana* induced by the larva *A. quadrisculpta* was unique and species specific.

Keywords: Host-parasitoid interaction; Host manipulation; Spider host; Ephialtini

Background

Parasitic organisms have often evolved the ability to manipulate the host phenotype, including its morphology, physiology and behaviour, for their own benefit (Moore 2002). Polysphinctine wasps (the *Polysphincta* genus-group sensu Gauld and Dubois 2006), which are all external parasitoids of spiders, exhibit a unique trait within the Ichneumonidae in terms of development (Fitton et al. 1987). Their larva is attached to the dorsal side of the spider's opisthosoma/prosoma, where it develops while the spider continues foraging. Shortly before pupation, some of the parasitoids (final instar larvae) manipulate the web-spinning activity of the host in order to establish effective protection against enemies and the environment (e.g. Eberhard 2000a, 2013; Matsumoto 2009; Korenko and Pekár 2011; Korenko et al. 2014). These effects of the larva are apparently due to chemical products that are introduced into the spider (Eberhard 2010).

A few studies have been devoted to the behavioural manipulation of orb web building spiders. Eberhard (2000a, b, 2001, 2013) and Sobczak et al. (2009) studied parasitoids associated with orb web building spiders from the family Tetragnathidae; Gonzaga et al. (2010) described the manipulation of spiders from the family Nephilidae; and Gonzaga and Sobczak (2007, 2011), Eberhard (2013) and Korenko et al. (2014) studied the manipulation of spiders from the family Araneidae. The studies revealed that the manipulated spider modifies

---

* Correspondence: korenko.stanislav@yahoo.com
[1] Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic
Full list of author information is available at the end of the article

the architecture of its web in various ways. The orb web is modified to the 'cocoon web' (termed by Eberhard (2000a, b) for the first time) when some of its components are reduced (e.g. web spiral, radii) and others are reinforced (e.g. radii, central hub, frame) or multiplied (e.g. threads). The cocoon web is stronger and effectively designed to provide more durable support for the wasp's cocoon than the normal web (e.g. Eberhard 2000a, b). The tetragnathid spider *Leucauge argyra* (Walckenaer, 1841) is manipulated by the larva of *Hymenoepimecis argyraphaga* Gauld, 2000 to build a web which consists of a low number of radial threads radiating in a plane from a central hub; the architecture of the cocoon web remains two-dimensional (hereafter 2D) (Eberhard 2000a, b, 2001). A similar 2D cocoon web is built by the related species *Leucauge roseosignata* Mello-Leitão, 1943 manipulated by *Hymenoepimecis japi* Sobczak, Loffredo, Penteado-Dias and Gonzaga, 2009 (Sobczak et al. 2009). A similar 2D architecture of the cocoon web, but protected by the 3D structure of the tangle positioned below the hub, was recently described in the spider hosts *Leucauge mariana* (Keyserling, 1881) manipulated by *Hymenoepimecis tedfordi* Gauld, 1991 (Eberhard 2013) and *Leucauge volupis* (Keyserling, 1893) manipulated by *Hymenoepimecis jordanensis* Loffredo & Penteado-Dias, 2009 (Gonzaga et al. 2014). In contrast, the larva of *Eruga gutfreundi* Gauld, 1991 induced the same host (*L. mariana*) to build a completely different three-dimensional cocoon web (hereafter 3D) (Eberhard 2013).

Cocoon webs of spiders from the families Araneidae and Nephilidae are mostly 3D. Three-dimensional cocoon webs are induced in araneid spider hosts in which the webs of unparasitised individuals are only 2D (Gonzaga and Sobczak 2011; Korenko et al. 2014). The cocoon web for the *Acrotaphus* wasp built by araneid hosts *Argiope argentata* (Fabricius, 1775) is 3D composed of non-sticky threads (Gonzaga and Sobczak 2011). Further, the wasps *Sinarachna pallipes* (Holmgren, 1860), *Polysphincta tuberosa* (Gravenhorst, 1829) and *P. boops* Tschek, 1868 manipulate spiders of the genus *Araniella* in a similar way (Korenko et al. 2014). All three species induced the production of a 3D structure instead of a 2D web, but thread density, thread concentration and the location of pupa on the cocoon web differed among species. The normal web of nephilid spiders consists of a 2D orb web and a 3D tangle of barrier threads at the side and its resting web is only 3D. Both the orb and the 3D tangle of the normal web are rebuilt by the manipulated spider to form the cocoon web, whose architecture is similar to the 3D resting web (Gonzaga et al. 2010; Korenko, unpublished data). The nephilid spider *Nephila clavipes* (Linnaeus, 1767) was manipulated by *Hymenoepimecis robertsae* Gauld, 1991 and *H. bicolor* (Brulle, 1846) to build a cocoon web which consisted of a hub-like platform (part of the rebuilt orb web), from which the cocoon was suspended, and a 3D structure of non-adhesive threads of variable density. The radii and the spiral of the orb web were mostly reduced, and the wasp's cocoon was attached to the reduced orb and the barrier threads on the side (Gonzaga et al. 2010). An interesting modification of the web architecture of orb web weavers of the genera *Cyclosa* (Araneidae), in which the 2D orb web retains its 2D structure after modification (the suppression of adhesive components and a change in the radii structure), was documented in wasps of the genus *Reclinervellus*, which used the web stabilimentum (the structure built by the spider serving as camouflage) as the same camouflage for its cocoon (Matsumoto and Konishi 2007). The number of descriptions of web architecture alterations induced by polysphinctine final instar larvae has increased in the last few decades, but no detailed study of the web alteration induced by the *Acrodactyla* wasp has been performed.

We studied the interaction between the parasitoid wasp *Acrodactyla quadrisculpta* (Gravenhorst, 1820) and its spider host *Tetragnatha montana* Simon 1874 and described in detail the manipulation of web architecture induced by the parasitoid larva. *A. quadrisculpta* is reported from most of the Holarctic, and the species is known to be associated with the following tetragnathid spiders: *T. montana*, *T. obtusa* Koch, 1837 and *T. extensa* (Linnaeus, 1785) (Nielsen 1937; Fitton et al. 1988). However, very little is known about its biology and its interaction with the spider host (only Nielsen 1937 and Belgers et al. 2013).

Methods

Collecting and field investigation

The spiders (*T. montana*) and polysphinctine parasitoids (*A. quadrisculpta*) were studied in a deciduous forest close to Fondotoce di Verbania (Italy, Lake Maggiore, 45° 56′ 16″ N, 8° 29′ 37″ E) on 30 and 31 October 2012 and in a Norway spruce stand (*Picea abies*) (The Netherlands, Blauwe Kamer, 51° 94′ 40″ N, 5° 61′ 88″ E) on 31 March 2012. Spiders were collected by beating tree canopies and undergrowth (30 to 200 cm above the ground) with a square-shaped beating net (1-m^2 area) placed beneath. The collected specimens were fixed in 70% or pure alcohol and identified to species/genus level using Nentwig et al. (2014) and classified according to foraging guild (orb web, tangle web weavers, foliage runners, ambushers and stalkers) (Uetz et al. 1999). The spider nomenclature follows the World Spider Catalog (2014). The identification of juvenile spider hosts was confirmed by rearing spiders to adulthood (Italian specimens) and through analysis of DNA from the remains of the spider (from Dutch specimens), using the procedures described by Miller et al. (2013). Parasitoid specimens were reared to adulthood for identification in the laboratory. Adult wasps were identified using Fitton et al.

(1988). The nomenclature of the polysphinctines follows Fitton et al. (1988) and Yu and Horstmann (1997). Voucher specimens were deposited in the personal collection of the first author and Kees Zwakhals (Netherland).

The composition of the potential host spectrum, the incidence of parasitism and the composition of the parasitoid community were recorded in the Italian locality. The incidence of parasitism was defined as the total number of cases of parasitised spiders divided by those collected by beating at the same site and time. The average parasitism incidence was the average of all three samples.

Behavioural study

Parasitised and unparasitised spiders of the genus *Tetragnatha* for laboratory study were collected by the same method as mentioned above. Specimens were taken alive to the laboratory for the investigation of their web building behaviour. Italian spider hosts were kept at room temperature (22°C ± 3°C) under a light/day regime of 12:12 and fed with flies *Drosophila melanogaster* Meigen, 1830; *Drosophila hydei* Sturtevant, 1921; small crickets *Acheta domestica* (Linnaeus, 1758); and larvae of mealworm *Tenebrio molitor* Linnaeus, 1758. Parasitised spiders from the Netherlands ($N = 3$) were kept at room temperature (20°C ± 2°C) under a light/day regime of 12:12 but were not fed in captivity.

The web architecture of both unparasitised spiders ($N = 20$) and parasitised spiders ($N = 22$ from Italy, $N = 3$ from the Netherlands) was analysed (web dimensionality, frequency of orb/resting web production, number of spirals and radii on orb web and its web orientation) and differences between them were identified. Spiders were placed singly into narrow plexiglass experimental arenas (frame 220 × 220 mm, depth 20 mm) with paper tape on four sides of the frame so that the spiders could build webs, or square glass experimental arenas (base 400 × 400 mm, height 550 mm) with a 3D construction (a cube-shaped frame with a side length of 30 cm) in the middle to provide support for webs. Spiders from the Netherlands were placed separately in small glass containers (450 ml, including a little moist moss) closed with a lid perforated with some small holes. Narrow experimental arenas where the web was built vertically were used for photo documentation, and large square arenas were used as control to recognize if there were any differences in spider behaviour between the two types of provided space (3D vs. 2D).

The web building behaviour of unparasitised spiders was recorded at 1- to 2-day intervals for 3 months. The web building behaviour of parasitised spiders was observed until the larva had consumed the spider and pupated. The web structures of both parasitised and unparasitised spiders were photographed using a Canon EOS 500 digital camera with Canon EFS 18- to 55-mm objective (Canon, Tokyo, Japan), 0.28 m/0.9 ft, and spider behaviour under the influence of the parasitoid larva and the most important parts of the parasitoid life history were recorded using a Canon HFX 10 camcorder with a 6× Model CM-3500 35-mm microscopic lens (Canon, Tokyo, Japan).

Measurement and statistics

Measurements of thread diameters were performed using NIS Elements Documentation Software on a Zeiss Stemi SV 11 microscope (Carl Zeiss, Thornwood, NY, USA) with a Nikon DS-2Mv camera (Melville, NY, USA). The diameter of the main and the side threads of the cocoon web and the diameter of the normal web threads taken from other parts of experimental arenas (taken before the period when the manipulation of the spider behaviour appeared) were measured. Obtained data were +1 log transformed. The *T*-test was used to reveal differences in thread diameter among threads sampled from different parts of the normal and altered web. The Kolmogorov and Smirnov test (KS) was used to test whether the data were sampled from a Gaussian distribution. The Student-Newman-Keuls method (SNK) was used as a *post hoc* test. The DiGraphPad InStat software v. 3.06 was used.

Results

Host community and incidence of parasitism

The orb web weaver *T. montana* was the most abundant spider species at the investigated Italian site, representing 53.6% of all collected spider individuals (from $N = 349$). The average incidence of parasitism of *T. montana* was 19.1% (Table 1). All parasitised spiders were juveniles with an average body length of 4.62 mm ($N = 50$, SD = 1.11). All parasitoid wasps reared from *T. montana* were *A. quadrisculpta* ($N = 9$).

Web architecture of unparasitized *T. montana*

Unparasitised spiders built an orb web (Figure 1a) which was typical of those of other species of *Tetragnatha*. The web was oriented horizontally ($N = 2$) in the cubical arena (when 3D space was provided) or vertically ($N = 18$) when only a narrow experimental arena was provided (only 2D space was provided). In the period of satiation (no capturing activity), spiders built the 'resting web', which consisted of several scattered single threads forming a sparse tangle structure in the corner of the arena (Figure 1b). A similar 'resting web' architecture (a few scattered threads) was also observed during spider moulting in captivity ($N = 2$). The normal orb web (for capturing) was attached by anchor threads, consisting of one reinforced thread anchored at several anchoring points to the frame of the experimental arena (Figure 1c).

Table 1 Relative spider host abundance (Ab.), average incidence of parasitism (PR) and reared wasp species

Family	Foraging guild	*N*	Ab. (%)	PR (%)	Associated parasitoid
Tetragnathidae	Orb web weavers	187	53.6	19.1	*Acrodactyla quadrisculpta*
Araneidae	Orb web weavers	6	1.7	?	*Zatypota picticollis*
Theridiidae	Tangle web weavers	63	18.1	23.5	*Zatypota percontatoria*
Anyphaenidae	Foliage runners	41	11.7	0	-
Clubionidae	Foliage runners	8	2.3	0	-
Thomisidae	Ambushers	29	8.3	0	-
Philodromidae	Ambushers	8	2.3	0	-
Salticidae	Stalkers	7	2.0	0	-
Total		349	100	12.5	

? - incidence of parasitism was not calculated because of the low number of collected spiders.

Interaction with parasitoid larva

The wasp's final instar larva induced subsequent unique changes in the webbing behaviour of *T. montana* (Table 2). The spider under manipulation built a specific cocoon web ($N = 12$). In all cases, the cocoon web contained one strong horizontally oriented thread - the 'main thread'; in 60% of cases, this thread was tensioned by a 'side thread' (Figure 1d). Both threads of the cocoon web were reinforced 50 to 60 times by a layer of silk (Additional file 1: Video 1, s 01). The building of the cocoon web started 8 to 9 h before the larva killed the spider host and began to suck it dry ($N = 3$). When the modified web was finished, the spider took a position at approximately the middle of the main thread close to

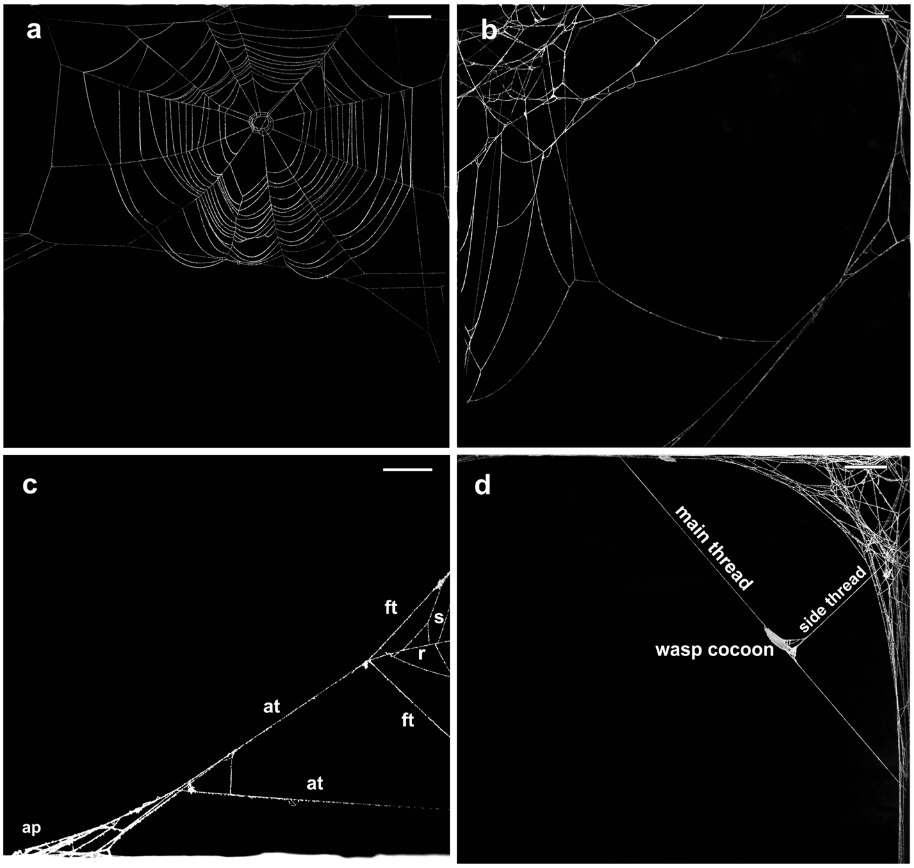


Figure 1 Normal orb web and induced cocoon web. Normal hunting orb web of *T. montana* **(a)**. Resting web when orb web was not presented **(b)**. Detail of anchoring of normal orb web (ap, anchor point; at, anchor thread; ft, frame thread; r, radial thread; s, spiral) **(c)**. Induced cocoon web consisting of the main thread and side thread **(d)**. Scale = 20 mm **(a, b)**, 5 mm **(c)** and 10 mm **(d)**.

Table 2 Behaviour table of host-parasitoid interactions in laboratory

Day	Time	Observed behaviour	Video
1st (30 December 2012)	21:00	Changes in spider behaviour; the spider was very active, webbing in several places of the experimental arena.	-
2nd	3:00	One thread was chosen by the spider. The thread was reinforced 58 times during the next 2 h. The spider rested, suspended on the thread or at the place where the thread was attached to the arena frame during the spinning of each silk layer.	s 01
	5:15	The spider took a position in the middle of the main thread of the cocoon web and died. The larva attached itself to the main thread by its dorsal tubercles. The larva began to consume the spider.	s 02
	10:40	The spider was completely consumed. The spider carcass was dropped onto the ground. The larva rested, suspended on the main thread of the cocoon web.	s 03
	12:00	The larva started to build a cocoon for pupation.	s 04
	20:00	The outer layer of the cocoon was finished, and the larva closed itself inside the cocoon. The larva spans the inner layers of the cocoon wall.	s 05
3rd	20:00	The larva finished the cocoon. After which, the larva exhibited low activity.	s 06
4th	8:00	The cocoon was completely finished, and the larva displayed no further activity.	s 06
15th	6:00	The adult wasp emerged from the cocoon.	s 07

the connection with the side thread if it was present and grasped the main thread with all its legs until it died (Additional file 1: Video 1, s 02). The larva, still attached to the opisthosoma of the already dead spider, hung itself on the main thread using its dorsal tubercles, preventing itself from falling to the ground (Additional file 1: Video 1, s 03). Seven pairs of dorsal tubercles appeared after the spider manipulation began. Tubercles were retracted repeatedly. When the spider was completely sucked dry and the spider remains were eventually discarded, the parasitoid larva (having almost doubled its size) took a position on the 'main thread' connected to it by its dorsal tubercles. Over the next 30 h, the larva spans its cocoon and later pupated inside (Additional file 1: Video 1, s 04–06). The cocoons enclosing pupae were fusiform, square in cross-section, and from white to yellowish brown (depending on the level of humidity in the experimental arena). The cocoon's long axis was along the axis of the main thread of the cocoon web. The cocoon walls were densely woven with a tight and smooth outer surface.

Adult wasps emerged after 10.2 days (SD = 0.75, $N = 6$) in Italy and 13.7 days (SD = 0.57, $N = 3$) in the Netherlands (Additional file 1: Video 1, s 07). The sex ratio of the reared wasps was 1:2 (female:male) in both Italy and the Netherlands.

Reinforcement of threads

The diameter of the main threads of the cocoon web was on average 21 μm (SD = 9.735, $N = 8$). It did not differ significantly to the diameter of the side threads of the cocoon web, which had an average diameter of 24 μm (SD = 8.734, $N = 8$; $t_{14} = 0.488$, $p = 0.63$). The diameter of normal web threads, taken from other parts of experimental arenas, averaged <5 μm (SD = 0.417, $N = 8$) and was significantly thinner than both the main threads and side threads of the cocoon web ($t_{14} = 4.888$, $p = 0.0002$ and $t_{14} = 6.150$, $p < 0.0001$) (Figure 2). All data groups passed the KS normality test. Variation between the group of control threads and groups of cocoon web threads was significantly greater than that expected by chance (SNK, $p < 0.05$). Variation between the group of main threads and the group of side threads was not significant (SNK, $p < 0.05$). The reinforcement of the main and side threads of the cocoon web was achieved by repeatedly adding silk lines on the already existing thread (Additional file 1: Video 1, s 01). The architecture of the anchoring of the cocoon webs (Additional file 1: Video 1, s 01) was similar to the anchoring of the frame threads in normal orb webs (for capturing) (Figure 1c). Both consisted of one

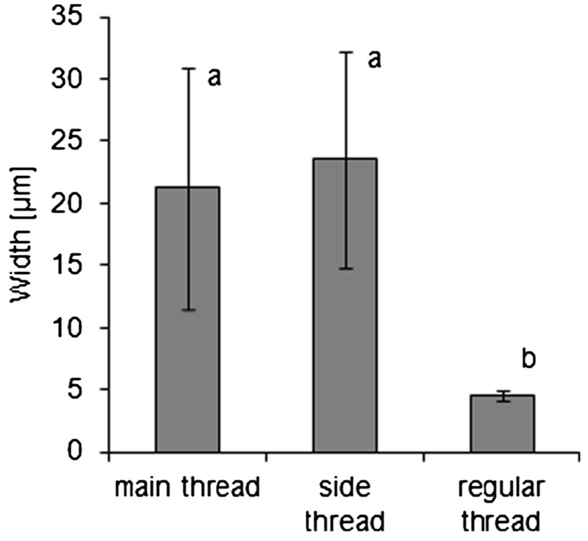


Figure 2 Differences in thread diameter among threads of the cocoon web and the normal web. Labels 'a' and 'b' mean the statistical significance of differences among groups.

reinforced thread anchored at several anchoring points to the frame of the experimental arena.

Discussion

We found that the wasp *A. quadrisculpta* was the most abundant spider parasitoid in the samples from the studied locality and was exclusively associated with the abundant web building spider *T. montana*, which represented more than 50% of the spiders in the samples. *A. quadrisculpta* is thought to be exclusively associated with spider hosts of the genus *Tetragnatha* (e.g. Nielsen 1937; Fitton et al. 1987, 1988; this study). Although araneid spiders of the genus *Araniella* were assumed to be hosts of *A. quadrisculpta* by Nielsen (1937), clear evidence linking it to this host is missing. Korenko et al. (2014) reported that all parasitoids reared on spiders of the genus *Araniella* collected during 4 years of study in Italy belonged to the genera *Sinarachna* and *Polysphincta*. On the basis of this fact, we suspect that *A. quadrisculpta* is capable of successfully attacking only spiders of the genus *Tetragnatha*, which includes several species with similar morphological, ecological and behavioural patterns. Polysphinctine wasps in Europe seem to be associated with species occurring abundantly (at least locally) (e.g. Korenko et al. 2011, 2014; Korenko et al., unpublished data). This was also observed in the studied wasp *A. quadrisculpta*. The association with abundant species seems to be effective and could be a consequence of a narrow host spectrum and the high specificity of the evolved adaptations to capture the particular spider host. We assume that the association with rare species, or species with low abundance in the community of potential hosts, could be leading to the extinction of the parasitoid population, because females, which do not find a sufficient number of suitable spider hosts, are not able to establish the next generation.

The final instar larva of *A. quadrisculpta* induced unique changes in spider web architecture that consisted of only one strong main thread, often but not always tensioned by one additional lateral thread supporting the wasp's cocoon. Similar cocoon webs, which consisted of only a few (sometimes only two) threads, were also observed in other tetragnathid spiders: *L. argyra* manipulated by *H. argyraphaga* (Eberhard 2000a) and *L. roseosignata* Mello-Leitão, 1943 manipulated by *H. japi* Sobczak et al., 2009 (Sobczak et al. 2009). Eberhard (2000a) described that the construction of the cocoon web induced by *H. argyraphaga* was nearly identical to the early steps in one subroutine of normal orb construction of *L. argyra*, and the other normal orb construction behaviour patterns were mostly repressed (Eberhard 2001). The cocoon web induced had, in most cases, no true hub (with hub loops) but rather non-spiral lines at the convergence of the radius lines, no frame lines and usually no tangle; however, in extremely reduced cases, the cocoon web consisted of only a single strong line with the cocoon suspended from the central portion (Eberhard 2000a, b, 2001; Eberhard, personal communication). These, the simplest observed cocoon webs induced by *H. argyraphaga*, had a general appearance similar to the cocoon web of *A. quadrisculpta* (one strong thread), but differed in several respects. The cocoon of *A. quadrisculpta* was horizontal, placed longitudinally along the horizontally oriented main thread. The main thread was present in all cocoon webs. In contrast, the cocoon of *H. argyraphaga* was suspended vertically on a line that was attached at the central point where the radial lines converged. The final architecture of the cocoon web induced by *H. argyraphaga* was more varied (Eberhard 2000a, b, 2001). In our study, the main thread of the cocoon web appeared to be morphologically and functionally similar to the frame thread of the normal orb web; both were strong structures that supported other parts of the web, such as the sticky spiral of the capture web (Additional file 1: Video 1, s 01) (Figure 1c).

The pupal cocoon of *A. quadrisculpta* is square in cross-section similar to those of some other polysphinctine wasps of the genera *Acrodactyla* (Fitton et al. 1987) and *Eruga* (Eberhard 2013). The morphology and position of the pupal cocoon are often species/genus/taxonomical group specific (e.g. Fitton et al. 1988; Matsumoto and Takasuka 2010; Korenko et al. 2014). This can be useful in the identification of wasp species, at least to genus level (Korenko et al. 2014; Korenko, unpublished data). In contrast, the coloration of cocoons varies. In our study, pupa cocoons of *A. quadrisculpta* placed in a dry environment were snow white (Additional file 1: Video 1, s 06), but cocoons in an environment with high humidity became orange-brown in colour (Additional file 1: Video 1, s 07).

Conclusions

The studied wasp *A. quadrisculpta* was associated only with the spider *T. montana*. This wasp species seems to be exclusively associated with orb web weaving spiders of the genus *Tetragnatha* from the family Tetragnathidae. The unique behavioural manipulation induced by the larvae of *A. quadrisculpta* was described for the first time. The architecture of the cocoon web induced by a particular wasp is species specific.

Additional file

Additional file 1: Video 1. Behavioural alteration of *T. montana* induced by *A. quadrisculpta* (the period from the first appearance of behavioural changes to the adulthood of the wasp). For a description of the behaviour, see Table 2.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SK carried out the collection of Italian specimens, conducted behavioural observations in the laboratory, participated in the video recording and photography, wrote the main text of the manuscript and participated in the statistical analysis. BK participated in the video recording and behavioural observations. JS carried out the laboratory rearing of animals and helped to draft the manuscript. KH participated in the statistical analysis. DB carried out the collection of Dutch specimens, conducted behavioural observations and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The study was supported by the European Science Foundation and Ministry for Education, Youth and Sport of the Czech Republic, project CZ.1.07/2.3.00/30.0040, and the Institutional Support Program for Long Term Conceptual Development of Research Institutions provided by the Ministry for Education, Youth and Sport of the Czech Republic.

Author details

[1]Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic. [2]Wageningen University, Droevendaalsesteeg 4, 6708 PB, Wageningen, Netherlands.

Received: 18 July 2014 Accepted: 29 April 2015

References

Belgers D, Zwakhals K, van Helsdingen P (2013) De bijzondere levensloop van de sluipwesp *Acrodactyla quadrisculpta* op de schaduwstrekspin *Tetragnatha montana* (Hymenoptera: Ichneumonidae, Araneae: Tetragnathidae). Nederl Faun Mededel 39:1–6

Eberhard WG (2000a) Spider manipulation by a wasp larva. Nature 406:255–256

Eberhard WG (2000b) The natural history and behavior of *Hymenoepimecis argyraphaga* (Hymenoptera: Ichneumonidae) a parasitoid of *Plesiometa argyra* (Araneae: Tetragnathidae). J Hymenopt Res 9:220–240

Eberhard WG (2001) Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitised by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). J Arachnol 29:354–366

Eberhard WG (2010) Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. Anim Behav 79:375–383

Eberhard WG (2013) The polysphinctine wasps *Acrotaphus tibialis*, *Eruga* ca. *gutfreundi*, and *Hymenoepimecis tedfordi* (Hymenoptera, Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. Ann Entomol Soc Am 106:652–660

Fitton MG, Shaw MR, Austin AD (1987) The Hymenoptera associated with spiders in Europe. Zool J Linn Soc-Lond 90:65–93

Fitton MG, Shaw MR, Gauld ID (1988) Pimpline ichneumon-flies. Handbooks for the Identification British Insects 7:1–110

Gonzaga MO, Sobczak JF (2007) Parasitoid-induced mortality of *Araneus omnicolor* (Araneae, Araneidae) by *Hymenoepimecis* sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. Naturwissenschaften 94:223–227

Gonzaga MO, Sobczak JF (2011) Behavioral manipulation of the orb-weaver spider *Argiope argentata* (Araneae: Araniedae) by *Acrotaphus chedelae* (Hymenoptera: Ichneumonidae). Entomol Sci 14:220–223

Gonzaga MO, Sobczak JF, Penteado-Dias AM, Eberhard WG (2010) Modification of *Nephila clavipes* (Araneae Nephilidae) webs induced by the parasitoids *Hymenoepimecis bicolor* and *H. robertsae* (Hymenoptera Ichneumonidae). Ethol Ecol Evol 22:151–165

Gonzaga MO, Moura RR, Pêgo PT, Bang DL, Meira FA (2014) Changes to web architecture of *Leucauge volupis* (Araneae: Tetragnathidae) induced by the parasitoid *Hymenoepimecis jordanensis* (Hymenoptera: Ichneumonidae). Behaviour, available online. doi:10.1163/1568539X-00003238

Korenko S, Pekár S (2011) A parasitoid wasp induces overwintering behaviour in its spider host. PLoS One 6:e24628

Korenko S, Michalková V, Zwakhals K, Pekár S (2011) Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid (Hymenoptera: Ichneumonidae). J Insect Sci 11:101

Korenko S, Isaia M, Satrapová J, Pekár S (2014) Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecol Entomol 39:30–38

Matsumoto R (2009) "Veils" against predators: modified web structure of a host spider induced by an ichneumonid parasitiod, *Brachyzapus nikkoensis* (Uchida) (Hymenoptera). J Insect Behav 22:39–48

Matsumoto R, Konishi K (2007) Life histories of two ichneumonid parasitoids of *Cyclosa octotuberculata* (Araneae), *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomol Sci 10:267–278

Matsumoto R, Takasuka K (2010) A revision of the genus *Zatypota* Förster of Japan, with descriptions of nine new species and notes on their hosts (Hymenoptera: Ichneumonidae: Pimplinae). Zootaxa 2522:1–43

Miller JA, Belgers JDM, Beentjes KK, Zwakhals K, van Helsdingen P (2013) Spider host (Arachnida, Araneae) and wasp parasitoids (Insecta, Hymenoptera, Ichneumonidae, Ephialtini) matched using DNA barcodes. Biodivers Data J 1:e992

Moore J (2002) Parasites and the behavior of animals. Oxford University Press, Oxford

Nentwig W, Blick T, Gloor D, Hänggi A, Kropf C (2014) Spiders of Europe. http://www.araneae.unibe.ch. Accessed on 20 Dec 2014.

Nielsen E (1937) A fourth supplementary note upon the life histories of the Polysphinctas (Hym. Ichneum.). Entomol Meddel 20:25–28

Sobczak JF, Loffredo APS, Penteado-Dias AM, Gonzaga MO (2009) Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. J Nat Hist 43:2691–2699

Uetz GW, Halaj J, Cady AB (1999) Guild structure of spiders in major crops. J Arachnol 27:270–280

World Spider Catalog (2014) World Spider Catalog. Natural History Museum Bern. http://wsc.nmbe.ch, version 15.5. Accessed 20 Dec 2014.

Yu DS, Horstmann K (1997) A catalogue of world Ichneumonidae (Hymenoptera). Mem Am Entomol Inst 58:1–1558

Supplement 7

Korenko S, Satrapova J, Zwakhals K. 2015. Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimlinae). Entomological Science 18, 383-388.

Citation report:
Web of Science^{TM}: 6
Scopus/Google Scholar: 6

ISI WOS:
Entomological Science
IF$_{(2015)}$ = IF = 1,144
Quartile $_{(2015)}$: Entomology – Q2

Entomological Science

Entomological Science (2015) **18**, 383–388 doi:10.1111/ens.12132

ORIGINAL ARTICLE

Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimplinae)

Stanislav KORENKO[1], Jitka SATRAPOVÁ[1] and Kees ZWAKHALS[2]

[1]*Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic; and* [2]*Arkel, The Netherlands*

Abstract

We found that the koinobiont ectoparasitoid wasp *Zatypota picticollis* is exclusively associated with three orb weaving spiders *Cyclosa conica*, *Mangora acalypha* and *Zilla diodia* from the family Araneidae. Under the influence of the parasitoid's final instar larva the spiders built a specific web architecture, which differed considerably from the capturing orb web. Manipulated webs of *C. conica* and *M. acalypha* lacked the spiral, stabilimentum and central hub, and the radials were reduced in number. The manipulated web of *Z. diodia* consisted of one strong horizontally oriented thread.

Key words: Araneidae, behavior, host spectrum, *Polysphincta* genus group, spider host.

INTRODUCTION

The polysphinctine wasps (*Polysphincta* spp. *sensu* Wahl & Gauld 1998) are exclusively external parasitoids of spiders (Nielsen 1923; Fitton *et al.* 1987). Their larvae are attached to the dorsal side of the spider's ophistosoma/prosoma, where they develop while the spider continues foraging. Shortly before pupation, the parasitoid's final instar larva manipulates the web-spinning activity of its host in order to establish a safe place for pupation that is effective against enemies and the environment (Fincke *et al.* 1990; Eberhard 2000a,b, 2001; Matsumoto 2009; Korenko & Pekár 2011). These effects are apparently caused by chemical products that larvae introduce into the spider (Eberhard 2010). Several studies have been devoted to the behavioral manipulation of orb web weaving spiders. Eberhard (2000a,b, 2001, 2013), Sobczak *et al.* (2009) and Belgers *et al.* (2013) studied parasitoids associated with orb web building spiders from the family Tetragnathidae. Gonzaga *et al.* (2010) described the manipulation of spiders from the family Nephilidae, and Matsumoto and Konishi (2007), Gonzaga and Sobczak (2007, 2011), Schmitt *et al.* (2012), Eberhard (2013) and Korenko *et al.* (2014) studied the manipulation of spiders from the family Araneidae.

Species of *Zatypota* are known mainly as ectoparasitoids of theridiid spiders (Nielsen 1923; Fitton *et al.* 1988; Gauld & Dubois 2006; Zwakhals 2006; Korenko & Pekár 2011; Korenko *et al.* 2011). Only three *Zatypota* species are really known to be associated with a spider host outside the family Theridiidae. *Zatypota anomala* (Holmgren, 1860) is known to be associated with cribellate tangle web weaving spiders belonging to the family Dictynidae. Vincent (1979) found this wasp associated with *Mallos pallidus* (Banks, 1904) in North America and the species was repeatedly reared from *Dictyna* spp. in Europe (Miller *et al.* 2013; S. Korenko, unpubl. data, 2010). Another exception is *Z. sulcata* (Matsumoto, 2010) from Japan, which attacks the sheet weaving spider *Turinyphia yunohamensis* (Bösenberg & Strand, 1906) (Linyphiidae) (Matsumoto & Takasuka 2010). *Zatypota picticollis* (Thomson, 1888) is known from

---

Correspondence: Stanislav Korenko, Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic.
Email: korenko.stanislav@yahoo.com

Received 28 March 2014; accepted 9 September 2014.

most parts of Europe (UK, France, Germany, the Netherlands, Switzerland, Poland, Spain), until now only known from *Zilla diodia* (Walckenaer, 1802) (Zwakhals 2006). This study presents data about the host spectrum of *Z. picticollis* and a description of the web architecture manipulation of three different spider hosts induced by the wasp's final instar larva.

MATERIALS AND METHODS

Web building spiders (Araneae: Orbiculariae) and their ectoparasitoids (Hymenoptera: Ichneumonidae: *Polysphincta* genus group) were collected from tree canopies (between 40 to 200 cm above ground) at five sites in the Piedmont region of northern Italy. Separate excursions were made each year from 2009 to 2012, each in the last week of October and the first week of November, and each lasting between 1 and 3 days. Collections were made at the following sites in Italy:

1. Province of Cuneo: in a commercial hazelnut and apple orchard (Azienda A. Rivetto) close to Ornato (44°60′55″N, 8°01′07″E) in 2009; in a commercial apricot orchard (Chiamina) close to Verzulo (44°58′26″N, 7°50′45″E) in 2009; in an organic hazelnut orchard and the surrounding forest in Bottonasco (44°24′47″N, 7°24′43″E) from 2009 to 2012; and in the forest edges with dominant *Corylus* sp., *Quercus* sp. and *Acer* sp. in Monterosso Grana (44°24′20″N, 7°19′17″E) in 2011.
2. Province of Verbano–Cusio–Ossola: deciduous forest on the shore of Lake Maggiore close to Fondotoce di Verbania (45°56′16″N, 8°29′37″E) in 2012.

At each site, the spiders were collected by beating tree branches and catching the spiders in a square shaped beating net (1 m^2 area) placed beneath the tree crown. At least three samples (each sample was made by beating 30 trees) were taken per site. The collected spiders were identified to generic level using Nentwig *et al.* (2013) and matched to species both on the basis of formerly known occurring species in the investigated localities (Isaia *et al.* 2010; S. Korenko, unpubl. data, 2012) and by rearing the collected specimens to adulthood. Hatched wasps were identified using Fitton *et al.* (1988) and Zwakhals (2006). The nomenclature of the polysphinctines follows Fitton *et al.* (1988) and Yu and Horstmann (1997). Voucher specimens are deposited in the collection of Kees Zwakhals (Arkel, the Netherlands).

Parasitized and unparasitized spiders were reared and studied in the laboratory. The web architecture of parasitized and unparasitized spiders was studied by placing spiders individually in experimental arenas (220 × 220 mm frame, 20 mm depth) with paper tape on four sides of the frame to provide space for webbing (large spiders), or square glass experimental arenas (100 × 100 mm base, 130 mm height) with an installed twig or frame providing the support for a spider web. Spiders were kept at room temperature (22 ± 3°C) under a natural light : dark regime and fed with a surplus of fruit flies *Drosophila melanogaster* Meigen, 1830. The web building activity of parasitized spiders was recorded until the larva killed and consumed the spider and pupated. The webs of unparasitized spiders and spiders under manipulation were recorded using a Canon EOS 500D digital camera with an EF-S 18–55 mm lens or a macro EF 100 mm f/2.8 L IS USM lens.

Table 1 Number of collected specimens (*N*) and number of parasitized specimens (*N*p) of three confirmed spider hosts

Year	*N*/*N*p *Cyclosa conica*	*Mangora acalypha*	*Zilla diodia*
2009	12/1	11/0	4/1
2010	8/1	5/0	1/0
2011	6/0	6/1	1/0
2012	8/2	13/0	4/1
Total	34/4	35/1	10/2

Seven spiders with a larva attached to their abdomen, *Cyclosa conica* (Pallas, 1772), *Mangora acalypha* (Walckenaer, 1802) and *Z. diodia*, with average body lengths of 2.8 mm, 2.3 mm and 2.4 mm, respectively $n = 4$, 1 and 2, were investigated (Table 1). One parasitized *Z. diodia* died at the larval stage; therefore, the number of observations of altered webs produced by manipulated spiders decreased to $n = 4$, 1 and 1. Next, two parasitoid specimens died at the pupal stage (after manipulation); therefore, only four parasitoid specimens were reared to adulthood, $n = 2$, 1 and 1.

RESULTS

Host association

Zatypota picticollis was reared exclusively from orb web weaving spiders of the family Araneidae. Araneid spiders represented 6% of all collected web building spiders (Table 2). *Zatypota picticollis* was reared from *C. conica*, *M. acalypha* and *Z. diodia*, which made up 13%, 13% and 4% of all Araneidae (Table 3). Eudominant spiders of the genus *Araniella*, which comprised 58% of all Araneidae, were not confirmed as a host of *Z. picticollis*.

Reared material

Italy, Province of Cuneo: Bottonasco, larva attached to *M. acalypha*, leg. 2.xi.2011, larva pupated 7.ii.2012, adult emerged 16.ii.2012 (female); larva attached to *C. conica*, leg. 1.xi.2012, larva pupated 16.xii.2012, adult emerged 26.xii.2012 (female). Oggeri, larva attached to *C. conica*, leg. 2.xi.2012, larva pupated 8.i.2013, adult emerged 17.i.2013 (male). Province of Verbano–Cusio–Ossola: Fondotoce di Verbania, larva attached to *Z. diodia*, leg. 30.x.2012, larva pupated 19.i.2013, adult emerged 29.i.2013 (female).

Changes in web architecture

In the laboratory, the unparasitized spiders *C. conica*, *M. acalypha* and *Z. diodia* ($n = 5$, 5 and 3) built a typical orb web for the capturing of prey (Fig. 1a,c) with 30–38, 16–29 and 32–38 radii. The web orientation was vertical in *C. conica* and *Z. diodia* and oblique or horizontal in *M. acalypha*. Three *C. conica* spiders built short linear silk decorations (stabilimentum) located at the center of web.

In the period of saturation (the period in which spiders do not eat and do not catch prey), they built "the resting web", which consists of several scattered single threads or used remnants of the old web as resting place.

The final instar larva of *Z. picticollis* (Fig. 1e) induced unique changes in the web building behavior of all three spider species, *C. conica* ($n = 4$), *M. acalypha* ($n = 1$) and *Z. diodia* ($n = 1$). Under the influence of the final instar larva, the spiders built a specific web architecture (Fig. 1b,d,f) which differed considerably from the capturing web (Fig. 1a,c). The manipulation led to diminished webs with two different types of structure: in both *C. conica* and *M. acalypha* the web lacked the spiral, and in *C. conica* also the stabilimentum. In addition, the number of radials was reduced and they consisted of several threads woven together, which made them stronger. In *C. conica*, the radials were quite concentrated with a considerable amount of empty space between the groups (Fig. 1b). In *M. acalypha*, the radial lines radiated more symmetrically (Fig. 1d) and the central hub was less reduced in size than in *C. conica*. The wasp's cocoon with the pupa inside was attached perpendicularly to the vertically oriented web (Fig. 2a,b). In *Z. diodia* the web was reduced to a single strong horizontal thread consisting of several lines woven together (Fig. 1f). The wasp's cocoon hung down from the thread (Fig. 2c).

The *Z. picticollis* cocoons were fusiform and white to brownish in color. The cocoon walls were densely woven with a tight and curled outer surface. Several easily visible pigmented spots (darker brown) were present in all studied cocoons at both the apical and basal ends (Fig. 2).

Table 2 Composition of web building spiders in tree canopies over the 4 years of investigation

Family	2009	2010	2011	2012	Total
Araneidae	79	41	90	48	258
Dictynidae	190	64	23	15	292
Linyphiidae	38	1	41	4	84
Tetragnathidae	17	2	17	65	101
Theridiidae	904	565	1183	629	3281
Total	1228	673	1354	761	4016

Table 3 Potential hosts for *Zatypota picticollis* from the family Araneidae in canopies over the 4 years of investigation

Species	2009	2010	2011	2012	Total
Agalenatae redi	0	0	1	0	1
Araneus diadematus	0	1	1	0	2
Araneus sturmi	0	0	0	6	6
Araneus sp.	2	0	13	3	18
Araniella displicata	0	0	43	0	43
Araniella opisthographa	29	0	8	1	38
Araniella sp.	18	26	11	13	68
Cyclosa conica	12	8	6	8	34
Gibaranea bituberculata	1	0	0	0	1
Gibaranea sp.	2	0	0	1	3
Mangora acalypha	11	5	6	12	34
Zilla diodia	4	1	1	4	10
Total	79	41	90	48	258

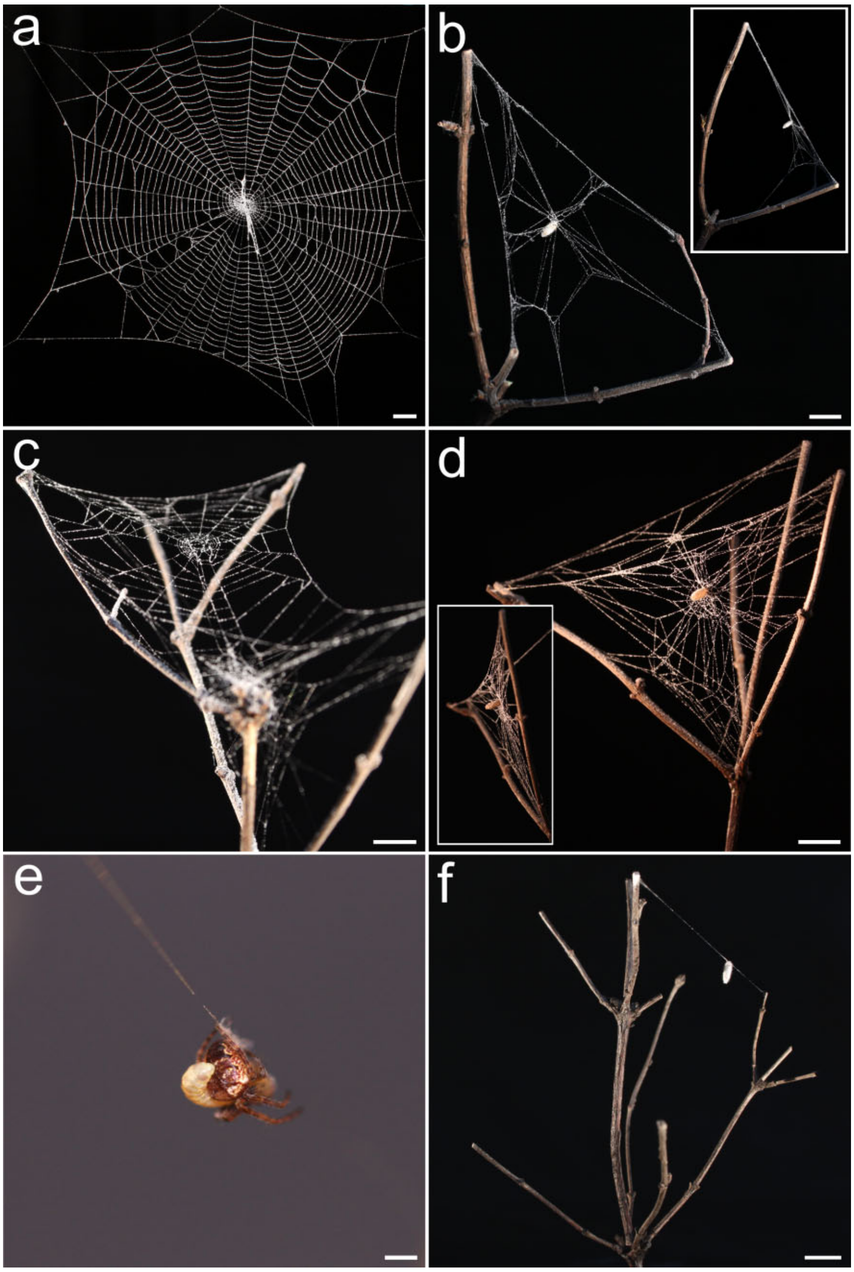


Figure 1 Webs of parasitized and unparasitized araneid spiders. (a) Capturing web of unparasitized *Cyclosa conica*; (b) manipulated web of parasitized *C. conica*; (c) capturing web of unparasitized *Mangora acalypha*; (d) manipulated web of parasitized *M. acalypha*; (e) *Zilla diodia* with final instar larva of *Zatypota picticollis*; (f) manipulated web of *Z. diodia* parasitized by *Z. picticollis*. Inner frames represent lateral view. Scale, 10 mm (a–d,f); 1 mm (e).

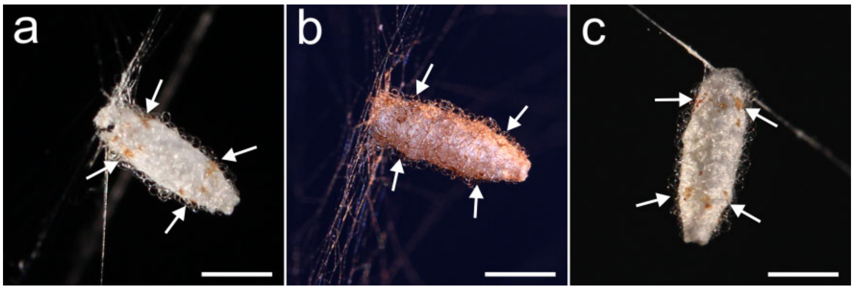


Figure 2 Cocoon of *Zatypota picticollis* hanging on manipulated web of three different hosts. Cocoon on web of (a) *Cyclosa conica*, (b) *Mangora acalypha* and (c) *Zilla diodia*. White arrows show pigmented spots. Scale, 2 mm.

DISCUSSION

Zatypota picticollis seems to be strictly associated with a group of orb web weaving spiders from the family Araneidae living in the canopies of trees. The hosts *C. conica* and *Z. diodia* are real arboreal spiders, whereas *M. acalypha* prefers undergrowth. *Mangora acalypha* was the eudominant spider species in undergrowth in the Bottonasco orchard and comprised more than 50% of all collected spider specimens (S. Korenko, unpubl. data, 2012), but less than 2% in tree crowns. *Zatypota picticollis* seems to be associated with medium sized arboreal orb web weaving spiders, but accepts other araneid spiders with similar webbing behavior, including spiders from the undergrowth. Surprisingly, *Z. picticollis* was not reared from the abundantly present arboreal spiders from the genera *Araniella* and *Araneus*, which formed 68% of all collected specimens in the family Araneidae. Only the parasitoid wasps *Sinarachna pallipes* (Holmgren, 1860) and *Polysphincta boops* Tschek, 1869 were reared from these genera (Korenko *et al.* 2014). Our results show that *Z. picticollis*, like many other polysphinctine wasps, is a strongly specialized parasitoid with highly specific demands with respect to its spider hosts.

There are considerable differences in web architecture induced by particular parasitoids. Spiders building two-dimensional webs (Araneidae, Tetragnathidae and Nephilidae) are manipulated in various ways. Some features of the manipulated web architecture are species specific; others are commonly used by various wasps (Eberhard 2013; Korenko *et al.* 2014). Some orb web weavers are manipulated to modify their two-dimensional orb web to a three-dimensional tangle structure (e.g. Gonzaga & Sobczak 2007; Eberhard 2013; Korenko *et al.* 2014). Other species only somewhat modify their two-dimensional webs; some parts are left out and others are reinforced (Eberhard 2001; Matsumoto & Konishi 2007; Sobczak *et al.* 2009). The final instar larvae of *Reclinervellus* sp. manipulate spiders of the genus *Cyclosa* in order to reduce the quantity of radials, but to increase their thickness; meanwhile, the debris decoration (stabilimentum) is used as camouflage for the wasp cocoon (Matsumoto & Konishi 2007). The cocoon of *Reclinervellus* is connected longitudinally to the cocoon web of *Cyclosa conica*; it is incorporated into the silk decoration (the other form of stabilimentum) or is connected to the radial threads (if the stabilimentum is not present) (Nielsen 1923). In contrast to this, the cocoon of *Z. picticollis* is connected perpendicularly to the reduced central hub of the manipulated *C. conica* web. This web lacks the stabilimentum, which is present in unmodified webs (both debris and silk decorations). The simple type of manipulated web, consisting of a single strong thread as found here in *Z. diodia*, has also been documented by Belgers *et al.* (2013) for the manipulation of *Tetragnatha montana* Simon, 1874 by *Acrodactyla quadrisculpta* (Gravenhorst, 1820). Eberhard (2000a,b, 2001) describes the influence of *Hymenoepimecis argyraphaga* Gauld, 2000 on the web structure of *Leucauge argyra* (Walckenaer, 1841). In this case, the spider repeats the spinning of only the basal parts of a web, resulting in a variable but very incomplete web structure. Similarly to Eberhard (2000a,b, 2001), we found that the manipulated web of *Z. picticollis* only consists of the early steps of normal orb web construction (frame lines and radials) and that the final steps (the building of the sticky spiral) are absent.

The morphology of cocoons built by final instar larvae for their safe pupation differs among wasp species and seems to be useful in the identification of wasp species (Korenko *et al.* 2014). The cocoon of European *Zatypota* wasps associated with three-dimensional web weavers (Theridiidae and Dictynidae) is of a very open construction of sparse loose whorls (the pupa is visible) (S. Korenko, unpubl. data, 2010). It is suggested that if the wasp pupa is sufficiently protected by a dense tangle of spider web threads, a strong cocoon wall is not necessary (Korenko *et al.* 2014). As the pupa of *Z. picticollis* is not protected by a three-dimensional tangle produced by the spider host, it has a strong cocoon wall.

ACKNOWLEDGMENTS

We thank Sanne Beikes from the ecological fruit growing company Cascina Rosso in Bottonasco for permitting us to study spiders and their parasitoids in their ecological orchards. We are also grateful to Marco Isaia, Alberto Chiarle and Mauro Paschetta from Università di Torino for help with collecting data. The study was supported by the European Science Foundation and Ministry for Education, Youth and Sport of the Czech Republic, project CZ.1.07/2.3.00/30.0040, and the Institutional Support Program for Long Term Conceptual Development of Research Institutions provided by the Ministry for Education, Youth and Sport of the Czech Republic.

REFERENCES

Belgers D, Zwakhals K, van Helsdingen P (2013) De bijzondere levensloop van de sluipwesp *Acrodactyla quadrisculpta* op de schaduwstrekspin *Tetragnatha montana* (Hymenoptera: Ichneumonidae, Araneae: Tetragnathidae). *Nederlandse Faunistische Mededelingen* **39**, 1–6.

Eberhard WG (2000a) Spider manipulation by a wasp larva. *Nature* **406,** 255–256.

Eberhard WG (2000b) The natural history and behavior of *Hymenoepimecis argyraphaga* (Hymenoptera: Ichneumonidae) a parasitoid of *Plesiometa argyra* (Araneae: Tetragnathidae). *Journal of Hymenoptera Research* **9,** 220–240.

Eberhard WG (2001) Under the influence: webs and building behavior of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). *Journal of Arachnology* **29,** 354–366.

Eberhard WG (2010) Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. *Animal Behaviour* **79,** 375–383.

Eberhard WG (2013) The polysphinctine wasps *Acrotaphus tibialis*, *Eruga* ca. *gutfreundi*, and *Hymenoepimecis tedfordi* (Hymenoptera, Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. *Annals of the Entomological Society of America* **106,** 652–660.

Fincke OM, Higgins L, Rojas E (1990) Parasitism of *Nephila clavipes* (Araneae, Tetragnathidae) by an ichneumonid (Hymenoptera, Polysphinctini) in Panama. *Journal of Arachnology* **18,** 321–329.

Fitton MG, Shaw MR, Austin AD (1987) The Hymenoptera associated with spiders in Europe. *Zoological Journal of the Linnean Society* **90,** 65–93.

Fitton MG, Shaw MR, Gauld ID (1988) *Pimpline Ichneumon-Flies: Hymenoptera, Ichneumonidae (Pimplinae): Handbooks for the Identification of British Insects, Vol. 7, Part 1*. Royal Entomological Society, London.

Gauld ID, Dubois J (2006) Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider ectoparasitoids. *Systematic Entomology* **31,** 529–564.

Gonzaga MO, Sobczak JF (2007) Parasitoid-induced mortality of *Araneus omnicolor* (Araneae, Araneidae) by *Hymenoepimecis* sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. *Naturwissenschaften* **94,** 223–227.

Gonzaga MO, Sobczak JF (2011) Behavioral manipulation of the orb-weaver spider *Argiope argentata* (Araneae: Araneidae) by *Acrotaphus chedelae* (Hymenoptera: Ichneumonidae). *Entomological Science* **14,** 220–223.

Gonzaga MO, Sobczak JF, Penteado-Dias AM, Eberhard WG (2010) Modification of *Nephila clavipes* (Araneae Nephilidae) webs induced by the parasitoids *Hymenoepimecis bicolor* and *H. robertsae* (Hymenoptera Ichneumonidae). *Ethology Ecology & Evolution* **22,** 151–165.

Isaia M, Beikes S, Paschetta M, Sarvajayakesavalu S, Badin G (2010) Spiders as potential biological controllers in apple orchards infested by *Cydia* spp. (Lepidoptera: Tortricidae). *Proceedings of the 24th European Congress of Arachnology*; 25–29 Aug 2008, Bern, pp 79–88.

Korenko S, Pekár S (2011) A parasitoid wasp induces overwintering behaviour in its spider host. *PLoS ONE* **6,** e24628.

Korenko S, Michalková V, Zwakhals K, Pekár S (2011) Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid *Zatypota percontatoria*. *Journal of Insect Science* **11,** 101.

Korenko S, Isaia M, Satrapová J, Pekár S (2014) Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). *Ecological Entomology* **39,** 30–38.

Matsumoto R (2009) “Veils” against predators: modified web structure of a host spider induced by an ichneumonid parasitiod, *Brachyzapus nikkoensis* (Uchida) (Hymenoptera). *Journal of Insect Behavior* **22,** 39–48.

Matsumoto R, Konishi K (2007) Life histories of two ichneumonid parasitoids of *Cyclosa octotuberculata* (Araneae): *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). *Entomological Science* **10,** 267–278.

Matsumoto R, Takasuka K (2010) A revision of the genus *Zatypota* Förster of Japan, with descriptions of nine new species and notes on their hosts (Hymenoptera: Ichneumonidae: Pimplinae). *Zootaxa* **2522,** 1–43.

Miller JA, Belgers JDM, Beentjes KK, Zwakhals K, van Helsdingen P (2013) Spider hosts (Arachnida, Araneae) and wasp parasitoids (Insecta, Hymenoptera, Ichneumonidae, Ephialtini) matched using DNA barcodes. *Biodiversity Data Journal* **1,** e992.

Nentwig W, Blick T, Gloor D, Hänggi A, Kropf C (2013) Spiders of Europe version 09.2013. [Cited 23 Mar 2014.] Available from URL: http://www.araneae.unibe.ch

Nielsen E (1923) Contributions to the life history of the pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*) (Hym. Ichneumon.). *Entomologiske Meddelelser* **14,** 137–205.

Schmitt M, Richter D, Göbel D, Zwakhals K (2012) Beobachtungen zur Parasitierung von Radnetzspinnen (Araneidae) durch *Polysphincta rufipes* (Hymenoptera: Ichneumonidae). *Arachnologische Mitteilungen* **44,** 1–6.

Sobczak JF, Loffredo APS, Penteado-Dias AM, Gonzaga MO (2009) Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. *Journal of Natural History* **43,** 2691–2699.

Vincent LS (1979) A new record for *Sinarachna anomala* (Hymenoptera: Ichneumonidae), an external parasitoid of *Mallos pallidus* (Araneae: Dictynidae). *Pan-Pacific Entomologist* **55,** 192–194.

Wahl D, Gauld ID (1998) The cladistics and higher classification of the Pimpliformes (Hymenoptera: Ichneumonidae). *Systematic Entomology* **23,** 265–298.

Yu DS, Horstmann K (1997) *A Catalogue of World Ichneumonidae (Hymenoptera)*. American Entomological Institute, Gainesville, FL.

Zwakhals K (2006) The European species of the genera *Zatypota* and *Sinarachna* (Hymenoptera: Ichneumonidae, Pimplinae, Polysphinctini). *Entomologische Berichten* **66,** 34–37.

Supplement 8

Korenko S. 2016. Web architecture alteration of the orb web weaving spider *Metellina merianae* (Araneae, Tetragnathidae) induced by the parasitoid *Megaetaira madida* (Ichneumonidae, *Polysphincta* group). Arachnologische Mitteilungen 52, 35-37.

Citation report:
Scopus/Google Scholar: 1

SCOPUS:
Arachnologische Mitteilungen
SJR$_{(2016)}$ = 0,374

Arachnologische Mitteilungen / Arachnology Letters 52: 35-37 Karlsruhe, September 2016

Web architecture alteration of the orb web weaving spider *Metellina merianae* (Araneae, Tetragnathidae) induced by the parasitoid *Megaetaira madida* (Ichneumonidae, *Polysphincta* group)

Stanislav Korenko

doi: 10.5431/aramit5207

Abstract. The polysphinctine wasp *Megaetaira madida* (Haliday, 1838) is a koinobiont ecto-parasitoid of spiders of the genus *Metellina*. Under the influence of the parasitoid's final instar larva, the spider host *M. merianae* (Scopoli, 1763) built a three-dimensional web architecture, which differed considerably from the capturing orb web. The alteration of spider web behaviour induced by a parasitoid larva in this host-parasitoid pair is described for the first time.

Keywords: behavioural manipulation, host-parasitoid interaction, spider web, wasp

Zusammenfassung. Durch den Parasitoid *Megaetaira madida* (Ichneumonidae, *Polysphincta*-Gruppe) induzierte Veränderungen im Netzbau von *Metellina merianae* (Araneae, Tetragnathidae). Die Schlupfwespe *Megaetaira madida* (Haliday, 1838) aus der *Polysphincta*-Gattungsgruppe ist ein koinobiontischer Ektoparasitoid von Spinnen der Gattung *Metellina*. Unter Einfluss des letzten Larvenstadiums des Parasitoiden baute die Wirtsspinne *M. merianae* (Scopoli, 1763) dreidimensionale Netze, deren Architektur erheblich von der der normalen Fangnetze abweicht. Die Veränderung des Netzbauverhaltens einer Spinne durch die Larve eines Parasitoiden wird erstmals beschrieben.

Koinobiont parasitoid wasps from the *Polysphincta* genus-group sensu Gauld & Dubois (2006) are all exclusively associated with spider hosts and their host range is taxonomically restricted (mostly to genus level) (Fitton et al. 1987). The female wasp temporally paralyses the spider and oviposits on the dorsal side of the spider's opisthosoma/prosoma, where the larva develops while the spider continues foraging. Shortly before pupation, the final stage larva can manipulate the web-spinning activity of the host in order to establish effective protection against enemies and an environment for parasitoid pupation (e.g. Eberhard 2000a, Korenko et al. 2014). This modified web which protects the parasitoid pupa after the spider's death is called the 'cocoon web', a term first introduced by Eberhard (2000a), in which some components of the normal web are reduced (e.g. the web spiral, radii) and others are reinforced (e.g. radii, the central hub, the frame) or multiplied (e.g. threads). These effects of the larva are apparently due to chemical products that are introduced into the spider, but the active compounds involved have not yet been identified (Eberhard 2010).

The polysphinctine parasitoid *Megaetaira madia* (Haliday, 1838) is distributed in several parts of the Palaearctic associated with spiders of the family Tetragnathidae (Yu et al. 2012). Its host range exclusively includes spiders of the genus *Metellina*, which build a typical orb web with a small hole in the hub (e.g. Roberts 1995). The spiders *Metellina mengei* (Blackwall, 1869), *Metellina merianae* (Scopoli, 1863) and *Metellina segmentata* (Clerck, 1757) have already been reported as hosts of *M. madida*, but host utilisation including web architecture modification induced by parasitoid final stage larvae has never been documented (Fitton et al. 1987, 1988). Several specimens of *M. madida* were reared by Nielsen (1923) in small tubes. Unfortunately, the small space in the tubes did not allow the observation of innate web building behaviour or its modification induced by the parasitoid larva. Nielsen observed that wasp cocoons were surrounded by a tangle of threads which were present in all parts of the tubes. Further, Nielsen collected one cocoon attached to a spruce twig in the field with no silk structure surrounding the cocoon. This resembles the situation in which the parasitized spider falls off the web in the period when the parasitoid larva reaches its final stage and is diverted from building any silk structure. Here, I present new host records and the first note on behavioural manipulation of a spider host by *M. madia* from Italy.

Material and methods

I collected parasitized spiders of the genus *Metellina* from the edge of a deciduous forest in Monterosso Grana (Province of Cuneo, Italy, 44°24'20'N, 7°19'17'E) over a two-day period at the end of October 2014. Spiders were collected by beating tree canopies and undergrowth (30 to 200 cm above the ground) with a square-shaped beating net (1-m^2 area) placed underneath. Each spider was visually inspected for the presence of a parasitoid larva.

The spider hosts were reared in plexiglass experimental arenas (frame 220 × 220 mm, depth 20 mm) with paper tape on four sides of the frame so that the spiders could build webs. The spiders were fed with a surplus of prey (small crickets and *Drosophila* flies). The web building activity of parasitized spiders was observed until the larva killed and consumed the spider and pupated. I used a Canon EOS 500D digital camera with an EF-S 18–55 mm lens to record the architecture of the cocoon web.

Results

Two parasitized *Metellina* spiders, one *M. segmentata* and one *M. merianae*, were collected on 29th October. The parasitoid larva on *M. segmentata* died after ten days in the laboratory and when the spider host moulted the shrivelled dead larva fell out with its exuvia. The parasitoid larva on *M. merianae* pupated on 28th December 2014 and the male wasp emerged on 12th January 2015. In the latter spider host, the architecture of the normal web and the modified cocoon web induced by the final stage larva of *M. madida* were observed.

Stanislav KORENKO, Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic;
E-mail: korenko.stanislav@gmail.com

submitted 10.5.2016, accepted 21.7.2016, online 14.8.2016

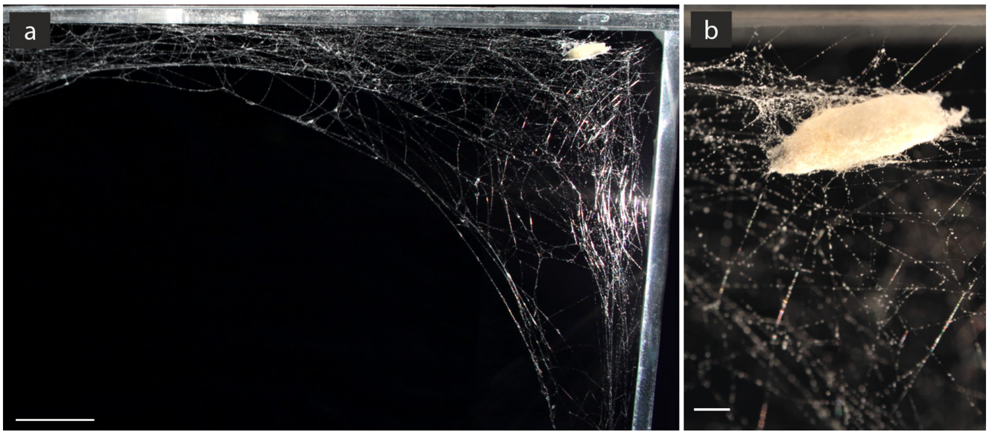


Fig. 1: a. Cocoon web built by a *Metellina merianae* spider host induced by the final stage larva of the parasitoid *Megaetaira madida*; **b.** detail of cocoon. Scale = 20 mm (a) and 2 mm (b)

The parasitized host *M. merianae* rebuilt its orb web several times until the larva reached its final stage, when the spider, under the influence of the parasitoid, built a unique three-dimensional (3D) structure in the upper part of the arena (Fig.1a). This 3D cocoon web had no clear counterpart in the webs of unparasitized spiders. The wasp cocoon was placed in an upper corner of the experimental arena with a high density of threads (Figs 1a, b).

Discussion

Several studies have been devoted to the behavioural manipulation of orb web building spiders from the family Tetragnathidae by polysphinctine wasps. Wasps of the Neotropical genus *Hymenoepimecis* associated with spiders of the genus *Leucauge* were observed to induce the construction of a two-dimensional (2D) cocoon web which consisted of a reduced number of radial threads radiating in a plane from a central hub; the cocoon was suspended from this central hub. This type of cocoon web is documented in *Hymenoepimecis argyraphaga* Gauld, 2000 associated with *Leucauge argyra* (Walckenaer, 1841) (Eberhard 2000a, 2000b, 2001) and *Hymenoepimecis japi* Sobczak, Loffredo, Penteado-Dias & Gonzaga, 2009 associated with *Leucauge roseosignata* Mello-Leitão, 1943 (Sobczak et al. 2009). A cocoon web with a similar 2D architecture, but protected by the 3D structure of a tangle positioned below the hub, was documented in the interaction between *Hymenoepimecis tedfordi* Gauld, 1991 and *Leucauge mariana* (Keyserling, 1881), and *Hymenoepimecis jordanensis* Loffredo & Penteado-Dias, 2009 and *Leucauge volupis* (Keyserling, 1893) (Gonzaga et al. 2015). It is interesting that the larva of the taxonomically distant Costa Rican wasp *Eruga gutfreundi* Gauld, 1991 induced its *Leucauge* host (*L. mariana*) to build a 3D cocoon web (Eberhard 2013). The cocoon web of the orb web weaving spider *Tetragnatha montana* Simon, 1874 induced by the final stage larva of the Palearctic wasp *Acrodactyla carinator* (Aubert, 1965) consisted of one highly reinforced main thread, tensioned mostly by a reinforced side thread (Korenko et al. 2015). *Acrodactyla carinator* was misidentified as Holarctic *Acrodactyla quadrisculpta* (Gravenhorst, 1820) in studies by Korenko et al. (2015) and Belgers et al. (2013) (material revised by K. Holy and K. Zwakhals). Additional investigation revealed that the cocoon web of *T. montana* induced by the larva of *A. quadrisculpta* has the same architecture as that induced by *A. carinator* (Korenko unpubl. data).

The cocoon web induced by *M. madida* resembled the cocoon web of *E. gutfreundi* from Costa Rica in the sense that both were 3D structures and the cocoons were oriented horizontally; however, the morphologies of the cocoons were considerably different (cocoon circular in cross-section covered by curled structure of *M. madida* vs. square in cross section with paper smooth surface in *E. gutfreundi*). The cocoon of *M. madida* had a densely-woven cocoon wall covered by curled fibres of various lengths and was circular in cross section (Fig. 1b), whereas the cocoon of *E. gutfreundi* had a paper-like smooth surface and was square in cross section (Eberhard 2013).

The wasp *M. madida* was formerly included in the genus *Acrodactyla*, also known as the the *madida* species-complex, but Gauld & Dubois (2006) excluded it because it lacked several features characteristic of the remaining *Acrodactyla* species. *Megaetaira madida* was the only valid species within the genus *Megaetaira*, but two other *Acrodactyla* species have recently been transferred to this genus (Matsumoto in press). Their relatedness to *M. madida* is also supported by the host range of *M. varicarinata* (Uchida & Momoi, 1958) associated with *Meta reticuloides* Yaginuma, 1958, which is related to the genus *Metellina*, the exclusive host of *M. madida* (Takasuka pers. comm.). Although wasps of both *Acrodactyla* and *Megaetaira* are associated with orb web building spiders from the family Tetragnathidae, their manipulation of the spider web architecture differs considerably. *Acrodactyla* wasps induce the production of a strong 2D cocoon web where the wasp cocoon is attached to the strongest main silk line (Korenko et al. 2015). In contrast, the cocoon web induced by *M. madida* is a densely woven 3D structure. However, both strategies are effective for protecting the parasitoid during the pupal stage and both are also used by other polysphinctine parasitoids. More observations of host parasitoid interactions are necessary to reveal further details of the host utilisation of this sparsely occurring spider parasitoid.

Acknowledgements

This study was supported by the Institutional Support Program for the Long Term Conceptual Development of Research Institutions provided by the Ministry for Education, Youth and Sport of the Czech Republic.

References

Belgers D, Zwakhals K & van Helsdingen P 2013 De bijzondere levensloop van de sluipwesp *Acrodactyla quadrisculpta* op de schaduwstrekspin *Tetragnatha montana* (Hymenoptera: Ichneumonidae, Araneae: Tetragnathidae). – Nederlandse Faunistische Mededelingen 39: 1-6

Eberhard WG 2000a Spider manipulation by a wasp larva. – Nature 406: 255-256 – doi: 10.1038/35018636

Eberhard WG 2000b The natural history and behavior of *Hymenoepimecis argyraphaga* (Hymenoptera: Ichneumonidae) a parasitoid of *Plesiometa argyra* (Araneae: Tetragnathidae). – Journal of Hymenoptera Research 9: 220-240

Eberhard WG 2001 Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). – Journal of Arachnology 29: 354-366 – doi: 10.1636/0161-8202(2001)029[0354%3AUTIWAB]2.0.CO%3B2

Eberhard WG 2010 Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. – Animal Behaviour 79: 375-383 – doi: 10.1016/j.anbehav.2009.10.033

Eberhard WG 2013 The Polysphinctine wasps *Acrotaphus tibialis*, *Eruga* ca. *gutfreundi*, and *Hymenoepimecis tedfordi* (Hymenoptera, Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. – Annals of the Entomological Society of America 106: 652-660 – doi: 10.1603/AN12147

Fitton MG, Shaw MR & Austin AD 1987 The Hymenoptera associated with spiders in Europe. – Zoological Journal of the Linnean Society 90: 65-93 – doi: 10.1111/j.1096-3642.1987.tb01348.x

Fitton MG, Shaw MR & Gauld ID 1988 Pimpline ichneumon-flies. – Handbooks for the Identification British Insects 7: 1-110

Gauld ID & Dubois J 2006 Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae), a taxonomic revision of spider ectoparasitoids. – Systematic Entomology 31: 529-564 – doi: 10.1111/j.1365-3113.2006.00334.x

Gonzaga MO, Moura RR, Pêgo PT, Bang DL & Meira FA 2015 Changes to web architecture of *Leucauge volupis* (Araneae: Tetragnathidae) induced by the parasitoid *Hymenoepimecis jordanensis* (Hymenoptera: Ichneumonidae). – Behaviour 152: 181-193 – doi: 10.1163/1568539X-00003238

Korenko S, Isaia M, Satrapová J & Pekár S 2014 Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). – Ecological Entomology 39: 30-38 – doi: 10.1111/een.12067

Korenko S, Korenková B, Satrapova J, Hamouzová K & Belgers D 2015 Modification of *Tetragnatha montana* (Araneae, Tetragnathidae) web architecture induced by larva of the parasitoid *Acrodactyla quadrisculpta* (Hymenoptera, Ichneumonidae, *Polysphincta* genus-group). – Zoological Studies 54(40): 1-7 – doi: 10.1186/s40555-015-0119-6

Matsumoto R (in press) Molecular phylogeny and systematics of the *Polysphincta* group of genera (Hymenoptera, Ichneumonidae, Pimplinae). – Systematic Entomology – doi: 10.1111/syen.12196

Nielsen E 1923 Contribution to the life history of the Pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*) (Hym. Ichneum.). – Entomologiske Meddelelser 14: 137-205

Roberts MJ 1995 Spiders of Britain and Northern Europe (Collins Field Guide). Harper Collins, London. 383 pp.

Sobczak JF, Loffredo APS, Penteado-Dias AM & Gonzaga MO 2009 Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. – Journal of Natural History 43: 2691-2699 – doi: 10.1080/00222930903244010

Yu DS, van Achterberg C & Horstmann K 2012 Taxapad 2012. Ichneumonoidea 2011. Taxonomy, biology, morphology and distribution. Ottawa, Ontario. – Internet: http://www.taxapad.com (May 4, 2016)

Supplement 9

Korenko S, Potopová V, Satrapová J, Pekár S. 2016. Life history of the spider parasitoid *Zatypota percontatoria* (Hymenoptera: Ichneumonidae). Entomological Science 19, 104-111.

Citation report:
Web of Science^{TM}: 0
Scopus/Google Scholar: 0

ISI WOS:
Entomological Science
IF$_{(2016)}$ = IF = 1,262
Quartile $_{(2016)}$: Entomology – Q2

Entomological Science (2016) **19**, 104–111 doi: 10.1111/ens.12171

ORIGINAL ARTICLE

Life history of the spider parasitoid *Zatypota percontatoria* (Hymenoptera: Ichneumonidae)

Stanislav KORENKO,[1] Vera POTOPOVÁ,[1] Jitka SATRAPOVÁ[1] and Stano PEKÁR[2]

[1] Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic and [2] Department of Botany and Zoology, Faculty of Sciences, Masaryk University, Brno, Czech Republic

Abstract

The life history of polysphinctine parasitoids is poorly understood. As a result, their effect on their host has not been evaluated. Here, we present the phenology of *Zatypota percontatoria*, an ectoparasitoid wasp of theridiid spiders, using rich field, semi-field and laboratory investigations of selected life-history parameters. The wasps overwintered as larva attached to the spider abdomen and the imagos of the first generation emerged at the beginning of May. The sex ratio (F/M) of overwintered wasp larvae was 1.4. The wasp spent an average of 6.4 days in the egg stage, 27.67 days in the larval stage and 9.6 days in the pupal stage. Adult longevity was on average 14.34 days. Oviposition occurred on average 8.44 days following maturity. The wasp laid on average 0.35 eggs/day. The total fecundity was on average 7.4 eggs per wasp and decreased with age (data from unmated females). The developmental rate increased with experimental temperature up to 28 °C. The lower developmental threshold for pupae was estimated at 6.6 °C. The sum of effective temperatures for pupae was estimated at 157.8 degree days. Using daily temperature records from the Czech Republic over the last 41 years, we estimated an average of 3.5 generations of *Z. percontatoria* per year. Mortality was 48% in semi-field conditions and 23% in the laboratory. The incidence of mortality was highest during egg and larval stages caused by the detachment of eggs or larvae during spider molting, the mortality of the spider-host and egg deposition on the spider prosoma.

Key words: Ephialtini, host–parasitoid interaction, phenology, *Polysphincta* genus group.

INTRODUCTION

Parasitic organisms have evolved many adaptations on the life histories of their specific hosts and the environments in which they coexist (Moore 2002). Polysphinctine parasitoids (*Polysphincta* genus group *sensu* Wahl & Gauld 1998) are koinobiont ectoparasitoids of spiders. Biologically and morphologically, polysphinctines belong to the most specialized Pimplinae, with larvae developing externally on an active spider host, which is killed and consumed shortly before pupation (Fitton *et al.* 1987). Polysphinctines are narrowly specialized on a specific host (often only on one host species). This arrangement requires the synchronization of parasitoid and host phenology (Fitton *et al.* 1987, 1988). Unfortunately, notes on the life history and phenology of polysphinctines are sporadic and largely incomplete (Fitton *et al.* 1987, 1988; Eberhard 2001; Matsumoto & Konishi 2007; Tanaka 2007; Korenko *et al.* 2011; Takasuka & Tanaka 2013).

Sphecid wasps are univoltine or bivoltine in the temperate zone, but plurivoltine in the tropics (Coville 1987). Similarly to other animals in the temperate zone, polysphinctine wasps are constrained by a shorter period of reproduction (from spring to autumn). Most polysphinctines overwinter as larvae and the first generation of imagos appears in early spring (Fitton *et al.* 1988). Depending on local climatic conditions they are either univoltine or bivoltine, but even more generations in one warm season can be expected (Fitton *et al.* 1988).

Zatypota percontatoria (Müller, 1776) has a Holoarctic distribution. It attacks only theridiid spiders of the

Correspondence: Stanislav Korenko, Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic.
Email: korenko.stanislav@yahoo.com

Received 5 December 2014; accepted 11 July 2015; first published 25 April 2016.

Theridion group, which includes several closely related species with similar morphology and biology: *Theridion varians* Hahn, 1833, *T. pinastri* L. Koch, 1872, *Phylloneta impressa* (L. Koch, 1881), *Neottiura bimaculata* (Linnaeus, 1767), *Platnickina tincta* (Walckenaer, 1802), *Heterotheridion nigrovariegatum* (Simon, 1873) and *Paidiscura pallens* (Blackwall, 1834) (Korenko *et al.* 2011). Interactions between *Z. percontatoria* and their theridiid host have been studied by Korenko *et al.* (2011) and Korenko and Pekár (2011). In these studies, the average prevalence of parasitism in Central Europe was observed to be 1.74–0.83%. The wasps responded adaptively to seasonal changes in host abundance and host body size and shifted host preference according to the availability of suitable hosts both during and between seasons. In spring and summer the highest percentage of parasitism was on *T. varians* and in autumn it was on *N. bimaculata* (Korenko *et al.* 2011). Further, Korenko and Pekár (2011) revealed plasticity in host manipulation, when alteration of the web architecture induced by the final instar larva was species specific. The spider host *N. bimaculata* produced a dense web, whereas *T. varians* built a cupola-like structure under manipulation by the final instar larva.

Zatypota percontatoria is one of the most common spider parasitoids in Europe (Fitton *et al.* 1987, 1988; Korenko *et al.* 2011). This species is univoltine in the Scottish Highlands, but at least bivoltine in England and other parts of Europe (Fitton *et al.* 1988). The aim of this study was to investigate the most important life history traits of *Z. percontatoria* and to estimate its phenology pattern in temperate zone conditions.

METHODS

Development under semi-field conditions

In October 2007, parasitized spiders ($n = 21$) were collected by means of a square-shaped beating tray (1 m^2 area) placed beneath the tree crown in an apple orchard in Brno, Czech Republic (49°09′37″N, 16°33′35″E). The spiders belonged to the *Theridion* group, which includes several closely related species with similar biology and web architecture (for details see Korenko *et al.* 2011). Spider hosts had an average prosoma length of 0.57 mm (SD = 0.07) and all wasp larvae were in the first instar ($n = 21$).

Spiders with parasitoids were placed singly in cylindrical containers (35 mm diameter, 40 mm height) with a layer of plaster of Paris at the bottom. The spiders were kept in outdoor conditions until May 2008 in a shaded place. Parasitized spiders were fed with a surplus of *Drosophila melanogaster* Meigen, 1830 on warm days (when the ambient temperature rose above 10 °C). Larval total body length was recorded weekly or in 1–2 day intervals if spiders were active and fed (on warm days). Molts of the wasp and the spider were also recorded. As it was impossible to identify larvae to instars according to molts, we used total body size as a proxy measure. The stage of the second instar began when the larva doubled in body length. The stage of the third instar began when the larva achieved maximum body length.

All emerged wasps were *Z. percontatoria*. The wasps were identified using Fitton *et al.* (1988). The nomenclature of the polysphinctines follows Yu and Horstmann (1997).

Ontogeny in the laboratory

To investigate larval development, living spiders with parasitoid larvae of the first instar attached to their abdomen were collected in October 2008 and 2009 by the same method as described above. Individuals were kept at a temperature of 22 ± 3.5 °C and under a 16 h light : 8 h dark photoperiod regime under laboratory conditions. Selected life span parameters were recorded in 42 individuals.

Oviposited eggs were inspected daily for the presence of segmentation that occurs in the first instar larva ($n = 5$). The larval stage was the period between the appearance of the first segmentation of the first instar and the end of pupation (i.e. up to when the pupal cocoon was finished). When adult wasps emerged ($n = 27$), they were placed singly in glass vials (85 mm height, 55 mm diameter), with a cotton ball soaked in 20% aqueous honey solution placed at the bottom.

Development related to temperature

Parasitized spiders were placed singly in containers (the same as above) in a climatic chamber for 90 days, or until the wasp emerged. The spiders were kept at experimental temperatures of 5, 10, 15, 20, 22, 26 and 30 °C and under a 12 h light : 12 h dark photoperiod regime. Humidity (not measured) was maintained by moistening the plaster with 2–3 drops of tap water at 1–3 day intervals (depending on the experimental temperature). One *Drosophila melanogaster* individual with a body length of up to 2 mm was offered as prey every three days. The duration of the pupal stage was recoded at all experimental temperatures, except for 5 °C, when larvae did not develop. Only the duration of the pupal stage was recorded, because only at this stage we were able to distinguish the beginning and termination of the stage.

A non-linear regression model was used to fit the data. We used the model devised by Lactin *et al.* (1995) to estimate the lower developmental threshold (LDT) and the optimum temperature (T_{opt}), defined here as the temperature at which the maximum developmental rate occurred. T_{opt} was found numerically by solving the first derivative of the function and finding the root. Then, the linear model on the interval 5 to 25 °C was used to estimate the sum of effective temperatures for the pupa. Analyses were performed in the R environment (R Core Team 2009).

Reproductive parameters

The sex ratio of the overwintering wasp population was estimated from all reared wasps collected in October 2007 ($n = 38$). This included 11 wasps reared outdoors and 27 wasps reared indoors. Reared wasp females were kept separately and did not mate. On the third day following emergence, three hosts (*Theridion* group spiders) were released into the vial. The number of offered hosts was kept constant for 30 days: as soon as one was parasitized it was replaced by a new unparasitized spider. The reproduction parameters of female wasps that produced progeny ($n = 10$) were recorded daily. The number of parasitized hosts, number of eggs laid and number of emerged wasps were recorded.

The change in fecundity rate (eggs/day) over time was modeled using an exponential model of the form $y = a(x - b)e^{-c(x - b)} + d$. The model was fitted using the nls function in R. The total number of eggs produced over time was modeled using Poisson regression (GLM-p).

Estimation of parasitoid phenology

The number of generations of *Z. percontatoria* in a season was calculated based on the estimated sum of effective temperatures and LDT using temperature records for the last 41 years. The estimation of the number of generations also considered the pre-reproductive period, when the emerged female wasp was not able to produce eggs (plus eight days per generation). This study also analyzed the occurrences of dry events during the life cycle of *Z. percontatoria*. In this work, the longest dry spell was considered to be the highest number of consecutive days registering a daily precipitation level less than or equal to 0.1 mm. The daily mean air temperatures and daily precipitation totals from 90 climatological stations uniformly covering the territory of the Czech Republic for years 1970–2010 were used. The selected stations represent different climatic conditions (warm, moderate and cold) in both lowland and highland regions and reflect differences between the maritime and continental weather regimes across the Czech Republic. It was assumed that LDT for egg and larval stages do not differ from estimated LDT for the pupal stage and that the sum of effective temperatures for full development (SET_{full}) is the sum of effective temperatures for egg (SET_e), larva (SET_l) and pupa (SET_p). SET_e and SET_l were estimated from data on SET_p based on the assumption of developmental rate isomorphy (Jarošík *et al.* 2002). The Surfer v8 software (Golden Software 2002) allows us to generate calculated data points (90 station observations) on a regular grid. We used this grid to generate a contour map of the spatial distribution of *Z. percontatoria* generation (gridding by the Kriging interpolation technique) across the Czech Republic.

Mortality

The mortality of larvae was recorded in both semi-field and laboratory conditions. In the laboratory, mortality was recorded for specimens collected in nature and for their haploid progeny. In progeny, mortality was recorded from oviposition to wasp maturity. Furthermore, the circumstances that caused mortality were recorded.

RESULTS

Life history under natural conditions

All wasp larvae ($n = 21$) overwintered as the first instar. The second instar ($n = 15$) appeared in the spring, between 8 and 21 April, when the average ambient temperature rose above 10 °C. At this time, 100% ($n = 15$) of surviving spiders molted. The third instar larvae killed and consumed the spider host and subsequently pupated in the spider web. On average, pupation occurred on 21 April ($n = 14$). The pupal stage in semi-field observations lasted on average 19.1 days (SD = 3.2, $n = 11$). On average, wasps emerged on 9 May. This was the first generation (Fig. 1).

Ontogeny in laboratory

The egg period lasted an average of 6.4 days (SD = 1.3, $n = 5$). The length of the larval stage (three instars combined) lasted an average of 27.7 days (SD = 8.3, $n = 12$). The length of the pupal stage lasted an average of 9.6 days (SD = 1.8, $n = 62$). Adult longevity was on average 14.3 days (SD = 7.4, $n = 32$). The proportions of the egg, larval and pupal stages (i.e. from egg to adult) were 15%, 63% and 22% of the total, respectively.

Development at different temperatures

No wasp larvae ($n = 6$) grew at 5 °C within three months. The pupal stage lasted an average of 42 days at 10 °C, but 7.7 days at 26 °C (Table 1). The developmental rate of pupa increased with the experimental temperature (Fig. 2), reaching a maximum at 28.3 °C. At 30 °C the developmental rate began to decrease. The LDT was estimated to be 6.58 °C. The optimal temperature was estimated to be 28.04 °C. SET_p was estimated to be 157.8 degree days.

Reproduction parameter

The sex ratio (F/M) of all wasps collected in October 2007 ($n = 38$) was slightly but not significantly biased towards females at 1.4 (exact binomial test, $P = 0.26$). Unmated females laid their first egg on the spider abdomen an average of 8.44 days after reaching maturity (SD = 2.70, $n = 9$). The eggs were whitish, conical and, on average, 0.43 × 0.16 mm in size ($n = 14$). The wasp laid an average of 0.35 eggs per day (SD = 0.17, $n = 9$), which decreased with age (Fig. 3). Female wasps ($n = 10$) laid all together 67 eggs on 63 spider hosts. In four cases, the female laid two eggs on the same spider individual. The total fecundity was on average 7.4 eggs (SD = 6.67, $n = 9$). Unmated female progeny were only males ($n = 12$). There was a positive relationship between fecundity and longevity (GLM-p, $\chi^2_1 = 24.4$, $P < 0.0001$, Fig. 4).

Estimation of parasitoid phenology

SET_e was estimated to be 107.6 degree days and SET_l was estimated to be 451.7 degree days. SET_{full} was estimated to be 717.1 degree-days. To this value we added the average length of the pre-reproductive period. Using temperature data from the last 41 years we estimated an average of 3.5 generations of *Z. percontatoria* per year. Annual variability in the number of generations of *Z. percontatoria* in the warm region characterized mostly by intensive agriculture can be seen in Figure 5. According to the spatial distribution of the frequencies of generation, the highest level of generation (3–4) was detected in the

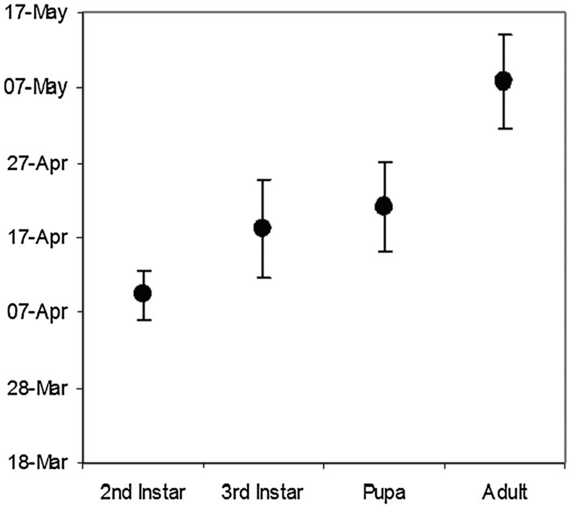


Figure 1 Observed phenology of the first generation in a semi-field experiment in spring 2007. Points are mean dates of emergence to a particular stage. Whiskers are ±SD.

Table 1. Mean and standard deviation (SD) of the duration of the pupal stage at seven experimental temperatures

T (°C)	Duration (days)	*n*	SD
5	–	6	–
10	42.0	8	6.42
15	25.5	6	4.79
20	13.3	6	0.47
22	9.1	51	1.13
26	7.7	6	0.47
30	8.1	7	1.73

–, Meaning no data (no wasp larvae grew at 5 °C).

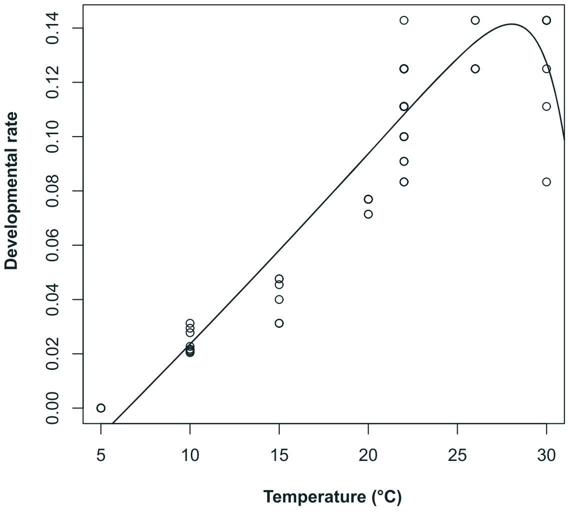


Figure 2 Relationship between the development rate (v) of pupae and the experimental temperature (T). The estimated non-linear model is of the form $v = e^{0.0064T} - e^{0.0064\times 35.45-\left(\frac{35.45-T}{1.6}\right)} - 1.04$.

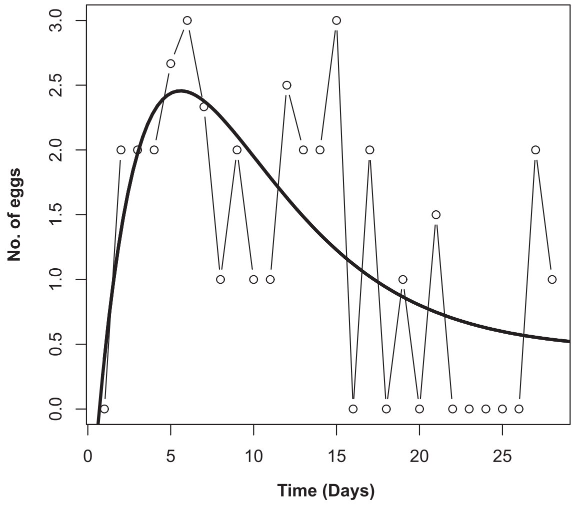


Figure 3 Change in production of eggs/day (y) and time (x). Plots mean average number of eggs. The exponential model is of the form $y = 1.208(x - 1.046)e^{-0.225(x-1.046)} + 0.452$.

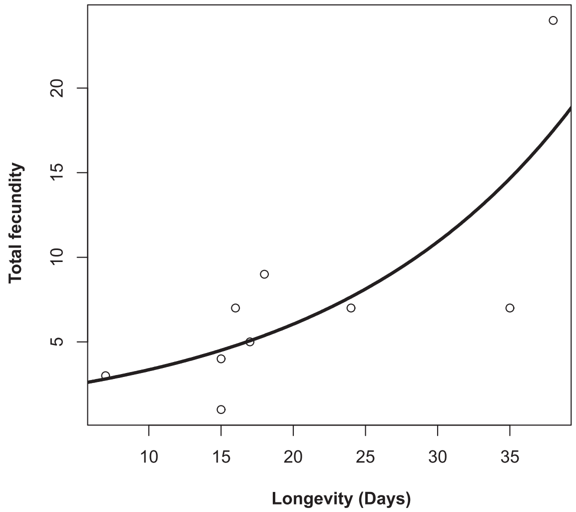


Figure 4 Relationship between fecundity (y) and female longevity (x) using a Poisson linear model of the form $y = e^{0.621 + 0.059x}$.

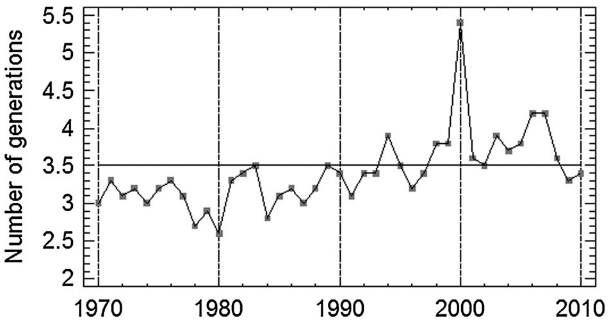


Figure 5 Temporal estimation of the number of generations of *Zatypota percontatoria* quantified by the daily mean air temperature in the warm region during years 1970–2010.

warm and moderate regions of the Czech Republic (the lowlands of the Elbe River valley, central Bohemia, southern Moravia, and the lowlands of south-eastern Bohemia). In contrast, the lowest level of generation occurred in cold regions, where drought occurred less than 15% of the time (Fig. 6).

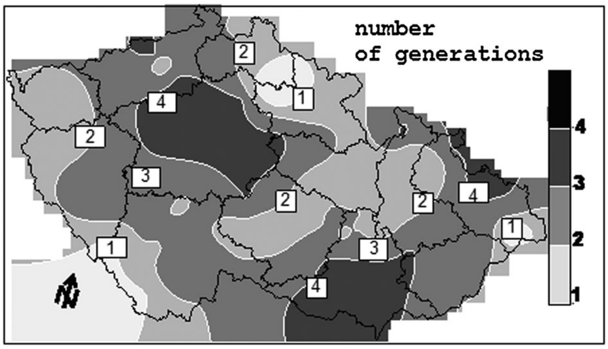


Figure 6 Spatial estimation of the number of generations over 41 years in the Czech Republic.

Mortality

Fifty-two percent of wasps reared in semi-field conditions and 77% of wasps reared in the laboratory reached adulthood ($n = 21$ and 84, respectively). Stage-specific mortality was 0.18 at the larval stage and 0.06 at the pupal stage. Twenty-seven percent of wasps from haploid progeny reached adulthood ($n = 63$). Stage-specific mortality was 0.41 at the egg stage, 0.59 at the larval stage and 0.27 at the pupal stage. Mortality occurred when the spider hosts died (32%), the egg dropped from the spider (23%), eggs or larvae were lost during spider molting (33%) or the larva died because it was laid on the prosoma (4%). The rearing temperature did not have a significant effect on pupal mortality (GLM-b, $\chi^2_5 = 2.3$, $P = 0.8$, Table 2).

DISCUSSION

Knowledge on the life history of polysphinctine wasps is very sparse and is mostly restricted to information on time from egg to adulthood or the duration of the pupal stage (e.g. Nielsen 1923; Fincke *et al.* 1990; Tanaka 2007; Fritzén, 2010, 2014; Korenko *et al.* 2014, 2015). The present study provides complex information on the life history and phenology of the polysphinctine spider parasitoid *Z. percontatoria* in climatic conditions in Central Europe using laboratory, semi-field and field data.

Hymenopteran parasitoid females that live longer lay more eggs over their life-span (Sandlan 1979). Their ovigeny index and life-span are negatively correlated (Jervis *et al.* 2001, 2003), suggesting that there is a cost to life-span of concentrating reproductive effort into early adult life (Jervis *et al.* 2001). The maximum fecundity of *Z. percontatoria* was observed in a female that laid 24 eggs during 38 days of longevity, but the eggs produced after the 25th day were not vital (they were transparent and shriveled after 1–2 days).

In Japan, the polysphinctine wasps *Reclinervellus masumotoi* Matsumoto & Konishi, 2007 and *R. tuberculatus* (Uchida, 1932) live sympatrically and attack the same spider host, presumably competing with each

Table 2. Mortality of parasitoid pupae at different temperatures under laboratory conditions

T (°C)	*n*	Mortality (%)
10	11	27
15	9	33
20	8	13
22	38	29
26	8	13
30	10	2

other (Matsumoto & Konishi 2007). The development of *R. masumotoi* larvae in spring is completed 10–20 days earlier than that of *R. tuberculatus*. This seems to be an advantage for *R. masumotoi* if both wasps oviposit on the same spider host. Indeed, *R. masumotoi* succeeded in developing because it consumed the spider mass such that younger larvae of *R. tuberculatus* were doomed to die (Matsumoto & Konishi 2007). The *Zatypota percontatoria* female also laid two eggs on one spider host several times in both the laboratory (this study) and in the field (S. Korenko, unpubl. data, 2014). Similarly to *Reclinervellus* larvae, only one larva of *Z. percontatoria* completed its development and pupated.

Polysphinctine wasps oviposit their eggs directly on the spider host. Their relatively large eggs (0.4 mm in length in *Z. percontatoria*) evidently provide sufficient resources and a high chance of surviving the initial stage of embryo development. The mortality of *Z. percontatoria* was highest at the egg stage (41% in haploid progeny). We suggest that this could be a consequence of the fact that older females reared in the laboratory produced unviable eggs. This waste egg production (non-viable eggs) was not observed in the natural population (S. Korenko, unpubl. data, 2013). The high mortality of eggs and larvae in laboratory rearing was observed several times in other polysphinctine species (S. Korenko, unpubl. data, 2008), but similarly to the present study, it was not known if the high mortality was a consequence of unnatural environmental conditions in the laboratory. We found that the rearing temperature did not have a significant effect on pupal mortality, but had a significant effect on development rate. Other variables responsible for mortality in laboratory rearing could be humidity and host diet, but empirical evidence is not available.

The phenology of polysphinctine wasps is poorly understood. It has been estimated on the basis of adult activity during a season (e.g. Fitton *et al.* 1988). Detailed study of the phenology of polysphinctines is missing; nevertheless, it is expected to be univoltine or bivoltine in the temperate zone. A bivoltine life cycle is documented in *Oxyrrhexis zephyrus* Fritzén, 2014 in Norway (Fritzén 2014) and in *Acrodactyla* wasps across Europe (Fitton et al. 1988). *Polysphincta rufipes* Gravenhorst, 1829 and *P. tuberosa* Gravenhorst, 1829 wasps are expected to be univoltine in the Scottish Highlands, but bivoltine in warmer parts of Europe (Fitton *et al.* 1988; S. Korenko, unpubl. data, 2014).

The phenology of *Z. percontatoria* was estimated using LDT, when empirical data from the laboratory were obtained only for pupal development, and LDT for egg and larval development were estimated as their proportions of full development, which was calculated from data on pupal development. Some inaccuracy could be expected in the presented results, because the rate of development in the egg and larval stages may differ from the rate in the pupal stage (S. Korenko, unpubl. data, 2009).

The existence of a plurivoltine life cycle for *Z. percontatoria* in Europe is also supported by our previous study (Korenko *et al.* 2011). *Zatypota percontatoria* wasps were reared from parasitized spiders in different periods of the warm season. Tanaka (2007) observed that the related species *Zatypota albicoxa* (Walker, 1874) continues to grow and reproduces under warm conditions and with an adequate food supply during the whole warm season in Japan. Takasuka and Tanaka (2013) confirmed four generations per year in *Z. albicoxa* and provided evidence that geographical differences in the seasonal life cycle may be caused by differences in local climatic conditions, but not by differences in seasonal host availability. In warmer south-western Japan the occurrence of newly formed pupae of *Z. albicoxa* was two months earlier than in colder northern Japan, while the season for growth and reproduction was four months longer (Takasuka & Tanaka 2013).

The phenology of *Z. percontatoria* changes with latitude and altitude and across time. Ambient temperature seems to be one of the most limiting factors for its developmental rate. *Zatypota percontatoria* is expected to be univoltine in the cold Scottish Highlands, bivoltine in England (Fitton *et al.* 1988) and plurivoltine in Central Europe (this study). On average, 3.5 generations per year were estimated for the Czech Republic (Central Europe) in the years 1970–2010. The highest numbers of generations were estimated for the decades with: (i) high positive temperature anomalies in spring–summer associated with below-normal precipitation (warm and dry condition during the 1990s and 2000s); (ii) both high positive temperature and precipitation anomalies in spring (warm and wet conditions between 1981 and 1988); and (iii) the highest deficit of water balance (2003, 1994, 2000 and 2007; years sorted by the highest deficit) (Potop *et al.* 2012).

The temporal model for the last four decades estimated 2.5–3 generations of *Z. percontatoria* per year in cold years and as many as 4–5 generations per year in extremely warm years. It is expected that low precipitation and other unknown factors could further limit the number of generations in extremely warm seasons. Further, the mean number of generations has increased with increasing mean ambient temperature in Central Europe over the last few decades (Brázdil *et al.* 2012). The spatial model showed variations in the number of generations of *Z. percontatoria* across the Czech Republic (between 1970 and 2010). It is also expected that areas with extreme climatic conditions such as cold highlands and extremely dry areas boast only lower numbers of generations, as in the Scottish Highlands, for example, where only one generation was observed (Fitton *et al.* 1988). The distribution of *Z. percontatoria* at high altitudes and in extremely dry areas could be limited by local conditions.

Phenological matching between parasitoid and host is essential for parasitoid survival, especially in such narrow specialists as polysphinctines. *Zatypota percontatoria* is associated with a small group of theridiid spiders in the temperate zone. As the host availability of a particular spider changes through the season, *Z. percontatoria* responds adaptively and shifts host to available host species. In spring and summer, the highest percentage of parasitism was observed on *T. varians* and, in autumn, on *N. bimaculata* (Korenko *et al.* 2011). Hence, climatic conditions (especially temperature) seem to be responsible for the number of generations per particular season.

ACKNOWLEDGMENTS

The study was supported by the European Science Foundation and Ministry for Education, Youth and Sport of the Czech Republic, project CZ.1.07/2.3.00/30.0040, and the Institutional Support Program for Long Term Conceptual Development of Research Institutions provided by the Ministry for Education, Youth and Sport of the Czech Republic.

REFERENCES

Brázdil R, Zahradníček P, Pišoft P, Štěpánek P, Bělínová M, Dobrovolný P (2012) Temperature and precipitation fluctuations in the Czech Lands during the period of instrumental measurements. *Theoretical and Applied Climatology* **110**, 17–34.

Coville RE (1987) Spider-hunting sphecid wasps. In: Nentwig W (ed) *Ecophysiology of Spiders*, pp 309–318. Springer-Verlag, Berlin.

Eberhard WG (2001) Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). *Journal of Arachnology* **29**, 354–366.

Fincke OM, Higgins L, Rojas E (1990) Parasitism of *Nephila clavipes* (Araneae, Tetragnathidae) by an ichneumonid (Hymenoptera, Polysphinctini) in Panama. *Journal of Arachnology* **18**, 321–329.

Fitton MG, Shaw MR, Austin AD (1987) The Hymenoptera associated with spiders in Europe. *Zoological Journal of the Linnean Society* **90**, 65–93.

Fitton MG, Shaw MR, Gauld ID (1988) *Pimpline Ichneumon – Flies: Hymenoptera, Ichneumonidae (Pimplinae): Handbooks for the Identification of British Insects* **7** Part 1. Royal Entomological Society, London.

Fritzén NR (2010) Natural history and description of *Zatypota kerstinae* sp.nov. (Hymenoptera: Ichneumonidae) reared from *Theridion palmgreni* Marusik et Tsellarius (Araneae: Theridiidae) in Finland. *Zootaxa* **2487**, 52–60.

Fritzén NR (2014) Natural history of *Oxyrrhexis zephyrus* sp.n. (Hymenoptera: Ichneumonidae), a parasitoid of *Enoplognatha serratosignata* (Araneae: Theridiidae), with notes on taxonomy and other host species of *Oxyrrhexis*. *Arthropoda Selecta* **23**, 135–144.

Golden Software (2002) Surfer software v. 8. Golden, CO.

Jarošík V, Honěk A, Dixon AFG (2002) Developmental rate isomorphy in insects and mites. *American Naturalist* **160**, 497–510.

Jervis MA, Heimpel GE, Ferns PN, Harvey JA, Kidd NAC (2001) Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *Journal of Animal Ecology* **70**, 442–458.

Jervis MA, Ferns PN, Heimpel GE (2003) Body size and the timing of egg production in parasitoid wasps: a comparative analysis. *Functional Ecology* **17**, 375–383.

Korenko S, Pekár S (2011) A parasitoid wasp induces overwintering behaviour in its spider host. *PLoS ONE* **6**, Article ID e24628. DOI:10.1371/journal.pone.0024628

Korenko S, Michalková V, Zwakhals K, Pekár S (2011) Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid *Zatypota percontatoria*. *Journal of Insect Science* **11**, Article ID 101. DOI:10.1673/031.011.10101

Korenko S, Isaia M, Satrapová J, Pekár S (2014) Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). *Ecological Entomology* **39**, 30–38.

Korenko S, Satrapova J, Zwakhals K (2015) Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimplinae). *Entomological Science* **18**, 383–388.

Lactin DJ, Holliday NJ, Johnson DL, Craigen R (1995) Improved rate model of temperature-dependent development by arthropods. *Environmental Entomology* **24**, 68–75.

Matsumoto R, Konishi K (2007) Life histories of two ichneumonid parasitoids of *Cyclosa octotuberculata* (Araneae): *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). *Entomological Science* **10**, 267–278.

Moore J (2002) *Parasites and the Behavior of Animals*. Oxford University Press, Oxford.

Nielsen E (1923) Contributions to the life history of the pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*) (Hym. Ichneumon.) *Entomologiske Meddelelser* **14**, 137–205.

Potop V, Možný M, Soukup J (2012) Drought at various time scales in the lowland regions and their impact on vegetable crops in the Czech Republic. *Agricultural and Forest Meteorology* **156**, 121–133.

R Core Team (2009) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from URL: http://www.R-project.org/

Sandlan K (1979) Host feeding and its effects on the physiology and behaviour of the ichneumonid parasite *Coccygomimus turionellae*. *Physiological Entomology* **4**, 383–392.

Takasuka K, Tanaka K (2013) Seasonal life cycle of *Zatypota albicoxa* (Hymenoptera: Ichneumonidae), an ectoparasitoid of *Parasteatoda tepidariorum* (Araneae: Theridiidae), in Southwestern Japan. *Pacific Science* **67**, 105–111.

Tanaka K (2007) Seasonal life cycle of *Zatypota albicoxa* (Hymenoptera: Ichneumonidae), an ectoparasitoid of *Achaearanea tepidariorum* (Araneae: Theridiidae), in northern Japan. *Entomological Science* **10**, 421–423.

Wahl D, Gauld ID (1998) The cladistics and higher classification of the Pimpliformes (Hymenoptera: Ichneumonidae). *Systematic Entomology* **23**, 265–298.

Yu DS, Horstmann K (1997) *A Catalogue of World Ichneumonidae (Hymenoptera)*. American Entomological Institute, Gainesville, FL.

Supplement 10

Takasuka K, Korenko S, Kysilková K, Štefánik M, Černecká Ľ, Mihál I, Dolejš P, Holý K. 2017. Host utilization of koinobiont spider-ectoparasitoids (Ichneumonidae, Ephialtini, *Polysphincta* genus-group) associated with *Cyclosa* spp. (Araneae, Araneidae) across the Palaearctic. Zoologischer Anzeiger 267, 8–14.

Citation report:
Web of Science^{TM}: 0
Scopus/Google Scholar: 0

ISI WOS:
Zoologischer Anzeiger
IF$_{(2016)}$ = IF = 1,200
Quartile $_{(2016)}$: Zoology – Q2

Zoologischer Anzeiger 267 (2017) 8–14

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Host utilization of koinobiont spider-ectoparasitoids (Ichneumonidae, Ephialtini, *Polysphincta* genus-group) associated with *Cyclosa* spp. (Araneae, Araneidae) across the Palaearctic

Keizo Takasuka[a], Stanislav Korenko[b,*], Kristýna Kysilková[b], Martin Štefánik[c], Ľudmila Černecká[d], Ivan Mihál[d], Petr Dolejš[e], Kamil Holý[f]

[a] *Graduate School of Agricultural Science, Kobe University, Rokkodai cho 1-1, Nada, Kobe, Hyogo 657-8501, Japan*
[b] *Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czechia*
[c] *Museum of Trenčín, Mierové námestie 46 (P.O.BOX 120), SK-912 50 Trenčín, Slovakia*
[d] *Institute of Forest Ecology, Slovak Academy of Sciences, Štúrova 2, 960 53 Zvolen, Slovakia*
[e] *Department of Zoology, National Museum-Natural History Museum, Cirkusová 1740, CZ-193 00, Praha 9, Horní Počernice, Czechia*
[f] *Research Institute of Crop Production, Drnovská 507, 161 06 Prague 6, Ruzyně, Czechia*

ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form
28 December 2016
Accepted 2 January 2017
Available online 3 January 2017

Keywords:
Spider host
Web decoration
Cocoon web
Reclinervellus nielseni
Zatypota picticollis

ABSTRACT

Most parasitoid wasps establish close interactions with their host taxa, and polysphinctines (ichneumonid koinobiont spider-ectoparasitoids) are no exception. Two polysphinctines, *Zatypota picticollis* and *Reclinervellus nielseni*, are, respectively, partial and exclusive parasitoids of *Cyclosa* spp. (Araneidae), which build an orb web with web decoration. The main aim was to investigate the host association of these wasps, including the behavioural alteration of spider hosts across two sides of the Palaearctic (Central Europe vs. Japan).

R. nielseni was associated with relatively common local species of *Cyclosa* both in Europe (*C. conica*), sharing these with *Z. picticollis*, and in Japan (*C. argenteoalba*). We also found a new alternative host spider, *Cyclosa laticauda*, in Japan.

Host web alterations were observed in all parasitoid-spider interrelationships. The alteration patterns of the two polysphinctines have a similar constructional plan that exploits pre-existing frame lines for the cocoon webs; however, they differ partially in their radii, which are either straight or zigzag shaped and in hub loops present or absent. This indicates that certain differences in the neurophysiologic changes in the spider host occur depending on the parasitoids. The fluffy decoration induced in *C. argenteoalba* manipulated by *R. nielseni* was not found in *C. conica* or *C. laticauda*, probably because of its non-innateness.

The cocoons of *R. nielseni* had four conspicuous long ribs making the cocoon quadrate in cross-section regardless of the host spider species or region; such ribs do not appear in two co-generic species.

1. Introduction

Several groups of parasitoid and predatory insects undergo their larval development by exclusively feeding on spiders. The most common associations with spider hosts are known within Hymenoptera. Some wasps develop individually or in small broods by feeding on the spider egg mass hidden in egg sacs (several lineages in Chalcidoidea and Ichneumonidae), while others are so tiny and highly specialised that they develop inside individual spider eggs (Scelionidae). In Aculeata, some predatory wasps hunt one large spider per offspring and then store it in mud chambers, existing galleries, or subterranean burrows (Pompilidae) (Grimaldi and Engel, 2005). Others gather several small spiders for offspring and then place them inside a shelter or cell (several genera in Sphecidae and Crabronidae) (Fitton et al., 1987). Koinobiont ectoparasitic mode of life associated with spiders is documented only sporadically in Aculeata (Souza et al., 2015; Grout and Brothers, 1982), but this mode of life has been well evolved in the *Polysphincta*

---

[*] Corresponding author.
E-mail addresses: korenko.stanislav@gmail.com, korenko.stanislav@yahoo.com (S. Korenko).

http://dx.doi.org/10.1016/j.jcz.2017.01.001
0044-5231/© 2017 Elsevier GmbH. All rights reserved.

genus group (hereafter polysphinctine) within Parasitica, Ichneumonidae, Ephialtini.

The polysphinctine larva is attached to the dorsal side of the spider's prosoma or abdomen depending on the lineage (Matsumoto, 2016), while the spider continues web building and foraging as usual (koinobiosis). When the parasitoid larvae reach the final stage, they kill and consume their spider hosts and pupate on the web. The immobile pupal stage seems to be a critical period of parasitoid ontogeny because loses the spider host acting as both a vehicle and a web-maintainer. Therefore, the penultimate instar larvae of several polysphinctine species modify web-building behaviour of spider hosts shortly before killing them. This modified web structure called a "cocoon web" establishes a safe place for larval pupation and protects it against enemies and natural elements. This larva-induced behaviour occurs apparently due to unidentified chemical products that are introduced into the spider body because artificially larva-removed spiders kept manipulative behaviour (Eberhard, 2000, 2010). Several studies have recently been devoted to the behavioural alteration of orb web weaving spiders from the families Tetragnathidae (Belgers et al., 2013; Eberhard, 2000, 2001, 2013; Korenko et al., 2015b; Sobczak et al., 2009), Nephilidae (Gonzaga et al., 2010), and Araneidae (Eberhard, 2010, 2013; Gonzaga and Sobczak, 2011; Kloss et al., 2016; Korenko et al., 2014; Matsumoto and Konishi, 2007; Schmitt et al., 2012; Takasuka et al., 2015).

In order to trace the evolutionary history of this fascinating phenomenon, the accumulation of case reports in various lineages is of fundamental importance. Here, we present the first records of two polysphinctines, *Zatypota picticollis* (Thomson, 1888) and *Reclinervellus nielseni* (Roman, 1923) associated with *Cyclosa conica* (Pallas, 1772) (Araneidae) from the Czech Republic and Slovakia, and the first host record of *R. nielseni* with *Cyclosa laticauda* (Bösenberg and Strand, 1906) in Japan (the primal host spider in Japan is *Cyclosa argenteoalba* (Bösenberg and Strand, 1906) (Matsumoto and Konishi, 2007; Takasuka et al., 2015)). We then document the modification of the web architecture of two *Cyclosa* spiders induced by these polysphinctine larvae and discuss differences in the host utilization of *C. conica* by these two polysphinctines and differences between the host utilization of *Cyclosa* spp. by *R. nielseni*. The life history traits of these parasitoids are also mentioned descriptively.

2. Materials and methods

2.1. Studied spider hosts

The genus *Cyclosa* from the family Araneidae is distributed around the world and includes 170 species (World Spider Catalog, 2016). The genus is characterized by a typical orb web that is often accompanied by conspicuous linear or circular web decorations near the hub. These are made of silk and/or detritus, including prey remnants, and are referred to as silk decoration (formally "silk stabilimentum") and debris decoration (e.g. Herberstein et al., 2000) (formally "detritus stabilimentum"), respectively; they serve mainly as camouflage devices (Eberhard, 2003; Gan et al., 2010; Gonzaga and Vasconcellos-Neto, 2012; Levi, 1977, 1999; Nakata, 2009; Roberts, 1995). The formally common term "stabilimentum" was derived from the belief that their function was to stabilize webs; however, today, this is now regarded as unlikely, as other functions have been reported depending on the species (Foelix, 2010; Walter and Elgar, 2012).

Cyclosa conica incorporates prey remnants and other debris into linear debris decorations (Levi, 1977); however, sometimes, it constructs pure silk decorations above and below the hub (Tso, 1998; Korenko, unpublished results). In contrast, *Cyclosa laticauda* was observed to have debris decorations above and below the hub (K. Takasuka, unpublished results), but such a description has never appeared in any scientific sources apart from a Japanese picture book (Shinkai, 2006). *Cyclosa argenteoalba* seldom exploits prey remnants for decorations but frequently constructs silk decorations above and below the hub (see Fig. 1c), which are thought to play a defensive role against predators (Nakata, 2009). This spider is also known to construct a simple "resting web" before moulting, exhibiting the absence of a capture region and a reduced numbers of radii with numerous fluffy decorations. Its cocoon web induced by *R. nielseni* resembles the resting web (Takasuka et al., 2015).

2.2. Field and rearing investigations

Cyclosa conica was inspected for the presence of parasitoid larvae on its abdomen. The larvae were attached antero-dorsally close to petioles hidden between abdomen and prosoma in *R. nielseni*, but dorsally or latero-dorsally in *Z. picticollis* (Takasuka et al., 2015; Korenko, unpublished results). Field work was conducted in 2015–2016: 1) on the banks of the Dubovy Brook with dominant willow in the Šumperk district of the Olomouc region, Czech Republic (49°45′01″N, 17°0′27″E), 2) in an ecotone between coniferous forest and a swamp close to the banks of the Teplá Vltava in the Volary environs of the Prachatice district, Czech Republic (48°54′38″N, 13°49′19″E), and 3) in a beech forest in the province of Zvolen, Kováčová, Slovakia (48°34′41″N, 19°5′35″E). The incidence of *R. nielseni* parasitising *C. conica* in Slovakia was investigated during several selected periods in 2015 and 2016 to reveal parasitoid activity in summer and autumn. In a two-hour investigation, spiders were collected by sweeping undergrowth and by beating of bushes and tree branches up to a height of two meters above ground.

The incidence of *R. nielseni* parasitising *C. argenteoalba* in Japan was investigated over four years from 2013 to 2016. Although preliminary data have already been presented in Takasuka et al. (2015), we describe them here in detail as well as newly obtained data. Specimens were collected from a series of shrines in the cities of Tamba and Sasayama, Hyogo prefecture, Japan, where *C. argenteoalba* and *R. nielseni* are present.

The parasitised spiders in Europe were taken to the laboratory, kept at room temperature (22 °C ± 3 °C) under a 12:12 light/day regime and fed with flies *Drosophila melanogaster* Meigen, 1830; *Drosophila hydei* Sturtevant, 1921 to obtain cocoon webs. They were placed individually into narrow plexiglass experimental arenas (frame 220 × 220 mm, depth 20 mm) with paper tape attached to four sides of the frame so that the spiders could build webs.

In the course of the collection survey of *R. nielseni* in Japan, we discovered by chance a penultimate instar parasitoid larva upon a specimen of *C. laticauda* sitting on a vertical orb web in Kawauchi-Tadanuhi Shrine, Sasayama city, Hyogo prefecture, Japan (35°05′54″N, 135°10′07″E). To remove the parasitised spider and its webs from the field, we used a round wire hoop held on a wooden stand; the anchor lines of the web were fastened to the hoop by means of cellophane adhesive tape. Unfortunately, however, in the act of transporting the spider to the laboratory prior to taking pictures, it started to exhibit altered behaviour and we thus have pictures only of the modified web.

Photographs were taken using an EOS 500D digital single-lens reflex camera, (Canon Inc.) with EF-S 18–55 mm/1:3.5–5.6 II (European material), and a DMC-GH1 digital single-lens camera (Panasonic Corporation) with Leica DG Macro-Elmarit 45 mm/F2.8 ASPH (Japanese material).

One specimen of *R. nielseni* from Slovakia is deposited in the collection of Kees Zwakhals (Netherland) and the remaining specimens obtained from the survey in Europe are deposited in the

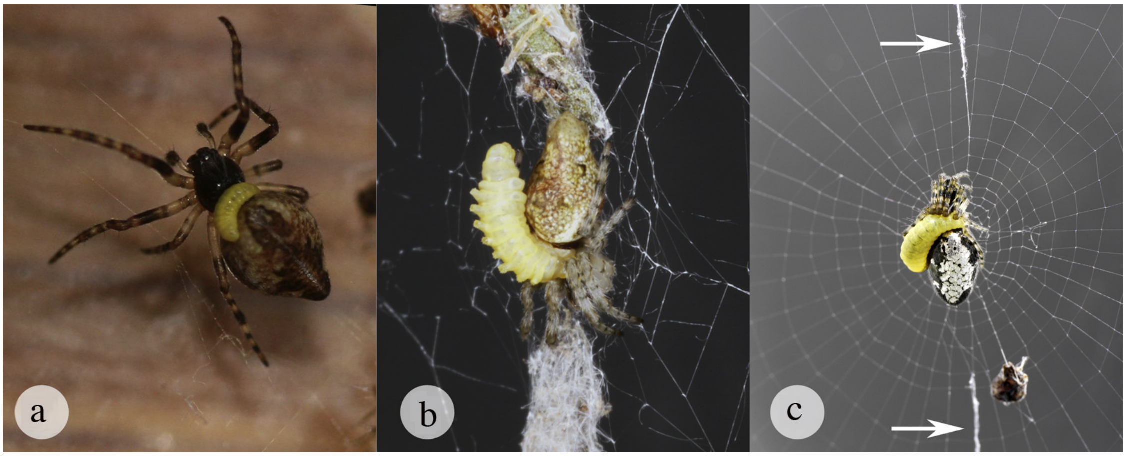


Fig. 1. Larvae of *R. nielseni* upon *Cyclosa* spp. (a) upon *C. conica* in Slovakia, (b) upon *C. laticauda* in Japan with debris decorations above and below, (c) upon *C. argenteoalba* in Japan with linear silk decorations above and below (white arrows).

Table 1
Incidence of the parasitism of *Z. picticollis* and *R. nielseni* upon *C. conica* in the locality of Kováčová, Slovakia, in 2015–2016 listed by date.

Collecting day	No. of spiders	No. of parasitoid larvae (parasitism incidence)	
		Z. picticollis	*R. nielseni*
12 July 2016	0	–	–
11 Aug. 2016	6[a]	–	–
12 Sept. 2016	42	0 (0.0%)	1 (2.3%)
11 Oct. 2015	19	0 (0.0%)	1 (5.2%)
29 Oct. 2015	22	1 (4.5%)	1 (4.5%)
Total	89	1 (1.1%)	3 (3.4%)

[a] Only spiders of the new generation with a body length between 2 and 3 mm (a suitable body length for parasitism) were collected.

collection of S.K. (Czech Republic). Specimens obtained from the survey in Japan are deposited in the collection of K.T. (Japan).

3. Results

3.1. Sampling outcomes and parasitism incidence

Two polysphinctines, *Z. picticollis* and *R. nielseni* (Fig. 1a) reared from *C. conica* were collected in the Czech Republic (N = 0 and 2) and Slovakia (N = 1 and 3) for the first time. The body size of the parasitised spiders was 3.5–7 mm. The observed incidence of parasitism in the Kovačova locality (Slovakia) in the end of summer and autumn was between 2.3–5.5% for *R. nielseni*, but no parasitised spiders were observed in summer. Only one spider was parasitised by *Z. picticollis* in October. We did not find any individuals of *C. conica* in the middle of summer 2016 (July). One of the first juvenile spiders of the new generation appeared in later summer (August). The second incidence of parasitism upon spiders with a suitable body size then occurred in September–October (Table 1).

We also obtained the first record of *R. nielseni* parasitising *C. laticauda* (Fig. 1b) in Japan (N = 1). In the investigated locality, 17 individuals of *C. argenteoalba*, three of them parasitised (Fig. 1c), were detected, whereas only one individual of *C. laticauda* was present with a parasitoid larva. Larvae of *R. nielseni* were characterized by a remarkable yellowish body colour regardless of host spider species (Fig. 1), in contrast to the larvae of co-generic species, which are whitish yellow in colour, and even those of other polysphinctines. The incidence of the parasitism of *R. nielseni* upon *C. argenteoalba* in Japan is shown in Table 2. We never observed any eggs on spiders or ovipositing females in this survey. All parasitised spiders were juveniles. Larvae were detected mainly in April and May, with an average incidence of 2.64%. The most intensive parasitisation occurred in the middle of (2015) or in late (2014, 2016) April. The detection of cocoons was restricted to the middle of (2016) or to late (2015) May. Meanwhile, almost no parasitisation occurred from summer (June) to early winter (November), except during August. The parasitisation in August was not accidental as it was found at three different shrines synchronously.

Table 2
Incidence of parasitism of *R. nielseni* upon *C. argenteoalba* spiders from spring to autumn at several shrines in the cities of Tamba and Sasayama (in total), Japan, from 2013 to 2016, listed by date. The number of spider individuals was not counted in the spring of 2013. The numbers of parasitoid cocoons with a pupa inside on cocoon webs are described in parentheses. The number of spiders was compensated (comp.) with the number of cocoons, when calculating the incidence of parasitism (e.g. (5 + 4)/(331 + 4) = 2.69% in 25 May 2015). We separated the total counts for spring (active season) and for summer/autumn (inactive season).

Collecting day	No. of spiders	No. of parasitoid larvae [+cocoons] (parasitism incidence)
18 Apr. 2014	387	5 (1.29%)
19 Apr. 2015	673	22 (3.27%)
25 Apr. 2013	NA	1 (NA)
26 Apr. 2014	401	11 (2.74%)
26 Apr. 2016	240	16 (6.67%)
2 May 2013	NA	1 (NA)
7 May 2014	393	3 (0.76%)
9 May 2013	NA	1 (NA)
13 May 2016	165	7 [+4] (6.51% comp.)
14 May 2014	259	3 (1.16%)
17 May 2013	NA	0 (NA)
25 May 2014	175	1 (0.57%)
25 May 2015	331	5 [+4] (2.69% comp.)
27 May 2016	114	2 (1.75%)
31 May 2013	NA	0 (NA)
Total (exclusive of 2013)	3138	75 [+8] (2.64% comp.)
20 Jun. 2016	70	0 (0.00%)
10 Aug. 2016	230	4 (0.17%)
2 Oct. 2013	125	0 (0.00%)
14 Oct. 2013	117	0 (0.00%)
30 Oct. 2013	346	0 (0.00%)
4 Nov. 2013	50	0 (0.00%)
Total	938	4 (0.004%)

3.2. Reared material

Czech Republic, region Olomouc: district Šumperk: a larva attached to *C. conica*: leg. 22. v. 2014, cocooned 27. vi. 2014, adult emerged 6. vii. 2014 (1♂, *R. nielseni*); district Prachatice: environs

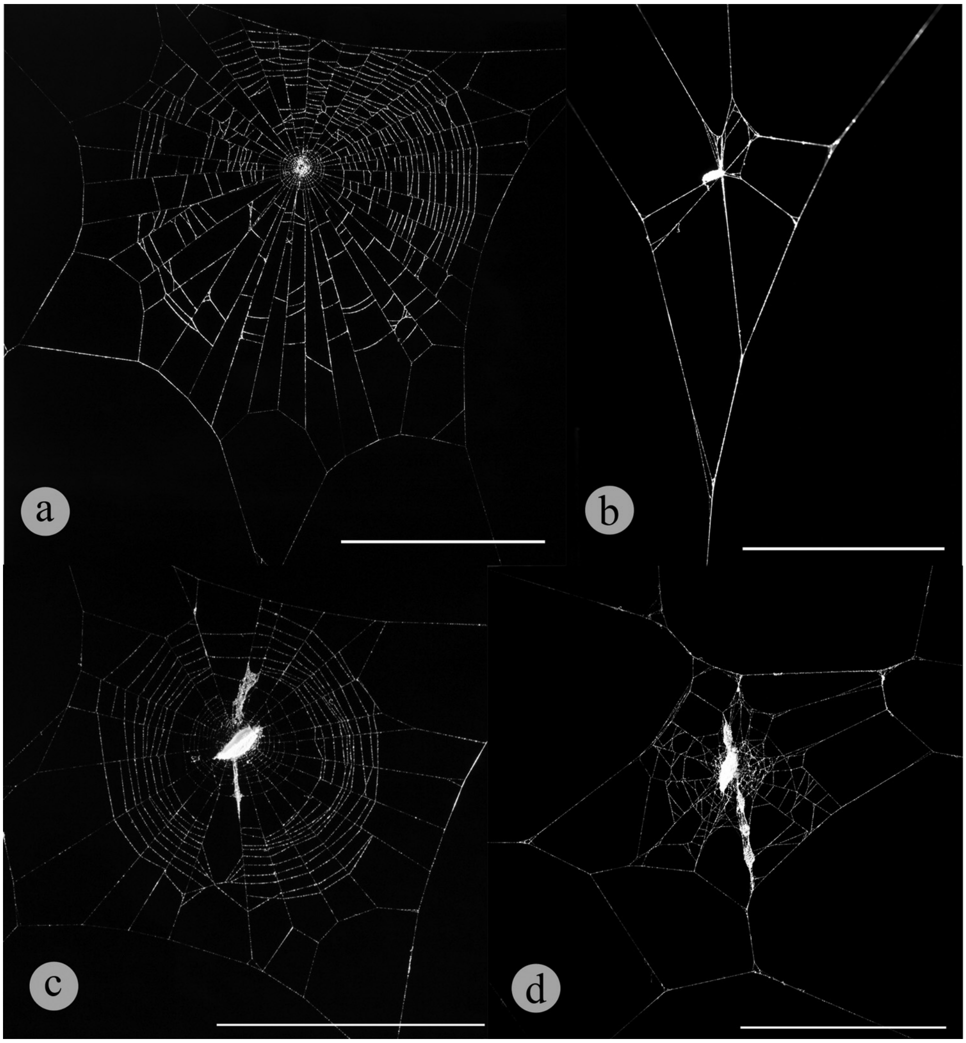


Fig. 2. An original orb web and cocoon webs of *C. conica* in Europe. All scales are 5 cm in length. (a) un-parasitised orb web without decoration, (b) cocoon web induced by *Z. picticollis* in Slovakia, (c) cocoon web induced by *R. nielseni* in Czech Republic, (d) cocoon web induced by *R. nielseni* in Slovakia.

Volary: a larva attached to *C. conica*: leg. 16. vi. 2016, cocooned 18. vi. 2016, adult emerged 27. vi. 2016 (1♀, *R. nielseni*). **Slovakia**, province Zvolen, region Kováčová, four larvae attached to *C. conica*: leg. 5. vi. 2013, cocooned 12. vi. 2013, adult emerged 17. vi. 2013 (1♀, R. *nielseni*); leg. 29. x. 2015, cocooned 2. ii. 2016, adult emerged 16. ii. 2016 (1♀, *R. nielseni*); leg. 12. ix. 2016, cocooned 6.xi. 2016, adult emerged 19. xi. 2016 (1♂, *R. nielseni*); leg. 29. x. 2015, cocooned 8. i. 2016, adult emerged 20. i. 2016 (1♀, *Z. picticollis*). **Japan**, Hyogo prefecture, Sasayama city, a larva attached to *C. laticauda*: leg. 13. v. 2016, cocooned 13. v. 2016, adult emerged 24. v. 2016 (1♂, *R. nielseni*).

3.3. Changes in web architecture

Before web modification, four parasitised *C. conica* built 2D orb webs with an average of 32.8 radii (N = 8 including the same individual repetitions, SD = 8.4) and 32.8 spiral lines in the upper part (N = 8 do., SD = 8.8) and 28.4 spiral lines in the lower part of the orb (N = 8 do., SD = 6.1). Most of the observed webs were with typical linear silk decorations without detritus above and below the hub (N = 7) and one web was without decoration (Fig. 2a).

The cocoon web of *C. conica* under influence of *Z. picticollis* was a modified 2D web in which spirals, silk decorations and hub loops were absent (Fig. 2b). The radii and pre-existing frame lines appeared to be woven together, seemingly making the 2D structure stronger, and the number of radii was dramatically reduced from 27 to 7.

The cocoon webs of *C. conica* under influence of *R. nielseni* were modified small 2D webs (N = 3); a downsized orb web with a spiral (Fig. 2c, N = 1) or a disordered orb web (Fig. 2d, N = 2). The number of spirals and radial lines were reduced irregularly but hub loops and white linear silk decorations (Fig. 2c) or debris decoration (Fig. 2d) above and below the hub remained. The reduced numbers of radii and pre-existing frame lines appeared to be woven together excluding radii of Fig. 2c.

Before web modification, the parasitised *C. laticauda* built a relatively small orb web with debris decorations above and below the hub, which were made of several prey remnants, but mostly of cotton-like white structures seemingly derived from a sort of seminal flocci (fluffy mass or tuft of plant seeds, Fig. 3 inset a) but no seed was detected. The cocoon web of *C. laticauda* under influence of *R. nielseni* was a modified 2D web in which the spiral was completely removed, while the white long debris decorations were kept as they were (Fig. 3). The hub consisted of the white debris decorations and hub loops, which were sustained by six radii internally and three frame threads externally. Pre-existing frame threads were attached at many points (Fig. 3 inset b) to the tape secured that day, which provided reinforcement for the cocoon web.

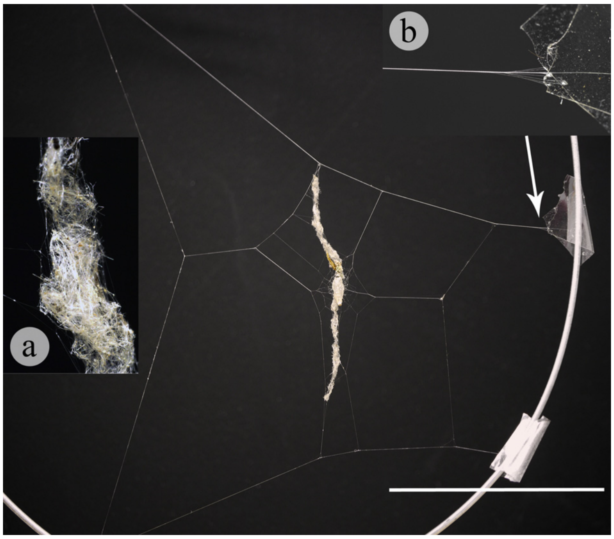


Fig. 3. A cocoon web of *C. laticauda* induced by *R. nielseni* in Japan; (inset a) magnification of white structures composing the debris decoration; (inset b) magnification of one of the distal ends of the frame line attached at many points to the tape after manipulation (frame lines were removed with the tapes that day).

3.4. Cocoon characteristics

The cocoon of *Z. picticollis* was attached perpendicularly to the hub of the vertically oriented web and was fusiform in shape with a distinct caudal orifice, and whitish brown in colour with dark brown pigmented spots arranged around both the apical and basal ends (Fig. 4a).

The cocoons of *R. nielseni* reared on *C. conica* were attached parallel to the web and were whitish brown in colour, spindle-shaped with a distinct caudal orifice, and made quadrate in cross-section by four longitudinal ribs ranging from the top to the distal end (N = 4, Fig. 4b, c). When the cocoon was attached outside of the web (in a plexiglass rearing tube), no ribs were present and it was circular in cross-section (N = 1, Fig. 4d).

The cocoon of *R. nielseni* reared on *C. laticauda* (N = 1, Fig. 4e) was identical to that of *R. nielseni* parasitising *C. conica* in Europe and *C. argenteoalba* in Japan (N > 50, Fig. 4f). A film clip of the formation of the ribbed cocoon induced by *R. nielseni* larvae parasitising *C. argenteoalba* is available at Takasuka (2015). The cocoons of two co-generic polysphinctines in Japan, *R. masumotoi* Matsumoto and Konishi, 2007 and *R. tuberculatus* (Uchida, 1932), parasitising *Cyclosa octotuberculata* Karsch, 1879 are also shown in Fig. 4g, h. They does not have any ribs and are thus circular in cross-section. The cocoons of *R. nielseni* formed indoors in this study, were whitish brown in colour (Fig. 4b, c, e), but the ones taken from the field (from *C. argenteoalba*) and the ones kept in tubes (from *C. conica*) were typically reddish brown (Fig. 4d, f), perhaps due to the high humidity. Reddish brown is thought to be the natural colour.

4. Discussion

4.1. Distribution and host utilization

Zatypota picticollis is known widely in nine countries in Europe (see Korenko et al., 2015a; Yu et al., 2012), including Slovakia, newly recorded in this study, but very uncommon species as a rule. We found the species in only one locality out of twenty localities in central Europe (Slovakia and Czech Republic) where polysphinctine parasitoids have been collected and studied by S.K. over a decade (unpublished results). In addition to *C. conica*, *Zatypota picticollis* is exceptionally associated with two other araneid genera such as *Zilla diodia* (Walckenaer, 1802) and *Mangora acalypha* (Walckenaer, 1802) (Korenko et al., 2015a).

Reclinervellus nielseni is widely distributed in the Palaearctic, from England to Japan, but occurs disjunctively on the western and eastern sides of the Palaearctic with host replacement (see below). Here, we present new distribution records for *R. nielseni* from Slovakia and Czech Republic in addition to existing records from eleven European countries (see Yu et al., 2012; Erzsébet, 1960). In the eastern Palaearctic, *R. nielseni* is recorded from the Kinki region of Japan (western central Japan) (Matsumoto and Konishi, 2007; Takasuka et al., 2015) and the Russian province of Primorsky Krai bordering China and North Korea (Kasparyan, 1976).

In this study, larvae of *R. nielseni* on *C. conica* were collected in spring (May to June) and end of summer and autumn (September and October) in Slovakia and the Czech Republic, and the latter larvae took over three months to complete larval development (see Section 3.2 "Reared material"). Putting our results and records provided by Fitton et al. (1988) from England, Fritzén (2005) from Finland and Nielsen (1923) from Denmark together, we can say that *R. nielseni* in European populations is associated exclusively with *C. conica* and has a bivoltine or plurivoltine life cycle from spring to autumn with larval overwintering. However, it must also be noted that summer reduction may occur, as in our investigated region.

On the other hand, investigations of many more spider individuals (*C. argenteoalba*) in Japan (Table 2) have shown that *R. nielseni* seems to be intensively active during April and May, similarly to a univoltine life cycle. However, the presence of four synchronous larvae in high summer is strong evidence of a second generation and even plural generations in Japan, as is the case in Europe. We assume that low populations of *R. nielseni* in the larval stage are able to survive unsuitable seasons (summer to winter) in Japan. Japanese population exclusively utilises *C. argenteoalba* including records by Matsumoto and Konishi (2007), but excluding one on *C. laticauda* recorded in this study.

4.2. Web alteration patterns

The alteration of web-building behaviour in host spiders by *Z. picticollis* and *R. nielseni* was recently studied by Korenko et al. (2015a) and Takasuka et al. (2015). However, we present some notes here which are deserving of discussion because the studied systems show uncommon relationships among the host spider–polysphinctine interactions i.e. two polysphinctines (*Z. picticollis* and *R. nielseni*) influencing the same spider (*C. conica*), and one polysphinctine (*R. nielseni*) influencing three different spiders (*C. conica*, *C. argenteoalba* and *C. laticauda*). Such anomalous relationships provide us with an opportunity to predict the proximal mechanism of web modification from a behavioural point of view.

The situation of two wasps influencing the same spider has already been observed in other polysphinctines. One of them, spider *Leucauge mariana* (Taczanowski, 1881) (Tetragnathidae) was altered its web-building behaviour by *Hymenoepimecis tedfordi* Gauld, 1991 and *Eruga* cf. *gutfreundi* in Costa Rica (Eberhard, 2013). The larva of the first one induces modification of the horizontal orb web into a more or less plane-like web, consisting of several reinforced radii and frame lines. The larva of the second one forces the spider to modify its orb web into a 3D tangle web with numerous radii converged centrally onto the cocoon and no frame lines.

In this study, both cocoon webs of *C. conica* induced by *Z. picticollis* and *R. nielseni* had a similar constructional plan that exploited pre-existing frame lines for the cocoon webs. However, the resulting cocoon webs were somewhat different; in *Z. picticollis*, the radii ran straight to the outer frames without any silk attachments and hub loops (Fig. 2b), also confirmed by Korenko et al. (2015a), while in *R. nielseni* in Slovakia, the radii ran in a somewhat zigzag fashion and hub loops remained (Fig. 2d), also observed by Nielsen

Fig. 4. Cocoons of *Z. picticollis* and *Reclinervellus* spp. (a) *Z. picticollis* upon *C. conica* in Slovakia with arrows indicating pigmented spots, (b) *R. nielseni* upon *C. conica* in Czech Republic, (c) *R. nielseni* upon *C. conica* in Slovakia, (d) *R. nielseni* upon *C. conica* without ribs cocooning in a tube, (e) *R. nielseni* upon *C. laticauda* in Japan, (f) *R. nielseni* upon *C. argenteoalba* in Japan obtained from the field, (g) *R. masumotoi* upon *C. octotuberculata* in Japan obtained from the field, (h) *R. tuberculatus* upon *C. octotuberculata* in Japan obtained from the field.

(1923). This indicates that certain differences in the neurophysiologic changes in the spider host occur depending on the parasitoids.

The web induced by the Czech *R. nielseni* (Fig. 2c) exhibited reduced numbers of spirals and radii, but this form is totally different from that induced in Slovakian *C. conica* (Fig. 2d), *C. argenteoalba* (Takasuka et al., 2015) and *C. laticauda* (Fig. 3) in that the fragile capture region of orb webs remained. Although diminished spirals may result in a smaller probability of insect or debris impact, cocoon webs ever known in any polysphinctines have never used capture materials and uniform spoke-like radii mean singly-woven silks (unreinforced). Two possibility may result in such variation; the one is an individual mismatching because the design properties of the spider's nervous system have certainly not evolved preferably according to the larval interference (Eberhard, 2010) or the other is a consequence from the insufficient amount of the manipulative compound if it has dose-dependent effect.

The cocoon web of *C. argenteoalba* induced by *R. nielseni* resembles the resting web according to the sharing of the reduced numbers of radii and the specific fluffy decorations on them (not silk decoration near the hub). This decoration reflects UV light making the web conspicuous to potential web-destroyers such as large flying insects and birds (Takasuka et al., 2015). Although hub loops and a decrease in the number of radii, which became somewhat zigzagged, were also observed in the cocoon webs of *C. conica* and *C. laticauda*, no decoration at all was newly attached to the radii. The conformity in several web substructures of the cocoon webs of three different *Cyclosa* hosts indicates that induced behaviours have a similar or identical constructional plan possibly derived from the spider's innate web building behaviour (e.g. resting web). The absence of decoration on radii in *C. conica* and *C. laticauda* cocoon webs is presumably because this decoration is not innate for these spider species.

4.3. *Cocoon morphology*

The cocoon of *R. nielseni* is noteworthy in having conspicuous long ribs that make the cocoon quadrate in cross-section (Fig. 4b–f) and which do not appear in two co-generic species (Fig. 4b,c,e,f). In a phylogenetic analysis based on molecular markers provided by Matsumoto (2016), *R. nielseni* was clustered inside a clade with *R. tuberculatus* being its sister lineage and with *R. masumotoi* being a paraphyletic lineage of this clade. Both, *R. tuberculatus* and *R. masumotoi* cocoons are strictly circular in cross-section. Therefore, the conspicuous ribs seem to have evolved independently as an autapomorphy in *R. nielseni*.

The ribs was first recognized by Nielsen (1923) in Denmark but it was newly found to be common to all *R. nielseni* regardless of host spider or region. One almost unribbed cocoon (Fig. 4d) was probably due to the rearing conditions in a small tube interfering with the cocooning process, indicating that the rib-forming behaviour is sensitive to disturbance. Such curious cocoon morphology is very similar to that of other polysphinctine genera, *Acrodactyla* and *Eruga* (Eberhard, 2013; Gauld and Dubois, 2006) and is so remarkable that it is valid as a generic trait (Gauld and Dubois, 2006) reflecting larval cocooning behaviour; however, its adaptive significance has never been clarified. A visual signalling function would be possible in *R. nielseni* because the purposes of the web decoration used in cocoon webs induced in *C. argenteoalba* (fluffy decoration to be conspicuous) by *R. nielseni* (Takasuka et al., 2015) and *C. octotuberculata* (debris decoration to be camouflaging) by two congeners are opposite. Although, we do not know how common debris decorations of *C. conica* and *C. laticauda* are, it seems not always.

Contribution of authors

Collected field data: all authors. Performed the laboratory observations, analyzed the data and wrote the paper: KT and SK.

Acknowledgements

K.T. was supported by a Sasakawa Scientific Research Grant (28-525) from The Japan Science Society and by the Hyogo Science and Technology Association. S.K. was supported by the Institutional Support Program for the Long Term Conceptual Development of Research Institutions provided by the Ministry of Education, Youth and Sports of the Czech Republic. Ľ.Č. and I.M. were supported by VEGA projects No2/0039/14 and 2/0035/13. P. D. was supported by Ministry of Culture of the Czech Republic (DKRVO 2016/15, National Museum, 00023272).

References

Belgers, D., Zwakhals, K., van Helsdingen, P., 2013. De bijzondere levensloop van de sluipwesp *Acrodactyla quadrisculpta* op de schaduwstrekspin *Tetragnatha montana* (Hymenoptera: Ichneumonidae, Araneae: Tetragnathidae). Nederlandse Faunistische Mededelingen 39, 1–6.

Eberhard, W.G., 2000. Spider manipulation by a wasp larva. Nature 406, 255–256.

Eberhard, W.G., 2001. Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). J. Arachnol. 29, 354–366.

Eberhard, W.G., 2003. Substitution of silk stabilimenta for egg sacs by *Allocyclosa bifurca* (Araneae: Araneidae) suggests that silk stabilimenta function as camouflage devices. Behaviour 140, 847–868.

Eberhard, W.G., 2010. New types of behavioral manipulation of host spiders by a parasitoid wasp. Psyche 950614, 1–4.

Eberhard, W.G., 2013. The Polysphinctine wasps *Acrotaphus tibialis*, *Eruga* ca. *gutfreundi*, and *Hymenoepimecis tedfordi* (Hymenoptera Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. Ann. Entomol. Soc. Am. 106, 652–660.

Erzsébet, N.B., 1960. Fürkészdarázs alkatúak I. (Ichneumonoidea I.) 72 ábrával Magyarország állatvilága, Fauna Hungariae, 54. (XI. kötet, 4. füzet). Akadémiai Kiadó (in Hungary).

Fitton, M.G., Shaw, M.R., Austin, A.D., 1987. The Hymenoptera associated with spiders in Europe. Zool. J. Linn. Soc. 90, 65–93.

Fitton, M.G., Shaw, M.R., Gauld, I.D., 1988. Pimpline ichneumon-flies. Handb. Identif. Br. Insects 7, 1–110.

Foelix, R.F., 2010. Biology of Spiders, third ed. Oxford University Press, NY, USA.

Fritzén, N.R., 2005. *Polysphincta nielseni* (Hymenoptera, Ichneumonidae, Pimplinae) new to Finland. Memoranda Soc. Fauna Flora Fennica 81, 49–51.

Gan, W.J., Liu, F.X., Zhang, Z.T., Li, D.Q., 2010. Predator perception of detritus and eggsac decorations spun by orb-web spiders *Cyclosa octotuberculata*: do they function to camouflage the spiders? Curr. Zool. 56, 379–387.

Gauld, I.D., Dubois, J., 2006. Phylogeny of the *Polysphincta* group of genera (Hymenoptera Ichneumonidae; Pimplinae), a taxonomic revision of spider ectoparasitoids. Syst. Entomol. 31, 529–564.

Gonzaga, M.O., Sobczak, J.F., 2011. Behavioral manipulation of the orb-weaver spider *Argiope argentata* (Araneae: Araniedae) by *Acrotaphus chedelae* (Hymenoptera: Ichneumonidae). Entomol. Sci. 14, 220–223.

Gonzaga, M.O., Vasconcellos-Neto, J., 2012. Variation in the stabilimenta of *Cyclosa fililineata* Hingston, 1932, and *Cyclosa morretes* Levi, 1999 (Araneae: Araneidae), in Southeastern Brazil. Psyche 2012, 1–10.

Gonzaga, M.O., Sobczak, J.F., Penteado-Dia, A.M., Eberhard, W.G., 2010. Modification of *Nephila clavipes* (Araneae Nephilidae) webs induced by the parasitoids *Hymenoepimecis bicolor* and *H. robertsae* (Hymenoptera Ichneumonidae). Ethol. Ecol. Evol. 22, 151–165.

Grimaldi, D., Engel, M.S., 2005. Evolution of the Insects. Cambridge University Press, Cambridge.

Grout, T.G., Brothers, D.J., 1982. Behaviour of a parasitic pompilid wasp (Hymenoptera). J. Entomol. Soc. South Afr. 45, 217–220.

Herberstein, M.E., Craig, C.L., Coddington, J.A., Elgar, M.A., 2000. The functional significance of silk decorations of orb-web spiders: a critical review of the empirical evidence. Biol. Rev. 75, 649–669.

Kasparyan, D.R., 1976. Review of Ichneumonids of the tribe Polysphinctini and Poemeniini (Hymenoptera, Ichneumonidae) of the Far East (in Russian). Tr. Zool. Inst. 67, 68–89.

Kloss, T.G., Gonzaga, M.O., Roxinol, J.A.M., Sperber, C.F., 2016. Host behavioural manipulation of two orb-weaver spiders by parasitoid wasps. Anim. Behav. 111, 289–296.

Korenko, S., Isaia, M., Satrapová, J., Pekár, S., 2014. Parazitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecol. Entomol. 39, 30–38.

Korenko, S., Satrapova, J., Zwakhals, K., 2015a. Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimlinae). Entomol. Sci. 18, 383–388.

Korenko, S., Korenková, B., Satrapova, J., Hamouzová, K., Belgers, D., 2015b. Modification of *Tetragnatha montana* (Araneae, Tetragnathidae) web architecture induced by larva of the parasitoid *Acrodactyla quadrisculpta* (Hymenoptera, Ichneumonidae, *Polysphincta* genus-group). Zool. Stud. 54, 40.

Levi, H.W., 1977. The American orb-weaver genera *Cyclosa*, *Metazygia* and *Eustala* north of Mexico (Araneae, Araneidae). Bull. Mus. Comp. Zool. 148, 61–127.

Levi, H.W., 1999. The Neotropical and Mexican orb weavers of the genera *Cyclosa* and *Allocyclosa* (Araneae Araneidae). Bull. Mus. Comp. Zool. 155, 299–379.

Matsumoto, R., Konishi, K., 2007. Life histories of two ichneumonid parasitoids of *Cyclosa octotuberculata* (Araneae), *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomol. Sci. 10, 267–278.

Matsumoto, R., 2016. Molecular phylogeny and systematics of the *Polysphincta* group of genera (Hymenoptera, Ichneumonidae, Pimplinae). Syst. Entomol. 41, 854–864.

Nakata, K., 2009. To be or not to be conspicuous: the effects of prey availability and predator risk on spider's web decoration building. Anim. Behav. 78, 1255–1260.

Nielsen, E., 1923. Contributions to the life history of the pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*) (Hym. Ichneumon.). Ent. Meddel. 14, 137–205.

Roberts, M.J., 1995. Spiders of Britain & Northern Europe. Harper Collins Publishers Bath.

Schmitt, M., Richter, D., Göbel, D., Zwakhals, K., 2012. Beobachtungen zur Parasitierung von Radnetzspinnen (Araneidae) durch *Polysphincta rufipes* (Hymenoptera: Ichneumonidae). Arachnol. Mitt. 44, 1–6.

Shinkai, E., 2006. Spiders of Japan. Bun-ichi Sogo Shuppan Co. Ltd., Tokyo, Japan (in Japanese).

Sobczak, J.F., Loffredo, A.P.S., Penteado-Dias, A.M., Gonzaga, M.O., 2009. Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. J. Nat. Hist. 43, 2691–2699.

Souza, H.d.S., essas, Y.F., Masago, F., dos Santos, E.F., Vasconcellos-Neto, J., 2015. *Paracyphononyx scapulatus* (Hymenoptera, Pompilidae), a koinobiont ectoparasitoid of *Trochosa* sp. (Araneae, Lycosidae). J. Hymenopt. Res. 46, 165–172.

Takasuka, K., Yasui, T., Ishigami, T., Nakata, K., Matsumoto, R., Ikeda, K., Maeto, K., 2015. Host manipulation by an ichneumonid spider-ectoparasitoid that takes advantage of preprogrammed web-building behaviours for its cocoon protection. J. Exp. Biol. 218, 2326–2332.

Takasuka, K., 2015. *Reclinervellus nielseni* sucking its host spider dry and weaving a cocoon on the web. Movie Archives of Anim. Behav. (data no.: momo150716rn01b). http://www.momo-p.com/showdetail-e.php?movieid=momo150716rn01b.

Tso, I.M., 1998. Stabilimentum-decorated webs spun by *Cyclosa conica* (Araneae, Araneidae) trapped more insects than undecorated webs. J. Arachnol. 26, 101–105.

Walter, A., Elgar, M.A., 2012. The evolution of novel animal signals: silk decorations as a model system. Biol. Rev. 87, 686–700.

World Spider Catalog, 2016. Version 17.0. Natural History Museum Bern, http://wsc.nmbe.ch, version 17.0, (Accessed 20 December 2016).

Yu, D.S., van Achterberg, C., Horstmann, K., 2012. Taxapad 2012–World Ichneumonoidea 2011. Taxonomy, Biology, Morphology and Distribution. Database on flash-drive. Ottawa, Ontario, Canada. http://www.taxapad.com, (Accessed 20 December 2016).

Supplement 11

Korenko S. 2017. First record from Italy of *Zatypota anomala* (Ichneumonidae, Ephialtini), a parasitoid of the cribellate spider *Dictyna pussila* (Araneae, Dictynidae). Arachnologische Mitteilungen 54, 1-4.

Citation report:
Scopus/Google Scholar: 0

SCOPUS:
Arachnologische Mitteilungen
SJR$_{(2016)}$ = 0,374

Arachnologische Mitteilungen / Arachnology Letters 54: 1-4
Karlsruhe, September 2017

First record from Italy of *Zatypota anomala* (Ichneumonidae, Ephialtini), a parasitoid of the cribellate spider *Dictyna pusilla* (Araneae, Dictynidae)

Stanislav Korenko

doi: 10.5431/aramit5401

Abstract. The polysphinctine wasp *Zatypota anomala* (Holmgren, 1860), a koinobiont ecto-parasitoid of spiders in the family Dictynidae, was recorded in Italy for the first time. Populations of both the wasp and its host, *Dictyna pusilla* Thorell, 1856, its host preference and the interaction between the parasitoid and the spider were documented in the field and by laboratory observations.

Keywords: ectoparasitoid, host parasitoid interaction, host range, host specialisation, spider web

Zusammenfassung. Erstnachweis von *Zatypota anomala* (Ichneumonidae, Ephialtini) für Italien, ein Parasitoid der cribellaten Spinne *Dictyna pusilla* (Araneae, Dictynidae). Die zu den Polysphinctinae gehörende Schlupfwespe *Zatypota anomala* (Holmgren, 1860), ein koinobionter Extoparasit von Spinnen der Familie Dictynidae, wurde erstmals für Italien nachgewiesen. Es werden Populationen der Wespe und ihres Wirtes, *Dictyna pusilla* Thorell, 1856, die Wirtspräferenz und Interaktionen zwischen Parasitoid und Spinne aus dem Freiland und dem Labor dokumentiert.

With 50 described species, the genus *Zatypota* comprises highly specialised koinobiont ecto-parasitoids of spiders and is the largest genus of the *Polysphincta*-group in the world (Gauld & Dubois 2006, Matsumoto & Takasuka 2010, Yu et al. 2012, Fritzén 2014). *Zatypota* probably includes as many species as in all other genera of the *Polysphincta* genus-group combined, and many undescribed species still exist in museum collections (e.g. Gauld & Dubois 2006). All *Zatypota* species are narrowly associated with a specific host spider species or a small group of closely related spider species. *Zatypota* wasps are mostly parasitoids of theridiid spiders (Nielsen 1923, Fitton et al. 1988, Gauld & Dubois 2006, Korenko & Pekár 2011, Korenko et al. 2011), but three *Zatypota* species are associated with spiders other than theridiids (Matsumoto & Takasuka 2010, Korenko et al. 2015, Vincent 1979).

Eight species from the genus *Zatypota* occur in Europe (de Jong et al. 2014, Fritzén 2010, 2014). Six of them – *Zatypota percontatoria* (Müller, 1776), *Z. bohemani* (Holmgren, 1860), *Z. discolor* (Holmgren, 1860), *Z. kerstinae* Fritzén, 2010, *Z. albicoxa* (Walker, 1874) and *Z. flamma* Fritzén, 2014) – are strictly associated with tangle web weavers from the family Theridiidae. *Zatypota percontatoria*, *Z. bohemani*, *Z. discolor* and *Z. kerstinae* are associated with spiders from the genus *Theridion*, or closely-related genera (e.g. *Phylloneta*, *Neottiura*) with similar habitus and behaviour (Fitton et al. 1987, 1988, Korenko et al. 2011, Korenko & Pekár 2011, Fritzén 2010, 2014). By contrast, *Z. albicoxa* and *Z. flamma* are associated with the genus *Parasteatoda* (e.g. Fitton et al. 1987, 1988, Fritzén 2014). *Zatypota picticollis* (Thomson, 1888) is associated with orb web weavers from the family Araneidae (Zwakhals 2006, Korenko et al. 2015), while the species studied here, *Zatypota anomala* (Holmgren, 1860), which is distributed across the Holarctic, is known to be associated with space web weavers from the family Dictynidae (Vincent 1979, Yu et al. 2012).

The aim of this study was to analyse *Z. anomala* populations in the canopies of an ecological fruit orchard in northern Italy and to observe the interaction between the parasitoid larva and its spider host in the laboratory.

Material and methods

Web building spiders (Araneae, Orbicularia) and their ecto-parasitoids (Hymenoptera, Ichneumonidae, *Polysphincta* genus group) were collected from tree canopies (between 40 cm and 200 cm above ground) in an organic hazelnut orchard in Bottonasco (44°25'13.9"N 7°23'40.2"E, 642 m a.s.l.) in the province of Cuneo (Piedmonte region, northern Italy) on the 4th November 2009.

Spider hosts and parasitoids at the larval stage attached to them were collected by beating tree branches and catching the spiders in a square shaped beating net (1 m^2 area) placed beneath the tree crown. Three samples (each sample was collected by beating 30 trees) were taken and preserved in 70 % alcohol. The collected spiders were identified to genus level using Nentwig et al. (2017) and matched to species both on the basis of formerly known occurring species in the investigated localities (Isaia et al. 2010, Korenko unpubl. data) and by rearing the collected specimens to adulthood.

Live unparasitised and parasitized spiders were collected by the same method (described above) and reared in the laboratory. Parasitoid larvae were reared to imagines. Hatched wasps were identified using Fitton et al. (1988) and Zwakhals (2006). The nomenclature of the wasps follows Yu et al. (2012). Voucher specimens were deposited in the collection of the author and in the collection of Kees Zwakhals (Netherlands).

The web architecture of non-parasitised (n = 12) and parasitised (n = 44) spiders was studied by placing spiders individually in square glass experimental arenas (100 × 100 mm base, 130 mm height) with an installed tree twig that provided three-dimensional space for the spider's webbing. Spiders were kept at room temperature (22 ± 3 °C) under a natural L:D regime and fed with a surplus of *Drosophila* flies. The webs of both unparasitised and parasitised spiders were recorded using a Canon EOS 500D digital camera with an EF-S 18–55 mm lens or a macro EF 100 mm f/2,8L IS USM lens.

Stanislav KORENKO, Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic;
E-mail: korenko.stanislav@gmail.com

submitted 17.2.2017, accepted 18.4.2017, online 30.4.2017

Results

Host community and incidence of parasitism. The family Dictynidae consisted almost exclusively of spiders of the genus *Dictyna* (reared adults identified as *D. pusilla*); only 1 % belonged to the genus *Nigma*. The space web weaver *D. pusilla* was one of the most abundant spider species in the investigated fruit orchard, representing 17.8 % of all collected spider individuals (from *n* = 1.069). The average incidence of parasitism of *D. pusilla* was 12 % (Tab. 1). The larvae were typically attached antero-dorsally close to the petiolus hidden between the abdomen and prosoma of the spider host. All parasitised spiders were juveniles with an average prosoma length of 0.64 mm (SD = 0.12, n = 44). Fifty-one percent of wasps reared in the laboratory reached the imaginal stadium. Mortality during ontogeny occured when the spider-hosts died (43 %), when the parasitoid died at the larval stage (33 %) and when the parasitoid died at the pupal stage (24 %). All parasitoid wasps reared from *D. pusilla* were *Zatypota anomala* (*n* = 22). The pupal stage in laboratory rearing lasted on average 11.9 days (SD = 1.39, n = 22). During this study, *Z. anomala* was recorded in Italy for the first time.

Reared material. Province of Cuneo: Bottonasco, organic hazel nut orchard (Cascina Rosa), larva attached to *Dictyna pusilla*, leg. 4.XI.2009 adult emerged 15.XII.2009–30.I.2010 (11 males, 11 females), leg. S. Korenko, det. K. Zwakhals & S. Korenko.

Fig. 1: Normal web of an unparasitised spider *Dictyna pusilla*. Arrow shows the typical spider resting position in the centre of the tangle.

Host parasitoid interaction. Unparasitised spiders built a typical cribellate (sticky) space web with a retreat formed from an aggregation of threads (Fig. 1). There was no observed modification of the spider web under the influence of the final stage parasitoid larva. Parasitized spiders took a position inside their retreat (100 %), where the larva killed the host, spun a cocoon, and pupated (Fig. 2). Cocoons were diaphanous, white, and sub-cylindrical, with a springy and very open construction of sparse loose whorls.

Discussion

The community of arboreal spiders in the studied fruit orchard was dominated by space web building spiders, and Dictynidae represented 17.8 % of all collected spiders. Dictynid spiders were relatively frequently parasitised by polysphinctine parasitoids (12 % average incidence of parasitism). The incidence of parasitism by polysphinctines among various spider species was mostly found to be low (often below 1–5 %) in Central Europe (Korenko et al. 2011), in Central America (Barrantes et al. 2008) and in Japan (Tanaka 2007), but several studies from northern Italy found relatively high parasitism rates, i.e. above 10 % (Korenko et al. 2014, 2015).

Zatypota anomala is known to be associated with cribellate tangle web weaving spiders belonging to the family Dictynidae (Aubert 1969). Vincent (1979) found this wasp to be associated with *Mallos pallidus* (Banks, 1904) in North America; and the species was repeatedly reared from *Dictyna* sp. and *D. pusilla* in Europe (Miller et al. 2013, this study). *Zatypota*

Tab. 1: Relative spider host abundance (Ab.), average incidence of parasitism (PR), and wasp species documented in the studied locality (Korenko et al. 2014, Korenko et al. 2015, Korenko unpubl. data, this study). Foraging guild classification follows Cardoso et al. (2011).

Host family	Foraging guild	n	Ab. (%)	PR (%)	Associated parasitoids
Araneidae	Orb web weavers	42	3,9	11	*Polysphincta tuberosa*, *P. boops*, *Zatypota picticollis*, *Sinarachna pallipes*
Tetragnathidae	Orb web weavers	12	1,1	0	
Dictynidae	Space web weavers	190	17,8	12	*Zatypota anomala*
Theridiidae	Space web weavers	611	57,2	4	*Zatypota percontatoria*
Linyphiidae	Sheet web weavers	2	0,2	0	
Mimetidae	Specialists	2	0,2	0	
Anyphaenidae	Hunters	52	4,9	0	
Clubionidae	Hunters	7	0,7	0	
Eutichuridae	Hunters	1	0,1	0	
Philodromidae	Hunters	139	13,0	0	
Salticidae	Hunters	1	0,1	0	
Thomisidae	Ambush hunters	10	0,9	0	
Total		**1069**	**100**	**5**	

Fig. 2: Web of a parasitised spider. Arrow shows wasp cocoon located at the spider's resting position.

anomala seems to be exclusively associated with the genus *Dictyna* in Europe (Miller et al. 2013, Gauld & Dubois 2006, Korenko unpubl. data, this study). A similarly narrow host specialisation can be found in Z. *kerstinae* known only from Finland, which is assumed to be associated only with *Theridion palmgreni* Marusik & Tsellarius, 1986 (Fritzén 2010). In contrast, *Zatypota percontatoria* attacks several closely related host species from the family Theridiidae (Korenko et al. 2011), while *Z. picticollis* from central and western Europe attacks three araneid species from three different genera, namely *Cyclosa conica* (Pallas, 1772), *Mangora acalypha* (Walckenaer, 1802) and *Zilla diodia* (Walckenaer, 1802) (Zwakhals 2006, Korenko et al. 2015).

Dictynids are cribellate spiders, i.e. spiders which use silk produced by a special silk spinning organ called the cribellum. Cribellate silk not only serves to capture prey, but could also protect the spider against predators or parasitoids. Presumable, *Zatypota anomala* developed a way to avoid this barrier and to use this silk mass for its own protection during the pupal stage. However, there is no other polysphinctine parasitoid which is known to be able to associate with any cribellate spider. The way in which *Zatypota albicoxa* lures the spider host, how it avoids being captured by the silk, and how it oviposits on the spider host hidden inside the tangle web was documented by Takasuka et al. (2009) and Takasuka & Matsumoto (2011). The related species *Z. albicoxa* decoys the spider out from its retreat by pulling on threads in such a way as to imitate prey captured on the edge of the web (Takasuka et al. 2009). This luring of the spider host, also called "ambush-style", would be expected in *Z. anomala*. Unfortunately, observation of oviposition by *Z. anomala* is missing and merits further investigation.

Zatypota species associated with space web weavers use the spider's innate 3D web as protection for their pupation. Some wasps are known to force their spider hosts to build an additional 3D structure and thereby to improve protection for wasp pupation (*Z. percontatoria* and *Z. discolor*) (Korenko & Pekár 2011, Korenko unpubl. data). Others, like Z. *kerstinae*, do not induce any changes in the host webbing (Fritzén pers. comm.). The studied species, *Z. anomala*, did not induce changes in web architecture, presumably because of the location of the parasitoid pupa inside the innate "sticky" space web of the *Dictyna* spider. The innate web of the dictynid host seems to provide sufficient protection for the parasitoid during its pupal stage, meaning that there is no reason to waste energy or resources on modifying it.

Acknowledgements

This study was supported by the Institutional Support Program for the Long Term Conceptual Development of Research Institutions provided by the Ministry for Education, Youth and Sport of the Czech Republic.

References

Aubert JF 1969 Les Ichneumonides ouest-paléarctiques et leurs hôtes. 1. Pimplinae, Xoridinae, Acaenitinae. Laboratoire d'Evolution des Etres Organises, Paris. 302 pp.

Barrantes G, Eberhard WG & Weng J-L 2008 Seasonal patterns of parasitism of the tropical spiders *Theridion evexum* (Araneae, Theridiidae) and *Allocyclosa bifurca* (Araneae, Araneidae) by the wasps *Zatypota petronae* and *Polysphincta gutfreundi* (Hymenoptera, Ichneumonidae). – Revista de Biología Tropical 56: 749-754

Cardoso P, Pekár S, Jocqué R & Coddington JA 2011 Global patterns of guild composition and functional diversity of spiders. – PLoS ONE 6(e21710): 1-10 – doi: 10.1371/journal.pone.0021710

Fitton MG, Shaw MR & Austin AD 1987 The Hymenoptera associated with spiders in Europe. – Zoological Journal of the Linnean Society 90: 65-93 – doi: 10.1111/j.1096-3642.1987.tb01348.x

Fitton MG, Shaw MR & Gauld ID 1988 Pimpline ichneumon-flies. Hymenoptera, Ichneumonidae (Pimplinae). – Handbooks for the Identification British Insects 7: 1-110

Fritzén NR 2010 Natural history and description of *Zatypota kerstinae* sp.nov. (Hymenoptera: Ichneumonidae) reared from *Theridion palmgreni* Marusik et Tsellarius (Araneae: Theridiidae) in Finland. – Zootaxa 2487: 52-60

Fritzén NR 2014 Two new species of the *Polysphincta* genus-group (Hymenoptera: Ichneumonidae: Pimplinae) reared from their spider (Araneae) hosts in Europe. – Zootaxa 3894: 117-130 – doi: 10.11646/zootaxa.3894.1.9

Gauld ID & Dubois J 2006 Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae), a taxonomic revision of spider ectoparasitoids. – Systematic Entomology 31: 529-564 – doi: 10.1111/j.1365-3113.2006.00334.x

Isaia M, Beikes S, Paschetta M, Sarvajayakesavalu S & Badino G 2010 Spiders as biological controller in apple orchards infested by *Cydia* spp. In: Nentwig W, Schmidt - Entling M & Kropf C (eds.) European Arachnology 2008. Contributions to Natural History. Natural History Museum, Bern. pp. 79-88

Jong Y de, Verbeek M, Michelsen V, Bjørn P de P, Los W, Steeman F, Bailly N, Basire C, Chylarecki P, Stloukal E, Hagedorn E, Wetzel FT, Glöckler F, Kroupa A, Korb G, Hoffmann A, Häuser C, Kohlbecker A, Müller A, Güntsch A, Stoev P & Penev L 2014 Fauna Europaea – all European animal species on the web. – Biodiversity Data Journal 2(e4034): 1-35 – doi: 10.3897/BDJ.2.e4034

Korenko S, Isaia M, Satrapová J & Pekár S 2014 Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). – Ecological Entomology 39: 30-38 – doi: 10.1111/een.12067

Korenko S, Michalková V, Zwakhals K & Pekár S 2011 Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid (Hymenoptera: Ichneumonidae). – Journal of Insect Science 11(101): 1-12 – doi: 10.1673/031.011.10101

Korenko S & Pekár S 2011 A parasitoid wasp induces overwintering behaviour in its spider host. – PLoS ONE 6(e24628): 1-5 – doi: 10.1371/journal.pone.0024628

Korenko S, Satrapova J & Zwakhals K 2015 Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimplinae). – Entomological Science 18: 383-388 – doi: 10.1111/ens.12132

Matsumoto R & Takasuka K 2010 A revision of the genus *Zatypota* Förster of Japan, with descriptions of nine new species and notes on their hosts (Hymenoptera: Ichneumonidae: Pimplinae). – Zootaxa 2522: 1-43

Miller J, Belgers J, Beentjes K, Zwakhals K & Helsdingen P van 2013 Spider hosts (Arachnida, Araneae) and wasp parasitoids (Insecta, Hymenoptera, Ichneumonidae, Ephialtini) matched using DNA barcodes. – Biodiversity Data Journal 1(e992): 1-20 – doi: 10.3897/BDJ.1.e992

Nentwig W, Blick T, Gloor D, Hänggi A & Kropf C 2017 araneae. Spiders of Europe. – Internet: http://www.araneae.unibe.ch (13.4.2017)

Nielsen E 1923 Contribution to the life history of the Pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*) (Hym. Ichneum.). – Entomologiske Meddelelser 14: 137-205

Takasuka K & Matsumoto R 2011 Lying on the dorsum: unique host-attacking behaviour of *Zatypota albicoxa* (Hymenoptera, Ichneumonidae). – Journal of Ethology 29: 203-207 – doi: 10.1007/s10164-010-0263-8

Takasuka K, Matsumoto R & Ohbayashi N 2009 Oviposition behavior of *Zatypota albicoxa* (Hymenoptera, Ichneumonidae), an ectoparasitoid of *Achaearanea tepidariorum* (Araneae, Theridiidae). – Entomological Science 12: 232-237 – doi: 10.1111/j.1479-8298.2009.00338.x

Tanaka K 2007 Seasonal life cycle of *Zatypota albicoxa* (Hymenoptera: Ichneumonidae), an ectoparasitoid of *Achaearanea tepidariorum* (Araneae: Theridiidae), in northern Japan. – Entomological Science 10: 421-423 – doi: 10.1111/j.1479-8298.2007.00231.x

Vincent LS 1979 A new record for *Sinarachna anomala* (Hymenoptera: Ichneumonidae), an external parasitoid of *Mallos pallidus* (Araneae: Dictynidae). – Pan-Pacific Entomologist 55: 192-164

Yu DS, Achterberg C van & Horstmann K 2012 Taxapad 2012. Ichneumonoidea 2011. Taxonomy, biology, morphology and distribution. Ottawa, Ontario. – Internet: http://www.taxapad.com (January 20, 2017)

Zwakhals K 2006 The European species of the genera *Zatypota* and *Sinarachna* (Hymenoptera: Ichneumonidae, Pimplinae, Polysphinctini). – Entomologische Berichten 66: 34-37

Supplement 12

Korenko S, Kysilková K, Černecká Ľ. 2017. Further records of two spider parasitoids of the genus *Polysphincta* (Ichneumonidae, Ephialtini) from Central Europe, with notes on their host interactions. Arachnologische Mitteilungen54: 28-32.

Citation report:
Scopus/Google Scholar: 1

SCOPUS:
Arachnologische Mitteilungen
SJR$_{(2016)}$ = 0,374

Arachnologische Mitteilungen / Arachnology Letters 54: 28-32 Karlsruhe, September 2017

Further records of two spider-parasitoids of the genus *Polysphincta* (Hymenoptera, Ichneumonidae, Ephialtini) from Central Europe, with notes on their host interactions

Stanislav Korenko, Kristýna Kysilková & Ľudmila Černecká

doi: 10.5431/aramit5406

Abstract. A polysphinctine wasp *Polysphincta longa*, associated with the orb web building spider *Araneus angulatus*, was recorded for the first time in Slovakia. Its congener *Polysphincta tuberosa* was recorded in association with a new spider host *Araneus sturmi*. New records of *Polysphincta* species from the Czech and Slovak Republics are presented. The final stage larvae of both, *P. longa* and *P. tuberosa*, induce a specific alteration in web architecture: the spider constructed a unique 3D tangle of silk – so called "cocoon web" – to protect the parasitoid during the pupal stage. The host range of wasps belonging to the genus *Polysphincta* in Europe is discussed.

Key words: behavioural alteration, diversity, spider host

Zusammenfassung. Neue Nachweise von zwei Spinnen-Parasitoiden der Gattung *Polysphincta* (Hymenoptera, Ichneumonidae, Ephialtini) aus Mitteleuropa, mit Beobachtungen ihrer Wirts-Interaktionen. Die Schlupfwespe *Polysphincta longa*, die mit der Radnetzspinne *Araneus angulatus* assoziiert ist, wurde erstmals in der Slowakei gefunden. *Polysphincta tuberosa* wurde erstmals an der Wirtsart *Araneus sturmi nachgewiesen*. Neue Vorkommen von *Polysphincta*-Arten in der Tschechischen Republik und der Slowakei werden präsentiert. Die Larven des letzten Stadiums von *P. longa* und *P. tuberosa* induzieren eine spezielle Veränderung der Netzarchitektur: die Spinnen bilden ein dreidimensionales ‚Kokonnetz' um die Puppe des Parasitoiden zu schützen. Die Wirtsspektren der *Polysphincta*-Arten in Europa werden diskutiert.

Polysphincta is a moderately large genus within the *Polysphincta* group of genera (Ephialtini, Ichneumonidae, Hymenoptera), which are koinobiont parasitoids (a parasitoid, whose host continues to feed and grow after parasitization) exclusively associated with spider hosts. The genus is presently represented by five valid species in Europe: *Polysphincta boops* Tschek, 1869, *P. longa* Kasparyan, 1976, *P. rufipes* Gravenhorst 1829, *P. tuberosa* Gravenhorst, 1829 and *P. vexator* Fitton, Shaw & Gauld, 1988 (Yu et al. 2012). All of them are known to be strictly associated with species of the family Araneidae (e.g. Fitton et al. 1988, Yu et al. 2012), but their host spectrum involves multiple species; with the exception of *P. longa* (see below) which uses a single host species (Fitton et al. 1988, Schmitt et al. 2012, Yu et al. 2012, Fritzén & Shaw 2014, Korenko et al. 2014). *Polysphincta longa* is probably widely distributed across Europe, but presumably is often misidentified as the morphologically similar species *P. boops* (Fritzén & Shaw 2014). *Araneus angulatus* Clerck, 1757 recently turned out to be an exclusive host of this species (Fritzén & Shaw 2014).

Interestingly, a host behavioural manipulation has evolved in the final stage larvae of these wasps. Shortly before killing the spider host, the final stage larvae of several polysphinctines manipulate the webbing behaviour of their hosts, which spin a special web structure called a 'cocoon web' in order to establish a safe place for pupation. The cocoon web, a term coined by Eberhard (2000), is a web construction which is built by the spider host under the influence of the parasitoid's final stage larva. The cocoon web is stronger than the normal web and presumably provides a more durable support for the wasp's cocoon (e.g. Eberhard 2000, Korenko et al. 2014). Some polysphinctine parasitoids make use of original structures of spider's normal web for protection during the pupal stage, as was documented for *P. rufipes* (Schmitt et al. 2012). No modification of spider web building behaviour of *P. longa* was observed by Fritzén & Shaw (2014).

Here we present new records for *P. longa* and *P. tuberosa* from Slovakia and the Czech Republic, the host records, and descriptions of web architecture modification induced by the parasitoid's final stage larva. The host range of wasps of the genus *Polysphincta* in Europe is also discussed.

Material and methods

Potential spider hosts for hymenopteran parasitoids of the genus *Polysphincta*, araneids from the genera *Araneus* and *Araniella*, were inspected for the presence of parasitoid larva during one to three hour excursions (1) in a beech forest at the Kováčová locality, in the province of Zvolen, Slovakia (48°34'41"N, 19°5'35"E, 490 m a.s.l.) on 12th September 2016, (2) at a forest edge of Veľké Lúky (Krásna Ves, in the province of Trenčín, Slovakia (48°51'33"N, 18°13'32"E, 400 m a.s.l.) on 13th September 2016 and (3) at a forest edge of Východná, in the province of Liptovský Mikuláš, Slovakia (49°02'53"N, 19°54'54"E, 750 m a.s.l.) on 16th September 2016. Further records from the Czech Republic are presented (4) from a forest ecotone in the Hradečno locality, in the province of Kladno (50°11'12"N, 13°58'48"E, 380 m a.s.l.) on 3rd October 2014, (5) from the Marschnerova louka Meadow locality in the Chřibská province, the Lužické hory Mts. (50°52'32"N, 14°28'28"E, 360 m a.s.l.) on 18th June 2015 and (6) from a peat bog Soumarské rašeliniště locality in the Volvary province (48°54'8"N, 13°49'51"E, 750 m a.s.l.) on 11th May 2016.

Spiders were collected by beating bushes and tree branches up to a height of two meters above ground. A square-shaped net (1 m^2 area) was used and each collected spider was inspected for the presence of parasitoid larva. A parasitized *Araneus angulatus* was reared in a glass arena with a 400 × 400 mm base, 550 mm height and with a Y-shaped twig installed across the arena to provide space for building a web. A parasitized *Araneus sturmi* (Hahn, 1831) was reared in glass arena with a 200 × 50 mm base and 200 mm height. Other parasitized spider hosts (*Araniella* spp. and *Araneus quadratus* Clerck, 1757) were reared in tubes with a 15 mm

---

Stanislav KORENKO, Kristýna KYSILKOVÁ, Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Praha 6, Suchdol, Czech Republic; E-mail: korenko.stanislav@gmail.com, kysilkova@af.czu.cz
Ľudmila ČERNECKÁ, Institute of Forest Ecology, Slovak Academy of Sciences, Štúrova 2, 960 53 Zvolen, Slovakia; E-mail: komata1@gmail.com

submitted 27.3.2017, accepted 19.7.2017, online 10.8.2017

diameter and 100 mm height. Laboratory reared *Drosophila* flies or houseflies (*Musca domestica* Linnaeus, 1758) were provided every three days until the spiders were killed by the parasitoid final stage larvae. The web building behaviour of manipulated *A. angulatus* and *A. sturmi* was observed until the larvae killed and consumed the spiders and then pupated. Photographs were taken using an EOS 500D single-lens reflex digital camera (Canon Inc.). Microscopic observations of spider silk were performed in various parts of the cocoon web of *A. angulatus* (the wall of the silk chamber, threads in the chamber surroundings and the dragline thread). Specimens are deposited in the collection of S.K. (Department of Agroecology and Biometeorology, CULS, Czech Republic) and Kamil Holý (Department of Entomology, Crop Research Institute, Czech Republic).

Results

Field observation

The orb web weaving spider *A. angulatus* is a relatively rare arboreal species. During our excursions, we found only one specimen, and it was parasitized by a polysphinctine larva. After rearing of the parasitoid larva to adulthood in the laboratory, the parasitoid was identified as *P. longa*. The wasp is recorded for the first time in Slovakia. Other araneid hosts, *Araniella* spp., *A. quadratus*, *A. sturmi* were observed in higher numbers at several localities beyond that of *A. angulatus*, but the presence of parasitoid larvae was sporadic (N = 8). All eight larvae on these araneid spiders were *P. tuberosa*.

Reared material

CZECH REPUBLIC. Šumava Mts: Volvary province, peat bog Soumarské rašeliniště, larva attached to juvenile *Araneus quadratus*, leg. 11.V.2016, adult emerged – no data (one female *P. tuberosa*), leg. Dolejš P., det. Korenko S.; Lužické hory Mts: Chřibská province, Marschnerova louka locality, in a forest ecotone, larva attached to juvenile *Araniella* sp., leg. 18.VI.2015, adult emerged 7.VII.2015 (one female *P. tuberosa*), leg. Dolejš P., det. Korenko S.; province of Kladno, in a forest ecotone in the Hradečno locality, larvae attached to juvenile *Araniella* sp., leg. 3.X.2014, adults emerged 12.XII.2014–22.I.2015 (two females, one male *P. tuberosa*), leg. Korenko S., det. Korenko S.

SLOVAKIA. Province of Zvolen, Kováčová, beech forest, larva attached to *A. angulatus*, leg. Černecká Ľ. & Korenko S., 12.IX.2016, cocooned 25.X.2016, adult emerged 5.XI.2016 (1 male, *P. longa*), det. Korenko S., rev. Holý K.; province of Trenčín, locality Veľké lúky close to Motešice, larva attached to *Araniella* sp., leg. Štefánik M. & Korenko S., 13.IX.2016, cocooned 5. and 25.XI.2016, adult emerged 19.XI. and 9.XII.2016 (2 females, *P. tuberosa*), det. Korenko S.; province of Liptovský Mikuláš, Východná locality, larva attached to *Araneus sturmi*, leg. Šestáková A., 16.IX.2016, cocooned 25.X.2016, adult emerged 5.XI.2016 (1 female, *P. tuberosa*), det. Korenko S., rev. Holý K.

Laboratory observation

The parasitized *Araneus angulatus* (body length 8 mm) was placed in an experimental arena containing a Y-shaped twig (20th September 2016). The larva sat transversely at the anterior apex of the spider's opisthosoma just above the pedicel (Fig. 1). The spider built only one strong silk line between the ends of the arms of the Y-shaped twig at an early stage. Most of the time, the spider sat on the bark at the end of a twig. Only one orb web was built (11th October 2016), when the spider captured prey and fed. The spider's capture web was 42 cm wide with 16 radial lines and 25/24 spiral lines in the upper/lower part of the orb. The web was kept by the spider for two days, then the fly was provided as prey and the web was damaged by the spider. After feeding, the spider did not build any other orb web. After two weeks (24th October 2016), the larva doubled its body length to

Fig. 1: *Araneus angulatus* parasitized by *P. longa*. The larva is located transversely at the anterior apex of the spider's opisthosoma just above the pedicel. Scale: 2 mm

Fig. 2: Cocoon web built by the *A. angulatus* under the influence of *P. longa* larva. Scale: 5 mm

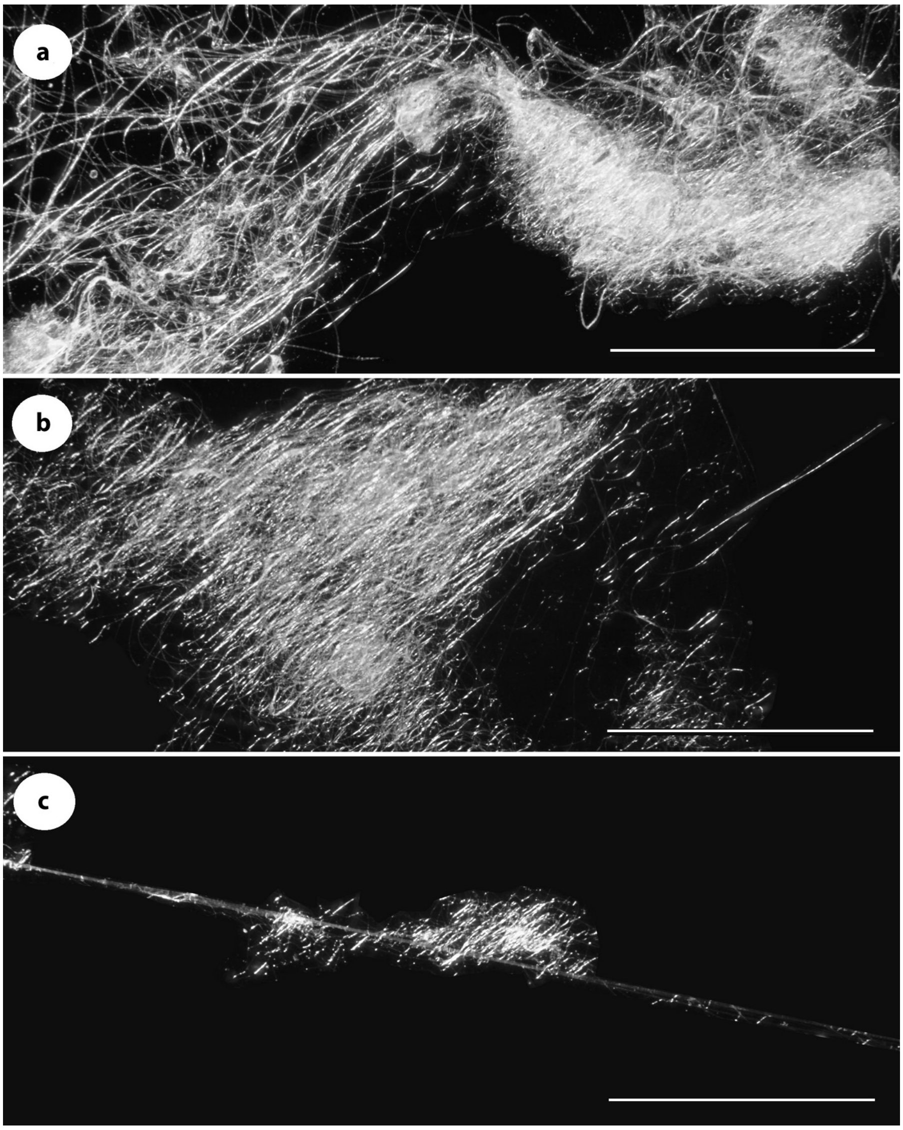


Fig. 3: Silk tufts produced by the spider under the influence of *P. longa* larva **a.** on the wall of the silk retreat; **b.** on threads in the surroundings of the retreat; **c.** on the dragline thread; Scales: 2 mm

9 mm and reached the final stage and induced changes in spider behaviour. Parasitized *A. angulatus* constructed a 3D tangle of silk (the cocoon web) at the end of the strong silk line in the top corner of the experimental arena (Fig. 2). The cocoon web of *P. longa* was decorated by various forms of silk tufts (Fig. 3), which were produced after modification of the spider's behaviour presumably induced by the larval effect. The tufts were produced in various parts of the cocoon web. The highest number of tufts was on a wall of the silk chamber, which surrounded the parasitoid pupa (Fig. 3a), on threads in the surroundings of the chamber (Fig. 3b) and on a frame thread (Fig. 3c).

The parasitoid larva paralysed and killed the spider, and built its cocoon at the centre of this 3D tangle. The cocoon was fusiform, white, sparsely spun with an opening at a distal end and oriented horizontally at an angle of 40°. On the next day (25th October 2016), the larva pupated and three days later meconium (the first excrement after pupation) appeared in the lower part of the cocoon (27th October 2016). One adult female emerged after 8 days (5th November 2016).

Araneus sturmi (body length 4.5 mm) had a parasitoid larva sitting transversely at the dorsal and posterior side of the spider's opisthosoma (Fig. 4). Under the influence of the final stage larva of *P. tuberosa* the spider built a unique three-dimensional (3D) structure in the upper corner of the experimental arena with a high density of threads (Fig.5). No tuft-like structure was observed. The cocoon was fusiform, yellowish white, sparsely spun with an opening at a distal end and oriented horizontally as for *P. longa*.

Discussion

Host utilisation and specificity of European *Polysphincta*

Our study supports the view of Fritzén & Shaw (2014) that *P. longa* is exclusively associated with *A. angulatus*. This arboreal spider is a relatively rare species and prefers natural forest habitats, where it builds a large orb web mostly in the higher strata of canopies. The host's rareness seems to be one of the reasons why *P. longa* has been seldom reared from hosts. In addition, *P. longa* is potentially more abundant in Europe than previously thought because Fritzén & Shaw (2014) re-examined material from several parts of Europe and revealed that *P. longa* was misidentified as *P. boops*, which is also associated with arboreal araneid spiders but attacks only the genus *Araniella* (e.g. Fitton et al. 1988, Fritzén & Shaw 2014, Korenko et al. 2014).

The hosts of both parasitoids occur in tree crowns but their microhabitat preferences, the sizes and orientations of their orb webs, and their body sizes differ considerably. Large

Fig. 4: *Araneus sturmi* parasitized by *P. boops*. The larva is attached at the dorsal side of the spider's opisthosoma. Scale: 2 mm

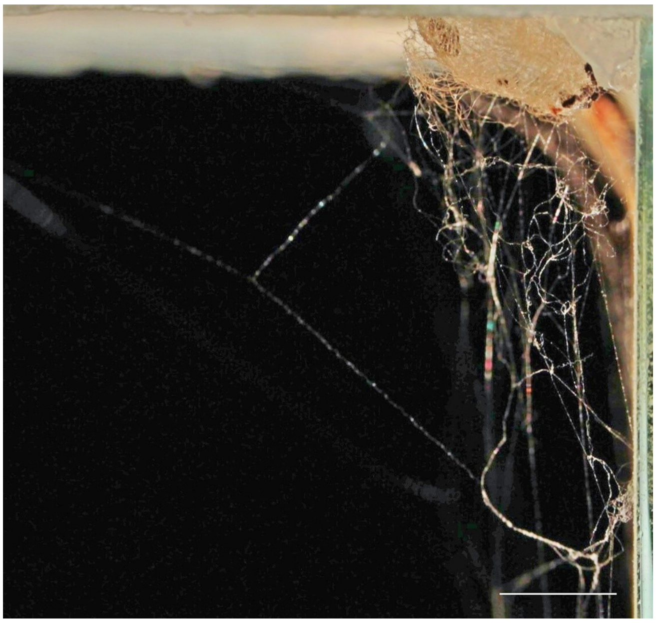


Fig. 5: Cocoon web built by *A. sturmi* under the influence of *P. tuberosa* larva. Scale: 5 mm

vertical orb webs of *A. angulatus* were located in high strata of the tree crown and were often constructed across two trees. In contrast, the much smaller *Araniella* species build a relatively small, mostly horizontally oriented orb web between tree twigs, sometimes covering only one tree leaf (Kůrka et al. 2015). Both parasitoids, *P. longa* and *P. boops*, share forest canopy habitats sympatrically, possibly causing the confusion. These two related parasitoid species presumable evolved their own host-searching behaviour towards closely-related but slightly different spider lineages.

The Holarctic *P. tuberosa*, morphologically similar to the Palaearctic *P. boops*, also prefers small arboreal araneid spiders, but its host range is much wider than that of *P. boops* (e.g. Fitton et al. 1988, Korenko et al. 2014). Although it attacks various taxa, their ecology (web architecture, habitat preference) is similar (Kůrka et al. 2015). Another *Polysphincta* occurring in Europe, *P. rufipes*, is widely distributed across the Palaearctic (Yu et al. 2012). The species attacks araneid spiders such as *Larinioides* or *Zygiella*, which build a protection chamber at the side of the orb web (e.g. Fitton et al. 1988, Schmitt et al. 2012). Another congener is *P. vexator* distributed in the British Isles and Scandinavia (Yu et al. 2012), which seems to be associated with grassy peat bogs and mosses, where its major host spider *A. quadratus* is common (Fitton et al. 1988). A single record reared from *Larinioides cornutus* (Clerck, 1757) is also present (Fitton et al. 1988).

In total, five wasp species of the genus *Polysphincta* occur so far in Europe. Their host range is restricted to the spider family Araneidae, but each host preference is varied (Tab. 1). The widest host range is documented in *P. tuberosa*, which attack three araneid genera (Fitton et al. 1988, Korenko et al. 2014). In contrast, *P. boops* seems to be strictly associated only with spiders of the genus *Araniella* (Korenko et al. 2014). *Polysphincta longa* seems to attack only *A. angulatus* (Fritzén & Shaw 2014, this study).

Manipulation of web-building behaviour

We observed, although only once, that the web-building behaviour of *A. angulatus* was modified by the final stage larva of *P. longa*. The spider built a unique structure corresponding to a cocoon web (a 3D tangle produced by the manipulated spider), which seems to serve to protect the parasitoid during the pupal stage. Our observation did not agree with Fritzén & Shaw (2014), who saw no modification of spider web building

Tab. 1: Host association of European spider-parasitoids of the genus *Polysphincta*. Values are percentage of host records (%) from reliable recent sources: Fitton et al. (1988), Schmitt et al. (2012), Fritzén & Shaw (2014), Korenko et al. (2014), Korenko (unpubl. data) and this study. *N* means total number of host records.

	Araniella		*Araneus*				*Larinioides*		*Zygiella*		
Polysphincta	*cucurbitina*	*ophistographa*	*angulatus*	*diadematus*	*quadratus*	*sturmi*	*sclopetarius*	*cornutus*	*x-notata*	*atrica*	N
boops Tschek, 1869	80	20									5
tuberosa Gravenhorst, 1829	43	19		31	4	1				1	70
longa Kasparyan, 1976			100								3
rufipes Gravenhorst 1829							22	70	9		23
vexator Fitton, Shaw & Gauld, 1988					96			4			26

behaviour. The explanation for this difference seems to lie in the size of the experimental arena. Fritzén & Shaw (2014) used a rearing arena of small size, whereas our observation was conducted in a large arena where the spider had enough space to build both a normal and a cocoon web.

The utilisation of spider web structures by a parasitoid was also documented in *P. rufipes* (the parasitoid uses the normal web structure – spider shelter built at the side of normal web) (Schmitt et al. 2012) and in *P. boops* and *P. tuberosa* (parasitoid induces building of unique cocoon web) (Korenko et al. 2014). Similar cocoon web architecture could also be expected in the other European species of this genus, *P. vexator*.

Considering all available data, the utilisation of a 3D web structure (for protection during pupal stage) seems to be typical for wasps of the genus *Polysphincta* in Europe. These protecting constructions can make use of the spider's normal structures (the spider retreat of *P. rufipes*) or can be achieved via a set of unique spider behaviours newly induced by the parasitoids (the 3D tangle of *P. boops*, *P. tuberosa* and *P. longa*). The cocoon web of *P. longa* uniquely contained many silk tufts of various forms which were produced by the spider after the parasitoid larva reached its final stage and modified the spider's behaviour. These structures were never observed in *P. boops* and *P. tuberosa* (Korenko et al. 2014, unpubl. data). Takasuka et al. (2015) found similar silk tufts on the cocoon webs of *Cyclosa argenteoalba* Bösenberg & Strand, 1906 under the influence of the parasitoid ichneumonid *Reclinervellus nielseni* (Roman, 1923). Takasuka et al. (2015) showed that tuft decoration reflects UV light, possibly to prevent damage caused by collision of large insects and birds. The same function is expected in the tufts present on the cocoon web induced by *P. longa*.

Acknowledgements

We thank Anna Šestáková and Petr Dolejš for photographing the parasitised spider and for help with the collection of material. The study was supported by the Institutional Support Program for Long Term Conceptual Development of Research Institutions provided by the Ministry for Education, Youth and Sport of the Czech Republic. ĽČ was supported by Vega grants 2/0039/14 and 2/0012/17.

References

Eberhard WG 2000 Spider manipulation by a wasp larva. – Nature 406: 255-256 – doi: 10.1038/35018636

Fitton MG, Shaw MR & Gauld ID 1988 Pimpline ichneumon-flies. – Handbooks for the Identification British Insects 7: 1-110

Fritzén NR & Shaw MR 2014 On the spider parasitoids *Polysphincta longa* Kasparyan and *P. boops* Tschek (Hymenoptera, Ichneumonidae, Pimplinae), with the first host records of *P. longa*. – Journal of Hymenoptera Research 39: 71-82 – doi: 10.3897/JHR.39.7591

Korenko S, Isaia M, Satrapová J & Pekár S 2014 Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). – Ecological Entomology 39: 30-38 – doi: 10.1111/een.12067

Kůrka A, Řezáč M, Macek R & Dolanský J 2015 Pavouci České republiky [Spiders of the Czech Republic]. Academia, Praha. 623 pp. [in Czech]

Schmitt M, Richter D, Göbel D & Zwakhals K 2012 Beobachtungen zur Parasitierung von Radnetzspinnen (Araneidae) durch *Polysphincta rufipes* (Hymenoptera: Ichneumonidae). – Arachnologische Mitteilungen 44: 1-6 – doi: 10.5431/aramit4401

Takasuka K, Yasui T, Ishigami T, Nakata K, Matsumoto R, Ikeda K & Maeto K 2015 Host manipulation by an ichneumonid spider-ectoparasitoid that takes advantage of preprogrammed web-building behaviours for its cocoon protection. – Journal of Experimental Biology 218: 2326-2332 – doi: 10.1242/jeb.122739

Yu DS, Achterberg C van & Horstmann K 2012 Taxapad 2012 Ichneumonoidea 2011. Taxonomy, biology, morphology and distribution. Ottawa, Ontario. – Internet: http://www.taxapad.com (February 10, 2017)

Supplement 13

Korenko S, Hamouzová K, Kysilková K, Kolářová M, Kloss TG, Takasuka K, Pekár S. Divergence in host utilisation in two spider parasitoids of the genus *Eriostethus* (Ichneumonidae, Ephialtini). In submission, Zoologischer Anzeiger.

Divergence in host utilisation by two spider ectoparasitoids within the genus *Eriostethus* (Ichneumonidae, Pimplinae)

Korenko S.[1], Hamouzová K.[1], Kysilková K.[1], Kolářová M.[1], Kloss T.G.[2], Takasuka K.[3], Pekár S.[4]

[1] Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic. E-mail: korenko.stanislav@yahoo.com
[2] Universidade Federal do Espírito Santo, Centro de Ciências Exatas, Naturais e da Saúde, Departamento de Biologia, Alegre, Espírito Santo, Brazil.
[3] Institute for Advanced Biosciences, Keio University, Kakuganji 246-2, Mizukami, Tsuruoka City, Yamagata, Japan.
[4] Department of Botany and Zoology, Faculty of Sciences, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic.

Correspondence

Stanislav Korenko, Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic.
E-mail: korenko.stanislav@gmail.com

Abstract

Polysphinctine wasps are exclusive parasitoids of spiders and their host utilisation strategy is almost taxonomically specific. Host utilisation of two Australasian parasitoid wasps of the genus *Eriostethus* was studied for the first time.

Our study revealed considerable differences in host-parasitoid interactions between *Eriostethus minimus* associated with 3D tangle web-building spiders from the family Theridiidae (new family record for *Eriostethus*) and *E. perkinsi* associated with 2D orb-web weaving spiders from the family Araneidae. A pupa of the former species was located in a spider retreat in the centre of unmodified 3D tangle web and the pupal cocoon had sparsely woollen and partially transparent wall. In contrast, a cocoon of the later species hanged in the centre of a modified irregular sparse 3D cocoon web and the pupal cocoon had a strong densely woven cocoon wall.

Our observations suggest trade-off in the robustness of cocoon walls and the intensity of alteration of spider web building behaviour induced by parasitoid final instar larva.

Highlights

Spider host utilisation by the genus *Eriostethus*

Key words

spider host; behavioural manipulation; web architecture; trade-off; *Millironia*

Introduction

One of the most spectacular parasite-host interactions with an extremely narrow host specialisation and remarkable adaptation to the host's biology have evolved within the hymenopteran *Polysphincta* group of genera, whose members are exclusively external koinobiont parasitoids of spiders (e.g. Fitton et al., 1987; Gauld and Dubois, 2006; Nielsen, 1923). The *Polysphincta* group of genera currently consists of 23 genera (Matsumoto, 2016) (hereafter polysphinctine) and more than 250 species found all over the world, but many species are still expected to be described or discovered.

Polysphinctine larvae are singly attached to the dorsal side of a spider's body, where they develop while the spider continues foraging. However, shortly before pupation, the final stage larva changes the web-spinning activity of its host dramatically in order to establish a safe place for pupation called “cocoon web" (e.g. Eberhard, 2000a, 2000b, 2001; Fincke et al., 1990; Korenko and Pekár, 2011), which protects pupa from enemies and unfavourable environment (Kloss et al., 2016a; Matsumoto, 2009). These effects of the larva are apparently realised by chemical products that are introduced into the spider (Eberhard, 2010; Kloss et al., 2017; Takasuka et al., 2015).

Polysphinctines are associated with spider hosts from various taxonomic groups and foraging guilds, but the host spectrum of each wasp species is known to be narrow: includes only a small group of taxonomically related species with a similar behaviour, or even only one species) (e.g. Eberhard, 2013; Gonzaga et al., 2015; Korenko et al., 2011). A number of studies on bionomics of polysphinctines have been accumulated particularly in Neotropical and Palaearctic regions, but none dealt with species of Indo-Australian genus *Eriostethus*.

Based on morphological characteristics provided by Gauld and Duboid (2006) the genus *Eriostethus* is a sister-group to *Flacopimpla*, and these are a sister-group to *Zatypota* and *Longitibia*. A recent phylogenetic study, based on several molecular markers, showed a weak relatedness of *Eriostethus rufus* (Uchida, 1932) to *Zatypota*, and suggested that the most related genus is *Sinarachna* (Matsumoto, 2016), which was considered as a distant group in analyses by Gauld and Dubois (2006). In the past, Baltazar (1964) split *Eriostethus* and designated a new genus, *Millironia*. The most important morphological characters by Baltazar’s criteria is the size of ocelli, which are much enlarged in *Millironia* probably as consequence of an adaptation to a crepuscular or nocturnal environment (Gauld, 1984a). On the other hand, Gauld (1984a) synonymized *Millironia* with *Eriostethus* based on intergrading generic characters other than ocelli. Currently accepted genus *Eriostethus* includes a moderately large-bodied species with pale coloured body, distributed mainly in the Indo-Australia and Japan (Yu et al., 2012).

A little is known on the host range of *Eriostethus* wasps and none of their interactions with the hosts are recorded. The genus seems to be exclusively associated with spiders from family Araneidae. Japanese species *Eriostethus rufus* is known to be associated with araneid orb-web weaving species *Neoscona scylloides* Bosenberg and Strand, 1906 (Masumoto et al., 2002) and *N. mellotteei* (Simon, 1895) (Matsumoto, 2016). Gauld (1984b) observed an undescribed New Guinea specimen attached to a remain of orb-web weaving jewel spider, presumably *Austracantha* sp. (mistyped as heteropteran "*Acanthosoma* sp."). Here we present observations on the biology of two species of *Eriostethus* from Australia for the first time. We focused on the host utilisation and the alteration of spider's web architecture induced by wasp's final stage larva.

Methods

The community of potential spider hosts was surveyed in tree canopies (between 40 cm to 200 cm above ground) at two suburban sites in Brisbane, suburb Gaythorne: bush land with dominant *Eucalyptus* spp. close to residential area (27°25'25''S, 152°57'30''E) and North Tamborine: bank of Cedar Creek, close to Tamborine National Park (27°54'27''S, 153°11'01''E) (Queensland, Australia). Seventeen one-day excursions were done between the beginning of July and end of October 2013. Spiders were collected individually by carefully searching tree branches and leaves for 2-3 hours in Gaythorne plus a one-day excursion to Cedar Creek site (8th November 2013). The collected spiders were inspected under a lens for presence of parasitoid larva and were identified to genus or species using Hawkeswood (2003) in laboratory. Adult wasps obtained after emerging from pupae were identified using Gauld (1984b). The nomenclature of the polysphinctines follows Yu et al. (2012). Voucher specimens of spiders and wasps are deposited in the collection of Kees Zwakhals (Netherlands) and the first author (Department of Agroecology and Biometeorology, Czech University of Life Sciences Prague, Czech Republic).

Rearing and the study of host behaviour alteration

Web-building behaviour of parasitised and unparasitised spiders was observed in both the field and the laboratory. Spiders were placed individually in experimental arenas and their behaviour was recorded in detail in the laboratory. Quadratic arenas (with 100 x 100 mm base, 130 mm height with an installed twig or frame providing the space for spider web) were used for rearing wasps from theridiid spiders (Theridiidae, 3D web weavers) and narrow cuboid arenas (with 200 x 50 mm base, 200 mm height) were used for araneid spiders (Araneidae, 2D web weavers). Spiders were kept at a room temperature of 22 ± 3°C, natural L:D regime (Australian winter) and fed with various insects (dipterans and hemipterans) collected in the field. The architecture of webs was observed daily until the larva killed and consumed the spider and pupated. Web architecture was recorded using a digital camera Canon EOS 500 with EF-S 18-55 mm lens or a macro lens EF 100 mm f/2,8L IS USM.

Results

In total, 1,824 tree crown dwelling spiders were collected in the investigated sites (Table 1). Spiders of the families Araneidae and Theridiidae were the most abundant representing 60 and 27% of inspected spiders, respectively. Only spiders from an araneid genus *Eriophora* and a theridiid genus *Parasteatoda* were parasitised by larvae of two *Eriostethus* wasp species on their abdomen. Two wasps were identified as *Eriostethus minimus* Gauld, 1984 and *Eriostethus perkinsi* (Baltazar, 1964) from spiders found in Gaythorn with the former also found in Cedar Creek.

Host association

The exclusive hosts of *Eriostethus minimus* were identified as *Parasteatoda* cf. *mundula* (adult females with 8 mm in length, collected in Gaythorn in October, N = 1) and *Parasteatoda* sp. (adult females with 8 mm in length, collected in Cedar creek in November, N = 1).

Eriostethus perkinsi was reared exclusively from juvenile araneid spiders of *Eriophora* sp. with body length 5.5-6 mm (N = 4). The parasitised spiders by a larva of *E. perkinsi* were observed in September (N = 2) and October (N = 2) with empty cocoons found in the end of October (N = 4).

Host manipulation

Normal (capture) webs of *P.* cf. *mundula* and *Parasteatoda* sp. were composed of a three-dimensional network of interconnected non-stick threads from which several gumfoot lines were attached to the vegetation in the surroundings, and a central retreat in which the spider rests (observation in nature). Parasitised females protected their egg sacs inside the retreat which was built of a dry leaf and/or detritus in the upper part of the web. There was not any modification of web architecture observed in species parasitized by *E. minimus*. After killing the host, the wasp larva built its cocoon inside the spider's retreat (in the field, N = 1), or in the centre of 3D web (in the laboratory, N = 1) where no retreat was hung.

Capture web of *Eriophora* spiders was a symmetrical vertical orb-web mostly built in shrubs and lower parts of tree crowns (Figure 1). Spiders were hidden in vegetation (e.g. base of tree twig, under bark) without the web during day and orb-web appeared only during night. *Eriophora* spider under the manipulation of final stage larva of *E. perkinsi* built an irregular 3D tangle web composed of non-sticky reinforced threads (Figure 2). The wasp cocoon hung from the centre of the modified web (Figure 2).

Morphology of wasp's cocoon

Cocoon of *E. minimus* was a diaphanous yellowish, fusiform, with a sparse construction and partially transparent wall (Figure 3). In contrast, cocoon of *E. perkinsi* was densely woven light whitish–brownish with opaque wall and a tight outer cover made of coarse fibres, characteristic by having several large projections of silk mass at the caudal end of cocoon (Figure 3).

Discussion

A few notes on the ecology of *Eriostethus* wasps including host records were available before this study was commenced. Only spiders from family Araneidae which build 2D orb-web were hitherto recorded as hosts for this genus (Gauld, 1984b; Masumoto et al., 2002; Matsumoto 2016). *Eriostethus rufus* is a sister lineage with *Sinarachna* spp. (but supported by low bootstrap value) (Matsumoto 2016) which seem to utilise araneid spiders exclusively (Fitton et al. 1987; Korenko et al. 2014). Therefore, we expected that species of *Eriostethus* are associated exclusively with araneid orb-web weavers but our study newly found that *E. minimus* was associated with theridiid 3D web building spiders, whereas *E. perkinsi* was associated with araneid spiders as expected. The architecture of cocoon webs, which seemingly protected the parasitoid's pupa of both species was 3D, but the density of 3D tangle and the presence/absence of spider's retreat differed between them.

During pupal stage *E. minimus* utilised the unmodified web of *Parasteatoda* with a retreat, while *E. perkinsi* induced the production of a completely new unique web structure in *Eriophora* host. Pupae of both wasp species seem to be protected by the cocoon web against environment and enemies sufficiently, but this protection is realised by different strategies. *Eriostethus perkinsi* invested an energy to manipulate the host spider into modifying 2D to 3D orb web and to produce a cocoon with a thick and opaque cocoon wall. In contrast, *E. minimus* took use of the structures of the spider's normal web (particularly the retreat) and produced a cocoon with a thin wall, indicating that spider's retreat would be quite beneficial to parasitoids for harbouring cocoon and saving energy. A similar trade-off between architecture of cocoon web and of pupal cocoon was observed in two genera of European polysphinctines associated with araneid spiders of genus *Araniella* (Korenko et al., 2014).

In addition to host behavioural manipulation by the larvae, a difference in original web of host spiders requires very different and specific set of oviposition behaviour to adult wasps. Several studies discovered specific oviposition behaviour of other polysphinctines against Theridiidae (Takasuka et al. 2009; Takasuka and Matsumoto 2011) and Araneidae (Gonzaga and Sobczak 2007; Kloss et al. 2016b) which are far from commonality resulting from entirely different web forms. Although we did not witness oviposition behaviour of studied *Eriostethus* spp., host record upon *Parasteatoda* spp. proves *E. minimus* to capture spiders protected by the 3D theridiid web by means of specific behaviour unlike other congeners upon araneid spiders.

Host spider spectrum among each polysphinctine genus or related genera is very constant at family-level (or sometimes genus-level) (Gauld and Dubois, 2006), indicating a remarkable difference in web forms (e.g. 2D orb vs. 3D tangle) will become an obstacle to host shift. A distant host shift thus seldom occurs but, once occurred, it would play a role of a new phylogenetic branching off. Noticeable multi-family utilisation within polysphinctine genus is known in *Hymenoepimecis* and *Acrotaphus* parasite upon Araneidae (vertical orb web) and Tetragnathidae (horizontal orb web), *Zatypota* upon Theridiidae (irregular 3D web), Dictynidae (irregular cribellate 3D web) and Linyphiidae (tent-shaped 3D sheet web), and *Acrodactyla* upon Linyphiidae and Araneidae. Although phylogenetic intrageneric relationship between host linages of genera *Hymenoepimecis* and *Acrotaphus* has been unknown, they utilise spiders with basically similar 2D orb web. On the other hand, *Zatypota* and *Acrodactyla* have morphologically species-complex reflected by host family (Gauld and Dubois 2006; Matsumoto 2016; Matsumoto and Takasuka 2010). Two distant types of host spiders utilised by *Eriostethus* thus indicates a phylogenetic differentiation within the genus like probably occur by *Zatypota* and *Acrodactyla*.

The shift between Araneidae and Theridiidae must make the wasps to face with unfamiliar environment to adapt, driving phylogenetic divergence. In addition to web form, is the hosts differ in circadian activity, diurnal or nocturnal. Gauld (1984a) suggested that species with large ocelli (character of synonymised *Millironia* sensu Baltazar (1964), including *E. perkinsi*) have nocturnal or crepuscular activity, supported by the fact that their araneid hosts build their orb-web during night and withdraw it in the daytime. Species with small or moderate sized ocelli (character of *Eriostethus* sensu stricto Baltazar (1964), including *E. minimus*) within *Eriostethus* seem to have diurnal activity and *Parasteatoda* utilised by *E. minimus* keeps building web during day and night. These ecological differences suggest the validity of Baltazar's treatment (1964) who erected the genus *Millironia* by seven species independent from "the true *Eriostethus*" based mainly on the size and position of eyes and ocelli, and presence/absence of occipital carina. A revision and additional phylogenetic analysis with re-consideration of morphological, ecological (especially host records) and molecular data is needed to resolve the puzzle.

Contribution of authors

Collected field data and performed the laboratory observations: SK. Analyzed the data and wrote the paper: all authors.

Acknowledgements

The study was supported the Institutional Support Program for Long Term Conceptual Development of Research Institutions provided by the Ministry for Education, Youth and Sport of the Czech Republic. Also, TGK is grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant numbers 300295/2016-2) and Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (grant number 1020/2015).

References

Baltazar, C.R., 1964. *Eriostethus* Morley and a new polysphinctine genus (Ichneumonidae, Hymenoptera). Pac. Insects. 6, 388–401.

Eberhard, W.G., 2000a. Spider manipulation by a wasp larva. Nature 406, 255–256.

Eberhard, W.G., 2000b. The natural history and behavior of *Hymenoepimecis argyraphaga* (Hymenoptera: Ichneumonidae) a parasitoid of *Plesiometa argyra* (Araneae: Tetragnathidae). J. Hym. Res. 9, 220–240.

Eberhard, W.G., 2001. Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). J. Arachnol. 29, 354–366.

Eberhard, W.G., 2010. Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. Anim. Behav. 79, 375–383.

Eberhard, W.G., 2013. The Polysphinctine wasps *Acrotaphus tibialis*, *Eruga* ca. *gutfreundi*, and *Hymenoepimecis tedfordi* (Hymenoptera, Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. Ann. Entomol. Soc. Am. 106, 652–660.

Fincke, O.M., Higgins, L., Rojas, E., 1990. Parasitism of *Nephila clavipes* (Araneae, Tetragnathidae) by an ichneumonid (Hymenoptera, Polysphinctini) in Panama. J. Arachnol. 18, 321–329.

Fitton, M.G., Shaw, M.R., Austin, A.D., 1987. The Hymenoptera associated with spiders in Europe. Zool. J. Linn. Soc. 90, 65–93.

Gauld, I.D., 1984a. An introduction to the Ichneumonidae of Australia, first ed. British Museum (Natural History), London.

Gauld, I.D., 1984b. The Pimplinae, Xoridinae, Acaenitinae and Lycorininae (Hymenoptera: Ichneumonidae) of Australia. Bull. Br. Mus. Nat. Hist. Zool. 49, 235–339.

Gauld, I.D., Dubois, J., 2006. Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae), a taxonomic revision of spider ectoparasitoids. Syst. Entomol. 31, 529–564.

Gonzaga, M.O., Moura, R.R., Pêgo, P.T., Bang, D.L., Meira. F.A., 2015. Changes to web architecture of *Leucauge volupis* (Araneae: Tetragnathidae) induced by the parasitoid *Hymenoepimecis jordanensis* (Hymenoptera: Ichneumonidae). Behaviour 152, 181–193.

Gonzaga, M.O., Sobczak, J.F., 2007. Parasitoid-induced mortality of *Araneus omnicolor* (Araneae, Araneidae) by *Hymenoepimecis* sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. Naturwissenschaften 94, 223–227.

Hawkeswood, T.J., 2003. Spiders of Australia: an introduction to their classification, biology and distribution. Pensoft Publishers, Sofia.

Kloss, T.G., Gonzaga, M.O., Oliveira, L.L., Sperber, C.F., 2017. Proximate mechanism of behavioral manipulation of an orb-weaver spider host by a parasitoid wasp. PLoS ONE 12(2), e0171336.

Kloss, T.G., Gonzaga, M.O., Roxinol, J.A.M., Sperber, C.F., 2016a. Host behavioural manipulation of two orb-weaver spiders by parasitoid wasps. Anim. Behav. 111, 286–296.

Kloss, T.G., Gonzaga, M.O., Roxinol. J.A.M., Sperber, C.F., 2016b. Attack behavior of two wasp species of the *Polysphincta* genus group (Hymenoptera, Ichneumonidae) on their orb-weaver spider hosts (Araneae, Araneidae). J. Insect Behav. 29, 315–324.

Korenko, S., Isaia, M., Satrapová, J., Pekár, S., 2014. Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecol. Entomol. 39, 30–38.

Korenko, S., Michalková, V., Zwakhals, K., Pekár, S., 2011. Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid (Hymenoptera: Ichneumonidae). J. Insect Sci. 11, 101.

Korenko, S., Pekár, S., 2011. A parasitoid wasp induces overwintering behaviour in its spider host. PLoS ONE 6(9), e24628.

Matsumoto, R., 2009. ''Veils'' against predators: Modified web structure of a host spider induced by an ichneumonid parasitiod, *Brachyzapus nikkoensis* (Uchida) (Hymenoptera). J. Insect Behav. 22, 39–48.

Matsumoto, R., 2016. Molecular phylogeny and systematics of the *Polysphincta* group of genera (Hymenoptera, Ichneumonidae, Pimplinae). System. Entomol. 41, 854–864.

Masumoto, T., Shimizu, I., Konishi, K., 2002. The first record of parasitism on *Neoscona scylloides* (Araneae: Araneidae) by *Eriostethus rufus* (Hymenoptera: Ichneumonidae). Acta Arachnol. 51, 5–6.

Matsumoto, R., Takasuka, K., 2010. A revision of the genus *Zatypota* Förster of Japan, with descriptions of nine new species and notes on their hosts (Hymenoptera: Ichneumonidae: Pimplinae). Zootaxa 2522, 1–43.

Nielsen, E., 1923. Contributions to the life history of the pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*) (Hym. Ichneum.). Entomol. Meddr. 14, 137–205.

Takasuka, K., Matsumoto, R., 2011. Lying on the dorsum: unique host-attacking behaviour of *Zatypota albicoxa* (Hymenoptera, Ichneumonidae). J. Ethol. 29, 203–207.

Takasuka, K., Matsumoto, R., Ohbayashi, N., 2009. Oviposition behavior of *Zatypota albicoxa* (Hymenoptera, Ichneumonidae), an ectoparasitoid of *Achaearanea tepidariorum* (Araneae, Theridiidae). Entomol. Sci. 12, 232–237.

Takasuka, K., Yasui, T., Ishigami, T., Nakata, K., Matsumoto, R., Ikeda, K., Maeto, K., 2015. Host manipulation by an ichneumonid spider-ectoparasitoid that takes advantage of preprogrammed web-building behaviours for pupal protection. J. Exp. Biol. 218, 2326–2332.

Yu, D.S., van Achterberg, C., Horstmann, K., 2012. Taxapad 2012. Ichneumonoidea 2011. Taxonomy, biology, morphology and distribution. http://www.taxapad.com (accessed 01.06.17).

Table 1. List of spider inspected for parasitism by *Eriostethus* wasps from Gaythorne bush land. R.b. means relative abundance of particular spider family. N means number of inspected spiders. P means observed parasitism incidence by *Eriostethus* wasps.

Family (R.b.)	**Genus/species**	**N**	**P (%)**
Agelenidae (0,.1)	unidentified	1	0
Amaurobiidae (0.3)	unidentified	6	0
Araneidae (60.0)	*Araneus dimidiatus* (L. Koch,)	121	0
	Araneus spp.	318	0
	Argiope spp.	6	0
	Cyclosa spp.	11	0
	Cyrtophora exanthematica (Doleschall)	102	0
	Cyrtophora hirta L. Koch	340	0
	Cyrtophora moluccensis Doleschall	22	0
	Eriophora spp.	32	18.7
	Nephila spp.	142	0
Tetragnathidae (1.3)	*Leucauge* spp.	16	0
	Tetragnatha spp.	8	0
Theridiidae (27.0)	*Anelosimus* spp.	337	0
	Parasteatoda spp.	27	3.7
	Theridion spp.	128	0
Uloboridae (11.3)	unidentified	207	0
Total		***1824***	***0.38***

Figure 1 Normal orb-web of *Eriophopra perkinsi*. Scale = 50 mm.

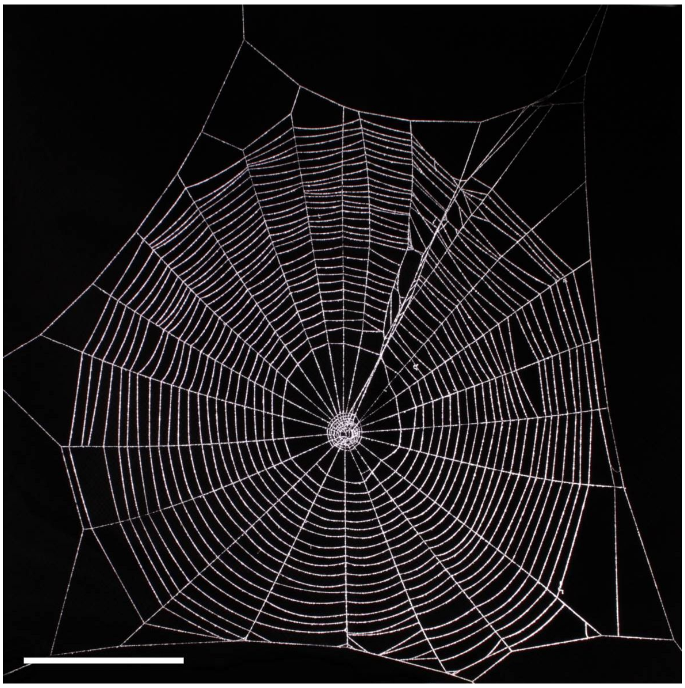


Figure 2 Cocoon web built by *Eriophora* spider manipulated by the final instar larva of *Eriostethus perkinsi*. Inset: detail of wasp cocoon hung in the centre of the cocoon web. Scale = 50 mm.

Figure 3 Cocoons built by the final instar larva of *Eriostethus* spp.: *E. perkinsi* from sparse 3D structure (upper picture). *E. minimus* from spider's retreat in dense 3D structure (lower picture). Scale = 2 mm.

Supplement 14

Korenko S, Di Giovanni F. Spider parasitoids of tribe Ephialtini (Ichneumonidae, Pimplinae) in Italy and their host association. In submission, North-Western Journal of Zoology.

Spider parasitoids of tribe Ephialtini (Hymenoptera, Ichneumonidae, Pimplinae) in Italy and their host association

Stanislav Korenko[1] and Filippo Di Giovanni[2]
[1]Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic.
[2] via F. Petrarca 43, 71122, Foggia, Italy.

Corresponding author: Stanislav Korenko, E-mail: korenko.stanislav@gmail.com**Keywords**: Checklist, *Polysphincta* group of genera, egg predator, koinobiont, spider host, new records, biodiversity
Running title: Spider parasitoids in Italy

Abstract

The list of Ephialtine wasps (Hymenoptera: Ichneumonoidea) of Italy associated with spider hosts is presented here. Until now, 28 species belonging to ten genera and two ecological groups in accordance with host type are recorded for Italy; the first group includes pseudo-parasitoids attacking spider egg sacs, the second one includes koinobiont ectoparasitoids attacking spiders. The host association of wasps occurring in Italy was summarised from published and unpublished data. *Zatypota discolor* (Holmgren, 1860) is new for Italy. *Zaglyptus varipes* (Gravenhorst, 1829) and *Schizopya podagrica* Gravenhorst, 1829 are recorded for the first time in South Italy and in Sardinia respectively.

Introduction

The association with spider hosts has been evolved as one of the most complex and intricate interaction of parasitoids. The largest group of parasitoids which are associated with spiders are pimpline wasps of the tribe Ephialtini. Twenty-six genera are known to parasitize spiders (Gauld & Dubois 2006, Yu et al. 2012). A phylogenetic study based on morphological characteristics conducted by Gauld & Dubois (2006) revealed that all groups of pimplines that use araneomorph spiders or their egg masses inside a silken sac as larval nourishment form a monophyletic clade within the Ephialtini. These spider parasitoids are clustered into two ecological groups in accordance with host type. The first one is a paraphyletic genus group which includes spider egg mass parasitoids (pseudo-parasitoids), the second one is the *Polysphincta* genus-group which includes true spider parasitoids (Yu et al. 2012). The first one thought to be the ancestral sister-group to the second one (Gauld & Dubois 2006).

Studies on biology of egg mass parasitoids is less than that in polysphinctines probably because of the invisibility or inconspicuousness of larvae inside spider egg sacs and the difficulty of identification of spider egg sacs. Attacking of two or more eggs is not recognized to be true parasitism in a narrow sense but called pseudo-parasitism instead (Dubois et al. 2002).

Wasps from *Polysphincta* genus-group (*sensu* Gauld & Dubois 2006) (Pimplinae, Ephialtini) consist exclusively of koinobiont ectoparasitoids of spiders. Larvae of these species keep their spider hosts alive during their development, attaching themselves externally to the opisthosoma or prosoma of spider. Many species are known to manipulate the behaviour of their hosts (e.g., Nielsen 1923, Eberhard 2000, Korenko et al. 2014, 2017a, 2017b, Kloss et al. 2017, Takasuka et al. 2017).

Polysphinctines as a whole are associated with spiders from different taxonomic groups and with different foraging techniques. The host spectrum of a particular wasp species is usually restricted to a small group of taxonomically closely related species with similar behaviour. In all species, where sufficient data are available, the hosts always belong strictly to a single family (e.g., Fitton et al. 1987, Korenko et al. 2011), except Australian taxon *Zatypota kauros* Gauld, 1984, which is known to attack spider hosts from at least two different families (Korenko et al. 2017b). Many genera are frequently encountered in the canopy where they are associated with orb-web weaving spiders inhabiting higher strata of the forests (e.g., Stüben et al. 2010, Di Giovanni et al. 2015a, Korenko et al. 2015b, 2017a). Polysphinctines show specific adaptations in many phenotypic traits, including complex behavioural repertoires that match the ecology of their hosts (e.g., Eberhard 2000). One set of such behavioural adaptations probably evolved in interaction with aggressive defence behaviour of the spider hosts and has presumably resulted in the restricted host ranges of these parasitoids (e.g., Dubois et al. 2002, Gauld & Dubois 2006).

Material and Methods

For each species, we reported the general distribution according to the division of Italy in four geographic regions as proposed by Minelli et al. (2005) and known regional records, based on data from literature. For literature data, the name used in the text is given in parentheses, if it differs from the valid species name. Both published and unpublished records have been summarised for Italy. Description of families occurred in investigated area and available host records were presented. Genera and species are listed in alphabetic order and included in two ecological groups in accordance with their host type (pseudo-parasitoids and true parasitoids). Generic records for Italy are indicated as **ITA**. For each region, the following abbreviations were used:

N: Friuli-Venezia Giulia (**FVG**), Veneto (**VEN**), Trentino-Alto Adige (**TAA**), Lombardy (**LOM**), Aosta Valley (**VAO**), Piedmont (**PIE**), Liguria (**LIG**), Emilia-Romagna (**EMR**)
S: Tuscany (**TOS**), Marche (**MAR**), Umbria (**UMB**), Lazio (**LAZ**), Abruzzo (**ABR**), Molise (**MOL**), Campania (**CAM**), Apulia (**PUG**), Basilicata (**BAS**), Calabria (**CAL**)
Si: Sicily (and smaller islands) (**SIC**)
Sa: Sardinia (and smaller islands) (**SAR**)

New available records have been added, based on further material from field samplings and public collections. Most of the new data originates from sampling efforts by the first author in Piedmont. The localities of new records are**:**

Province of Cuneo: (1) a commercial hazelnut and apple orchard (Azienda A. Rivetto) close to Ornato (44°36'19.8"N, 8°00'38.7"E), (2) a commercial apricot orchard (Chiamina) close to Verzulo (44°35'14.2"N, 7°29'50.1"E), (3) an organic hazelnut orchard and the surrounding forest in Bottonasco (44°25'14.2"N, 7°23'39.8"E), (4) forest edges with dominant *Corylus* sp., *Quercus* sp. and *Acer* sp. in Monterosso Grana (44°24’20”N, 7°19’17”E), (5) Terme di Valdieri, bush in rocky slope in bank of torrent Valasco (44°12'17.2"N, 7°15'48.7"E).

Province of Verbano–Cusio–Ossola: (6) a deciduous forest on the bank of Lake Maggiore close to Fondotoce di Verbania (45°56’16”N, 8°29’37”E).

Province of Turin: (7) close to Macello, bank vegetation of torrent Chisone (44°50'11.6"N, 7°23'51.2"E).

The material is deposited in the following collections:

- MZUR: private collection of F. Di Giovanni, Museo di Zoologia, Università degli Studi di Roma "Sapienza" (Rome, Italy).
- DISAAA: P.L. Scaramozzino's collection, Dipartimento di Scienze Agrarie, Alimentari e Agro-alimentari, Università degli Studi di Pisa (Pisa, Italy).
- CULS: private collection of S. Korenko, Department of Agroecology and Biometeorology, Czech University of Life Sciences Prague (Prague, Czech Republic).

Results and Discussion

In total, 28 species belonging to ten genera and two ecological groups in accordance with host type of Ephialtini wasps associated with spiders have been summarised from Italy. Since the checklist of Italian fauna of Ichneumonidae (Scaramozzino 1995), 10 species have been added to the list (Bauer 2002, Bordoni 2003, Korenko et al. 2014, Korenko et al. 2015a, Korenko et al. 2015b, Di Giovanni et al. 2015a, Di Giovanni et al. 2015b, Korenko 2016, Korenko 2017) and for 9 species known distribution in Italy has been extended (Bauer 2002, Korenko et al. 2014, Zwakhals & Turrisi 2014, Corcos et al. 2017). The presence of two species previously recorded generally for Italy, *Acrodactyla degener* (Haliday, 1838) and *Oxyrrhexis carbonator* (Gravenhorst, 1829), has been confirmed (Bauer 2002, this study).

In this study, *Zatypota discolor* (Holmgren ,1860) is new for Italy (Piedmont). Two species, *Zaglyptus varipes* (Gravenhorst, 1829) and *Schizopya podagrica* Gravenhorst, 1829, are recorded for the first time in South Italy (Tuscany) and in Sardinia respectively.

Spider egg parasitoids (pseudo-parasitoids)

This paraphyletic genus complex currently consists of three genera and 94 species all over the world (Yu et al. 2012). All three genera were already documented from Italy (Scaramozzino 1995). Wasps are parasitoids of spider egg mass and thought to be the ancestral sister-group to the *Polysphincta* genus-group (Gauld & Dubois 2006).

Clistopyga Gravenhorst, 1829

Widely distributed genus, with 34 species all over the world except Australia (Yu et al. 2012). Six species occur in Europe (van Achterberg et al. 2017). There is only sparse information on their biology and hosts. *Clistopyga incitator* (Fabricius, 1793) is associated with egg sacks of the spider *Segestria senoculata* (Linnaeus, 1758) (Segestriidae), which is found in holes and crevices in stone walls and under tree bark (Nielsen 1929).

Clistopyga incitator (Fabricius, 1793) N S Si Sa

[**TAA**: Bauer 2002; **LOM**: Di Giovanni et al. 2015a; **PIE**: Pisica & Pagliano 1982; **LIG**: Kirchner 1867 (=*Clistopyga haemorrhoidalis*); **TOS**: Corcos et al. 2017 (Siena, Arbia, 27-28.IV.2012, yellow pan trap (1 female)). **SIC**: Zwakhals & Turrisi 2014; **SAR**: Martelli & Arru 1958].

New records. **FVG**: Udine, Musi, 11-23.VII.1989, P.L.Scaramozzino leg., F. Di Giovanni det. (2 females) (DISAAA); Udine, Palazzolo dello Stella, Nogali Braide, Bosco Brussa, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (4 males) (MZUR); Udine, Carlino, proprietà Villabruna, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 male, 3

females) (MZUR); Udine, Marano Lagunare, proprietà Villabruna, 21.VII.-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 male, 5 females) (MZUR). **VEN**: Venezia, Mestre, impianto Bosco di Carpenedo, 22.VII-04.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 male) (MZUR); Venezia, Mirano, fraz. Villamagno, Bosco del Parauro, 22.VII-04.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR); Treviso, Gaiarine, loc. Francenigo, Bosco Otello, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 male) (MZUR); Treviso, Meolo, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR); Treviso, Cessalto, S.Maria di Campagna, Bosco S.Marco, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR). **PIE**: Cuneo, Barbaresco, San Lorenzo, vineyard, 18.V-07.VI.2016, F. Di Giovanni det. (3 females) (DISAAA); Same locality, 07-27.VI.2016, F. Di Giovanni det. (1 female) (DISAAA); Same locality, 27.VI-18.VII.2016, F. Di Giovanni det. (3 females) (DISAAA). **TOS**: Pisa, Crespina, La Serra, vineyard, 13.VI.2013, A.Loni leg., F. Di Giovanni det. (2 females) (DISAAA). Same locality, 02.VII.2013, F. Di Giovanni det. (1 male, 1 female) (DISAAA); Same locality, 27.VIII. 2013, F. Di Giovanni det. (2 females) (DISAAA); Same locality, 08.X.2013, F. Di Giovanni det. (8 females) (DISAAA); Pisa, Crespina, Poggio al Casone, vineyard, 27.VIII.2013, A.Loni leg., F. Di Giovanni det. (1 female) (DISAAA); Same locality, 08.X.2013, F. Di Giovanni det. (1 female) (DISAAA). **MAR**: Ancona, Selva di Gallignano, 20.IX.2010, G.Giovagnoli leg., F. Di Giovanni det. (1 female) (MZUR). **SIC**: Isole Eolie, Vulcano, 28.VI-18.VII.2008, M.Mei leg., F. Di Giovanni det. (3 females) (MZUR); Isola di Pantelleria, Khaddiuggia, 19-28.VIII.2014, F.Di Giovanni leg. & det., on *Plumbago* sp. (1 female) (MZUR). **SAR**: Sassari, Villanova Monteleone, Sa Serra, 23.VII.2009, P.Niolu leg., F. Di Giovanni det. (1 female) (MZUR).
Notes. Specimens of *C. incitator* from Italy often display extensively light coloration with respect to European specimens, with head and body extensively red-marked to completely red, and face often completely yellow (var. *haemorrhoidalis* Gravenhorst, 1829).

Tromatobia Förster, 1869
The genus includes 33 species with three subspecies distributed worldwide (Holarctic and Neotropical, partly Oriental, Australasian and Afrotropical regions) (Yu et al. 2012). They have been reared alone or in broods from egg sacs of various spiders (Fitton et al. 1988). Wasps of this genus seem to be associated with egg masses mainly of the family Araneidae but also of Tetragnathidae and Linyphiidae (Fitton et al. 1987, 1988). Association with egg sacs of the wandering spiders from family Philodromidae was observed in *T. lineatoria* (Fitton et al. 1987). Ovipositing female does not harm the female spiders guarding egg sacs, and at least in *T. lineatoria* few young spiders were observed hatching from parasitized egg sacs (Fitton et al. 1988).

Tromatobia lineatoria (Villers, 1789) N S Si Sa
[**LOM**: Di Giovanni et al. 2015a; **LIG**: Kirchner 1867 (=*Pimpla oculatoria*; =*Ichneumon lineatorius*); **TOS**: Rivosecchi & Bettini 1958 (=*Pimpla oculatoria*); **LAZ**: Rivosecchi & Bettini 1958 (=*Pimpla oculatoria*); **SIC**: Zwakhals & Turrisi 2014; **SAR**: Costa 1886 (=*Pimpla oculatoria rubella*)]
New records. **FVG**: Udine, Precenicco, Bosco Bando, 09-21.VI.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR); Udine, Marano Lagunare, proprietà Villabruna, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR); Udine, Carlino, proprietà Villabruna, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (2 females)

(MZUR); Udine, Carlino, Bosco Bolderatis, 09-21.VI.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR). **VEN**: Verona, near Monte, Stramonte, 45°34'N 10°49'E, 05.V.2014, 500m, P.Cerretti, F.Di Giovanni & G.Lo Giudice leg., F. Di Giovanni det. (2 females) (MZUR); Venezia, Mestre, Bosco di Zaher, 22.VII-04.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (8 females) (MZUR); Venezia, Portogruaro, fraz. Lison, Bosco del Merlo, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (2 females) (MZUR); Venezia, Concordia Sagittaria, fraz. Sindacale, Bosco delle Lame, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (6 females) (MZUR); Treviso, Meolo, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (5 females) (MZUR); Treviso, Cessalto, S.Maria di Campagna, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (7 females) (MZUR); Treviso, Gorgo al Monticano, Bosco di Cavalier, 09-19.V.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR); Same locality, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni det. (1 female) (MZUR); Treviso, Gaiarine, loc. Francenigo, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (2 females) (MZUR); Treviso, Gaiarine, loc. Francenigo, Bosco Crasere, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR). **PIE**: Cuneo, Barbaresco, San Lorenzo, vineyard, 18.V-07.VI.2016, F. Di Giovanni det. (1 male, 3 females) (DISAAA). **TOS**: Pisa, Crespina, Poggio al Casone, vineyard, 08.X.2013, A.Loni leg., F. Di Giovanni det. (1 female) (DISAAA). **LAZ**: Roma, Pomezia, cork oaks, 23.IX.2010, M.Mei leg., F. Di Giovanni det. (1 female) (MZUR); Frosinone, Serrone, 15-16.VII.2010, light trap, M.Gigli leg., F. Di Giovanni det. (1 male) (MZUR). **ABR**: Pescara, Pianella, 10-18.VIII.2014, A.Morelli leg., F. Di Giovanni det. (1 female) (MZUR). **SIC**: Palermo, Riserva di Capo Gallo, 12.IV.2013, A.Reshchikov leg., F. Di Giovanni det. (1 male) (MZUR). **SAR**: Oristano, 15.VIII.1977, F. Di Giovanni det. (1 female) (DISAAA); Same locality, 10.I.1978, F. Di Giovanni det. (1 female) (DISAAA).
Notes: specimens from Sardinia are reported as belonging to the subspecies *rubella* Costa, 1886 by Yu et al. (2012). Scaramozzino (1995) correctly indicated *rubella* as synonym of *T. lineatoria* (Villers, 1789) (see also Scaramozzino 1994).

Tromatobia ornata (Gravenhorst, 1829) N Si
[**SIC**: Sichel 1860 (=*Pimpla ornata*), Zwakhals & Turrisi 2014]
New records. **FVG:** Udine, Precenicco, Bosco Bando, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 females) (MZUR); Udine, Carlino, proprietà Villabruna, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR); Udine, Carlino, Bosco Pra Quain e Venchiaratis, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 females) (MZUR); Udine, S. Giorgio di Nogaro, fraz. Zellina, Bosco Boscat, 09-21.VI.2013, Malaise trap, F.Di Giovanni leg. & det. (1 females) (MZUR). **VEN**: Verona, near Monte, Stramonte, 45°34'N 10°49'E, 05.V.2014, 500m, P.Cerretti, F.Di Giovanni & G.Lo Giudice leg., F. Di Giovanni det. (1 females) (MZUR); Venezia, Portogruaro, fraz. Lison, Bosco del Merlo, 10-22.VI.2013, Malaise trap, F.Di Giovanni leg. & det. (2 females) (MZUR); Treviso, Cessalto, Bosco Olmè, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR); Treviso, Mansuè, Bosco di Basalghelle, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (2 females) (MZUR). **PIE**: Cuneo, Barbaresco, San Lorenzo, vineyard, 18.V-07.VI.2016, F. Di Giovanni det. (9 females) (DISAAA); Same locality, 07-27.VI-2016, F. Di Giovanni det. (1 female) (DISAAA); Same locality, 27.VI-18.VII.2016, F. Di Giovanni det. (5 females) (DISAAA).

Tromatobia ovivora (Boheman, 1821) N Si

[**TAA**: Bauer 1936 (=*Pimpla angens*), Giordani Soika 1942 (=*Pimpla ovivora*); **LOM**: Di Giovanni et al. 2015a; **SIC**: De Stefani 1894 (=*Pimpla angens*), De Stefani 1895 (=*Pimpla angens*)]
New records. **PIE**: Cuneo, Barbaresco, San Lorenzo, vineyard, 18.V-07.VI.2016, F. Di Giovanni det. (1 female) (DISAAA).

Tromatobia variabilis (Holmgren, 1856) N
[**TAA**: Bauer 2002]

Zaglyptus Förster, 1869
Genus includes 23 described species with eight subspecies distributed worldwide (Yu et al. 2012). They are parasitoids of both spiders and eggs of wandering spiders e.g. Eutichuridae, Clubionidae and Salticidae (Fitton et al. 1987). *Z. varipes* was reared from egg chambers of the wandering spiders *Clubiona* spp. (Clubionidae) (Nielsen 1935, Fitton et al. 1988), *Cheiracanthium erraticum* (Walckenaer, 1802) (Eutichuridae) and in rare case *Sitticus floricola* (Koch, 1837) (Salticidae) (Nielsen 1935). Wasp's larvae develop on both the egg mass and the paralysed adult spider. Nielsen (1935) described that it was not consistent which the eggs of *Z. varipes* were deposited on spider's egg cocoon or on the paralysed spider itself and observed 24 cases of *C. erraticum* being parasitized by *Z. varipes* without spider's egg masses. All parasitized female of *C. erraticum* hiding in their chambers were stung to death and about 2 to 4 (up to 8) eggs were laid (Nielsen 1935).

Zaglyptus multicolor (Gravenhorst, 1829) N S Si
[**ITA**: Aubert 1969; **TAA**: Smits van Burgst 1915, 1918 (=*Polysphincta multicolor*), Giordani Soika 1942 (=*Polysphincta multicolor*); **LOM**: Masi 1948 (=*Polysphincta multicolor*), Di Giovanni et al. 2015a; **TOS**: Corcos et al. 2017 (Siena, San Giovanni d'Asso, 13-16.IX.2012, yellow pan trap (1 female); Siena, Monteroni d'Arbia, 21-24.XI.2012 (1 female)). **SIC**: De Stefani 1895 (=*Pimpla ephippium*), Zwakhals & Turrisi 2014]
New records. **FVG**: Udine, Marano Lagunare, proprietà Villabruna, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 females) (MZUR); Udine, Carlino, proprietà Villabruna, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 females) (MZUR); Udine, Carlino, Bosco Bolderatis, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR). **VEN**: Venezia, Portogruaro, fraz. Lison, Bosco del Merlo, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR); Venezia, Concordia Sagittaria, fraz. Sindacale, Bosco delle Lame, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR). Treviso, Meolo, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (4 males) (MZUR); Treviso, Cessalto, Bosco Olmè, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 male) (MZUR); Treviso, Mansuè, Bosco di Basalghelle, 23.VII-05.VIII.2013, Malaise trap, F.Di Giovanni leg. & det. (1 female) (MZUR). **PIE**: Cuneo, Barbaresco, San Lorenzo, vineyard, 27.VI-18.VII.2016, F. Di Giovanni det. (1 male) (DISAAA); Torino, Chiaverano, Bienca, X-XI.1985, Casale leg., F. Di Giovanni det. (1 female). **TOS**: Pisa, Crespina, La Serra, vineyard, 08.X.2013, A.Loni leg., F. Di Giovanni det. (5 females) (DISAAA); Pisa, Crespina, Ceppaiano, vineyard, 08.X.2013, A.Loni leg., F. Di Giovanni det. (3 females) (DISAAA); Pisa, Crespina, Poggio al Casone, vineyard, 08.X.2013, A.Loni leg., F. Di Giovanni det. (1 female) (DISAAA). **LAZ**: Frosinone, Serrone, 28.VIII.2010, 650m, black light trap, M.Gigli leg., F. Di Giovanni det. (1 male, 1 female) (MZUR). **SIC**: Isole Eolie, Vulcano, 28.VI-18.VII.2008, M.Mei leg., F. Di Giovanni

det. (1 female) (MZUR); Palermo, Riserva di Capo Gallo, 12.IV.2013, A.Reshchikov leg., F. Di Giovanni det. (1 male) (MZUR).

Zaglyptus varipes (Gravenhorst, 1829) N S Si
[**TAA**: Smits van Burgst 1914, 1915, 1918 (=*Polysphincta varipes*); **LOM**: Di Giovanni et al. 2015a; **SIC**: Zwakhals & Turrisi 2014]
New records. **TOS**: Pisa, Crespina, Poggio al Casone, vineyard, 27.VIII.2013, A.Loni leg., F. Di Giovanni det. (1 female) (DISAAA); Pisa, Crespina, La Serra, vineyard, 08.X.2013, A.Loni leg., F. Di Giovanni det. (5 males, 2 females) (DISAAA); Pisa, Crespina, Ceppaiano, vineyard, 08.X.2013, A.Loni leg., F. Di Giovanni det. (2 females) (DISAAA).

True parasitoids of spiders (*Polysphincta* genus-group)
Gauld & Dubois (2006) divided the *Polysphincta* genus-group into seven taxonomical groups, four of which occur in Italy.

Acrodactyla Haliday, 1838
This moderately large genus currently includes thirty-two described species in the Holarctic and the Indo-Australian regions (Yu et al. 2012). Seven of them occurr in Europe (van Achterberg et al. 2017). Townes (1969) divided the genus *Acrodactyla* sensu lato into three taxonomical groups. Each of these taxonomical groups has several unique morphological and ecological characteristics, including specific host range. The *Madida* genus-group already have been re-classified as the genus *Megaetaira* (Gauld & Dubois 2006). However, there is still a lack of strict taxonomical evidence with respect to the other two genus-groups: the *degener* species-group (*Acrodactyla* s. str., e.g. *Acrodactyla degener*), which includes wasps associated with sheet-web weaving spiders from the family Linyphiidae (e.g. Aubert 1969, Fitton et al. 1988, this study) and the *quadrisculpta* species-group (e.g. *A. quadrisculpta*), which includes wasps associated with orb-web weaving spiders from the genus *Tetragnatha* (Tetragnathidae) (Nielsen 1937, Aubert 1969, Howell & Pienkowski 1972, Fitton et al. 1988, Korenko et al. 2015a).

Acrodactyla carinator (Aubert, 1965) N
[**PIE**: Korenko et al. 2015a (mis. as *Acrodactyla quadrisculpta*)]
New records. PIE: Province of Verbano Cusio Ossola, Fondotoce di Verbania, bank of Lago Maggiore, larvae attached to *Tetragnatha montana*, leg. 28.X.2014, adults emerged 20.-21.XI.2014 (2 males), leg. Korenko S., det. Holý K. (CULS).

Acrodactyla degener (Haliday, 1838) N
This species has been reported generically for North Italy in the checklist of Scaramozzino (1995).
New records. FVG: Udine, Precenicco, Bosco Bando, 21.VII-03.VIII.2013, Malaise trap, F.Di Giovanni leg., A.Sasha det. (1 female) (MZUR); **PIE**: Province of Verbano Cusio Ossola, Fondotoce di Verbania, bank of Lago Maggiore, larva attached to *Neriene montana*, leg. 28.X.2014, adult emerged 23.XII.2014 (1 male), leg. Korenko S., det. Holý K. (CULS).

Acrodactyla quadrisculpta (Gravenhorst, 1820) N

[**ITA**: Gravenhorst 1820, Kirchner 1867 (=*Tryphon quadrisculptus*), Aubert 1969 (=*Colpomeria quadrisculpta*), Townes et al. 1965; **PIE**: Kirchner 1867 (=*Ichneumon quadrisculptus*)]
New records. PIE: Province Torino, close to Macello, bank of torrent Chisone, larvae attached to *Tetragnatha* sp., leg. 26.VIII.2014, adults emerged 08.IX.2014, 11.IX.2014 (1 male, 1 female), leg. Korenko S., det. Holý K. (CULS).

Megaetaira Gauld & Dubois, 2006
The genus is represented by one uncommon but presumable widely distributed species in Europe, including Great Britain. It was formerly known as *Acrodactyla madida*, but excluded from the *Acrodactyla* genus because it lacks several features of the remaining *Acrodactyla* species (Gauld & Dubois 2006). *Megaetaira madida* is associated with spiders of the genus *Metellina* from Tetragnathidae (*Metellina mengei* (Blackwall, 1869), *Metellina merianae* (Scopoli, 1863), and *Metellina segmentata* (Clerck, 1757)) (Fitton et al. 1988, Korenko 2016). Several details on its biology are provided by Nielson (1923) under the former synonym *Polysphincta clypeata*.

Megaetaira madida (Haliday, 1838) N
[**TAA**: Bauer 2002 (=*Acrodactyla madida*); **PIE**: Korenko 2016]

Oxyrrhexis Förster, 1869
This small Holarctic genus comprises four described species (Yu et al. 2012), associated with spiders from family Theridiidae (e.g. Fritzén & Fjellberg 2014). Only *Oxyrrhexis carbonator* was documented from Italy. This species is known to be associated with tangle web weavers from family Theridiidae: *Steatoda bipuncatata* (Linnaeus, 1758) in North Europe (Fritzén & Fjellberg 2014), and *Steatoda borealis* (Hentz, 1850) in North America (Townes & Townes 1960).

Oxyrrhexis carbonator (Gravenhorst, 1807) N
[**TAA**: Bauer 2002]

Polysphincta Gravenhorst, 1829
This large genus includes 27 described species, distributed widely in Holarctic and Neotropical regions (Yu et al. 2012). The genus is presently represented by five valid taxa in Europe (van Achterberg et al. 2017). All of them are known to be strictly associated with the family Araneidae (e.g. Fitton et al. 1988, Yu et al. 2012), and their host spectrum seems to be specific (Schmitt et al. 2012, Fritzén & Shaw 2014, Korenko et al. 2014, 2017a).

Polysphincta boops Tschek, 1869 N
[**TAA**: Bauer 2002; **PIE**: Korenko et al. 2014]

Polysphincta longa Kasparyan, 1976 N
[**VEN**: Di Giovanni et al. 2015b; **LOM**: Di Giovanni et al. 2015a (mis. as *Polysphincta boops*)]
New records. PIE: Cuneo, Barbaresco, San Lorenzo, vineyard, 07-27.VI.2016, F. Di Giovanni det. (1 female) (DISAAA).
Notes: *P. boops* and *P. longa* have been probably confused in the past due to the morphological similarities (Fritzén & Shaw 2014). Both species are associated with orb web

building spiders occurring in tree crowns, but *P. boops* is exclusively associated with spiders of genus *Araniella* (e.g. Fitton et al. 1988, Fritzén & Shaw 2014, Korenko et al. 2014), whereas *P. longa* is associated only with *Araneus angulatus* Clerck, 1757.

Polysphincta rufipes Gravenhorst, 1829 N
[**TAA**: Bauer 1936]

Polysphincta tuberosa Gravenhorst, 1829 N Sa
[**ITA**: Gravenhorst 1829, Blanchard 1840, Aubert 1969; **TAA**: Bauer 2002; **VAO**: Bauer 2002; **PIE**: Korenko et al. 2014]. This species has been reported for Sardinia in the checklist of Scaramozzino (1995).
New records. PIE: Province of Cuneo, Bottonasco, organic apple orchard, larva attached to *Araniella sp.*, leg. 29.X.2014, adult emerged 21.I.2015 (1 female), leg. Korenko S., det. Holý K. (CULS).

Schizopyga Gravenhorst, 1829, subg. ***Schizopyga*** Gravenhorst, 1829
The genus includes 12 species (Yu et al. 2012), which are associated with wandering spiders from the families Miturgidae (*Cheiracanthium* spp.), Clubionidae (*Clubiona* spp.) and Gnaphosidae (*Drassodes lapidosus* (Walckenaer, 1802)) (Bignell 1894, Nielsen 1935, Fitton et al. 1988, Shaw 2006).

Schizopyga circulator (Panzer, 1800) N
This species has been reported for North Italy by Scaramozzino (1995).

Schizopyga frigida Cresson, 1870 N
[**TAA**: Bauer 2002]

Schizopyga pictifrons (Thomson, 1877) N
This species has been reported for North Italy by Scaramozzino (1995) (as *Dreisbachia pictifrons*).

Schizopyga podagrica Gravenhorst, 1829 N S Si Sa
[**ITA**: Blanchard 1840, Townes et al. 1965, Aubert 1969; **TAA**: Bauer 2002; **EMR**: Kirchner 1867; **SIC**: Zwakhals & Turrisi 2014]
New records. **SAR**: Oristano, 20.VII.1978, F. Di Giovanni det. (1 female) (DISAAA).

Sinarachna Townes, 1960
A small genus with five described species and one subspecies from the northern hemisphere. Only two species are documented in Europe (van Achterberg et al. 2017): *Sinarachna nigricornis* (Holmgren, 1860) widely distributed in the Palaearctic, and *S. pallipes* (Holmgren, 1860), widely distributed in the Holarctic, with a subspecies *S. pallipes strigis* (Howard, 1892) in Nearctic (Yu et al. 2012).
Wasps of the genus *Sinarachna* seem to be associated exclusively with the family Araneidae; *S. nigricornis* is known to be associated with *Araneus diadematus* (Clerck, 1757) and *Araneus sturmi* (Hahn, 1831) (Araneidae) (Fitton et al. 1988). Host association with *Theridion* sp. (Fitton

et al. 1988) was corrected as *Araneus* sp. by Shaw (1994). *S. pallipes* is known to be associated with *Araneus diadematus*, *Araneus quadratus* Clerck, 1757 (Araneidae) (Nielsen 1928, Aubert 1969), *Larinioides cornutus* (Clerck, 1757) (Aubert 1969) and spiders from genus *Araniella* (Korenko et al. 2014). Host association with spiders from family Linyphiidae were also recorded by Nielsen (1923) but the species in this paper turned out to be a misidentification of *Acrodactyla degener* (Nielsen 1928). A recent study on the parasitoids of arboreal spiders in Italy additionally found strong host associations between *S. pallipes* and spiders of the genus *Araniella* (Araneidae): *A. cucurbitina* (Clerck, 1757), *A. displicata* (Hentz, 1847), *A. opisthographa* (Kulczyński, 1905) (Korenko et al. 2014, 2017a).

Sinarachna nigricornis (Holmgren, 1860) N
[**TAA**: Bauer 2002]

Sinarachna pallipes (Holmgren 1860) N
[**PIE**: Korenko et al. 2014]
New records. PIE: Province of Cuneo, Bottonasco, organic apple orchard (Cascina Rosa), larva attached to *Araneus* (*Atea*) sp., leg. 29.X.2014, adult emerged 28.I.2015 (1 male), leg. Korenko S., det. Holý K. (CULS).

Zatypota Förster, 1869
This is the largest genus within the *Polysphincta* genus-group, comprising at least 50 described species and probably many undescribed ones in museum collections (Gauld & Dubois 2006, Matsumoto & Takasuka 2010, Yu et al. 2012, Fritzén 2014). *Zatypota* wasps of different zoogeographical zones have several unique taxonomical characteristics, e.g. the dorsal absence of the occipital carina in Neotropical species, this never mediodorsally narrowly interrupted; or the lack of an epomia (an oblique ridge crossing the transverse furrow on the side of the pronotum) in Afrotropical species (Gauld & Dubois 2006). All *Zatypota* species are narrowly associated with a specific spider host or with a small group of closely related species from one family, with the only known exception of the Australian *Z. kauros* Gauld, 1984 (Korenko et al. 2017b). *Zatypota* species are known mainly as parasitoids of theridiid spiders (Nielsen 1923, Fitton et al. 1988, Gauld & Dubois 2006, Korenko & Pekár 2011, Korenko et al. 2011). Only four *Zatypota* species are associated with spiders other than theridiids: *Zatypota sulcata* Matsumoto, 2010 in Japan is associated with Linyphiidae (Matsumoto & Takasuka 2010), *Zatypota picticollis* (Thomson, 1888) in Europe is associated with Araneidae (Korenko et al. 2015b), and *Zatypota anomala* (Holmgren, 1860) in the Holarctic region is associated with Dictynidae (Fitton et al. 1987). A fourth species, *Zatypota kauros* in Australia, is oligophagous and attacks spiders from several host families (Theridiidae, Araneidae and Nephilidae) (Korenko et al. 2017b). Species with different morphology and host association may be separated as a distinct genus, but extensive revision of this genus is required.
Eight wasps from this genus occur in Europe (Fritzén 2010, Yu et al. 2012, Fritzén 2014); six of them (*Z. percontatoria, Z. bohemani, Z. discolor*, *Z. kerstinae, Z. albicoxa,* and *Zatypota flamma*) seem to be strictly associated with tangle web weavers from the family Theridiidae. *Zatypota percontatoria, Z. bohemani, Z. discolor* and *Z. kerstinae* are associated with spiders from the genus *Theridion*, or closely-related genera (e.g. genera *Phylloneta*, *Neottiura*) with similar habitus and behaviour which comprise the "*Theridion* group" (Fitton et al. 1987, 1988,

Korenko & Pekár 2011, Korenko et al. 2011, Fritzén 2010). In contrast, *Z. albicoxa* and *Z. flamma* seem to be strictly associated with the genus *Parasteatoda* (e.g. Fitton et al. 1987, 1988, Fritzén 2014). Two *Zatypota* wasps, *Z. anomala* and *Z. picticolis*, occurring in Europe, are associated with spiders out of the family Theridiidae. *Zatypota anomala* (Holmgren, 1860) is associated with cribellate tangle web weaving spiders belonging to the family Dictynidae. This Holarctic species was transferred from the genus *Sinarachna* to the genus *Zatypota* by Gauld & Dubois (2006) and recently grouped with *Z. kauros*, which is also not associated only with spiders from family Theridiidae (Korenko et al. 2017b). Vincent (1979) found this wasp to be associated with *Mallos pallidus* (Banks, 1904) in North America and the species was repeatedly reared from *Dictyna* sp. and *Dictyna pusilla* Thorell, 1856 and *Nigma flavescens* (Walckenaer, 1830) in Europe (Miller et al. 2013, Korenko 2017, Korenko unpubl.). *Zatypota picticollis* (Thomson, 1888) is a species known from Central and Western Europe (Zwakhals 2006, Korenko et al. 2015b), which attacks orb web weaving spiders from the family Araneidae (Korenko et al. 2015b). The Araneid spiders *Cyclosa conica*, *Mangora acalypha* (Walckenaer, 1802) and *Zilla diodia* (Walckenaer, 1802) are known to be its hosts (Zwakhals 2006, Korenko et al. 2015b, Takasuka et al. 2017). The final instar larva of *Z. picticolis* was found to induce unique changes in web architecture of all three host spiders. Under the influence of the final instar larva, the spiders build a specific web architecture which differes considerably from the capture web. In *C. conica*, the web lacks any sticky spiral, reduction of radials and absence of stabilimentum (Korenko et al. 2015b).

Zatypota albicoxa (Walker, 1874) N
[**LOM**: Di Giovanni et al. 2015a; **LIG**: Bordoni 2003]
New records. FVG: Udine, Palazzolo dello Stella, Nogali Braide, Bosco Brussa, 21.VII-03.VIII.2013, Malaise trap, F. Di Giovanni leg. & det. (1 female) (MZUR); Udine, Precenicco, Bosco Bando, 21.VII-03.VIII.2013, Malaise trap, F. Di Giovanni leg. & det. (1 female) (MZUR); Udine, Muzzana del Turgnano, Selva di Avronchi, 09-21.VI.2013, Malaise trap, F. Di Giovanni leg. & det. (1 female) (MZUR); Udine, Porpetto, Bosco Sgobitta, 21.VII-03.VIII.2013, Malaise trap, F. Di Giovanni leg. & det. (1 female) (MZUR). **VEN:** Venezia, Mestre, Bosco di Carpenedo, 22.VII-04.VIII.2013, Malaise trap, F. Di Giovanni leg. & det. (2 females) (MZUR); Venezia, Mestre, Bosco dell'Osellino, 22.VII-04.VIII.2013, Malaise trap, F. Di Giovanni leg. & det. (2 females) (MZUR); Venezia, Mirano, fraz. Villamagno, Bosco del Parauro, 22.VII-04.VIII.2013, Malaise trap, F. Di Giovanni leg. & det. (1 female) (MZUR); Venezia, Portogruaro, fraz. Lison, Bosco del Merlo, 23.VII-05.VIII.2013, Malaise trap, F. Di Giovanni leg. & det. (2 females) (MZUR); Treviso, Gaiarine, fraz. Francenigo, Bosco Otello, 10-22.VI.2013, Malaise trap, F. Di Giovanni leg. & det. (1 female) (MZUR); Same locality, 23.VII-05.VIII.2013, Malaise trap, F. Di Giovanni leg. & det. (1 female) (MZUR). **PIE**: Province of Cuneo, Chiamina, commercial apricot orchard close to Verzulo, larva attached to *Parasteatoda* sp., leg. 05.XI.2009 adult emerged 13.XII.2009 (1 female), leg. Korenko S., det. Zwakhals K. (CULS).

Zatypota anomala (Holmgren, 1860) N
[**PIE**: Korenko 2017]
New records. PIE: Province of Verbano Cusio Ossola, Fondotoce di Verbania, bank of Lago Maggiore, larva attached to *Dictyna pussila*, leg. 28.X.2014, adult emerged 01.XII.2014 (1 male), leg. Korenko S., det. Holý K. (CULS).

Zatypota bohemani (Holmgren, 1860) N S

[**TOS**: Corcos et al. 2017 (Siena, Vescona Chiesa, 26-29.VIII.2012, yellow pan trap (1 male))]. This species has been reported for North Italy in the checklist of Scaramozzino (1995).

New records. TOS: Pisa, Crespina, Poggio al Casone, vineyard, 08.X.2013, A.Loni leg., F. Di Giovanni det. (1 female) (DISAAA); Pisa, Crespina, La Serra, vineyard, 08.X.2013, A.Loni leg., F. Di Giovanni det. (1 female) (DISAAA); Pisa, Crespina, Ceppaiano, vineyard, 08.X.2013, A.Loni leg., F. Di Giovanni det. (2 females) (DISAAA).

Zatypota discolor (Holmgren, 1860) N

New records. PIE: Province of Cuneo, Bottonasco, organic apple orchard; larva attached to *Phylloneta* sp., leg. 4.XI.2009 adult emerged 29.XII.2009 (1 male), leg. Korenko S., det. Zwakhals K. (CULS); larvae attached to *Phylloneta* sp. and *Theridion* sp., leg. 29.X.2014, adults emerged 01-23.II.2015 (1 male, 2 female), leg. Korenko S., det. Holý K. (CULS). Chiamina, commercial apricot orchard close to Verzulo; larvae attached to *Pylloneta* sp., leg. 05.XI.2009 adults emerged 16.XII.2009 – 20.I.2010 (6 males, 7 females), leg. Korenko S., det. Zwakhals K. (CULS). Monterossa Grana, decidious forest ecoton; larvae attached to *Phylloneta* sp. sp., leg. 02.XI.2011 adults emerged 16.II.2012 – 06.III.2012 (3 female), leg. Korenko S., det. Zwakhals K. (CULS).

Zatypota percontatoria (Müller, 1776) N Si

[**TAA**: Smits van Burgst 1914 (=*Polysphincta percontatoria*), Bauer 2002; **LOM**: Di Giovanni et al. 2015a; **SIC**: Zwakhals & Turrisi 2014]

New records. PIE: Province of Cuneo, Bottonasco, organic apple orchard, larvae attached to *Theridion varians*, *T. tinctum*, *Theridion* sp., leg. 04.XI.2009 adults emerged 16.XII.2009 – 20.I.2010 (8 males, 17 females), leg. Korenko S., det. Zwakhals K., Korenko S. (CULS); 02.XI.2011 adults emerged 05.II.2012 – 20.III.2012 (9 males, 6 females), leg. Korenko S., det. Zwakhals K., Korenko S. (CULS); larva attached to *Parasteatoda tepidariorum*, leg. 02.XI.2011 adult emerged 7.II.2012 (1 male), leg. Korenko S., det. Zwakhals (CULS); larva attached to *Theridion* sp., leg. 29.X.2014, adult emerged 28.I.2015 (1 male), leg. Korenko S., det. Holý K. (CULS). Azienda A. Rivetto close to Ornato, organic apple and huzlenut orchard, larvae attached to *Theridion varians*, *Theridion* sp*.*, *Theridion pinastri*, leg. 03-07.XI.2009 adults emerged 26.XII.2009 – 20.I.2010 (6 males, 4 females), leg. Korenko S., det. Zwakhals K., Korenko S. (CULS). Valdieri, bush in rocky slope in bank of torrent Valasco, larva attached to *Theridion* sp*.*, leg. 27.VIII.2014, adult emerged 12.XI.2014 (1 female), leg. Korenko S., det. Holý K. (CULS); Province of Verbano Cusio Ossola, Fondotoce di Verbania, bank of Lago Maggiore, larva attached to *Theridion* sp*.*, leg. 30.X.2012, adult emerged 9.I.2013 (1 female), leg. Korenko S., det. Zwakhals K. (CULS); larva attached to *Theridion varians*, leg. 28. X.2014, adult emerged ?.XII.2014 (1 male), leg. Korenko S., det. Holý K. (CULS). **SIC**: Palermo, Riserva di Capo Gallo, 12.IV.2013, A. Reshchikov leg., F. Di Giovanni leg. (1 male) (MZUR).

Zatypota picticollis (Thomson, 1888) N

[**FVG**: Di Giovanni et al. 2015b; **PIE:** Korenko et al. 2015b]

New records. PIE: Province of Verbano Cusio Ossola, Fondotoce di Verbania, bank of Lago Maggiore; larva attached to *Zilla dioda*, leg. 28.X.2014, adult emerged 19-30.XII.2014 (1 male, 1 female), leg. Korenko S., det. Holý K. (CULS).

References

van Achterberg, K., Taeger, A., Blank, S.M., Zwakhals, K., Viitasaari, M., Yu, D.S.K., de Jong, Y. (2017): Fauna Europaea: Hymenoptera – Symphyta & Ichneumonoidea. Biodiversity Data Journal 5: e14650.

Aubert, J.F. (1969): Les Ichneumonides ouest-palearctiques et leurs hotes. 1. Pimplinae, Xoridinae, Acaenitinae. Laboratoire d'Evolution des Etres Organises, Paris, 302 pp.

Bauer, R. (1936): Beitrag zur Ichneumoniden Fauna von San Valentino alla Muta (Val Venosta). Bollettino della Società Entomologica Italiana 68: 103–111.

Bauer, R. (2002): Bemerkungen ueber die Ichneumoniden der Alpen. Teil IV (Hymenoptera, Ichneumonidae). Entomofauna 23 (8): 93–107.

Bignell, G.C. (1894): Two new species of Ichneumonidae from Devonshire. Entomologist's Monthly Magazine 30: 255–256.

Blanchard, E. (1840): Histoire naturelle des insectes. Orthoptères, Néuroptères, Hémiptères, Hyménoptères, Lépidoptères et Diptères. Avec une introdution renfermant l'anatomie et al physiologie des animaux articulés par M. Brullé. Lacour et Ce., Paris, 672 pp.

Bordoni, A. (2003): Observations on *Zatypota albicoxa* (Walker) (Hymenoptera, Ichneumonidae) and his host *Achaearanea luunata* (Clerck) (Araneae, Theridiidae). Doriana 8 (339): 1–4.

Corcos, D., Inclán, D.J,, Cerretti, P., Mei, M., Di Giovanni, F., Birtele, D., Rosa, P., De Biase, A., Audisio, P., Marini, L. (2017): Environmental heterogeneity effects on predator and parasitoid insects vary across spatial scales and seasons: a multi-taxon approach. Insect Conservation and Diversity, early view: 1-10. Suppl. material at doi: 10.1111/icad.12249

Costa, A. (1886): Notizie ed osservazioni sulla geo-fauna Sarda. Memoria quinta. Risultamento delle ricerche fatte in maggio 1885. Atti della Reale Accademia delle Scienze Fisiche e Matematiche 2 (7): 1–24.

De Stefani, T. (1894): Imenotteri di Sicilia raccolti nel territorio di Santa Ninfa in Provincia di Trapani. Naturalista Siciliano 13: 211–219.

De Stefani, T. (1895): Catalogo degli Imenotteri di Sicilia. Naturalista Siciliano 14: 169–182.

Di Giovanni, F., Cerretti, P., Mason, F., Minari, E., Marini, L. (2015a): Vertical stratification od ichneumonid wasp communities: the effects of forest structure and life-history traits. Insect Science 22: 688–699.

Di Giovanni, F., Reshchikov, A., Riedel, M., Diller, E., Schwarz, M. (2015b): New records of Ichneumonidae (Hymenoptera) for the Italian fauna. Biodiversity Data Journal 3: e5057.

Dubois, J., Rollard, C., Villemant, C., Gauld, I.D. (2002): The phylogenetic position of parasitoids of spiders within Pimplinae (Hymenoptera, Ichneumonidae). pp. 27–35. In: Samu F., Szinetár,

C. (eds.), Proceedings of the 20th European Colloquium of Arachnology, Plant Protection Institute and Berzsenyi College Press, Budapest, 356 pp.

Eberhard, W. (2000): Spider manipulation by a wasp larva. Nature 406: 255–256.

Fitton, M.G., Shaw, M.R., Austin, A. (1987): The Hymenoptera associated with spiders in Europe. Zoological Journal of the Linnean Society 90 (1): 65–93.

Fitton, M.G., Shaw, M.R., Gauld, I.D. (1988): Pimpline ichneumon-flies. Hymenoptera, Ichneumonidae (Pimplinae). Handbooks ofr the identification of British insects, vol.7, part 1. Royal Entomological Society of London, London, 110 pp.

Fritzén, N.R. (2010): Natural history and description of *Zatypota kerstinae* sp.nov. (Hymenoptera: Ichneumonidae) reared from *Theridion palmgreni* Marusik et Tsellarius (Araneae: Theridiidae) in Finland. Zootaxa 2487: 52–60.

Fritzén, N.R. (2014): Two new species of the *Polysphincta* genus-group (Hymenoptera: Ichneumonidae: Pimplinae) reared from their spider (Araneae) hosts in Europe. Zootaxa 3894 (1): 117–130.

Fritzén, N.R., Fjellberg, A. (2014): Natural history of *Oxyrrhexis zephyrus* sp.n. (Hymenoptera: Ichneumonidae), a parasitoid of *Enoplognatha serratosignata* (Araneae: Theridiidae), with notes on taxonomy and other host species of *Oxyrrhexis*. Arthropoda Selecta 23 (2): 135–144.

Fritzén, N.R., Shaw, M.R. (2014): On the spider parasitoids *Polysphincta longa* Kasparyan, and *P. boops* Tschek (Hymenoptera, Ichneumonidae, Pimplinae), with the first host records of *P. longa*. Journal of Hymenoptera Research 39: 71–82.

Gauld, I.D., Dubois, J. (2006): Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider parasitoids. Systematic Entomology 31 (3): 529–564.

Giordani Soika, A. (1942): Contributo alla conoscenza degli Icneumonidi della Venezia Tridentina. Studi Trentini di Scienze Naturali 23: 135–142.

Gravenhorst, J.L.C. (1820): Monographia Ichneumonum Pedemontanae Regionis. Memorie della Reale Accademia delle Scienze di Torino 24: 275–388.

Howell, J.O., Pienkowski, R.L. (1972): Notes on the biology of a spider parasite *Colpomeria kincaidii* Ashmead (Hymenoptera: Ichneumonidae). Entomophaga 17 (1): 5–7.

Kirchner, L. (1867): Catalogus Hymenopterorum Europae. Vindobonae, 285 pp.

Kloss, T.G., Gonzaga, M.O., de Oliveira, L.L., Sperber, C.F. (2017): Proximate mechanism of behavioral manipulation of an orb-weaver spider host by a parasitoid wasp. PLoS ONE 12 (2): e0171336. doi:10.1371/journal.pone.0171336

Korenko, S. (2016): Web architecture alteration of the orb web weaving spider *Metellina merianae* (Araneae, Tetragnathidae) induced by the parasitoid *Megaetaira madida* (Ichneumonidae, *Polysphincta* group). Arachnologische Mitteilungen 52: 35–37.

Korenko, S. (2017): First record from Italy of *Zatypota anomala* (Ichneumonidae, Ephialtini), a parasitoid of the cribellate spider *Dictyna pusilla* (Araneae, Dictynidae). Arachnologische Mitteilungen 54: 1–4.

Korenko, S., Pekár, S. (2011): A parasitoid wasp induces overwintering behaviour in its spider host. PloS ONE 6 (9): e24628.

Korenko, S., Michalková, V., Zwakhals, K., Pekár, S. (2011): Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid *Zatypota percontatoria*. Journal of Insect Science 11 (101): 1–12.

Korenko, S., Isaia, M., Satrapová, J., Pekár, S. (2014): Parazitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecological Entomology 39: 30–38.

Korenko, S., Korenková, B., Satrapova, J., Hamouzová, K., Belgers, D. (2015a): Modification of *Tetragnatha montana* (Araneae, Tetragnathidae) web architecture induced by larva of the parasitoid *Acrodactyla quadrisculpta* (Hymenoptera, Ichneumonidae, *Polysphincta* genus-group). Zoological Studies 54 (40): 1–7.

Korenko, S., Satrapova, J., Zwakhals, K. (2015b): Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimplinae). Entomological Science 18: 383–388.

Korenko, S., Kysilková, K., Černecká, Ľ. (2017a): First record from Slovakia *of Polysphincta longa* Kasparyan (Ichneumonidae, Ephialtini) narrowly specialised spider parasitoid of *Araneus angulatus* Clerck. Arachnologische Mitteilungen, accepted.

Korenko, S., Pekár, S., Walter, G.H., Korenková, V., Hamouzová, K., Kolářová, M., Kysilková, K., Spasojevic, T., Klopfstein, S. (2017b): One generalist or several specialist species? Wide host range and diverse manipulations of the hosts' web building behaviour in the true spider parasitoid *Zatypota kauros* (Hymenoptera: Ichneumonidae). Zoological Journal of Linnean Society, resubmission required by editor.

Martelli, G.M., Arru, G.M. (1958): Ricerche preliminari sull'entomofauna della quercia da sughera (*Quercus subera* L.) in Sardegna. Bollettino di Zoologia Agraria e di Bachicoltura 1: 5–49.

Masi, L. (1948): Reperti d'Imenotteri parassiti e note varie. Bollettino della Società Entomologica Italiana 78: 13–15.

Matsumoto, R., Takasuka, K. (2010): A revision of the genus *Zatypota* Förster of Japan, with descriptions of nine new species and notes on their hosts (Hymenoptera: Ichneumonidae: Pimplinae). Zootaxa 2522: 1–43.

Miller, J.A., Belgers, J.D.M., Beentjes, K.K., Zwakhals, K., van Helsdingen, P. (2013): Spider hosts (Arachnida, Araneae) and wasp parasitoids (Insecta, Hymenoptera, Ichneumonidae, Ephialtini) matched using DNA barcodes. Biodiversity Data Journal 1: e992.

Minelli, A., Ruffo, S., Vigna Taglianti, A. (2005): Le province faunistiche italiane. pp. 37–39. In: Ruffo, S., Stoch, F. (eds.), Checklist e distribuzione della fauna italiana. 10.000 specie terrestri e delle acque interne. Memorie del Museo Civico di Storia Naturale di Verona, 2° serie, Sezione Scienze della Vita 16, Verona, 308 pp.

Nielsen, E. (1923): Contributions to the life history of the pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*). Entomologiske Meddelelser 14: 137–205.

Nielsen, E. (1928): A supplementary note upon the life histories of the Polysphinctas (Hym., Ichneum.). Entomologiske Meddelelser 16: 152–158.

Nielsen, E. (1929): A second supplementary note upon the life histories of polysphinctas (Hym. Ichneum.). Entomologiske Meddelelser 16: 366-368.

Nielsen, E. (1935): A third supplementary note upon the life histories of the Polysphinctas (Hym. Ichneum.). Entomologiske Meddelelser 19: 191–215.

Nielsen, E. (1937): A fourth supplementary note upon the life histories of the Polysphinctas (Hym. Ichneum.). Entomologiske Meddelelser 20: 25–28.

Pisica C., Pagliano, G. (1982): Ichneumonid flies (Ichneumonidae, Hymenoptera) from the fauna of Italy. Analele Stiintifice ale Universitatii "Al. I. Cuza" din Iasi, Sect. Iia 28: 89–94.

Rivosecchi, L., Bettini, S. (1958): Contributo alla conoscenza dei predatori delle ova di *Latrodectus tredecimguttatus* Rossi. Rivista di Parassitologia 19 (4): 249–266.

Scaramozzino, P.L. (1994): Nota preliminare sui tipi delle specie di Ichneumonidae, Evaniidae e Gasteruptionidae descritte da Achille Costa. Hy-Men 5: 17–19.

Scaramozzino, P.L. (1995): Hymenoptera Ichneumonidae. In: Minelli, A., Ruffo, S., La Posta, S. (eds.), Checklist delle specie della fauna italiana 94, Calderini, Bologna.

Schmitt, M., Richter, D., Göbel, D., Zwakhals, K. (2012): Beobachtungen zur Parasitierung von Radnetzspinnen (Araneidae) durch *Polysphincta rufipes* (Hymenoptera: Ichneumonidae). Arachnologische Mitteilungen 44: 1–6.

Shaw, M.R. (1994): Parasitoid host ranges. In: Hawkins, B.A., Sheehan, W. (eds.), Parasitoid community ecology. Oxford University Press, 528 pp.

Shaw, M.R. (2006): Notes on British Pimplinae and Poemeniinae (Hymenoptera: Ichneumonidae), with additions to the British list. British Journal of Entomology and Natural History 19 (4): 217–238.

Sichel, J. (1860): Liste des Hyménopterès recueillis en Sicilie par M.E. Bellier de la Chavignerie pendant les mois d'Aout à Septembre 1859. Annales de la Société Entomologique de France 8: 749–764.

Smits van Burgst, C. A. L. (1914): Ichneumonidae (Hym.) captured in the environs of Bozen (Tyrol) in June 1913. Deutsche Entomologische Zeitschrift 1914: 325–333.

Smits van Burgst, C.A.L. (1915): Ichneumonidae gesammelt in der Umgebung von Bozen (Tirol) im Juni 1914. Deutsche Entomologische Zeitschrift 1915: 701–702.

Smits van Burgst, C.A.L. (1918): Verzeichnis der in der Ichneumonen-Sammlung der Niederlande vorhandenen Genera und Species der Familie Ichneumonidae. Ginneken (Luctor et Emergo), 48 pp.

Stüben, P.E., Behne, L., Floren, A., Günther, H., Klopfstein, S., López, H., Machado, A., Schwarz, M., Wägele, J.W., Wunderlich, J., Astrin, J.J. (2010): Canopy fogging in the Canarian laurel forest of Tenerife and La Gomera. Weevil News 51: 1–21.

Takasuka, K., Korenko, S., Kysilková, K., Štefánik, M., Černecká, L., Mihál, I., Dolejš, P., Holy, K. (2017): Host utilization of koinobiont spider-ectoparasitoids (Ichneumonidae, Ephialtini, *Polysphincta* genus-group) associated with *Cyclosa* spp. (Araneae, Araneidae) across the Palaearctic. Zoologischer Anzeiger 267: 8–14.

Townes, H.K. (1969): The genera of Ichneumonidae. Part 1. Ephialtinae to Agriotypinae. Memoirs of the American Entomological Institute 11: 1–300.

Townes, H.K., Townes, M. (1960): Ichneumon-flies of America north of Mexico 2. Subfamilies Ephialtinae, Xoridinae, Acaenitinae. United States National Museum Bulletin 216: 1–676.

Townes, H.K., Momoi, S., Townes, M. (1965): A catalogue and reclassification of the eastern Palearctic Ichneumonidae. Memoirs of the American Entomological Institute 5: 1–661.

Vincent, L.S. (1979): A new record for *Sinarachna anomala* (Hymenoptera: Ichneumonidae), an external parasitoid of *Mallos pallidus* (Araneae: Dictynidae). Pan-Pacific Entomologist 55: 192–194.

Yu, D.S., Achterberg van, C., Horstmann, K. (2012): Taxapad 2012, Ichneumonoidea 2011. Database on flash-drive. Ottawa, Ontario, Canada. Online version at <www.taxapad.com>

Zwakhals, K. (2006): The European species of the genera *Zatypota* and *Sinarachna* (Hymenoptera: Ichneumonidae, Pimplinae, Polysphinctini). Entomologische Berichten 66 (2): 34–37.

Zwakhals, K., Turrisi, G.F. (2014): Contribution to the knowledge of Ichneumonidae from Sicily. III. Pimplinae and Poemeniinae (Hymenoptera). Bollettino della Società Entomologica Italiana 146 (1): 41–46.

Supplement 15

Korenko S, Spasojevic T, Pekár S, Walter GH, Korenková V, Hamouzová K, Kolářová M, Kysilková K, Klopfstein S. One generalist or several specialist species? *Zatypota kauros* as an emerging model system to study parasitoid specialization and speciation (Hymenoptera: Ichneumonidae). Manuscript in submission, Frontiers in Zoology.

One generalist or several specialist species? *Zatypota kauros* as an emerging model system to study parasitoid specialization and speciation (Hymenoptera: Ichneumonidae)

Korenko Stanislav*[1], Spasojevic Tamara[2], Pekár Stano[3], Walter Gimme. H.[4], Korenková Vlasta[5], Hamouzová Kateřina[1], Kolářová Michaela[1], Kysilková Kristýna[1], Klopfstein Seraina[2]

[1] Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic
[2] Naturhistorisches Museum Bern, Department of Invertebrates, Bernastr. 15, CH-3005 Bern, Switzerland
[3] Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
[4] School of Biological Sciences, The University of Queensland, Brisbane QLD 4072, Australia
[5] Institute of Biotechnology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Praha 4, Prague, Czech Republic
* Corresponding author: korenko.stanislav@gmail.com

Running title: One generalist or several specialist spider parasitoid species?

Abstract

Parasitoid wasps of the *Polysphincta* genus group are usually highly specialised on their spider hosts and most of them are known to manipulate their hosts into building a special web in which the parasitoid pupates. The trophic niche and plasticity of host use was investigated in the koinobiont parasitoid *Zatypota kauros* Gauld from Queensland, Australia. We found behaviour of this parasitoid wasp unusual, unlike in other spider parasitoids. It attacks hosts from different families, each differing widely in their web-building behaviours. Molecular analysis revealed that the taxon *Zatypota kauros* contained at least two divergent mitochondrial lineages. Lineage A was associated exclusively with spiders of the genus *Anelosimus* (Theridiidae) and did not induce any changes in the structure of its host's tangle webs. Lineage B was associated with spiders of the genus *Cyrtophora* (Araneidae), all of which weave tent webs. Lineage C has a broader host range, including spiders of both families Araneidae and Theridiidae. Unique manipulations could be observed in the web-building behaviours of the different host groups. However, nuclear data from two ribosomal genes and three introns did not support the mitochondrial lineages or host groups, indicating either maternal effects or very recent and/or incomplete speciation in this taxon. Given their wide host range and intriguing interactions with their hosts, the *Z. kauros* complex thus represents a promising system to study parasitoid speciation.

Keywords: behavioural manipulation – co-evolution – interactions – koinobionts – speciation – silk– wasp – web architecture

Introduction

The order Hymenoptera contains the most diversified parasitoids, taxonomically and biologically (Eggleton and Belshaw 1992). Many parasitoid species have been reported having a wide range of hosts represented by insects or spiders from different families or even orders (Yu and Horstmann 2012). However, parasitoids that attack predators are host-specific. These specialists are from the high trophic levels and have potentially high impact on species at lower trophic levels. Due to the intimate nature of the associations evolved between the parasitoids and their hosts, diversification in this group is believed to have been accompanied by host shifts in most cases (e.g., Forbes et al. 2009). Yet, detailed data on the intricate host-parasitoid interactions in many parasitoid groups is too scarce to draw sound conclusions about the role of host shifts in their diversification.

The *Polysphincta* genus group (*sensu* Gauld and Duboi 2006) (Ichneumonidae, Pimplinae, Ephialtini) consists exclusively of koinobiont ectoparasitoids of spiders. Larvae of these species keep their spider hosts alive during their development and are known to manipulate the behaviour of their hosts (e.g., Nielsen 1923; Eberhard 2000; Korenko et al. 2014). They attach themselves externally to the opisthosoma or prosoma of spiders, where they manage to remain through several moult cycles of their hosts. They probably evolved from species that feed, as larvae, on spider eggs (Gauld et al. 2002), and the transition to juvenile or adult spiders probably occurred in a species that attacked a sedentary female spider guarding its egg sac (Godfray 1994).

Polysphinctines are described highly host-specific and show specific adaptations in many phenotypic traits, including complex behavioural repertoires that match the ecology of their hosts (e.g., Eberhard 2000). One set of such behavioural adaptations probably evolved in interaction with aggressive defence behaviour of the spider hosts and has presumably resulted in the restricted host ranges of these parasitoids (e.g., Gauld and Dubois 2006; Dubois et al. 2012). Even though polysphinctines as a whole are associated with spiders from different taxonomic groups with different foraging techniques, the host spectrum of a particular wasp species is usually restricted to a small group of taxonomically closely related species with similar behaviours. In all the species where sufficient data are available, the hosts always belong strictly to a single family (e.g., Fitton et al. 1987; Korenko et al. 2011).

The final instar larvae of polysphinctines have evolved the ability to manipulate the web-spinning behaviour of the spider host shortly before the wasp pupates. They cause the spider to construct a safe shelter for the wasp pupa that protects it from enemies (Eberhard 2000, 2001; Fincke et al. 1990; Matsumoto 2009; Korenko and Pekár 2011; Korenko et al. 2014). Several studies described behavioural manipulations of web-building spiders by polysphinctine parasitoids all over the world, in Japan (Matsumoto 2009; Matsumoto and Konishi 2007; Matsumoto and Takasuka 2010), Central America (Eberhard 2000, 2001, 2010, 2013; Weng and Barrantes 2007), South America (Sobczak et al. 2009; Gonzaga et al. 2010; Gonzaga and Sobczak 2007, 2011, 2015; Kloss et al. 2016), and Europe (Schmitt et al. 2012; Belgers et al. 2013; Korenko 2016; Korenko and Pekár 2011; Korenko et al. 2014, 2015; Korenko et al. 2015).

Parasitoid specificity and interactions between polysphinctine wasps and spiders in Australia have not yet been investigated. Only four genera and 18 species of the *Polysphincta* genus group have been documented from this continent (Gauld, 1984), but this number will certainly increase given that several undescribed species are already known. Here, we described for the first time, the host-parasitoid interactions in *Zatypota kauros* Gauld, 1984; endemic species in Australia. We focus on the host range of this parasitoid and the final instar larvae behavioural manipulation of the hosts. We found a great variability in both instances. By a combination of behavioural and molecular analyses, we investigate whether the taxon currently known as *Z. kauros* represents a single, polyphagous and behaviourally plastic species, or whether it constitutes a complex of more specialized lineages.

Materials and methods

Field sampling

The host spectrum, i.e. the potential and parasitized hosts of *Z. kauros*, was surveyed in tree canopies (between 40 cm to 200 cm above ground) at two suburban sites in Brisbane (Queensland, Australia): Gaythorne (bush land with dominant *Eucalyptus* spp. close to residential areas, 27°25'25'S, 152°57'30'E) and Enoggera (bush land on the bank of a small creek (Ferguson Park), 27°25'12'S, 152°59'19'E). We assumed that potential hosts are only web-building spider species, which is the case in almost all *Zatypota* species (Fitton et al. 1987; Matsumoto and Takasuka 2010).

Seventeen one-day excursions were conducted once a week between the beginning of July and the end of October 2013. The collected spiders were identified to genus with the aid of Hawkeswood's (2003) key and inspected under a hand lens for parasitoid presence. The parasitism rate was defined as the proportion of parasitized spiders in the population at a given time. All parasitized and some unparasitised spiders were taken to the laboratory for further examination and rearing of the adult wasps. Parasitoid wasps were identified using the key of Gauld (1984). Voucher specimens of spiders and wasps are deposited in the collections of Kees Zwakhals (Arkel, The Netherlands) and at the Department of Agroecology and Biometeorology, CULS (Prague, Czech Republic). The body length (prosoma + ophistosoma) of both unparasitised and parasitized spider was measured using an ocular ruler in a stereomicroscope.

Molecular methods

Genomic DNA was isolated from the hind legs of 26 *Z. kauros* individuals from different hosts and 14 specimens of other *Zatypota* species (collected in various parts of the world, see Table S1) preserved in 90% ethanol using the PrepGEM DNA isolation kit for insects (ZyGEM) or the Qiagen QIAamp DNA Micro kit (Qiagen) according to manufacturer protocols. Prior to isolation, the legs were cut in small pieces. Polymerase chain reaction (PCR) was performed using primers for the 5' portion of the mitochondrial cytochrome c oxidase subunit I (CO1) gene (Table 1). Given the potential of several cryptic species in *Zatypota kauros* indicated by the mitochondrial results, we also investigated nuclear diversity in a subset of specimens from each mitochondrial lineage. We first targeted the ribosomal RNA genes 28S and ITS2 which both proved uninformative. We thus used transcriptome data from ten ichneumonid wasps to develop primers for introns in protein-coding genes. These are the first intron markers used in this family (Table 1, gene names according to the annotation of the *Nasonia* genome).

PCR reactions either contained 8 µl DNA sample, 0.2 µl Phusion Hot Start II DNA Polymerase, 2 µl 10 uM primer (final concentration 1000 nM), 4 µl 5x Phusion HF Buffer, 0.4 µl 10 nM dNTPs and 5.4 µl RNAse free water (for most of the CO1 amplifications) or 1.5 µl DNA, 12.5 µl of GoTaq® Green Hot Start Master Mix, 2 µl primer (10µM), and 6.5 µl nuclease free water (for ITS2, 28S, introns and some of the CO1 amplifications) The primer annealing temperatures used for the PCR temperature protocol are given in table 1. PCR products were either purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research) and sequenced on an ABI Prism 310 automated sequencer using Big Dye Terminator technology (Applied Biosystems), or directly submitted for sequencing to LGC Genomics (Berlin). The sequences have been deposited on GenBank under accession nos. KU904368 – KU904393 (Table S1).

Pairwise distances and phylogenetic analyses

Additional sequences of *Zatypota* and outgroup species were downloaded from Genbank and from the BOLD database. All CO1 sequences were aligned with Muscle (Edgar 2004) after translation into amino acids using Mega 6.06 (Tamura et al. 2013). Alignment was straight-forward because no indels were detected. The alignment can be downloaded from TreeBASE (Gen Bank accession numbers: MF085406-MF085443). Uncorrected pairwise distances (p-distances) were calculated in Mega 6.06 with pair-wise deletion.

Phylogenetic analyses were performed under a Bayesian approach in MrBayes 3.2.2 (Ronquist et al. 2012). We used a “mixed” substitution model (integrating over the GTR model space; Huelsenbeck et al. 2004) and gamma-distributed among-site rate variation, including a proportion of invariant sites. For the COI gene, separate substitution models were included for the combined first and second codon positions versus the third codon positions. The four independent MCMC runs with one cold and three heated chains were run for 10,000,000 generations and sampled every 1,000th generation. As a conservative burn-in, we used half of the generations. Convergence was judged from the average standard deviation of split frequencies (ASDSF) for the topology parameter and the potential scale reduction factor (PSRF) for the scalar parameters (ASDSF < 0.005, PSRF < 1.001 for all parameters). All analyses were run on the University of Bern Linux Cluster UBELIX.

Laboratory study of web-building manipulation

The web-building behaviour of parasitised (cocoon web) and unparasitised (normal web) spiders was studied in the laboratory. "Cocoon web" refers to the unique web built by the spider host under the manipulation of the final instar larva (Eberhard 2000). This web serves the parasitoid as a safe refuge during the pupal stage, which is a critical period of parasitoid development. Spiders were placed individually in experimental arenas (at least 100 x 100 mm base, 130 mm height) with an installed twig or frame providing the space for the web. Spiders were kept at room temperature (22 ± 3°C) and natural light, and were fed with a surplus of fruit flies (*Drosophila melanogaster* Meigen) and various insects collected in the field. The architecture of the webs built by parasitised spiders was recorded on video until the larva killed and consumed the spider and pupated. Spider parasitoids were allowed to pupate in the cocoon web and were kept at room temperature for the next two weeks to let the adult wasps emerge. Forty-three out of the 60 parasitised specimens emerged as adult wasps; they were killed in 90% ethanol for further analysis. The remaining specimens were identified to species based on a comparison of the morphology of the larval stage. The web architecture of unparasitised spiders was observed for at least two weeks as a control (N = 5 – 20 per spider host). The web-building behaviour of both unparasitised and parasitised spiders was recorded using a digital camera Canon EOS 500 with a EF-S 18-55 mm lens or a macro lens EF 100 mm f/2,8L IS USM.

Results

Parasitisation in the field

Numerous juvenile and adult web-building spiders (N = 1,557, Fig. 1) were collected individually by carefully searching tree branches, and 1,446 were identified as potential hosts (spiders from families known to be accepted by polysphinctines) for *Z. kauros* (Table 2). We found 71 spiders parasitized by a polysphinctine larva, 60 of which were *Z. kauros*. The taxon *Z. kauros* was associated with juvenile web-building spiders belonging to five different species from three different subfamilies of family Araneidae (Araneinae, Cyrtophorinae and Nephilinae) and family Theridiidae. The body lengths of *Z. kauros* hosts were on average 2.55 mm (SD = 0.28, N = 60, Table 2).

Molecular analyses

To investigate if the observed, broad host range is indeed realized by a single species, we examined mitochondrial and nuclear diversity in the parasitoids. Both the phylogenetic analysis and the pairwise distances of the mitochondrial data partition suggest that the taxon currently known as *Z. kauros* consists of a complex of two or three putative species, lineages A, B and C (Fig. 2). Lineages B and C might or might not constitute a monophyletic clade; the CO1 analysis is ambiguous in this question, as the European species *Zatypota picticollis* (Thomson) clusters with B, but with low support. Pairwise distances between lineages A versus B and C amount to 1.7 - 4.3%, while B and C are separated by 2.3 – 3.2%. Intra-lineage distances are considerably smaller (maximal 0.6% within A, 0.3% in B, 0.8% in C). The distances between the three lineages are in general larger than the distances between lineages B and C and the European species *Z. picticollis* (2.1-2.6% and 2.3-3.2%, respectively). Wasp individuals reared from spiders from subfamily Araneinae were not nested to any mitochondrial lineage because we did not isolate enough DNA, but behavioural data on host parasitoid interaction are presented. The nuclear data from five different markers proved mostly inconclusive (Fig. 3) due to very low variability and the three mitochondrial lineages were not recovered in any of the gene trees. All genes showed low variability (1–3 variable sites in the introns, and three indels in one of the introns), and many of the variable positions appeared as heterozygous in one or both lineages, indicating either standing variation or gene flow (Supplementary Fig. S2).

Host associations per lineage and phenology

Lineage A wasps attacked exclusively juveniles or adults of tangle-web weavers from the family Theridiidae, with the highest parasitism rate in July when most hosts were still juvenile. In the following months, an increasing proportion of the observed theridiid spider hosts were adult females protecting their egg sacs (Fig. 4A). By contrast, lineage B wasps attacked only tent-web weavers from the subfamily Cyrtophorinae (family Araneidae) while lineage C was reared from Theridiidae and three subfamilies of Araneidae (Araneinae, Cyrthophorinae, Nephilinae). The most frequently parasitized spiders were araneids from June to August and juvenile nephilids from September to October (Fig. 4B). Wasps of lineage C shifted host preferences according to seasonal changes in the abundance of hosts of suitable body length (Fig. 5).

Behavioural observations

We observed the web building behaviour of spiders and its manipulation by parasitoids in 58 parasitized spiders in the laboratory. Wasps of lineage A did not induce any change in the web architecture of their tangle-web-weaving hosts (Theridiidae), as shown by a comparison of the webs built by parasitized and unparasitised spiders (Fig. 6). The tangle webs built by *Anelosimus* spiders consisted of a sparse 3D web with a shelter in the centre, usually at the base of three twigs. Under the influence of the final instar larva of the parasitoid, the spiders stayed in this retreat, where they died and were consumed, and the parasitoid larvae invariably built their cocoon and pupated at the entrance of this shelter (N = 19).

Parasitoids of lineage B attacked araneids of the subfamily Cyrtophorinae. In our experiments, *Cyrtophora hirta* L. Koch built a typical tent web with a horizontal sheet and a funnel-like shelter at its centre surrounded by a 3D tangle (Fig. 7A). Under manipulation by the final-instar larva of the wasp, *C. hirta* removed the horizontal sheet and all threads surrounding the shelter, and the wasp cocoon was invariably placed at the entrance of the shelter (N = 16, Figs 7A, B). The tent-web building spider *C. exanthematica* Doleschall also built a tent web consisting of a horizontal sheet and a 3D tangle, but without a funnel shelter. Under manipulation by the parasitoid, it removed the horizontal sheet and threads. The wasp cocoon was made within the sparse tangle which remained in all cases (N = 4).

The other araneid spiders (unidentified Araneinae) built a typical araneid orb-web (Fig. 8A). Under manipulation by the wasp, they built unique webs consisting of a reduced number of radial threads originating from the central hub where the wasp cocoon was attached in a perpendicular position to the plane of the web (N = 12, Fig. 8B). Wasps reared from Araneinae subfamily were not nested to any mitochondrial lineage (see methods).

Wasps of lineage C exhibited high plasticity in host utilisation and induced different modifications in the web architecture of spiders from different families. Seven wasps were found to attack *Nephila plumipes* Latreille which built orb webs with sparse tangled threads at both edges of the web plane (Fig. 8C). In five out of the seven cases, the spider host completely consumed the web and built a 3D structure into which the wasp placed its pupa in the centre, the apical end of the cocoon directed to the ground (Fig. 8D). In one case, the web was only partially consumed and a reduced number of radial threads served as support for the parasitoid pupa, which was placed in a perpendicular position on the side of the centre of the orb web (Fig. 8E). The same was in one spider host, which did not alter the web at all (Fig. 8F).

Discussion

Host ranges in the *Z. kauros* complex and related species

Here we present the evidence that the taxon currently known as *Z. kauros* attacks a wide variety of spider hosts. The wasps induce a variety of changes in the web-building behaviour of the affected spiders. These results are surprising both with respect to the taxonomic width of the host range and the diversity of host ecologies. The genus *Zatypota* includes more than 50 described species (Gauld and Dubois 2006; Matsumoto and Takasuka 2010; Yu et al. 2012; Fritzén 2010), and most of them are associated with particular species of spiders in the family Theridiidae, which build three-dimensional webs. However, our knowledge is fragmentary and mostly focussed on the European taxa. The only known *Zatypota* wasp species attacking hosts other than a theridiid species are *Z. anomala* (Holmgren, 1860), *Z. sulcata* Matsumoto, 2010*, Z. picticollis* and now *Z. kauros*. The Japanese species *Z. sulcata* is associated with the sheet-web weaving spider *Turinyphia yunohamensis* from the family Linyphiidae (Matsumoto and Takasuka 2010). The Holarctic *Z. anomala* attacks cribellate tangle-web weaving spiders of the genus *Dictyna* in Europe (Korenko 2017) and *Mallos pallidus* (Banks) in North America (Vincent 1979), both from the family Dictynidae. The European *Z. picticollis* is associated with the orb-web weavers *Mangora acalypha* (Walckenaer), *Cyclosa conica* (Pallas) and *Zilla diodia* (Walckenaer) from the family Araneidae (Korenko et al. 2015).

The CO1 analysis (Fig. 3) suggests that *Z. picticollis* and *Z. anomala* are closely related to *Z. kauros*. The morphological phylogeny provided by Gauld and Dubois (2006) also grouped *Z. kauros* with *Z. anomala* (but did not include the other species in question). Matsumoto and Takasuka (2010) mention that the Japanese species *Z. sulcata* is closely related to *Z. kauros* in morphology. This species group within the genus *Zatypota* thus seems to have switched to hosts of different families which build different web types from the hosts of the other *Zatypota* species. A possible scenario for the evolutionary history of the group would assume a hypothetical Old World ancestor that colonised several regions of the world, in which they diversified and which includes *Z. kauros* in Australia. More intensive taxon sampling and additional host records are needed to track the evolutionary history of switches between host groups in the genus *Zatypota*, but our analysis already points to a key aspect in the radiation of this species genus.

Host manipulation by wasps of the *Z. kauros* species complex

Four distinct forms of manipulation of the spider's web architecture were induced by *Z. kauros* larvae: 1) no alteration of the web (by lineage A and partially lineage C) in theridiid hosts of the genus *Anelosimus*); 2) alteration and reinforcement of a 2D web (in araneid hosts of the subfamily Araneinae, lineage was not designated); 3) alteration of the 3D web and isolation of the pupal cocoon by removing threads close to the site of pupation (in araneid hosts of subfamily Cyrtophorinae, lineage B and partially lineage C), and 4) alteration of a 2D orb web to a 3D structure (lineage C in araneid hosts of subfamily Nephilinae). All four forms of host manipulation are already known within polysphinctines, three of them in the genus *Zatypota*. Host attack with no alteration of the web architecture is known in *Z. anomala* which is associated with cribellate tangle-web weavers of the family Dictynidae (Vincent 1969; Korenko 2017). This lack of manipulation could be explained by the 3D tangle web of their hosts already being sufficiently safe for both the spider and the parasitiod pupa, so host manipulation is unnecessary. Manipulation 2) has been documented in *Z. picticollis* from Europe, whose cocoon web is identical with that of *Z. kauros* lineage B in association with orb-web building spiders; the cocoon of *Z. kauros* is even very similar to that of *Z. picticollis*, with unique pigmented spots at the basal and apical ends (Korenko et al. 2015). Manipulation 3) has been documented in the tent-web building spider *Manogea porracea* Koch under the manipulation by *Hymenoepimecis sooretama* Sobczak, Loffredo, Penteado-Dias & Gonzaga in Brazil (Sobczak et al. 2009). The utilisation of a host that builds tent webs and the associated behaviour manipulation, involving the same reduction of threads and resulting isolation of the cocoon on different continents, presumably evolved convergently in this unrelated parasitoid species. The presence of this convergence might point to the importance of host biology in the evolution of the wasp's manipulation strategies. Finally, Gonzaga et al. (2010) documented the manipulation of *Nephila clavipes* (Linnaeus) by *Hymenoepimecis bicolor* (Brulle) and *H. robertsae* Gauld in Brazil. In both these species, the parasitoid pupa was protected by a 3D silk structure as in manipulation 4), but they did not show the high variability in the hosts' responses as we have seen in *Z. kauros*.

Even though each of the observed manipulations has been recorded so far, they were all found to be species-specific, with each wasp species causing only one form of web-architecture alteration (e.g., Fritzen 2010; Korenko et al. 2014, 2015; Korenko and Pekár 2012). The high plasticity in both host range and host manipulation in *Z. kauros* is thus unusual and it might suggest to be a complex of several, more specialized species.

How many species does the *Z. kauros* complex contain?

Several studies have used CO1 sequences to study parasitoid of herbivore species diversity, and described that species assumed to be polyphagous are, on the contrary, represented by complexes of more specialized cryptic species (Smith et al., 2008; Rafter et al. 2013; König et al. 2015). Our CO1 results suggest that the species currently known as *Z. kauros* consists of several distinct lineages with only partially (lineage C) overlapping host-ranges; however, these lineages could not be confirmed by the nuclear markers studied here. An initial morphological investigation (SK and Kees Zwakhals, personal communication) found no clear morphological characters to distinguish the mitochondrial lineages from one another, nor among specimens reared from the three host families. Colouration is highly variable in this taxon, as already noted in the original description (Gauld 1984), but it seems to co-vary only partially with the mitochondrial lineage or the hosts. A more detailed, quantitative study of the morphology might still reveal diagnostic characters. In any case, the highly similar morphology suggests a young age of the taxa in this complex, but this conclusion is contradicted by the high divergence levels found in the CO1 sequences, which are typical for species-level divergences in wasps (e.g., Quicke et al. 2012; Klopfstein 2014). If we assume that substitution rates from other groups can be taken as a reasonable guide, such a divergence would be indicative of a common ancestor about 500,000 years ago (Brower 1994; Papadopoulou et al. 2010). Furthermore, at least lineages A and B are further apart from each other than B is from the European species *Z. picticollis,* from which both lineages differ clearly in morphology (Zwakhals 2006), although they are very similar in terms of their host manipulation behaviour and cocoon morphology (Korenko et al. 2015). The broad oligophagy and high plasticity in host manipulation in lineage C suggests that there might be additional diversity hidden within this lineage.

On the other hand, the molecular data from our study is highly inconclusive. This is despite the fact that we included the ITS2 marker, which has proven useful in previous studies on species delimitation in ichneumonid wasps (Klopfstein 2014, Klopfstein et al. 2016), and we even developed new intron markers to study the nuclear diversity of the taxon. The limited variation present in the nuclear markers did not follow any clearly discernible pattern with respect to mitochondrial lineages or host groups, but only a small number of specimens could be studied. Additional molecular data, preferable microsatellite data, and maybe even full-genome sequences from specimens reared from different hosts will be necessary to clarify the number of species present in this lineage.

The only clear evidence for the presence of multiple species thus stems from mitochondrial DNA, which is only maternally inherited and whose population biology can be influenced by endosymbiontic bacteria (Hurst and Jiggins 2005; Klopfstein et al. 2016); it might thus not accurately reflect the species history. Unless the mitochondrial lineages indeed turn out to represent biological species, the high correspondence between mitochondrial lineage and host choice is difficult to explain. One might speculate about maternal effects or associative learning, such as post-emergence imprinting, which has been shown to play a role in speciation in other parasitoid groups (Beltman and Meltz 2005; Kester and Barbosa 1991; König et al. 2015).

The *Z. kauros* complex as a model system for parasitoid speciation

Speciation in parasitoids is often accompanied by host shifts, although the exact mechanisms and sequence of events often remain elusive (Forbes et al. 2009; König et al. 2015; Shaw 2002). Closely related parasitoid species typically still attack phylogenetically or at least ecologically similar hosts or even retain some overlap in their host ranges (Shaw 1994, 2002). The *Zatypota kauros* complex is unique among koinobiont spider parasitoids in that it attacks hosts of different, ecologically divergent families, some of which are tangle-web weavers, while others create orb-webs. As we show here, it manipulates them into building a diverse array of special cocoon webs for the parasitoids to pupate in, a width of behavioural repertoires not reported from any other parasitoid species. It remains to be shown how changes in host choice, hunting behaviour, and physiological interactions with the host concur in this system to create such ecological diversity, and how this diversity pertains to molecular divergence and species status in the parasitoids. All these aspects make the *Z. kauros* complex an intriguing and highly promising system to study parasitoid speciation. More in-depth behavioural studies, including host choice and mate choice experiments, and molecular investigations at the population level would be auspicious next steps.

References

Belgers, D., K. Zwakhals, and P. van Helsdingen. 2013. De bijzondere levensloop van de sluipwesp *Acrodactyla quadrisculpta* op de schaduwstrekspin *Tetragnatha montana* (Hymenoptera: Ichneumonidae, Araneae: Tetragnathidae) [The life cycle of *Acrodactyla quadrisculpta* on *Tetragnatha montana* (Hymenoptera: Ichneumonidae, Araneae: Tetragnathidae)]. Nederl. Faun. Meded. 39: 1–6.

Belshaw, R., and D. L. J. Quicke. 1997. A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). Mol. Phylogenet. Evol. 7: 281–293.

Beltman, J. B., and J. A. Metz. 2005. Speciation: more likely through a genetic or through a learned habitat preference? Proc. R. Soc. Lond., B, Biol. Sci. 272: 1455–63.

Brower, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. U. S. A. 91: 6491–6495.

Dubois, J., C. Rollard, C. Villemant, and I. D. Gauld. 2002. The phylogenetic position of parasitoids of spiders within Pimplinae (Hymenoptera, Ichneumonidae). In: Samu F, Szinetár Cs, eds. Proc. 20th European Coll. Arachnol. Szombathely 22-26 July 2002, 27–35.

Eberhard, W. G. 2000. Spider manipulation by a wasp larva. Nature 406: 255–256.

Eberhard, W. G. 2001. Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenopera, Ichneumonidae). J.of Arachnol. 29: 354–366.

Eberhard, W. G. 2010. New types of behavioral manipulation of host spiders by a parasitoid wasp. Psyche 950614: 1–4.

Eberhard, W. G. 2013. The Polysphinctine wasps *Acrotaphus tibialis*, *Eruga* ca. *gutfreundi*, and *Hymenoepimecis tedfordi* (Hymenoptera, Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. Ann. Entomol. Soc. Am. 106: 652–660.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32: 1792–1797.

Eggleton, P., and R. Belshaw. 1992. Insect parasitoids: An evolutionary overview. Philos. Trans. R. Soc. Lond. Ser. B-Biol. Sci. 337: 1–20.

Fincke, O. M., L. Higgins, and E. Rojas. 1990. Parasitism of *Nephila clavipes* (Araneae, Tetragnathidae) by an ichneumonid (Hymenoptera, Polysphinctini) in Panama. J.Arachnol. 18: 321–329.

Fitton, M.G., M. R. Shaw, and A. D. Austin. 1987. The Hymenoptera associated with spiders in Europe. Zool. J. Linn. Soc. 90: 65–93.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.

Forbes, A. A., L. L. Stelinski, T. H. W. Powell, J. J. Smith, and J. L. Feder. 2009. Sequential sympatric speciation across trophic levels. Science 323: 776–779.

Fritzén, N. R. 2010. Natural history and description of *Zatypota kerstinae* sp.nov. (Hymenoptera: Ichneumonidae) reared from *Theridion palmgreni* Marusik et Tsellarius (Araneae: Theridiidae) in Finland. Zootaxa 2487: 52–60.

Gauld, I. D. 1984. The Pimplinae, Xoridinae, Acaenitinae and Lycorininae (Hymenoptera: Ichneumonidae) of Australia. Bull. br. Mus. nat. Hist. Entomol. 49: 235–339.

Gauld, I. D., and J. Dubois. 2006. Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae), a taxonomic revision of spider ectoparasitoids. Syst. Entomol. 31: 529–564.

Gauld, I. D., D. B. Wahl, and G. R. Broad. 2002. The suprageneric groups of the Pimplinae (Hymenoptera: Ichneumonidae): a cladistic re-evaluation and evalutionary biological study. Zool. J. Linn. Soc. 136: 421–485.

Godfray, H. C. J. 1994. Parasitoids. Behavioral and evolutionary ecology. Princenton Univ. Press, Princenton, NJ.

Gonzaga, M. O., and J. F. Sobczak. 2007. Parasitoid-induced mortality of *Araneus omnicolor* (Araneae, Araneidae) by *Hymenoepimecis* sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. Naturwissenschaften 94: 223–227.

Gonzaga, M. O., and J. F. Sobczak. 2011. Behavioral manipulation of the orb-weaver spider *Argiope argentata* (Araneae: Araniedae) by *Acrotaphus chedelae* (Hymenoptera: Ichneumonidae). Entomol. Sci. 14: 220–223.

Gonzaga, M. O., R. R. Moura, P. Teixeira, and F. A. Meira. 2015. Changes to web architecture of *Leucauge volupis* (Araneae: Tetragnathidae) induced by the parasitoid *Hymenoepimecis jordanensis* (Hymenoptera: Ichneumonidae). Behaviour 152: 181–193.

Gonzaga, M. O., J. F. Sobczak, A. M. Penteado-Dias, and W. G. Eberhard. 2010. Modification of *Nephila clavipes* (Araneae Nephilidae) webs induced by the parasitoids *Hymenoepimecis bicolor* and *H. robertsae* (Hymenoptera Ichneumonidae). Ethol. Ecol. Evol. 22: 151–165.

Hawkeswood, T. J. 2003. Spiders of Australia: An Introduction to Their Classification, Biology and Distribution. Pensoft, Sofia-Moscow.

Huelsenbeck, J. P., B. Larget, and M. E. Alfaro. 2004. Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. Mol. Biol. Evol. 21: 1123–1133.

Hurst, G. D. D., and. F. M. Jiggins. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc. R. Soc. Lond. Ser. B-Biol. Sci. 272: 1525–1534.

Kester, K. M., and P. Barbosa. 1991. Postemergence learning in the insect parasitoid, *Cotesia congregata* (Say) (Hymenoptera: Braconidae). J. Insect Behav. 4: 727–742.

Klopfstein, S. 2014. Revision of the Western Palaearctic Diplazontinae (Hymenoptera, Ichneumonidae). Zootaxa 3801: 1–143.

Klopfstein, S., C. Kropf, and H. Baur. 2016. *Wolbachia* endosymbionts distort DNA barcoding in the parasitoid wasp genus *Diplazon* (Hymenoptera: Ichneumonidae). Zool. J. Linn. Soc. 177: 541–557. DOI 10.1111/zoj.12380.

Kloss, T. G., M. O. Gonzaga, J. A. M. Roxinol, and C. F. Sperber. 2016. Host behavioural manipulation of two orb-weaver spiders by parasitoid wasps. Anim. Behav. 111: 289–296.

Korenko, S. 2016. Web architecture alteration of the orb web weaving spider *Metellina merianae* (Araneae, Tetragnathidae) induced by the parasitoid *Megaetaira madida* (Ichneumonidae, *Polysphincta* group). Arachnol. Mitt. 52: 35–37.

Korenko, S. 2017. First record from Italy of *Zatypota anomala* (Ichneumonidae, Ephialtini), a parasitoid of the cribellate spider *Dictyna pussila* (Araneae, Dictynidae). Arachnol. Mitt. 54: 1–4.

Korenko, S., and S. Pekár. 2011. A parasitoid wasp induces overwintering behaviour in its spider host. *PLoS ONE* 6: e24628.

Korenko, S., V. Michalková, K. Zwakhals, and S. Pekár. 2011. Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid (Hymenoptera: Ichneumonidae). J Insect Sci. 11: 101.

Korenko, S., M. Isaia, J. Satrapová, and S. Pekár. 2014. Parazitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecol. Entomol. 39: 30–38.

Korenko, S., J. Satrapova, and K. Zwakhals. 2015. Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimlinae). Entomol. Sci. 18: 383–388.

Korenko, S., B. Korenková, J. Satrapová, K. Hamouzová, and D. Belgers. 2015. Modification of *Tetragnatha montana* (Araneae, Tetragnathidae) web architecture induced by larva of the parasitoid *Acrodactyla quadrisculpta* (Hymenoptera, Ichneumonidae, *Polysphincta* genus-group). Zool. Stud. 54: 40.

König, K., E. Krimmer, S. Brose, C. Ganter, I. Buschlüter, C. König, S. Klopfstein, I. Wendt, H. Baur, L. Krogmann, and J. L. M. Steidle. 2015. Does early learning drive ecological divergence during speciation processes in parasitoid wasps? Proc. R. Soc. Lond. Ser. B-Biol. Sci. 282: 20141850.

Mardulyn, P., and J. B. Whitfield. 1999. Phylogenetic signal in the COI, 16S, and 28S genes for inferring relationships among genera of Microgastrinae (Hymenoptera; Braconidae): evidence of a high diversification rate in this group of parasitoids. Mol. Phylogenet. Evol. 12: 282–294.

Matsumoto, R. 2009. ‘‘Veils’’ against predators: Modified web structure of a host spider induced by an ichneumonid parasitiod, *Brachyzapus nikkoensis* (Uchida) (Hymenoptera). J. Insect Behav. 22: 39–48.

Matsumoto, R., and K. Konishi. 2007. Life histories of two ichneumonid parasitoids of *Cyclosa octotuberculata* (Araneae), *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomol. Sci. 10: 267–278.

Matsumoto, R., and K. Takasuka. 2010. A revision of the genus *Zatypota* Förster of Japan, with descriptions of nine new species and notes on their hosts (Hymenoptera: Ichneumonidae: Pimplinae). *Zootaxa* 2522: 1–43.

Miller, J. A., J. D. M. Belgers, K. K. Beentjes, K. Zwakhals, and P. van Helsdingen. 2013. Spider hosts (Arachnida, Araneae) and wasp parasitoids (Insecta, Hymenoptera, Ichneumonidae, Ephialtini) matched using DNA barcodes. Biodivers. Data J. 1: e992.

Nielsen, E. 1923. Contributions to the life history of the pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*) (Hym. Ichneumon.). Entomol. medd. 14: 137–205.

Papadopoulou, A., I. Anastasiou, and A. P. Vogler. 2010. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. Mol. Biol. Evol. 27: 1659–1672.

Quicke, D. L. J., M. Mori, A. Zaldivar-Riverón, N. M. Laurenne, and M. R. Shaw. 2006. Suspended mummies in *Aleiodes* species (Hymenoptera: Braconidae: Rogadinae) with descriptions of six new species from western Uganda based largely on DNA sequence data. J. Nat. Hist. 40: 2663–2680. DOI 10.1080/00222930601121288

Quicke, D. L. J., M. A. Smith, D. H. Janzen, W. Hallwachs, J. Fernandez-Triana, N. M. Laurenne, A. Zaldívar-Riverón, M. R. Shaw, G. R. Broad, S. Klopfstein, S. Shaw, and et al. 2012. Utility of the DNA barcoding gene fragment for parasitic wasp phylogeny (Hymenoptera: Ichneumonoidea): data release and new measure of taxonomic congruence. Mol. Ecol. Resour. 12: 676–685. DOI: 10.1111/j.1755-0998.2012.03143.x

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget , L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. Mrbayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61: 539–542.

Rafter, M. A., J. P. Hereward, and G. H. Walter. 2013. Species limits, quarantine risk and the intrigue of a polyphagous invasive pest with highly restricted host relationships in its area of invasion. Evol. Appl. 6: 1195–1207.

Schmitt, M., D. Richter, D. Göbe, and K. Zwakhals. 2012. Beobachtungen zur Parasitierung von Radnetzspinnen (Araneidae) durch *Polysphincta rufipes* (Hymenoptera: Ichneumonidae) [Observations on the parasitation of orb-weaving spiders (Araneidae) by *Polysphincta rufipes* (Hymenoptera: Ichneumonidae)]. Arachnol. Mitt. 44: 1–6.

Shaw, M. R. 1994. Parasitoid host ranges. In: Hawkins, B.A., and W. Sheehan eds. Parasitoid community ecology. Oxford University Press, Oxford, 111–144.

Shaw, M. R. 2002. Host ranges of *Aleiodes* species (Hymenoptera: Braconidae), and an evolutionary hypothesis. In: Melika, G., and C. Thuroczy eds. Parasitic wasps: evolution, systematics, biodiversity and biological control. Budapest: Agroinform, 322–327.

Smith, M. A, J. J. Rodriguez, J. B. Whitfield, A. R. Deans, D. H. Janzen, W. Hallwachs, and P. D. N. Hebert. 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. Proc. Natl. Acad. Sci. U. S. A. 105: 12359–12364.

Sobczak, J. F., A. P. S. Loffredo, A. M. Penteado-Dias, and M. O. Gonzaga. 2009. Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. J. Nat. Hist. 43: 2691–2699.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30: 2725–2729.

Yu, D. S., C. van Achterberg, and K. Horstmann. 2012. *Taxapad 2012. Ichneumonoidea 2011. Taxonomy, biology, morphology and distribution*. Ottawa, Ontario. Available at http://www.taxapad.com. Accessed January 15, 2017

www.globalamphibians.org.

Vincent, L. S. 1979. A new record for *Sinarachna anomala* (Hymenoptera: Ichneumonidae), an external parasitoid of *Mallos pallidus* (Araneae: Dictynidae). Pan-Pacific Entomol. 553: 192–194.

Weng. J.-L., and G. Barrantes. 2007. Natural history and larval behavior of the parasitoid *Zatypota petronae* (Hymenoptera: Ichneumonidae). J. Hymenopt. Res. 16: 326–335.

Zwakhals, K. 2006. The European species of the genera *Zatypota* and *Sinarachna* (Hymenoptera: Ichneumonidae, Pimplinae, Polysphinctini). Entomol. Ber. 66: 34–37.

Table 1. List of primers and their annealing temperatures used in the molecular study.

Gene	Primer	Sequence 5'-3'	Reference	Annealing temperature
CO1	*Lep_Fw*	ATTCAACCAATCATAAAG ATATTGG	Miller *et al.*, 2013	50 °C
	LCO	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.,* 1994	50-51 °C
	HCO2198_Rv	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.,* 1994	50-51 °C
28S	*D2-D3 fwd*	AAGAGAGAGTTCAAGAGTACGTG	Belshaw and Quicke, 1997	52 °C
	D2-D3 rev	TAGTTCACCATCTTTCGGGTCCC	Mardulyn and Whitfield, 1999	52 °C
ITS2	*fwd*	TGTGAACTGCAGGACACATG	Quicke *et al.*, 2006	50-51 °C
	rev	ATGCTTAAATTTAGGGGGT	Quicke *et al.*, 2006	50-51 °C
EOG7M10F6 intron 4	*fwd*	CCTGGATTTATCAGAGCTCG	this study	54-59 °C
	rev	GACCATATTGTATCGGACAT	this study	54 °C
EOG7F5BR9 intron 2	*fwd*	GCTTACAGGAACAAACTCGA	this study	56 °C
	rev	GCATCTTTCGTATTGTCGTTCG	this study	56-60 °C
EOG7F5BR9 intron 3	*fwd*	GCTTACAGGAACAAACTCGA	this study	56 °C
	rev	GCCAACGAAAATCCGAGAAC	this study	56-60 °C

Table 2. Host associations and sample sizes of *Z. kauros* lineages. Average host length in mm. N_{tot} stands for the total number of collected spiders, N_{par} stands for number of parasitised spiders. * number represents all spiders of the genus *Nephila*, including *N. plumipes*.

Lineage	Host Family/Subfamily	Host species	Host length (mm)	N_{tot}	N_{par}
A/C	Theridiidae	*Anelosimus* sp.	2.29	422	19
B	Araneidae/Araneinae	Unid. araneinae	2.25	330	12
B/C	Araneidae/Cyrtophorinae	*Cyrtophora hirta*	2.7	323	17
		C. exanthematica	2.6	98	4
C	Araneidae/Nephilinae*	*Nephila plumipes*	2.9	273*	8

Figure 1. Number of unparasitised and parasitized arboreal spiders inspected during four months of field sampling. N indicates the number of collected spiders; Np indicates the number of spiders parasitized by *Zatypota kauros*.

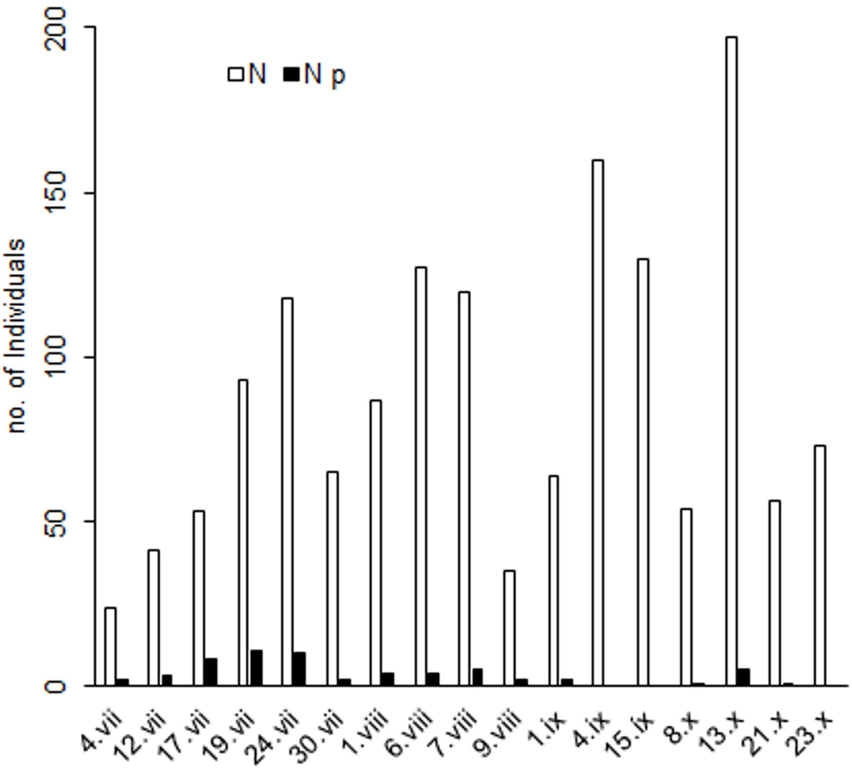


Figure 2. Majority-rule consensus based on CO1 sequences of the genus *Zatypota* including the *Zatypota kauros* complex, obtained using partitioned Bayesian inference. Spider host species and family are shown at the tips. The numbers at the nodes represent posterior probabilities, the scale bar is in substitutions per site.

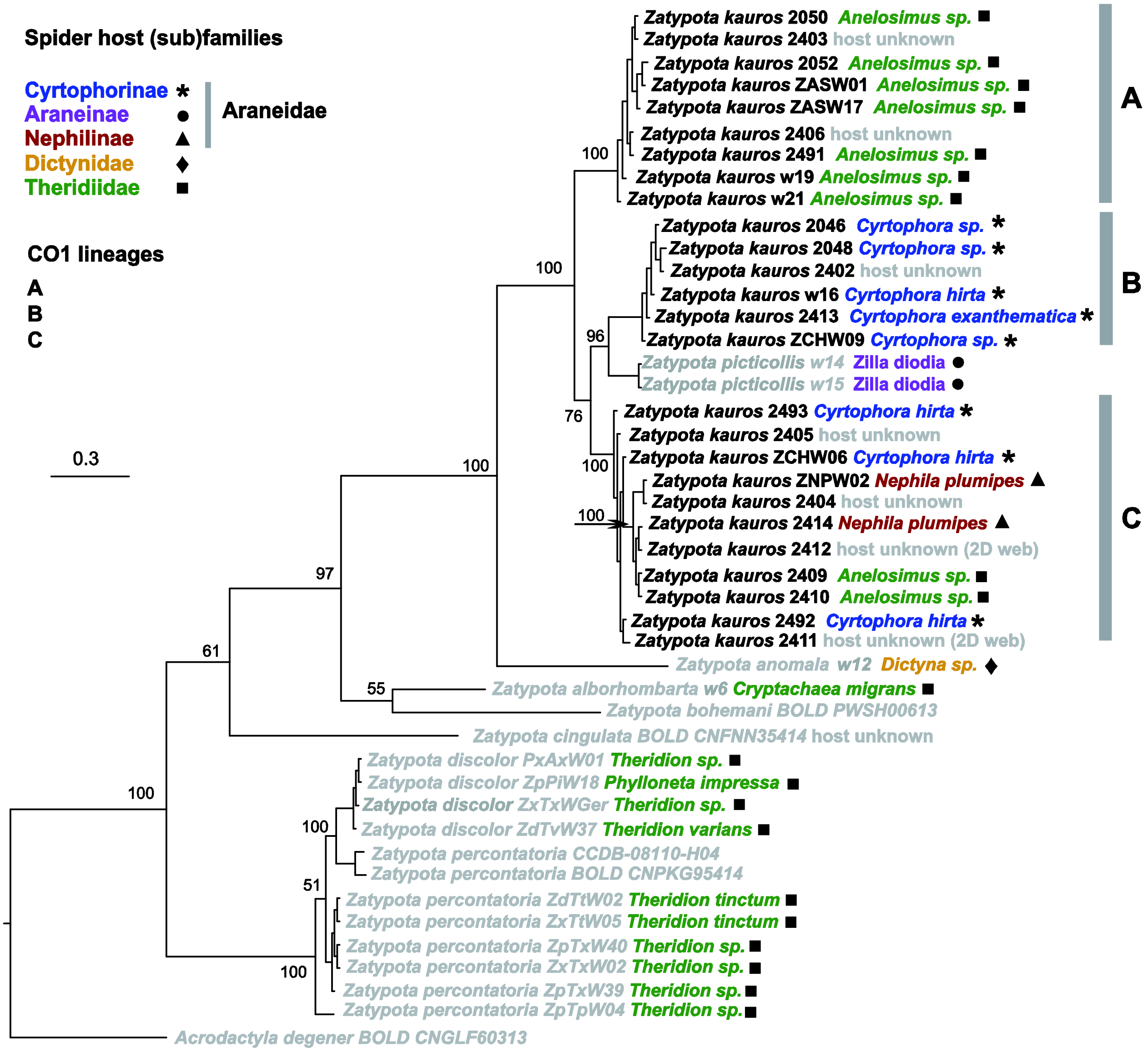


Figure 3. Majority-rule consensus trees of five nuclear markers in specimens of the *Zatypota kauros* complex, obtained using partitioned Bayesian inference. Spider host species and mitochondrial lineages are shown at the tips. The numbers at the nodes represent posterior probabilities, the scale bar is in substitutions per site.

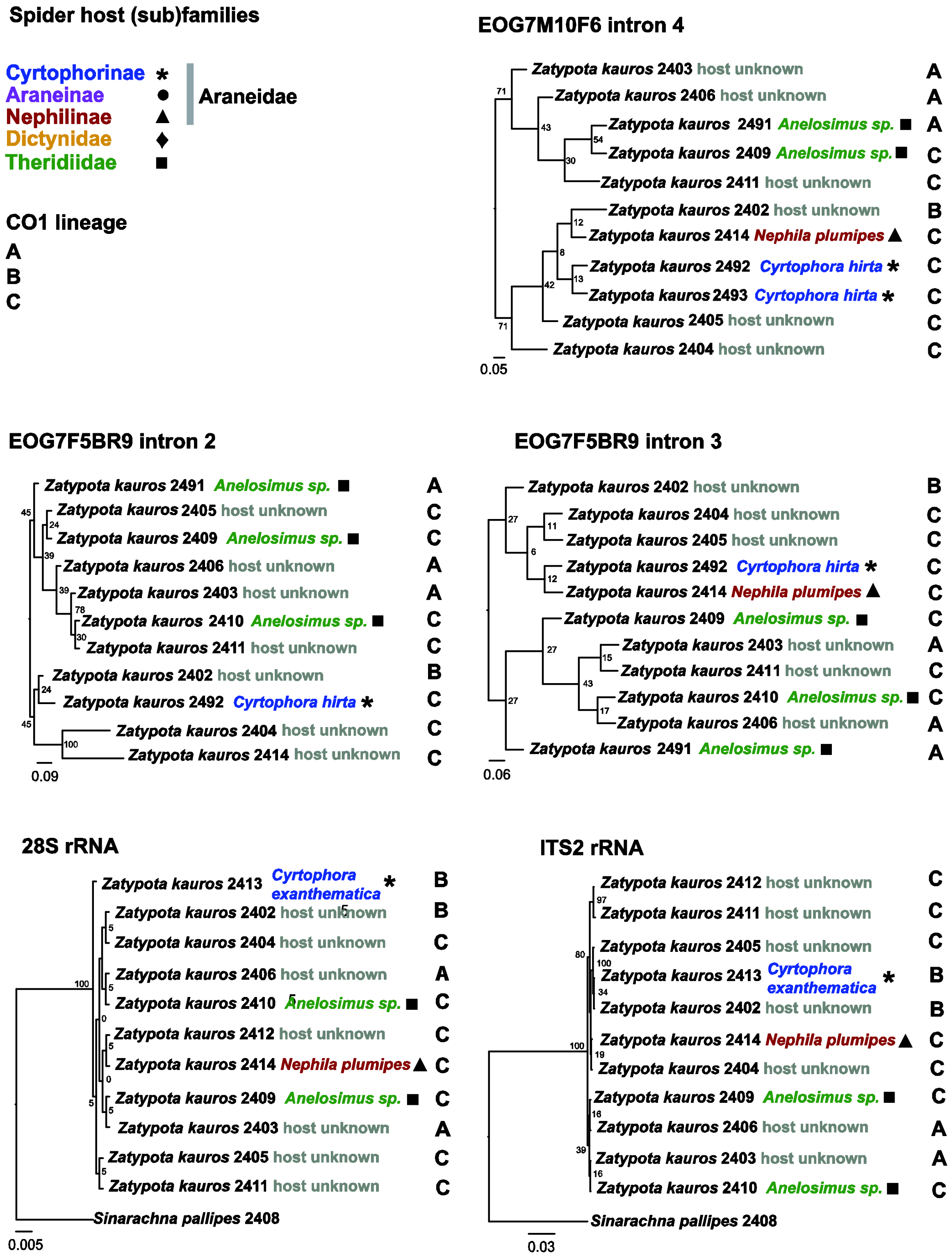


Figure 4. Change in the frequency of spider hosts parasitised by *Zatypota kauros* lineage A (a), and lineage B/C in combination (b) from July to October 2013.

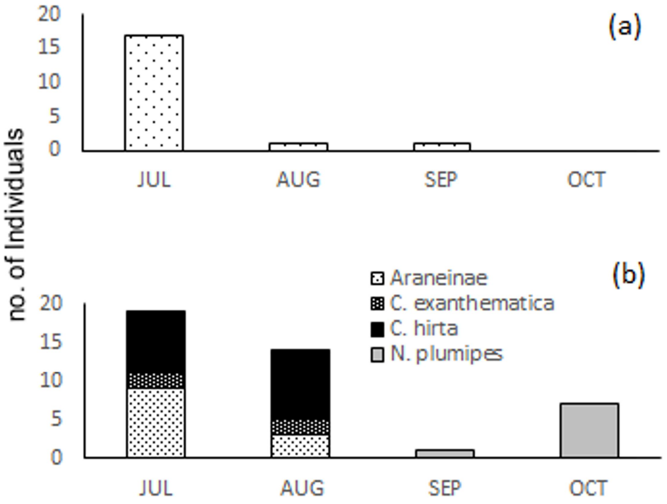


Figure 5. Comparison of average body length of *Cyrtophora hirta* and *Nephila plumipes* spider hosts parasitized by *Zatypota kauros* lineage B/C combination between July and October 2013 compared with the expected body length preference calculated from the average of all actually parasitized spiders (dotted line). *Nephila* spiders are separated into adults and juveniles.

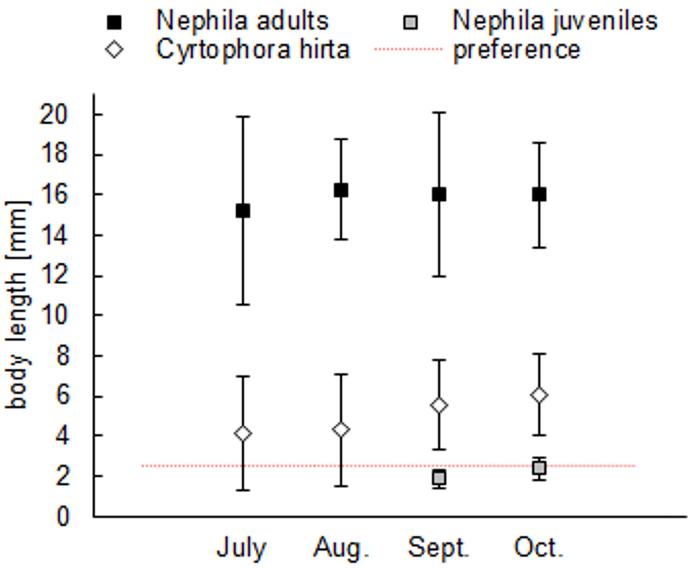
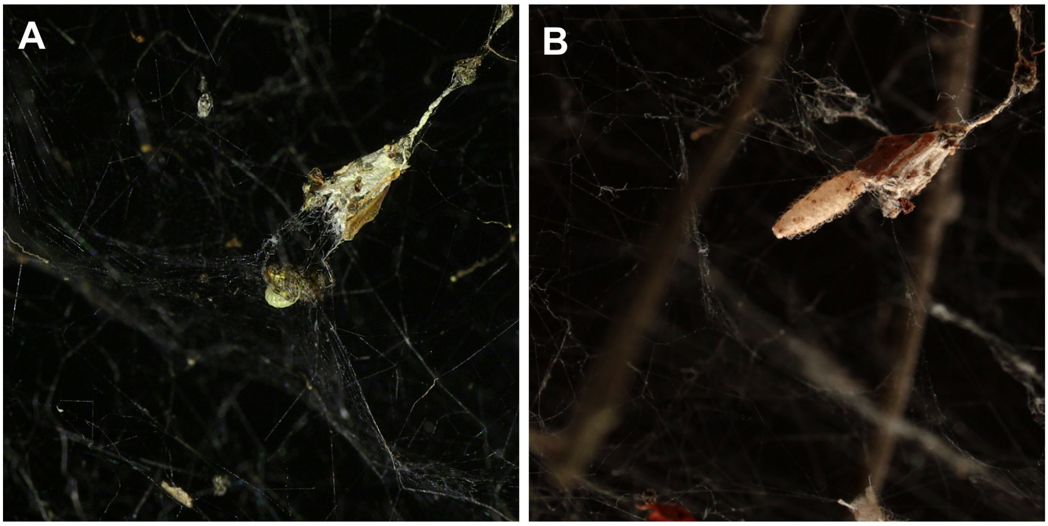


Figure 6. Unmodified tangle web of *Anelosimus* sp. with a shelter at the base of three twigs. Arrow shows wasp cocoon placed at the entrance of the shelter.

Figure 7. Web architecture alteration in *C. hirta* induced by final-instar larva of *Zatypota kauros*. The parasitised spider removes the sheet web from the surroundings of the funnel-like shelter (a).Wasp cocoon placed at the entrance of the funnel-like shelter (b).



Supplement 16

Supplement 16: Corresponding author’s statement in connection to the habilitation thesis.

Corresponding author's statement in connection to the habilitation thesis

Paper (author, title, journal):

Kehlmaier Ch, Michalko R, Korenko S. 2012. *Ogcodes fumatus* (Diptera: Acroceridae) reared from *Philodromus cespitum* (Araneae: Philodromidae), and first evidence of *Wolbachia* in Acroceridae. Annales Zoologici 62(2), 281-286

The undersigned who is corresponding author on the paper above, hereby confirms that candidate **Stanislav Korenko** has contributed to the work as stated below:

The percentage of candidate's contribution to the paper:

☐ Less than 10% ☒ 10-25% ☐ 25-50% ☐ 50-75%

Contribution of the candidate:

The candidate contributed to conception and design of the manuscript in section dealing with host behaviour alteration induced by parasitoid larva, provided a photography, and revising manuscript critically before submission.

Corresponding author
Dr. Christian Kehlmaier