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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

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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.

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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*

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

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%)

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.

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
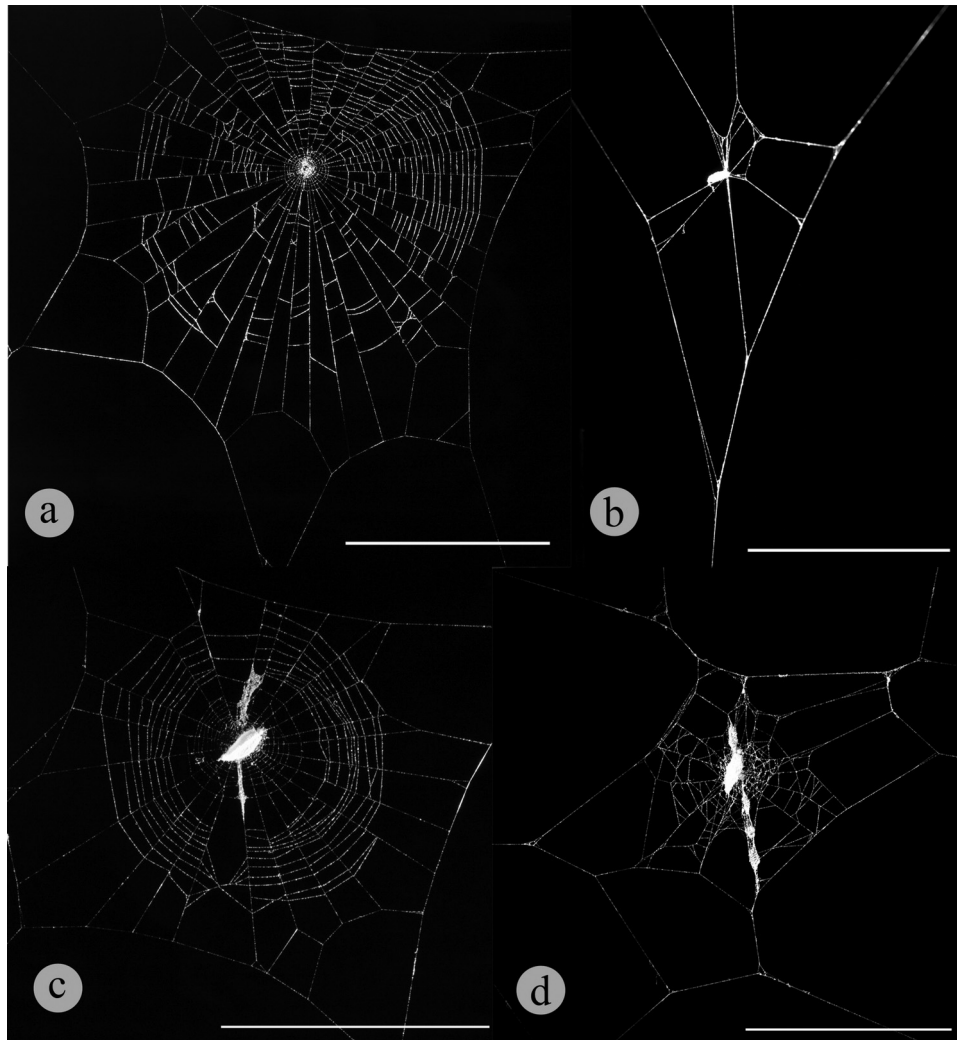
Fig. 2. An original orb web and cocoon webs of *C. conica* in Europe. All scales are 5 cm in length. (a) un-parasitised orb web without decoration, (b) cocoon web induced by *Z. picticollis* in Slovakia, (c) cocoon web induced by *R. nielseni* in Czech Republic, (d) cocoon web induced by *R. nielseni* 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.
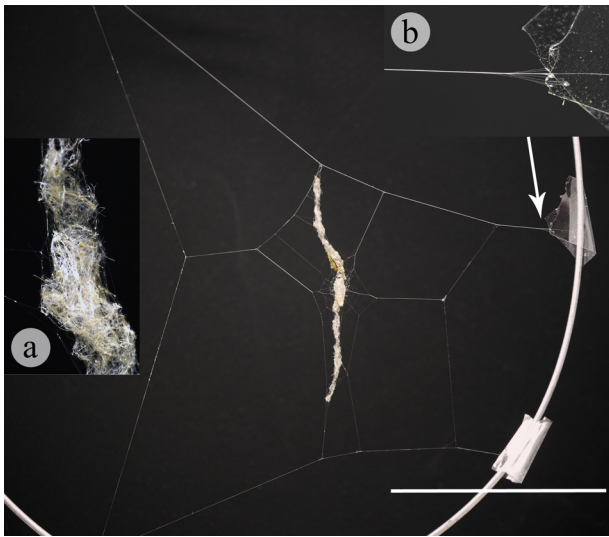
Fig. 3. A cocoon web of *C. laticauda* induced by *R. nielseni* in Japan; (inset a) magnification of white structures composing the debris decoration; (inset b) magnification of one of the distal ends of the frame line attached at many points to the tape after manipulation (frame lines were removed with 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 do 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.

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