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

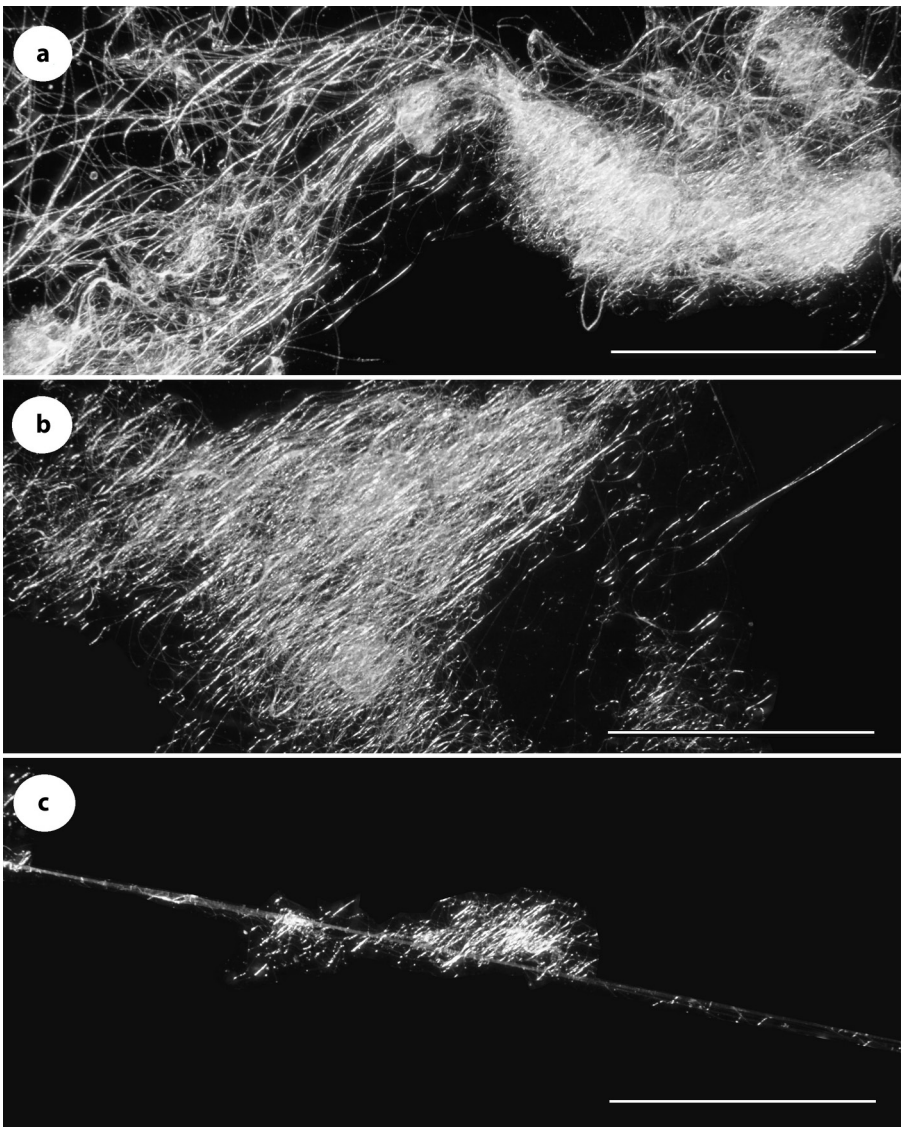

Fig. 3: Silk tufts produced by the spider under the influence of *P. longa* larva **a.** on the wall of the silk retreat; **b.** on threads in the surroundings of the retreat; **c.** on the dragline thread; Scales: 2 mm

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

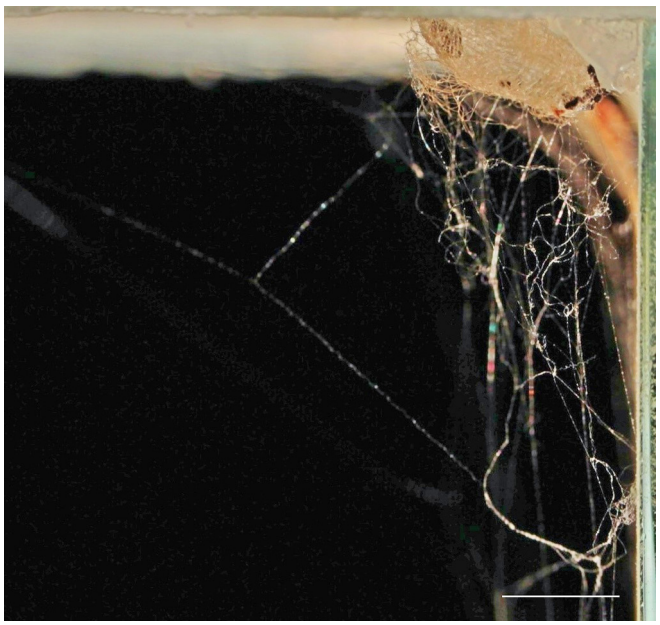

Fig. 5: Cocoon web built by *A. sturmi* under the influence of *P. tuberosa* larva. Scale: 5 mm

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)