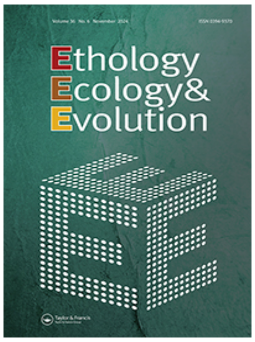

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Unveiling behavior modification induced by the *Ticapimpla* Darwin wasp (Ichneumonidae)

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Polysphinctine ichneumonid wasps comprise a group of koinobiont ectoparasitoids, in which all species complete their life cycle by developing the larval stage on an active host spider. Although the larvae of most species are known to manipulate the web-building behavior of the host spider during the pre-pupal stage, there is still a lack of biological understanding and host identity for six out of the 25 genera that form the group, including *Aravenator, Chablisea, Dreisbachia, Lamnatibia, Pterinopus*, and *Ticapimpla*. In this study, we offer the first insights into the biology of *Ticapimpla* wasps. We described the web modifications induced by *T. carinata* in their host spider *Spilasma duodecimguttata*, comparing webs built by parasitized and non-parasitized individuals of *S. duodecimguttata*, collected in a continuous old-growth forest in the Central Amazon. In addition, we provide the placement of *Ticapimpla carinata* in the molecular phylogeny of the *Polysphincta* group of genera. We observed a distinct difference between the webs of unparasitized *S. duodecimguttata* and those of individuals parasitized by *T. carinata*. Modified webs were characterized by a reduced number of radii and the absence of sticky spirals, resembling patterns seen in other Araneidae species parasitized by

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polysphinctine wasps. Furthermore, we provide robust support that *Ticapimpla carinata* forms a monophyletic clade with species of the genus *Acrotaphus* and discuss host specificity in polysphinctine group.

KEY WORDS: Amazon forest, biodiversity, orb-weaver spider, parasitoid, systematics, spider hosts.

INTRODUCTION

The ability to manipulate host behavior in order to promote the success of the life cycle is a widely recognized adaptation in various groups of parasitic organisms (Thomas et al. 2005). Darwin wasps, which belong to the *Polysphincta* group of genera (Ichneumonidae Pimplinae) (Gauld & Dubois 2006), are examples of parasitoids that developed this ability (Eberhard 2000; Gonzaga et al. 2017; Weinersmith 2019). The *Polysphincta* group of genera (hereafter, polysphinctines) constitutes a monophyletic lineage in which all members are solitary koinobiont ectoparasitoid wasps of spiders (Matsumoto 2016). These wasps are known for their parasitoid larvae that, during the pre-pupal stage, frequently induce the host spider to produce a modified web soon before parasitoid consuming it completely, and subsequently, they produce their pupa within the modified web (Gonzaga et al. 2017; Eberhard & Gonzaga 2019).

The modified web induced by polysphinctine wasps is known as the "cocoon web" and is often characterized by the absence of capture elements and a reduced, typically more durable and stable structure, where the cocoon of parasitoid is positioned in the center (Eberhard 2001; Takasuka et al. 2015; Korenko et al. 2022). This architecture prevents debris and flying insects from becoming entangled in the web, thus preventing structural collapse and promoting the pupa survival (Kloss et al. 2016). Besides this generalized structure, induced cocoon webs by some species exhibit specific variations in structure. For instance, there are cocoon webs with the presence of silk structures or shelters within the web, which can reduce the pupa's exposure to natural enemies (Matsumoto 2009). In other cases, cocoon webs may feature a tangle of threads around the orb web, creating a barrier structure on the webs (Gonzaga et al. 2010; Eberhard 2021). The variation in these modified webs can only be recognized through the identification of parasitoid larvae developing on the hosts, making the description of these interactions extremely useful for understanding potential adaptations between these parasitoids and their host spiders.

Polysphinctine wasps are diverse and widely distributed worldwide, comprising 25 genera and over 300 valid species, including seven fossil ones (Eberhard & Gonzaga 2019; Gaione-Costa et al. 2022; Khoramabadi et al. 2022; Kloss et al. 2022; Pádua 2022; Takasuka & Broad 2024). Although there is extensive knowledge of the group's systematics (Fitton et al. 1988; Gauld & Dubois 2006; Matsumoto 2016; Takasuka et al. 2018), most of the information is based on specimens collected through trapping methods, which do not allow the host recognition. Furthermore, information related to biology and host manipulation capability remains largely restricted to a few genera, especially *Hymenoepimecis* Viereck, *Polysphincta* Gravenhorst, and *Zatypota* Förster (Gonzaga et al. 2017). In contrast, six of the 25 genera, including *Aravenator* Momoi, *Chablisea* Gauld & Dubois, *Dreisbachia* Townes, *Lamnatibia* Palacio & Sääksjärvi, *Pterinopus* Townes, and *Ticapimpla* Gauld, remain completely unexplored in terms of their biology until date (Eberhard & Gonzaga 2019; Takasuka & Broad 2024). Moreover, the most recent molecular phylogeny (Matsumoto 2016) does not include some of the exclusive Neotropical genera, which hinders the understanding of the evolutionary relationships among these genera.

Among the genera with unknown information about their biology, *Ticapimpla* stands out as a Neotropical genus with only five valid species (Gauld 1991; Loffredo & Penteado-Dias 2008; Palacio et al. 2010; Pádua et al. 2019). Morphologically, individuals of *Ticapimpla* are similar to those of the genera *Acrotaphus* Townes and *Hymenoepimecis* (Gauld & Dubois 2006), but molecular studies on species of the *Ticapimpla* genus are scarce (see Matsumoto 2016; Spasojevic et al. 2021).

Considering the importance of studying genera with limited biological and phylogenetic information to understand the specificity and evolution of wasps in the polysphinctine group, the aim of this study was to provide the first insights into the biology of *Ticapimpla* wasps in the Brazilian Amazon and to place *Ticapimpla carinata* (Palacio et al. 2010) (Ichneumonidae) in the molecular phylogeny of the *Polysphincta* group of genera. We present, for the first time, information on the prevalence and behavior modification induced by *T. carinata* in *Spilasma duodecimguttata* (Keyserling 1879) spiders (Araneidae) and meticulously placed the *Ticapimpla* genus and their closer groups within the *Polysphincta* group of genera.

MATERIAL AND METHODS

Field collections

Field collections were performed during two expeditions conducted in October and November of 2022 and in May and June of 2023, in the km 41 site, a continuous old-growth evergreen Terra Firme forest located inside the Area of Relevant Ecological Interest of Biological Dynamics of Forest Fragments Project (ARIE-PDBFF), situated approximately 80 km north of Manaus, Brazil, in Central Amazonia (02°24’S, 59°43’W; 132 m asl). The area receives annual rainfall ranging from 1900 to 3500 mm, and the mean annual air temperature is approximately 26 °C (Laurance et al. 2002, 2010, 2017; Ferreira et al. 2005). We systematically collected all individuals of *S. duodecimguttata* from the central region (30 × 30 m) of the 32 permanent plots of the Amazon Fertilization Experiment (AFEX), covering a total sampling area of 28,800 m^2.

In addition, to improve the understanding of position of *Ticapimpla* in the polysphinctine phylogeny with the inclusion of tropical genera, we collected individuals of *Trichonephila clavipes* (Linnaeus) parasitized by the wasp *Hymenoepimecis bicolor* (Brullé), a genus not considered in the previous molecular phylogeny (see Matsumoto 2016; Spasojevic et al. 2021). Including *Hymenoepimecis* is important because Gauld and Dubois (2006) suggested, based on morphological phylogeny, that this genus is closely related to *Ticapimpla*. Therefore, this inclusion was essential for understanding the evolutionary relationships and diversification patterns within the group. We collected the individuals in April of 2023 in an Atlantic Forest urban area (20°45’S, 42°51’W; 713 m asl) located on the campus of the Universidade Federal de Viçosa, State of Minas Gerais, Brazil.

Study taxa

Ticapimpla carinata and *H. bicolor* were identified by Diego G. Pádua. *Ticapimpla carinata* is characterized mainly by having tarsal claw with a more or less broad auxiliary tooth, with inner margin strongly concave (tarsal claw simple in male); epicnemial carina short, present only ventrally; occipital carina forming a strongly raised flange in the occiput; fore wing blackish, with a weakly yellowish band between junction of vein R1 up to pterostigma until middle of the vein M or very faintly yellowish with apex and area adjacent to pterostigma of fore wing clearly blackish; hind leg orange, with distal 0.4 of tibia and tarsus black; metasoma orange with tergites VI+ black (Pádua et al. 2019).

Hymenoepimecis bicolor is distinguished mainly from other *Hymenoepimecis* species by having sternite I with a high, laterally compressed protuberance (sometimes low, rounded swelling protuberance) posteriorly; ovipositor 1.0–1.3 times longer than the hind tibia; fore wing hyaline (sometimes yellowish), with apex blackish; metasoma orange, with posterior margins of tergites II-V narrowly black and tergites VI+ black (Pádua et al. 2015). Amongst the parasitoid wasp individuals collected in the field, we analyzed the specimens of *Ticapimpla* (one male and one female) deposited in the Invertebrate Collection at the Instituto Nacional de Pesquisas da Amazônia (INPA) (Márcio Luíz de Oliveira, curator); and one specimen of *H. bicolor* (one female), which was processed for molecular analyses.

Spilasma duodecimguttata was identified by Adalberto J. Santos. *Spilasma duodecimguttata* is widespread recognized in Latin America, with the distribution described from Honduras to the state of Rio de Janeiro, Brazil (Levi 1995). These spiders often build their webs close to the ground, but some individuals also can be observed in higher strata of understory vegetation (A. Gaione-Costa personal observation). Eberhard (2020) describes its webs as a horizontal web, with sticky threads, pulled up at the hub, and above the hub, there are a median granular shelter, with sand grains, with a conical shape, which have a lateral flap. This flap is usually closed by the spider when it feels threatened. Spider voucher specimens were deposited in the arachnid collection at Centro de Coleções Taxonômicas at Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil (Adalberto José Santos, curator).

Host behavioral modifications

We described the web modifications induced by *T. carinata* on their host spider *S. duodecimguttata*, based on two parasitized individuals collected during samplings conducted in the Amazon Fertilization Experiment (AFEX) plots. Parasitized individuals were collected in the field and maintained in plastic cages. Since restrictions on cage size may influence web architecture, we kept the webs in large cages (55 × 36 × 40 cm) specifically to avoid this type of issue with cocoon web architecture. Plastic cages were kept under natural conditions within the study area, as well as in the Laboratory of Ecology and Behavior at Universidade Federal de Viçosa (25 ± 1 °C). Inside the cage, we attached the web shelter in dry twigs until they built the cocoon web and were killed by the parasitoid larva. Cocoons were removed from the webs and maintained in plastic tubes until the emergence of the adult parasitoid. The two pupae were collected and kept under controlled laboratory conditions (25 ± 1 °C, 70 ± 10% RH). One male and one female parasitoid emerged after 14 days. We analyzed eight webs built by unparasitized females in the field and two webs built by parasitized spiders. All webs were covered with cornstarch and photographed (see Eberhard 1976).

DNA extraction, amplification, and sequencing

We extracted the genomic DNA of three larvae, two adults of *T. carinata* and one adult of *H. bicolor*. We removed the larvae from the spider host abdomen and extracted a leg from adults to macerate in a microtube in contact with liquid nitrogen. We transferred the samples to 2 mL tubes with Lysing Matrix E and macerated for 1 min at 6 m/sec in FastPrep™ bead beater (MP Biomedicals, EUA). Genomic DNA was extracted using the ZymoBIOMICS DNA Miniprep kit (Zymo Research®, USA), following the manufacturer's protocol, and quantified using spectrophotometry in NanoDrop (Thermo Scientific®, USA). We amplified three genomic regions: the mitochondrial gene cytochrome c oxidase subunit I (COI), the large nuclear ribosomal subunit D2 (28S), and the translation elongation factor 1-α (tef1). Primers used to amplify each region are the same used by Matsumoto (2016), designed by Campbell et al. (1993) (28S), Folmer et al. (1994) (COI), Belshaw and Quicke (1997) and Klopfstein et al. (2011) (tef1), and the sequences are described in Table S1 in Supplemental Data. Polymerase chain reactions (PCRs) were performed in a final volume of 25 µL, following the conditions described in Matsumoto (2016) and adapting the temperatures suggested by the KAPA Taq ReadyMix KK1006 protocol (Merck, Darmstadt, Germany). Sequences were generated by Macrogen Korea (https://www.macrogen.com/). We edited and assembled sequences into contigs in Geneious Prime® 2023.2.1 and compared all sequences using a BLASTn tool at the NCBI database to confirm their identity (Altschul et al. 1990).

Phylogenetic analyses

We assembled the sequences and aligned each genomic region separately in Geneious Prime® 2023.2.1 using the MUSCLE algorithm, and they were employed to select the evolutionary models for the phylogenetic analyses. For selecting models and data partition, we used MrModelTest2 v 2.4 (Nylander 2004) on PAUP v 4.0a (Swofford 2003). The concatenated dataset consisted of seven data partitions. Based on Akaike Information Criterion (AIC) scores, we utilized the same data partition and evolutionary models as definitions to perform Maximum Likelihood and Bayesian Inference analyses (for 28S: SYM + I + G; COI and tef1: GTR + I + G).

We aligned the sequences from this study (*Ticapimpla* and *Hymenoepimecis*) with sequences from 20 polysphinctine genera from previous studies deposited in the NCBI-GenBank database (Table S2 in Supplemental Data) (Quicke et al. 2009; Matsumoto 2016; Bennett et al. 2019; Spasojevic et al. 2021; Bukowski et al. 2022), covering 88% of the 25 described genera. Considering previous analyses (Gauld & Dubois 2006; Matsumoto 2016), we incorporated species from six pimpline genera into our study as outgroups: *Tromatobia*, *Zaglyptus*, *Clistopyga*, *Acropimpla* (Ephialtini), *Apechthis* (Pimplini), and *Delomerista* (Delomeristini). Following Gauld and Dubois (2006), we kept the genera *Zaglyptus* + *Clistopyga* as a sister to a monophyletic *Polysphincta* group of genera. Additionally, adhering to Matsumoto's (2016) approach, we included the undetermined species of *Delomerista* Förster (Ichneumonidae) and *Apechris rufata* (Gmelin) (Ichneumonidae) as more remote outgroups.

Maximum Likelihood analyses were conducted with IQ-TREE multicore v. 1.6.12 (Hoang et al. 2018; Nguyen et al. 2020). We used 1,000 Ultrafast bootstrap replicates to generate a consensus tree. Bayesian Inference analyses were performed with MrBayes v. 3.2.7.a (Ronquist et al. 2012). Two parallel runs with four chains of Markov Chain Monte Carlo (MCMC) were run until converging, with a split frequency < 0.01, lasting five million generations. Trees were saved every 1,000 generations, and 25% of their topologies were discarded at the burn-in phase. A consensus tree was generated and viewed using FigTree v. 3.5.9 (Rambaut 2017). After comparing both tree topologies and numerical values, we showed at each branch of the ML tree the values higher than the threshold settled (bootstrap percentages: ML > 50%; posterior probabilities: BI > 0.50) and scaled the nucleotide substitutions per site at the bottom. Since we detected intraspecific genomic variation among the isolates of *Ticapimpla* (different branch lengths in clade III), we calculated the percentage of identity among the sequences for each genomic region (Table S3 in Supplemental Data). Trees were edited using Inkscape (www.inkscape.org).

RESULTS

Prevalence of parasitoidism

We collected 175 adult or subadult females, 12 adult or subadult males and 22 juveniles of *S. duodecimguttata* (totaling 115 females, four males, and 10 juveniles in 2022; 60 females, eight males, and 12 juveniles in 2023). We observed that, of all adult or subadult females collected, only three females (2.60%) collected in 2022 and two females (3.33%) collected in 2023 were parasitized by wasp larvae, while no male was parasitized. In addition, we did not observe any cocoon webs in the field.

Web modifications

Among the five parasitized spiders collected in the field, we described the cocoon web built by two of them. The remaining three parasitized individuals were collected with early-stage larvae and preserved in 70% alcohol for molecular analyses. We observed in the field that unparasitized individuals of *S. duodecimguttata* built a tangled web with some radially arranged lines above the horizontal orb, pulling the orb upward the hub to form a cone (see Levi 1995; Eberhard 2020; Fig. 1a). Moreover, in some situations, a tangle of threads was pulled at one of the cone's radius, generating an inclined standing for the web. The horizontal orb contains numerous sticky spirals that efficiently intercept insects (Fig. 1a). Additionally, this host species constructs a central shelter formed by debris mixed with silk (Fig. 1b-c). The shelter has a kind of door that the spider can retract with its front legs in the presence of danger. This behavior entirely isolates the spiders inside the shelters. We observed that the webs are typically located close to the ground, but they also occur in the forest understory (up to 1.5 m).

We observed that both cocoon webs showed the same pattern. The modified web was formed by a structure with a complete absence of sticky spirals, a reduced number of radii, and increased reinforcing radii (V radii, Fig. 1b-d). Furthermore, it was noticeable that, unlike normal webs, the modified webs are anchored to the branch structure by reinforced threads, and the parasitized spiders inserted several lateral threads, increasing the number of connection points of the web to the twigs (Fig. 1b-d). This was especially evident in the wire anchoring the twig shelter. In the modified webs, they are bifurcated in the middle of the wire, forming a triangular structure in the anchorage (Fig. 1c). After building a cocoon web, we observed that parasitized individuals remained inside the retreat and partially closed the entrance before being killed by the parasitoid larva (Fig. 1c). Moreover, we observed that the larva (Fig. 1e) constructs a very sparsely woven cocoon that adheres to the internal silk lining within the shelter, being practically imperceptible (Fig. 1f).

Phylogeny

The three genomic regions resulted in a final alignment with 1,617 bp (COI: 523; 28S: 541; tef1: 553 bps) and 79 taxa, containing 435 parsimony-informative sites. The same topology was obtained in both Maximum Likelihood (ML) and Bayesian Inference (BI) approaches for most clades (Fig. 2). We observed that *Ticapimpla* formed a monophyletic branch with high support (ML: 100%; BI: 1.0). The sequences of *Ticapimpla* exhibit high percentage of identity across each genomic region, indicating that all the isolates likely originate from *T. carinata*, even though there is intraspecific genomic variation among the isolates (Table S3 in Supplemental Data). Additionally, we observed that the newly incorporated genera in our phylogenetic analyses, *Ticapimpla*, *Hymenoepimecis*, *Eruga* Townes & Townes, *Flacopimpla*, and *Longitibia* He & Ye, which were not considered in the molecular phylogeny of Matsumoto (2016), were strongly supported as constituents of the derived clades, belonging to the subgroup *Polysphincta* sensu Matsumoto (2016).

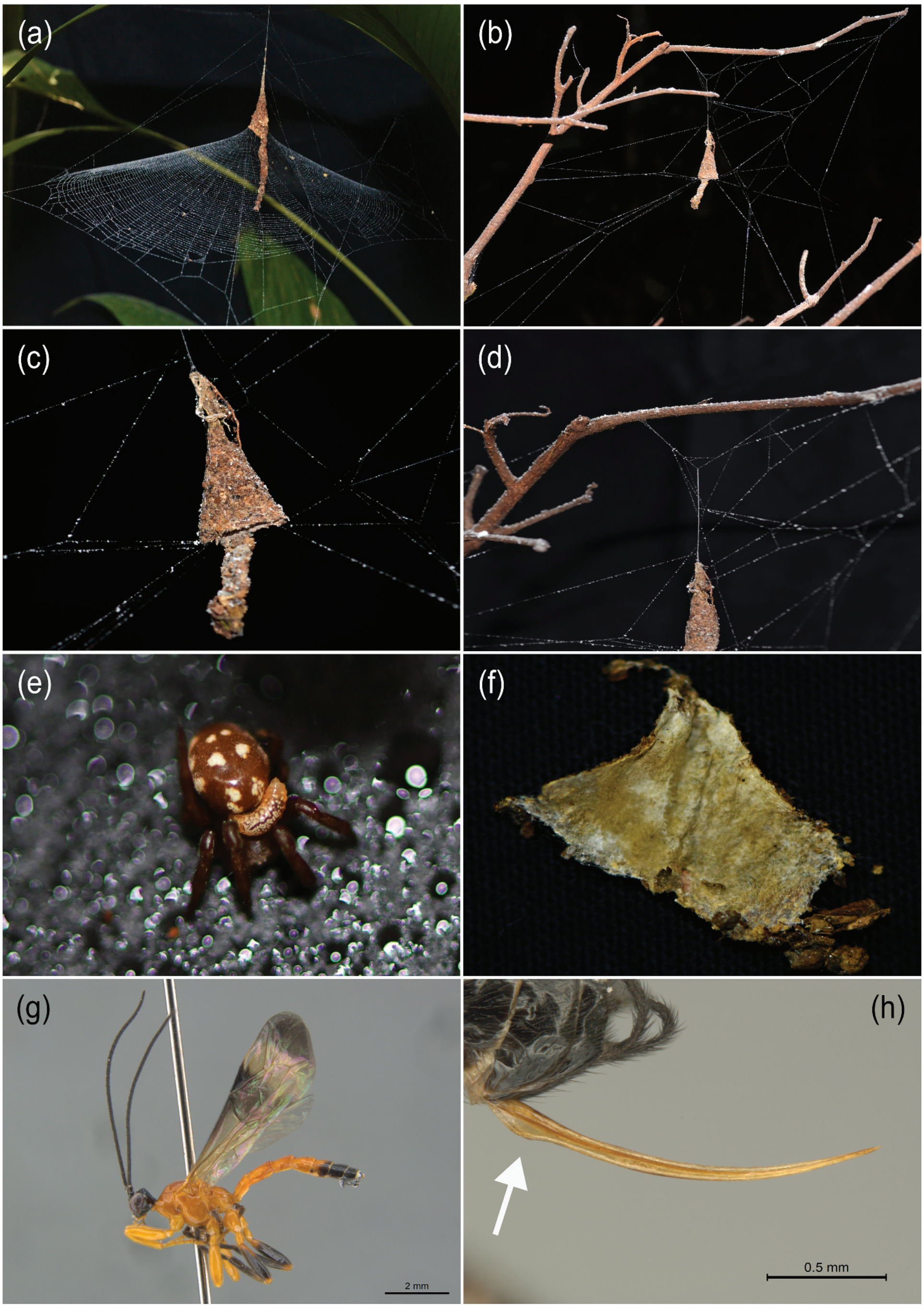

Fig. 1. — Webs of *S. duodecimguttata* (Araneidae) (a) web of a non-parasitized female spider; (b) modified web constructed by a parasitized spider by the last instar larvae; (c) web shelter constructed by a modified spider that was almost completely sealed off by the spider before falling victim to parasitoid larvae; (d) lines of cocoon web attached to vegetation by many points; (e) parasitized female of *S. duodecimguttata* by larvae of *T. carinata*; (f) open shelter of the modified web of *S. duodecimguttata* showing a very sparsely woven cocoon that adheres to the inner silk lining within the shelter; (g) adult male of *T. carinata*; (h) expanded ovipositor of the female of *T. carinata*, highlighted by the white arrow.

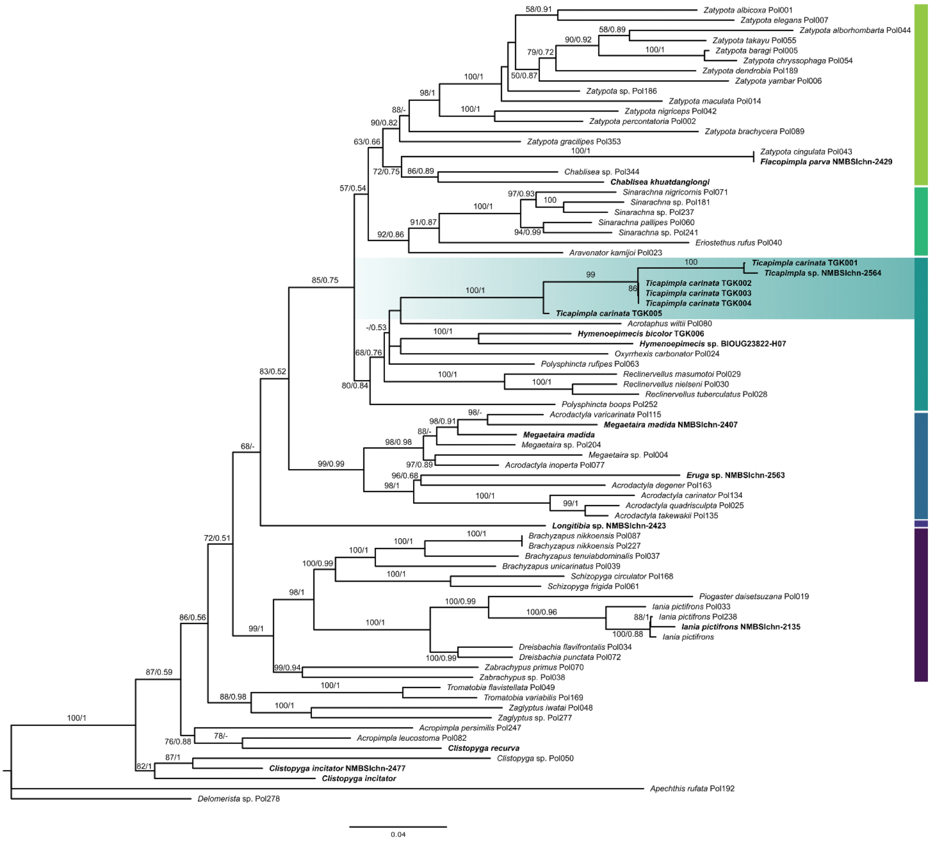

Fig. 2. — Molecular phylogeny of polysphinctine ichneumonid wasps. We showed the maximum likelihood topology, with six specimens collected at Brazilian Amazon and Atlantic forests and the specimens available at GenBank-NCBI databases, totaling 79 taxa. The analysis was based on concatenated sequences of 28S, COI, and tef1 markers. Numerical values on branches indicate the percentages of bootstraps followed by posterior probabilities. Values under 50% and 0.50 were suppressed. Bar on bottom show nucleotide substitutions per site. Bold names indicate new specimens that were not considered in phylogeny of Matsumoto (2016).

Nonetheless, we observed in our analyses that the subgroup *Polysphincta* (Matsumoto 2016) could be subdivided into three clades with high support values (Fig. 2). The most derived, clade I, was formed by the genera *Zatypota*, *Chablisea*, and *Flacopimpla*. The clade II was formed by *Sinarachna* Townes, *Eriostethus* Morley, and *Aravenator*, and the clade III by *Ticapimpla*, *Acrotaphus*, *Hymenoepimecis*, *Oxyrrhexis* Förster, *Polysphincta*, and *Reclinervellus* He & Ye. Also, we observed that despite not being a basal polysphinctine genus, the placement of the Oriental genus *Longitibia* within the polysphinctine phylogeny remains uncertain (ML: 68%; BI: 0.52), but it might constitute a clade with unique characteristics (clade V).

Additionally, as initially defined in the phylogeny by Matsumoto (2016), we have found conclusive evidence supporting the recognition of *Schizopyga* Gravenhorst and *Acrodactyla* Haliday subgroups. In this study, we adopted the understanding presented by Shaw (2006) and Matsumoto (2016), which maintains the genus *Dreisbachia* as distinct from the *Schizopyga* genus. As a result, we observed that the *Schizopyga* subgroup (clade VI) includes the genera *Brachyzapus* Gauld & Dubois, *Schizopyga*, *Piogaster* Perkins, *Iania* Matsumoto, *Dreisbachia* Townes, and *Zabrachypus* Cushman, whereas the *Acrodactyla* subgroup (clade IV) encompasses the genera *Acrodactyla, Megaetaira* Gauld & Dubois, and *Eruga*.

DISCUSSION

We provided, for the first time, key information on the biology and parasitoid cycle of a polysphinctine wasp belonging to the genus *Ticapimpla*. We observed that, similarly to the other 14 polysphinctine wasp genera with documented biological information (Eberhard & Gonzaga 2019), *Ticapimpla* has the ability to induce behavioral modifications in its host spider, which may be associated with increased survival probabilities for those parasitoids (Kloss et al. 2016).

We observed that individuals of the host spider *S. duodecimguttata* parasitized by *T. carinata* (Fig. 1e, g) construct modified webs with a reduced number of radii and the absence of sticky spirals, a pattern similar to that observed in various species of the Araneidae family parasitized by polysphinctine wasps (Gonzaga et al. 2017; Eberhard & Gonzaga 2019). The suppression of the retention structure may be associated with the accumulation of debris or large possible preys in the modified web, which could result in the collapse of the structure during the development of the pupa (Gonzaga et al. 2010; Kloss et al. 2016), probably decreasing the parasitoid's chances of survival.

In the modified web, we observed that the shelter remains an integral part of web, serving as a structure utilized by the parasitoid during its larval developmental stage (Fig. 1b). Notably, individuals of *S. duodecimguttata* demonstrate a behavioral pattern of sealing the shelter entrance upon the approach of any perceived threat, effectively preventing potential predators from accessing the spider. We observed that parasitized individuals of *S. duodecimguttata* almost completely sealed the entrances of their shelters before being killed by *T. carinata* larvae ($n = 2$) (Fig. 1c). This behavior may be crucial in reducing the likelihood of predation or hyperparasitoidism of pupae during their development. Kloss et al. (2016) observed that pupae of polysphinctine wasps could be preyed upon by spiders that access cocoon webs. However, Pádua et al. (2022) found that some hyperparasitoids are capable of attacking polysphinctine wasp pupae within web shelters. This suggests that the behavior of closing shelters may be more effective in mitigating the impact of predators than that of hyperparasitoids.

We observed that *T. carinata* forms a monophyletic sister group with the genus *Acrotaphus*, as previously suggested by Spasojevic et al. (2021). *Ticapimpla* and *Acrotaphus* are forming the clade III together with *Polysphincta boops* + {*Reclinervellus* + [*Polysphincta rufipes* + (*Hymenoepimecis* + *Oxyrrhexis*)]} (Fig. 2). Gauld and Dubois (2006) suggested *Polysphincta complex* + [*Ticapimpla* + (*Acrotaphus* + *Hymenoepimecis*)] in the clade denominated *Polysphincta* genus-complex (or clade F) with the genera *Polysphincta* based in the morphological phylogeny. In our study, the genera *Oxyrrhexis* and *Reclinervellus* are parts of *Polysphincta* genus-complex clade, and in Gauld and Dubois (2006), the two genera were in different clades (D and E).

The clade III was formed by parasitoids exclusively associated with spiders of family Araneidae, as the genera *Polysphincta, Reclinervellus*, and *Acrotaphus* (Fig. 2). Nevertheless, we observed support for the inclusion of the genus *Oxyrrhexis* in this clade, which was associated with Theridiidae and Titanoecidae spiders (Fritzén &

Fjelberg 2014; Gadallah & El-Hennawy 2017; Eberhard & Gonzaga 2019), indicating a lower host specificity within this clade compared to other clades (Gonzaga et al. 2024). Similarly, we observed low patterns of host specificity in clades I and II. In clade II, the genus *Eriostethus* is associated with spiders from the families Araneidae and Theridiidae (Korenko et al. 2018), while *Sinarachna* is associated with Araneidae (Nielsen 1923; Townes & Townes 1960; Eberhard & Gonzaga 2019; Takasuka 2021; Korenko et al. 2022). In clade I, *Flacopimpla* is exclusively associated with Theridiidae, whereas *Zatypota* is associated with spiders from the families Dictynidae, Theridiidae, and Araneidae (Eberhard & Gonzaga 2019; Sobczak et al. 2019; Villanueva-Bonilla et al. 2021; Korenko et al. 2022). Taken together, this suggests that the formation of these subgroups is likely not associated with the development of high host specificity.

The polysphinctine group displayed two well-supported clades with differences regarding the group of hosts attacked, and the oviposition and larval development site on the hosts (Matsumoto 2016; Takasuka et al. 2018). Takasuka et al. (2018) showed that the ancestral clade, *Schizopyga* clade Matsumoto (2016) or clade VI of this study, parasitizes ground-dwelling RTA-spiders (a group united by the retrolateral tibial apophysis on the tibia of the male pedipalp), and lay their eggs in the spider's cephalothorax. The derived clade included several genera that showed an expanded ovipositor and laid eggs in the abdomen of Araneoidea spiders (Takasuka et al. 2018). We observed that *T. carinata* showed an expanded ovipositor and laid their eggs in the abdomen of the host spider (Fig. 1h), which supports that this Neotropical genus can actually be included in the most derived clade of polysphinctine Takasuka et al. (2018). Altogether, our results have contributed to a more comprehensive understanding of the ecology and phylogeny of polysphinctine wasps, enhancing our knowledge of the diversity and evolution of these parasitoids.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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ETHICAL STANDARD

Fieldwork was conducted with the permission of the System of Authorization and Information on Biodiversity (SISBIO/ICMBio Authorization No. 83292–1, Brazil). It complied with Brazil's current legal and ethical requirements for animal welfare.

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SUPPLEMENTAL DATA

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REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215(3):403–410. doi:10.1016/S0022-2836(05)80360-2

Belshaw R, Quicke DLJ. 1997. A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). Mol Phylogenet Evol. 7(3):281–293. doi:10.1006/mpev.1996.0400

Bennett AM, Cardinal S, Gauld ID, Wahl DB. 2019. Phylogeny of the subfamilies of Ichneumonidae (Hymenoptera). J Hymen Res. 71:1–156. doi:10.3897/jhr.71.32375

Bukowski B, Ratnasingham S, Hanisch PE, Hebert PD, Perez K, DeWaard J, Tubaro PL, Lijtmaer DA, Bossart JL. 2022. DNA barcodes reveal striking arthropod diversity and unveil seasonal patterns of variation in the southern Atlantic Forest. PLoS ONE. 17(4): e0267390. doi:10.1371/journal.pone.0267390

Campbell BC, Steffen-Campbell JD, Werren JH. 1993. Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. Insect Mol Biol. 2:225–237. doi:10.1111/j.1365-2583.1994.tb00142.x

Eberhard WG. 1976. Photography of orb webs in the fields. Bull Br Arachnol Soc. 3(7):200–204.

Eberhard WG. 2000. Spider manipulation by a wasp larva. Nature. 406(6793):255–256. doi:10.1038/35018636

Eberhard WG. 2001. Under the influence: webs and building behavior of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyraphaga* (Hymenoptera, Ichneumonidae). J Arachnol. 29(3):354–366. doi:10.1636/0161-8202(2001)029[0354:UTIWAB2.0.CO;2

Eberhard WG. 2020. Spider webs: behavior, function, and evolution. Chicago (IL): The University of Chicago Press.

Eberhard WG. 2021. Zombie spiders and ecdysone: manipulation of *Allocyclosa bifurca* (Araneae: Araneidae) behavior by a parasitic wasp. J Arachnol. 49(2):250–252. doi:10.1636/JoA-S-19-046

Eberhard WG, Gonzaga MO. 2019. Evidence that *Polysphincta*-group wasps (Hymenoptera: Ichneumonidae) use ecdysteroids to manipulate the web-construction behaviour of their spider hosts. Biol J Linn Soc. 127(2):429–471. doi:10.1093/biolinnean/blz044

Ferreira SJF, Luizão FJ, Dallarosa RLG. 2005. Throughfall and rainfall interception by an upland forest submitted to selective logging in Central Amazonia. Acta Amazonica. 35 (1):55–62. doi:10.1590/S0044-59672005000100009

Fitton MG, Shaw MR, Gauld ID. 1988. Handbooks for the identification of British insects Pimpline Ichneumon-flies: Hymenoptera, Ichneumonidae (Pimplinae), 7. London (UK): Royal Entomological Society of London.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech. 3:294–299.

Fritzén NR, Fjelberg 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. doi:10.15298/arthsel.23.2.05

Gadallah NS, El-Hennawy HK. 2017. First record of the genus *Oxyrrhexis* Foerster, 1869 (Hymenoptera: Ichneumonidae: Pimplinae, Ephialtini) for the fauna of Egypt, with an unexpected new host record. Zootaxa. 4318(1):187–194. doi:10.11646/zootaxa.4318.1.11

Gaione-Costa A, Pádua DG, Delazari ÍM, Santos ARS, Kloss TG. 2022. Redescription and oviposition behavior of an orb-weaver spider parasitoid *Hymenoepimecis cameroni* Townes, 1966 (Hymenoptera: Ichneumonidae). Zootaxa. 5134(3):415–425. doi:10.11646/zootaxa.5134.3.5

Gauld ID. 1991. The Ichneumonidae of Costa Rica. Memoirs of the American entomological institute, 47. Logan (UT): American Entomological Institute.

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

Gonzaga MO, Kloss TG, Sobczak JF. 2017. Host behavioural manipulation of spiders by Ichneumonid wasps. In: Vieira C, Gonzaga M, editors. Behaviour and ecology of spiders. New York (NY): Springer; p. 417–437. doi:10.1007/978-3-319-65717-2

Gonzaga MO, Moura RR, Gaione-Costa A, Kloss TG. 2024. Patterns of host specificity in interactions involving behavioral manipulation of spiders by Darwin wasps. In: Podos J, Healy S, editors. Advances in the study of behavior. Vol. 56. United States: Academic Press; p. 89–154. doi:10.1016/bs.asb.2024.02.002

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(2):151–165. doi:10.1080/03949371003707836

Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol. 35(2):518–522. doi:10.1093/molbev/msx281

Khoramabadi AM, Talebi A, Broad GR, Zwakhals K. 2022. A study of the genus *Zabrachypus* Cushman, 1920 (Hymenoptera Ichneumonidae Pimplinae) from Iran with descriptions of two new species. J Zool. 105(1):29–36. doi:10.19263/REDIA-105.22.05

Klopfstein S, Quicke DLJ, Kropf C, Frick H. 2011. Molecular and morphological phylogeny of *Diplazontinae* (Hymenoptera, Ichneumonidae). Zool Scr. 40:379–402. doi:10.1111/j.1463-6409.2011.00481.x

Kloss TG, Gonzaga MO, Roxinol JAM, Sperber CF. 2016. Host behavioural manipulation of two orb-weaver spiders by parasitoid wasps. Anim Behav. 111:289–296. doi:10.1016/j.anbehav.2015.11.001

Kloss TG, Pádua DG, Almeida SS, Penteado-Dias AM, Mendes-Pereira T, Sobzack JF, Lacerda FG, Gonzaga MO. 2022. A new Darwin wasp (Hymenoptera: Ichneumonidae) and new records of behavioral manipulation of the host spider *Leucauge volupis* (Araneae: Tetragnathidae). Neotrop Entomol. 51:821–829. doi:10.1007/s13744-022-00991-6

Korenko S, Černecká L, Dorková M, Sýkora J, Gajdoš P. 2022. *Sinarachna nigricornis* and genus-specific host utilization of *Araneus* spiders by the genus *Sinarachna* (Hymenoptera: Ichneumonidae). J Arachnol. 50(1):51–55. doi:10.1636/JoA-S-21-012

Korenko S, Hamouzová K, Kysilková K, Kolárová M, Kloss TG, Takasuka K, Pekár S. 2018. Divergence in host utilisation by two spider ectoparasitoids within the genus *Eriostethus* (Ichneumonidae, Pimplinae). Zool Anz. 272:1–5. doi:10.1016/j.jcz.2017.11.006

Korenko S, Sýkora J, Černecká L, Gajdoš P, Purgat P, Černecký J, Holý K, Heneberg P, Agnarsoson I. 2022. Elevation gradient affects the distribution and host utilisation of *Zatypota anomala* (Hymenoptera, Ichneumonidae) associated with mesh web weaving spiders (Araneae, Dictynidae). J Hymen Res. 93:89–100. doi:10.3897/jhr.93.91513

Laurance SGW, Laurance WF, Andrade A, Fearnside PM, Harms KE, Vicentini A, Luizão RCC. 2010. Influence of soils and topography on Amazonian tree diversity: a landscape-scale study. J Veg Sci. 21(1):96–106. doi:10.1111/j.1654-1103.2009.01122.x

Laurance WF, Camargo JLC, Fearnside PM, Lovejoy TE, Williamson GE, Mesquita RCG, Meyer CFJ, Bobrowiec PED, Laurance SGW. 2017. An Amazonian rainforest and its fragments as a laboratory of global. Biol Rev. 93(1):223–247. doi:10.1111/brv.12343

Laurance WF, Lovejoy TE, Vasconcelos HL, Bruna EM, Didham RK, Stouffer PC, Gascon C, Bierregaard RO, Laurance SG, Sampaio E. 2002. Ecosystem decay of Amazonian forest fragments: a 22-year investigation. Conserv Biol. 16(3):605–618. doi:10.1046/j.1523-1739.2002.01025.x

Levi HW. 1995. Orb-weaving spiders *Actinosoma, Spilasma, Micrepeira, Pronous*, and four new genera (Araneae: Araneidae). Bull Mus Comp Zool. 154(1):153–213.

Loffredo APS, Penteado-Dias AM. 2008. First record of *Ticapimpla vilmae* Gauld, 1991 (Hymenoptera, Ichneumonidae, Pimplinae) from Brazil. Braz J Biol. 68(4):911–911. doi:10.1590/S1519-69842008000400032

Matsumoto R. 2009. “Veils” against predators: modified web structure of a host spider induced by an Ichneumonid parasitoid, *Brachyzapus nikkoensis* (Uchida) (Hymenoptera). J Insect Behav. 22(1):39–48. doi:10.1007/s10905-008-9152-1

Matsumoto R. 2016. Molecular phylogeny and systematics of the *Polysphincta* group of genera (Hymenoptera, Ichneumonidae, Pimplinae): molecular phylogeny of the *Polysphincta* group. Syst Entomol. 41(4):854–864. doi:10.1111/syen.12196

Nguyen TH, Sondhi S, Ziesel A, Paliwal S, Fiumera HL. 2020. Mitochondrial-nuclear coadaptation revealed through mtDNA replacements in *Saccharomyces cerevisiae*. BMC Evol Biol. 20(1):128. doi:10.1186/s12862-020-01685-6

Nielsen E. 1923. Contributions to the life history of the pimpline spider parasites (*Polysphincta, Zaglyptus, Tromatobia*) (Hymenoptera: Ichneumonidae). Entomol Medd. 14:137–205.

Nylander JAA. 2004. MrModeltest v2. Sweden: Evolutionary Biology Centre, Uppsala University.

Pádua DG. 2022. First record of the Darwin wasp *Hymenoepimecis* Viereck, 1912 (Hymenoptera: Ichneumonidae: Pimplinae) from Argentina and Bolivia, with description of a new species. Zootaxa. 5169(1):49–60. doi:10.11646/zootaxa.5169.1.4

Pádua DG, Kloss TG, Tavares MT, Santos BF, Araujo RO, Schoeninger K, Sobczack JF, Gonzaga MO. 2022. Hyperparasitoids of polysphinctine Darwin wasps (Hymenoptera: Ichneumonidae) in South America. Austral Entomol. 61(2):170–186. doi:10.1111/aen.12593

Pádua DG, Oliveira ML, Onody HC, Sobczak JF, Sääksjärvi IE, Gómez IC. 2015. The Brazilian Amazonian species of *Hymenoepimecis* Viereck, 1912 (Hymenoptera: Ichneumonidae: Pimplinae). Zootaxa. 4058(2):175–194. doi:10.11646/zootaxa.4058.2.2

Pádua DG, Sääksjärvi I, Monteiro R, Oliveira M. 2019. New records of *Ticapimpla* Gauld, 1991 (Hymenoptera: Ichneumonidae: Pimplinae) from Brazil and French Guiana, with taxonomic notes. Biodivers Data J. 7:e38141. doi:10.3897/BDJ.7.e38141

Palacio E, Broad GR, Sääksjärvi IE, Veijalainen A. 2010. Western Amazonian *Ticapimpla* (Hymenoptera: Ichneumonidae: Pimplinae): four new species from Colombia, Ecuador, and Peru, with a key to species of the genus. Psyche: J Entomol. 2010:161595. doi:10.1155/2010/161595

Quicke DL, Laurenne NM, Fitton MG, Broad GR. 2009. A thousand and one wasps: a 28S rDNA and morphological phylogeny of the Ichneumonidae (Insecta: Hymenoptera) with an investigation into alignment parameter space and elision. J Nat Hist. 43:1305–1421. doi:10.1080/00222930902807783

Rambaut A. 2017. FigTree: tree figure drawing tool version 1.4.3.2009. Available from: http://tree.bio.ed.ac.uk/software/figtree/ [Accessed 2023 Dec 1].

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 32: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 61:539–542. doi:10.1093/sysbio/sys029

Shaw MR. 2006. Notes on British Pimplinae and Poemeniinae (Hymenoptera: Ichneumonidae), with additions to the British list. Br J Entomol Nat Hist. 19(4):217–238.

Sobczak FJ, Arruda IDP, Pádua DG, Villanueva-Bonilla GA. 2019. Parasitism in *Theridion* sp. (Araneae: Theridiidae) by *Zatypota riverai* Gauld, 1991 (Hymenoptera: Ichneumonidae: Pimplinae). J Arachnol. 47(2):266–270. doi:10.1636/JoA-S-18-103

Spasojevic T, Broad GR, Sääksjärvi IE, Schwarz M, Ito M, Korenko S, Klopfstein S. 2021. Mind the outgroup and bare branches in total-evidence dating: a case study of pimpliform Darwin wasps (Hymenoptera, Ichneumonidae). Syst Biol. 70(2):322–339. doi:10.1093/sysbio/syaa079

Swofford DL. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sunderland (MA): Sinauer Associates. doi:10.1111/j.0014-3820.2002.tb00191.x

Takasuka K. 2021. The northernmost record of *Eriostethus rufus* (Uchida, 1932) (Hymenoptera, Ichneumonidae) with an indication of new host, *Trichonephila clavata* (Koch, 1878) (Araneae, Araneidae) and its web manipulation. Entomol Commun. 3:ec03015. doi:10.37486/2675-1305.ec03015

Takasuka K, Broad GR. 2024. A bionomic overview of spider parasitoids and pseudo-parasitoids of the ichneumonid wasp subfamily Pimplinae. Contrib Zool. 93(1):1–106. doi:10.1163/18759866-BJA10053

Takasuka K, Fritzén NR, Tanaka Y, Matsumoto R, Maeto K, Shaw MR. 2018. The changing use of the ovipositor in host shifts by ichneumonid ectoparasitoids of spiders (Hymenoptera, Ichneumonidae, Pimplinae). Parasite. 25:1–17. doi:10.1051/parasite/2018011

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 behaviour for its cocoon protection. J Exp Biol. 218(15):2326–2332. doi:10.1242/jeb.122739

Thomas F, Adamo S, Moore J. 2005. Parasitic manipulation: where are we and where should we go? Behav Processes. 68(3):185–199. doi:10.1016/j.beproc.2004.06.010

Townes H, Townes M. 1960. Ichneumon-flies of America North of Mexico pt. 2: subfamilies Ephialtinae, Xoridinae, and Acaenitinae. New York (NY): Bulletin of the United States National Museum. doi:10.5479/si.03629236.216.1-2

Villanueva-Bonilla GA, Faustino ML, dos Santos WR, Pereira LC, Pádua DG, Sobczak JF. 2021. Behavioral manipulation of a “Trashline Orb-weaving spider” *Cyclosa fililineata* (Araneidae) by the parasitoid wasp *Zatypota riverai* (Ichneumonidae: Pimplinae). J Arachnol. 49(1):146–150. doi:10.1636/JoA-S-20-043

Weinersmith KL. 2019. What’s gotten into you?: a review of recent research on parasitoid manipulation of host behavior. Curr Opin Insect Sci. 33:37–42. doi:10.1016/j.cois.2018.11.011