

SHORT COMMUNICATION

Behavioral manipulation of a “Trashline Orb-weaving spider” *Cyclosa fililineata* (Araneidae) by the parasitoid wasp *Zatypota riverai* (Ichneumonidae: Pimplinae)

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Abstract. In this study, we report a new interaction of the wasp *Zatypota riverai* Gauld, 1991 (Ichneumonidae) parasitizing the spider *Cyclosa fililineata* Hingston, 1932 (Araneidae) and we describe the modified spider web. Our results show that parasitized spiders build modified webs that are clearly different from normal capture webs. This modified web presents several additional lines at the center of the web, forming a disk-like structure that was also observed in modified webs of other host orb-weaving spiders. To our knowledge, *Z. riverai* (this study) and *Z. kauros* Gauld, 1984 are the only two species of the genus *Zatypota* that use spiders from different families as host. However, the unexpected host *C. fililineata* could be an accidental host, since in the same region there are two of the typical hosts, the theridiid spiders *Anelosimus baeza* Agnarsson, 2006 and *Theridion* sp.

Keywords: Cocoon web, *Polysphincta* genus group, koinobiont ectoparasitoid, Neotropical.

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Interactions between spiders and wasps of the *Polysphincta* genus group (*sensu* Gauld & Dubois 2006), which act as koinobiont ectoparasitoids of spiders, are a well-known phenomenon (e.g., Kloss et al. 2017; Sobczak et al. 2019a). Many of these interactions involve changes in spider web-building behavior, resulting in modified webs (cocoon webs) with architectures that increase parasitoid larval survival within the cocoon (Gonzaga et al. 2010, 2016). The most typical characteristics of modified webs include reduction in the number of spirals (Gonzaga & Sobczak 2007) or the production of additional reinforced web lines surrounding the cocoon (Eberhard 2013; Korenko et al. 2014). To the best of our knowledge, only one experimental study has effectively shown that cocoon webs reduce the risk of predation by ants on the parasitoid pupae (Matsumoto 2009). Other studies mention potential benefits of the cocoon web. Larval mortality by predation or abiotic factors may be reduced due to stronger threads (e.g., Takasuka et al. 2015), or, reinforcement in the number of threads attached to the vegetation may increase resistance to abiotic factors (Pádua & Kloss 2020). These have not been tested experimentally.

Currently, the *Polysphincta* group consists of more than 250 species described among 25 accepted genera (Matsumoto 2016; Yu et al. 2016; Pádua et al. 2020a, b). The host range of polysphinctines is remarkably narrow at the genus level and generally specific to host species; for example, the genus *Hymenoepimecis* Viereck, 1912 attacks exclusively orbicular spiders (summarized in Pádua et al. 2016). In contrast, the genus *Zatypota* Förster, 1869 are parasitoid wasps of four spider families of different web-building habits (Matsumoto & Takasuka 2010; Korenko et al. 2015; Gonzaga et al. 2016; Korenko 2017). In the last 15 years, several studies have described how spiders have been manipulated behaviorally by wasps of the *Polysphincta* group. Most involve changes in typical web-building behavior,

resulting in a web with a different structure compared to a normal one (Gonzaga et al. 2016). This is thought to maximize the probability of survival of the parasitoid wasp inside the cocoon (e.g., Gonzaga & Sobczak 2007; Matsumoto 2009; Eberhard 2010a).

The genus *Zatypota* has 52 valid species worldwide (Yu et al. 2016; Sobczak et al. 2019b). The vast majority of species act as parasitoids of Theridiidae (Matsumoto & Takasuka 2010; Korenko et al. 2011; Sobczak et al. 2019b) but records of interactions with other spider families have been reported (Vincent 1979; Matsumoto & Takasuka 2010; Takasuka et al. 2017; Korenko 2017; Korenko et al. 2015, 2018). Interactions between *Zatypota* wasps and spiders involving behavioral manipulation have been described since 2007. For example, the females of *Theridion evexum* Keyserling, 1884 parasitized by *Zatypota* sp. are manipulated to add a greater amount of web threads to the shelter, to increase the cocoon protection against heavy rain (Weng & Barrantes 2007). The theridiids *Anelosimus octavius* Agnarsson, 2006 and *A. near studiosus* parasitized by the wasp *Z. near solanoi* were also observed adding a greater number of horizontal lines in the center of the tangle and to enclose entire web with an unusual top sheet, which is not normal in non-parasitized spider webs (Eberhard 2010a). Modification in webs has also been described in host species that build orb-webs. For example, the manipulated webs of *Cyclosa conica* (Pallas, 1772) and *Mangora acalypha* (Walckenaer, 1802) (Araneidae) parasitized by *Z. picticollis* (Thomson, 1888) have no spiral or stabilimentum and the radials have been reduced in number (Korenko et al. 2015).

The genus *Cyclosa* Menge, 1866 (Araneae, Araneidae) is commonly known as “trashline orb-weaving spider” because the spiders decorate their webs with different materials such as silk, plant debris, prey scraps and even ovisacs (Neet 1990; Tan & Li 2009; Tan et al. 2010). *Cyclosa fililineata* Hingston, 1932 has a restricted distribution

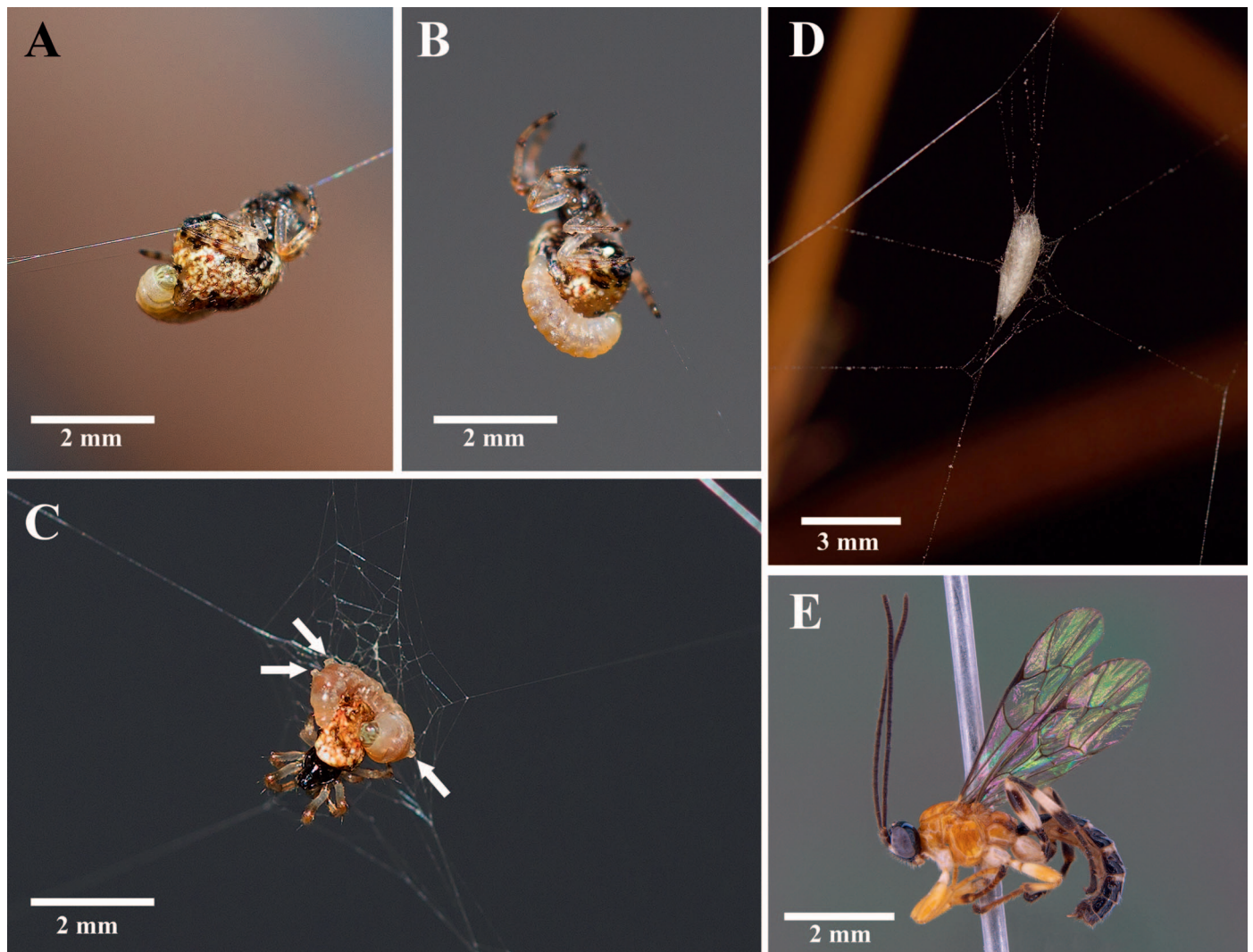


Figure 1.—Female of *Cyclosa fililineata* (Araneidae) parasitized by *Zatyptota riverai* (Ichneumonidae). A. and B. Larva instar II of the wasp adhering to the abdomen of the spider host. C. Larva instar III feeding on the host spider (white arrows indicate the retractile tubercles). D. Cocoon of *Z. riverai* adhered to the cocoon web. E. Adult male wasp of *Z. riverai*.

in the Neotropical region, being found from Panama to northern Argentina (WSC 2020). They are relatively small spiders (total length of males is 2.4 mm, females 4.7 mm) with light brown abdomen and small dark markings (Levi 1999). This species builds a web with a vertical line of debris that obscures the outline of the spider at the hub, which can reduce the intensity of predation (Gonzaga & Vasconcellos-Neto 2005, 2012). Kloss et al. (2016) recently reported behavioral manipulation of *C. fililineata* when attacked by the parasitoid wasp *Polysphincta* nr. *purcelli*. In this study we report for the first time an alteration of web architecture in the spider *C. fililineata* induced by the final instar larva of a *Zatyptota riverai* wasp. This study was conducted in the municipalities of Mulungu (04°18'40"S, 038°58'05"W; 840 m.a.s.l.) and Pacoti (4°13'21.10"S, 38°53'35.80"W; 900 m.a.s.l.), in the state of Ceará, Brazil. These two locations represent some of the few remnants of the Atlantic Forest surrounded by a semiarid region of the Caatinga biome, making them a biodiversity hotspot. The average annual temperature is 20.8 °C and the average annual rainfall is 1221 mm (Araújo et al. 2007) for both study sites.

We performed a visual search for parasitized individuals of *C. fililineata* along the forest edges of Maciço de Baturité in July 2019.

When we found a parasitized spider, we placed a red rope at a point near the web as a reference to follow the development of the parasitoid larva. In the field, to make the strands of webs more visible, we added a little cornstarch to later photograph them. Subsequently, in the laboratory we counted the number of radii and spirals in the photographs for further comparisons. The parasitoid specimens that emerged from the cocoons were fixed in 70% pure ethanol and refrigerated. Voucher specimens of the parasitoid were deposited in the Invertebrate Collection of Instituto Nacional de Pesquisas da Amazônia (INPA, M.L. Oliveira, curator). The spider was identified by Dr. Antonio Brescovit, and it was deposited in Laboratório Especial de Coleções Zoológicas in Instituto Butantan, São Paulo (IBSP, A.D. Brescovit, curator).

We collected 49 unparasitized adult and subadult *C. fililineata* and one parasitized adult female *C. fililineata* with a single second instar larva of *Z. riverai* attached to the posterior-lateral portion of its ophisthosoma (Figs. 1A, B). The head of the larva pointed toward the posterior part of the ophisthosoma (Figs. 1A, B). Three days after observing the parasitized spider, the larva reached the final (third) instar (Fig. 1C). This final instar presented eight retractile tubercles (Fig. 1C). When it reached this last instar, the larva continues to

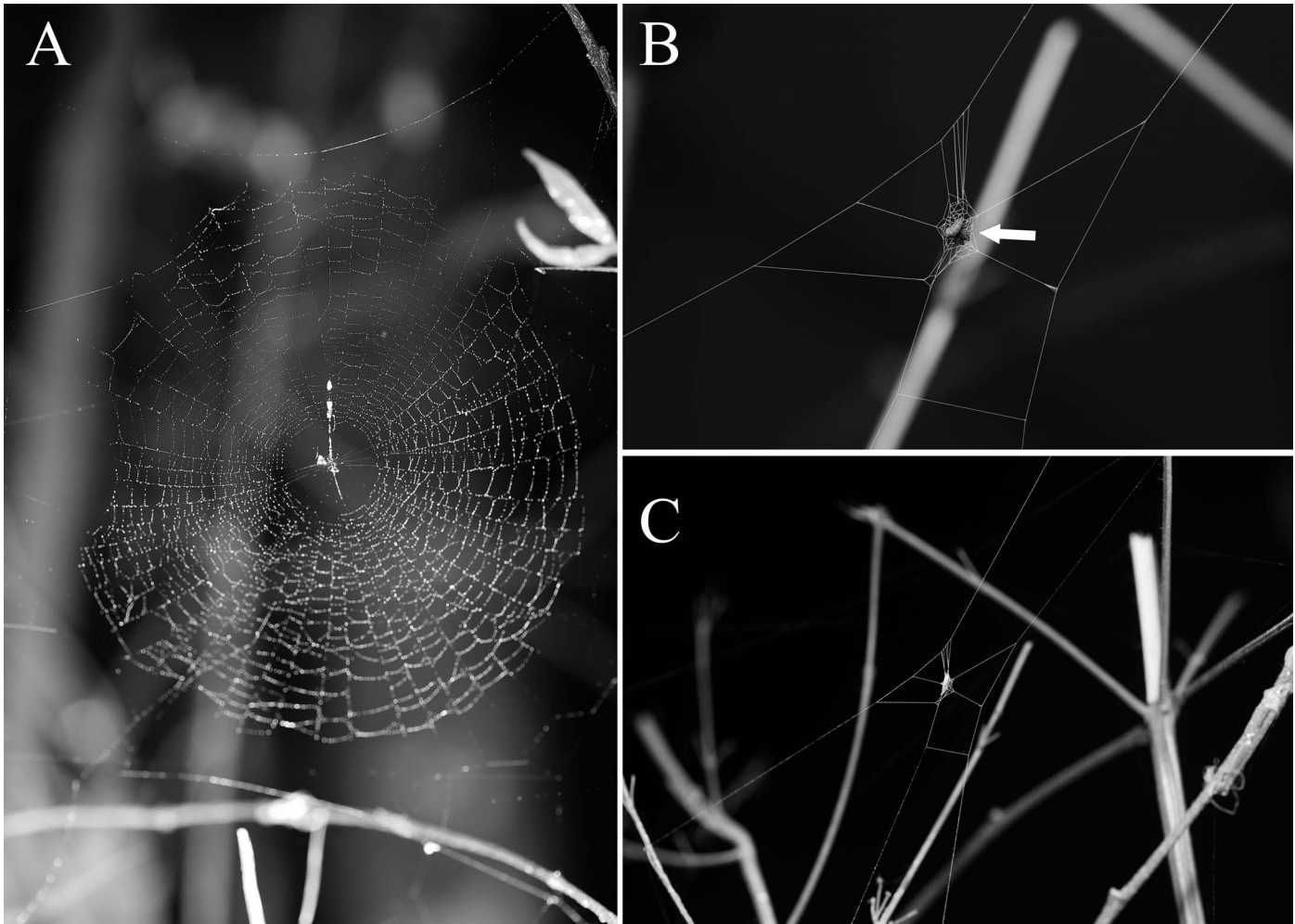


Figure 2.—*Cyclosa fililineata* webs. A. Normal web of *C. fililineata*. B. Cocoon web of *C. fililineata* parasitized by *Zatypota riverai* (white arrow showing the central hub of the web with a few lines to support the parasitoid larva). C. Cocoon web with the cocoon in the center of the web.

consume the host hemolymph until the host dies and becomes desiccated (Fig. 1C). After that, the larva discarded the spider remains and began to build the cocoon using the tubercles to attach its body to the already modified web lines (Fig. 1C). Once the larva was situated in the center of the modified web, the larva began to add several silk strands starting at the posterior portion of its body. After approximately 20 hours, the larva was fully enclosed within the cocoon (Fig. 1D). The cocoon was built upright in the center of the cocoon web and its coloration changes from light gray to dark gray over time (Fig. 1D). After eight days of pupation, an adult male wasp (Fig. 1E) emerged from the apical portion of the cocoon.

Unparasitized webs ($n = 49$) showed the typical architecture of an orbweb (Fig. 2A) and had on average 41.28 ± 5.5 SD radii and 29.36 ± 4.9 SD spirals. The orientation of the normal web was vertical and this had a line of vertical debris located in the center of the web (Fig. 2A).

In the case of the parasitized spider, the web had a much more simplified architecture considerably different from the normal prey capture web (Fig. 2B, C). This modified web was vertically oriented and contained two main support lines consisting of several superposed and radiating lines which apparently made them stronger. From these two main support lines, there are 11 additional lines that converge in the center of the modified web. Of these lines, the top five are much closer to each other than the rest of the lines (Fig. 2B). We

observed other additional lines in the center of the cocoon web forming a disk-like structure where clearly the parasitoid larva was held by the retractable tubercle hooks (Fig. 2B).

Previous studies have reported *Z. riverai* parasitizing two theridiid spiders, *Anelosimus baeza* Agnarsson, 2006 and an undetermined species of *Theridion* Walckenaer, 1805, so it was believed that this species has specificity for spiders in this family (Sobczak et al. 2017, 2019a). However, in the present study, we show for the first time the interaction and behavioral manipulation of the araneid *C. fililineata* by the parasitoid wasp *Z. riverai*. To our knowledge, *Z. riverai* (this study) and *Z. kauros* Gauld, 1984 (Korenko et al. 2018) are the only two species of the genus *Zatypota* that use spiders from more than one family as host. On the one hand, the unexpected host, *C. fililineata*, could be an accidental host, since in the same region there are two of the typical hosts, the theridiids *A. baeza* and *Theridion* sp. (Sobczak et al. 2017, 2019a). A similar event happened with *Z. picticollis* parasitizing *Mangora acalypha* (Walckenaer, 1802) (Korenko et al. 2015), the latter of which prefers undergrowth, but which can occur only accidentally on tree branches, where typical hosts of *Z. picticollis* (*C. conica* and *Zilla didia* (Walckenaer, 1802)) occur. Consequently *M. acalypha* may have been accidentally parasitized (Korenko, unpublished data). On the other hand, Korenko et al. (2018) mention that the species *Z. kauros* contains three divergent mitochondrial lineages and each of them has specificity for a group of

host spiders, which indicates maternal ancestry more or less reflecting host preference. It is possible that *Z. riverai* also has similar mitochondrial characteristics to *Z. kauros* that partly explain the different host utilization, making these two species promising for studies of specialization and speciation (Korenko et al. 2018). However, further genetic studies would be needed to confirm our hypothesis.

Our results also show that spiders parasitized by *Z. riverai* build modified webs very different from normal capture webs. In previous studies, where spider orb webs modified by parasitoid wasp were recorded, it has been suggested that the reduction of radii and spirals, as observed in the present study, decreases the chances of insect interception or debris accumulation, which would result in web rupture and damage to the cocoon (Eberhard 2010a; Korenko et al. 2015; Takasuka et al. 2015). The decrease in the number of radii and spirals of the modified orb webs was also observed in *C. fililineata* spiders parasitized by wasp *Polysphincta* nr. *purcelli* and *Cylosa morretes* Levi, 1999 parasitized by *Polysphincta janzeni* Gauld, 1991 (Kloss et al. 2016). Quite peculiarly, the cocoon web we recorded in *C. fililineata* looks much more like the pattern for *C. morretes* cocoon webs (parasitized by *P. janzeni*) than the *C. fililineata* cocoon web itself (parasitized by *P. nr. purcelli*). This is due to the fact that *C. morretes* cocoon webs also present several additional lines resulting in “V radii” at the center of the web (Kloss et al. 2016). These “V radii” have also been observed in cocoon webs of other orb-weaving spider species such as *Allocyclosa bifurca* (McCook, 1887) (Araneidae) parasitized by the wasp *Polysphincta gutfreundi* Gauld, 1991 (Eberhard 2010b), *Leucauge mariana* (Taczanowski, 1881) and *L. argyra* (Walckenaer, 1841) (Tetragnathidae) parasitized by *Hymenopimecis tedfordi* Gauld, 1991 and *H. argyraphaga* Gauld, 2000 respectively (Eberhard 2001, 2013), *C. conica* parasitized by *Z. picticollis* (Korenko et al. 2015) and *C. argenteoalba* Bösenberg & Strand, 1906 (Takasuka et al. 2019). All of these studies suggest that the “V radii” structure could serve as a physical complement to stability in modified webs and could reduce the risk of rupture by abiotic factors (Kloss et al. 2016). However, there are modified orbicular webs without “V radii” that function properly as a refuge for the parasitoid (e.g., Messas et al. 2017). This makes future studies necessary to compare the stability of cocoon webs with and without “V radii”.

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