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SHORT COMMUNICATION

Parasitism in *Theridion* sp. (Araneae: Theridiidae) by *Zatypota riverai* Gauld, 1991 (Hymenoptera: Ichneumonidae: Pimplinae)

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Abstract. The hymenopteran genus *Zatypota* Förster, 1869 (Ichneumonidae: Pimplinae, Ephialtini) comprises highly specialized koinobiont ectoparasitoids of spiders and is the largest genus of the *Polysphincta* group of genera in the world, with more than 50 described species. The vast majority of species of *Zatypota* are parasitoids of the spider family Theridiidae. In this study, we present information about a new interaction between the parasitoid spider wasp *Zatypota riverai* Gauld, 1991 and the host spider *Theridion* sp. Walckenaer, 1805 (Theridiidae) with information about host weight selection. We collected 102 non-parasitized adult and subadult females of *Theridion* sp. and six spiders with larvae of *Z. riverai* attached to host's abdomen. The pupal development takes about 8–11 days, though the development time of the pupa varies with the sex of the wasp. All larvae collected in the field completed their life cycle on the host spiders, even though all of the hosts were small, indicating that the host biomass was sufficient for larval development and no larger-sized spiders are needed. Moreover, larger *Theridion* probably pose a greater risk because they are more likely to be successful at wasp predation, even if they offer a greater resource to the larva.

Keywords: Biodiversity, Ephialtini, parasitoid wasps, *Polysphincta* group.

Spiders are among the most common and abundant predators of terrestrial ecosystems (Turnbull 1973; Coddington & Levi 1991). In this way, spiders constitute an important resource in the diet of many predators, parasites and also parasitoids (Gonzaga & Sobczak 2007). Ichneumonid wasps of the *Polysphincta* genus group (*sensu* Gauld & Dubois 2006) (hereafter polysphinctine wasps) are well known to act exclusively as koinobiont ectoparasitoids of spiders (Gauld & Dubois 2006). This monophyletic group is currently formed by 24 genera (Gauld & Dubois 2006; Palacio et al. 2007; Matsumoto 2016) with more than 200 species described (Yu et al. 2012). The host range of these wasps is remarkably narrow and often species-specific; some polysphinctine genera (e.g., *Hymenoepimecis*, *Acrotaphus*) usually attack orb-weaver spiders (Sobczak et al. 2014; Pádua et al. 2016), whereas others are specialized on spiders that construct three-dimensional webs (e.g., *Zatypota*) (e.g., Korenko & Pekár 2011; Fritzén 2014).

The genus *Zatypota* Förster, 1869 comprises highly specialized koinobiont ectoparasitoids of spiders and is the largest genus of the polysphinctine wasps in the world, with more than 50 described species (Yu et al. 2012; Korenko 2017). The vast majority of species of *Zatypota* are parasitoids of spiders in the family Theridiidae (Korenko & Pekár 2011; Korenko et al. 2011). There are only three *Zatypota* species that attack spiders of other families: (1) *Zatypota sulcata* Matsumoto, 2010 associated with the sheet-web spider *Turinyphia yunohamensis* (Bösenberg & Strand, 1906) (Linyphiidae) in Japan (Matsumoto & Takasuka 2010); (2) *Zatypota picticollis* (Thomson, 1888) associated with the orb-web spiders *Mangora acalypha* (Walckenaer, 1802), *Cyclosa conica* (Pallas, 1772), and *Zilla diodia* (Walckenaer, 1802) (Araneidae) in Europe (Korenko et al. 2015); and (3) *Zatypota anomala* (Holmgren, 1860) associated with cribellate tangle-web spiders of the genus *Dictyna* Sundevall, 1833 (Korenko 2017) and *Mallos pallidus* (Banks, 1904) (Vincent 1979) (Dictynidae) in the Holarctic. Recently, Sobczak et al. (2017a) described for the first time the male of *Z. riverai* Gauld, 1991, in addition to providing new information about this wasp parasitizing the spider *Anelosimus baeza* Agnarsson, 2006 (Theridiidae) in Brazil.

The genus *Theridion* Walckenaer, 1805 (Theridiidae) is a cosmopolitan group of spiders that currently consists of 586 described species (World Spider Catalog 2018). *Theridion* sp. is a small-sized spider, with an orange brown cephalothorax, dark brown bands on the legs and a lighter brown abdomen. *Theridion* sp. individuals do not construct webs, but build a shelter made with a leaf and silk. The leaf is wrapped by silk threads and remains suspended, close to the ground, by silk threads in the vegetation. Inside this refuge, the spider deposits up to four egg sacs and provides maternal care. Currently there are some records of species of *Theridion* being parasitized by *Zatypota* wasps in the temperate (Fitton et al. 1987; Gauld & Dubois 2006) and Neotropical region (Weng & Barrantes 2007; Barrantes et al. 2008). In this study, we present new information about the biology of a new interaction between the parasitoid spider wasp Z*atypota riverai* and the host spider *Theridion* sp. (Theridiidae) with information about host weight selection.

This study was conducted in Mulungu, Ceará State, Brazil (4°18′40″S, 38°58′05″W, altitude of 840 m) (Fig. 1A), a municipally in the APA (Área de Proteção Ambiental) of the Maciço de Baturité. This area, considered a biodiversity hotspot, is peculiar because it forms an island of mountainous, semideciduous tropical forest surrounded by a semi-arid region of Caatinga Biome. The mean annual temperature is 20.8° C, the mean annual rainfall is 1221 mm (Araújo et al. 2007) and the humidity is 50%, showing diel variation (Sobczak et al. in prep.). This area is an important locale for studies involving the interaction between polysphinctine wasp groups of the Brazilian Northeast.

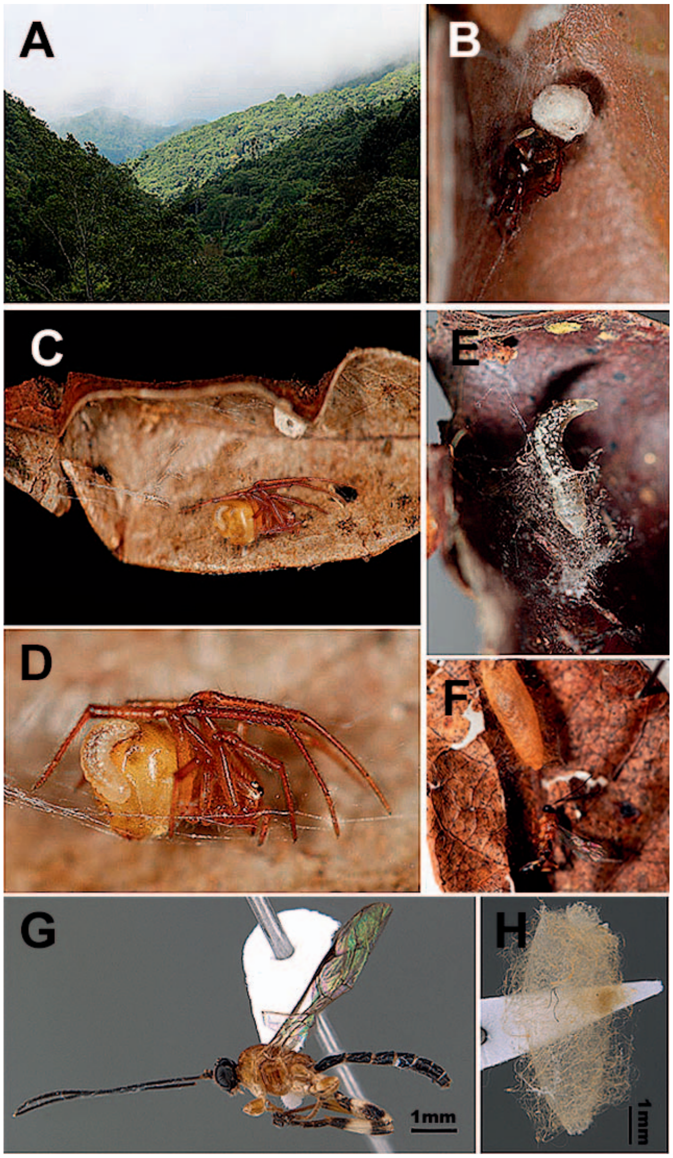

Figure 1.—Studied area and adult female of *Theridion* sp. (Theridiidae) parasitized by *Zatypota riverai* Gauld, 1991 (Ichneumonidae: Pimplinae). (A) Semideciduous tropical forest in Mulungu, Ceará State, Brazil; (B) Larval instar I of the wasp adhering to the abdomen of a spider accompanying an egg sac; (C) Larval instar II; (D) Larval instar III; (E) Larval instar IV; (F) Adult attacking the host; (G) Adult of *Z. riverai*; (H) Empty cocoon.

Adult females of *Theridion* sp. with larvae of *Z. riverai* attached to the abdomen, as well as unparasitized adults and subadults, were located on trails inside the forest in October of 2017 in "sítio Gameleiras" in the municipality of Mulungu. The parasitized spiders collected were kept in plastic containers (8 × 10 × 10 cm) that were sealed with a thin screen. The spiders were fed daily with *Drosophila* sp. flies and a nutrient solution (Zanata & Vasconcellos-Neto, unpublished data). In the laboratory, all parasitized and non-parasitized spiders were weighed on an analytical balance before they were fed (Gonzaga & Sobczak 2011). To determine the weight of the parasitized spiders, the weight of a first or second instar *Z. riverai* larva (previously determined; unpublished data) was subtracted from the weight of the parasitized spider+larva. Spiders were then classified into three types based on the recorded weight (small, medium, large). To evaluate if host weight selection exists, we performed a Chi-square test to compare the total availability of spiders in each weight class with the total parasitized spiders. For this test, the *P*-value was calculated using the Monte Carlo simulation (Hope 1968) with 5000 replicates. Later, to evaluate if differences exist in the development time between females and males of the parasitoid wasp, we used a T test. We used the free software R (R Core Team 2016) with the package "rmngb" and an alpha value of 0.05 for the analyses.

Digital images of adult wasp and cocoon were taken using a DMC4500 digital camera attached to a Leica M205A stereomicroscope and combined by using the software Leica Application Suite V4.10.0. In the field, the digital images were taken using a Canon EOS 6D digital camera with a 105 mm macro lens.

The parasitoid specimens that emerged from the cocoons were fixed in alcohol 70% and refrigerated. Voucher specimens of the parasitoid were deposited in the Invertebrate Collection of Instituto Nacional de Pesquisas da Amazônia (INPA, curator M. L. Oliveira). 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 102 unparasitized adult and subadult *Theridion* sp. and six parasitized adult females of *Theridion* sp. with first instar (Fig. 1B) or second instar (Fig. 1C) larvae of *Z. riverai* attached to the lateral portion of their abdomens.

No parasitized spider had more than a single larva of *Z. riverai* (Fig. 1D). Larvae were located from the posterior to the anterolateral dorsal section of the abdomen. The last larval instar (Fig. 1E) has eight dorsal retractile tubercles, each with several hooks on its surface. The larva is capable of retracting each tubercle inside its body independently. When it reaches this last instar, the larva continues consuming the hemolymph of the host and increases near three times its size. Host death occurs within the leaf that was previously used as a shelter.

After killing the host, the larva discarded the spider carcass and started to construct the cocoon using the tubercle hooks to affix its body to the silk wall, attached to the inner surface of the sheet that the spider used as shelter. The larva performed repetitive back and forth movements with the head adding silk strands between the leaf walls and the center of the leaf. The total time of cocoon construction lasted about 12 hours. The cocoon remained upright and attached to the shelter leaf. Soon after the construction, the cocoon acquired a light-yellow color (Fig. 1F), turning dark orange after two days.

The pupal stage lasted 8–11 days. However, we observed that the development time of the pupa varies with the sex of the wasp. Most of the females ($n = 3$) emerged after 11 days, while males ($n = 2$) emerged in eight days (T= 6.862, df = 2.8698, $P = 0.007295$). Adult wasps emerged (Fig. 1G) by cutting the silk threads of the apical portion of the cocoon (Fig. 1H). The adult hymenopteran stayed in the cocoon web for approximately one hour ($n = 2$), stretching its wings before flying. All the wasps emerged from the cocoon under temperatures higher than 25° C.

The weights of the 108 spiders recorded were from 0.0007 to 0.0351 grams. Based on the frequency of distribution of these weights (Fig. 2A), spiders between 0.0007 and 0.0133 grams would correspond to small spiders; spiders between 0.014 and 0.0266 grams are medium; and spiders weighing more than 0.0267 grams would be large spiders. The six parasitized spiders had weights between 0.0055 and 0.0122 grams, placing them in the "small" class. There were no significant differences between the observed and expected value of parasitized spiders in the three weight classes (X^2: 0.582; $P = 0.7473$; in 5000 simulations; Fig. 2B).

Unfortunately, in the *Theridion* – *Z. riverai* interaction, we did not observe differences between modified webs and normal webs in the

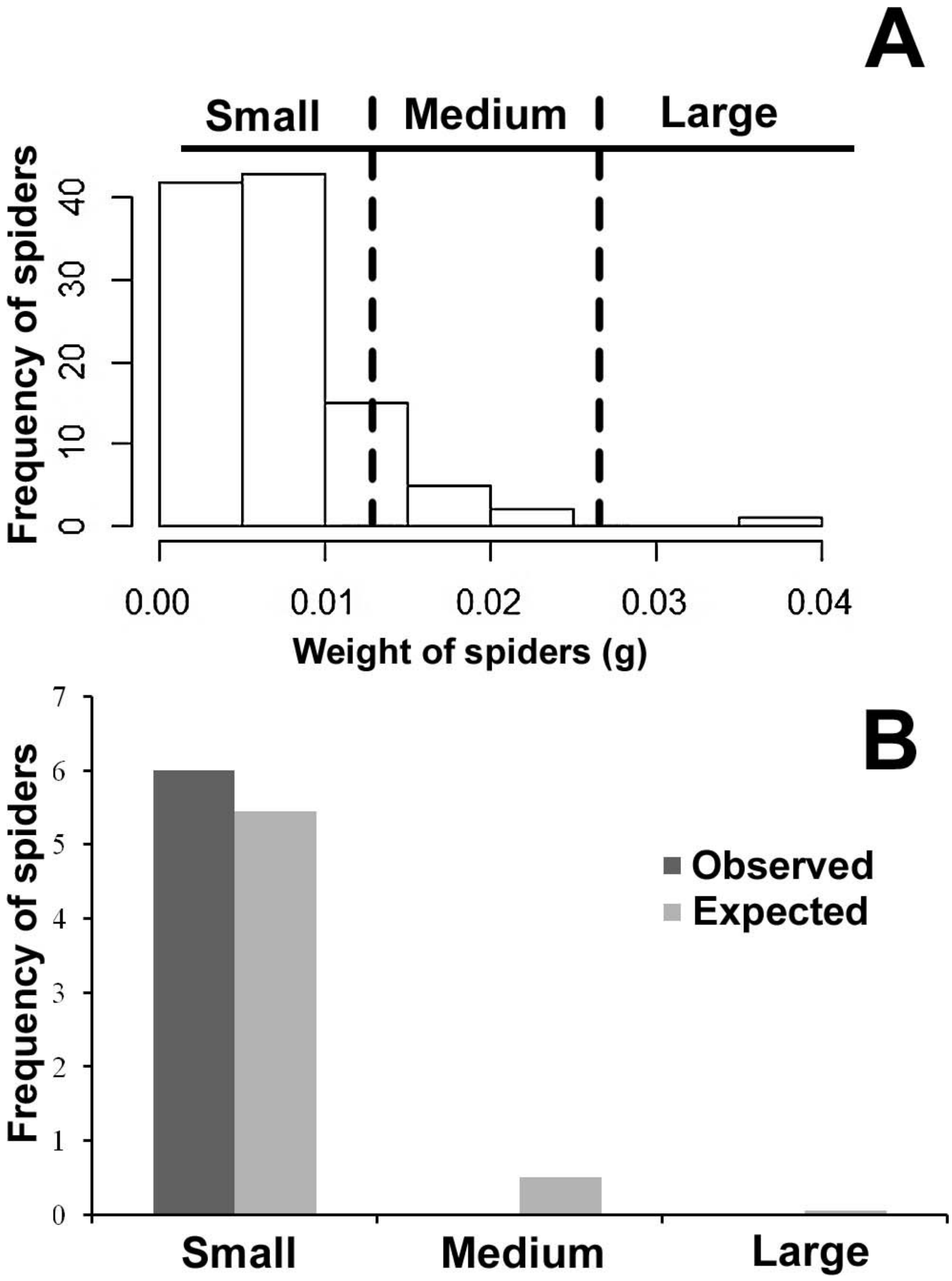

Figure 2.—Individuals of *Theridion* sp. (Theridiidae) collected in field. (A) Frequency and weight range of *Theridion* sp. individuals; (B) Observed and expected frequencies of *Theridion* sp. parasitized by *Zatypota riverai* Gauld (Ichneumonidae: Pimplinae).

field. In fact, in the interaction *Anelosimus baeza*–*Z. riverai*, the general structure of the modified web was apparently similar to the normal web, but with greater amount of silk strands in the central part of the modified web (Sobczak et al. 2017a).

The placement of the larva of *Z. riverai* is quite similar to that of larvae of other *Zatypota* species, such as *Z. petronae* Gauld, 1991 on *Theridion evexum* Keyserling, 1884 and *Z. riverai* on *A. baeza* (Weng & Barrantes 2007; Sobczak et al. 2017a). The head of the larva points toward the posterior part of the spider's abdomen in all of these species, as well as *Flacopimpla varelae* Gauld, 1991 (Ichneumonidae: Pimplinae) parasitizing *Achaearanea tingo* Levi, 1963 (Theridiidae) (Sobczak et al. 2017b). As mentioned by other authors, the significance of the larva's placement on the host spider is still unclear, but it may be useful in identifying the parasitoid taxon (Sobczak et al. 2017a).

Biological aspects of parasitic wasp larvae such as larval development time or the time required to construct the pupae, unfortunately, are not always recorded due to larval or spider mortality. In our study, we were able to record the pupal development time (8–11 days) which was not different from that recorded for *Z.*

anomala (nine days on average) (Korenko 2017) and for *Z. percontatoria* (Müller, 1776) (9.6 days on average) under laboratory conditions (Korenko et al. 2016). In other individuals of *Z. riverai* parasitizing *A. baeza* (Sobczak et al. 2017a), the development time was nine days. On the other hand, in our study the pupal construction time was shorter (12 hours) when compared to *Z. petronae* (18 hours) (Weng & Barrantes 2007).

Although we did not find *Z. riverai* preferences for a specific spider size, all parasitized spiders were relatively small. Similar results were recorded by Gonzaga & Sobczak (2011) when the parasitoid wasp *Acrotaphus chedelae* Gauld, 1991 attacks *Argiope argentata* (Fabricius, 1775) (Araneidae). The same authors also reported that *Hymenoepimecis* Viereck, 1912 wasps usually choose small host spiders because larger spiders probably represent a greater risk as predators of wasps even if the spiders offer greater resource to the larva (Gonzaga & Sobczak 2007; Gonzaga et al. 2010). In our study, all larvae completed their development, indicating that host spider biomass was sufficient. Additional observations are still necessary to determine if there is a specific pattern in host size selection.

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