

## Natural History and Larval Behavior of the parasitoid *Zatypota petronae* (Hymenoptera: Ichneumonidae)

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*Abstract.*—The koinobiont ectoparasitoid *Zatypota petronae* Gauld (Ichneumonidae) parasitizes medium-sized immatures of the cobweb spider *Theridion evexum* Keyserling (Theridiidae). *Zatypota petronae* apparently attacks the spider inside its retreat. An egg is glued on the antero-lateral dorsal section of the spider's abdomen. First-instar larvae remain partially inside the egg chorion which is attached to the spider's abdomen. In later instars, a layer of a brownish material (saddle), to which the 7<sup>th</sup> and 8<sup>th</sup> abdominal segments of the larva adhere ventrally, anchors the larva to the spider. In the last instar the saddle includes the egg chorion and the shed exoskeletons of previous instars. A row of retractile, dorsal protuberances, crowned with hooklets, is present on abdominal segments 1 to 8 of the final-instar larva. The larva uses the hooklets to grab silk lines of the retreat of the spider's web. Hanging on the spider's web the larva kills the spider and sucks out its body tissues. Then the larva pushes vigorously laterally with its head against the spider's corpse, and alternately presses the corpse against the saddle. These movements, in combination with peristaltic movements, free the larva from the saddle that falls to the ground with the dead spider. The larva then constructs its pupal cocoon. Prior to cocoon construction, the larva induces the spider to reinforce the retreat by adding more threads. Parasitism rate and host behavior are also described.

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The polysphinctine pimelines are koinobiont ectoparasitoids of spiders in several families (Nielsen 1923, 1932, Fincke et al. 1990, Hanson and Gauld 1995, Gauld et al. 1998). Nielsen (1923, 1932) described in detail the behavior of the larvae and hosts of several European polysphinctine species. The parasitism rates and life cycle of *Hymenoepimecis robertsae* Gauld on the neotropical tetragnathid *Nephila clavipes* (L.) was described by Fincke et al. (1990). However, larval behavior of neotropical polysphinctine wasps has been described in detail for only one species, *H. argyraphaga* Gauld on the tetragnathid *Plesiometa argyra* (Walker) (Eberhard 2000a, 2000b, 2001).

Parasitoid wasps of the cosmopolitan speciose polysphinctine genus *Zatypota* Förster parasitize spiders in at least five families (Dictynidae, Agelenidae, Tetragnathidae, Araneidae and Theridiidae) (Shaw 1994, Gauld et al. 1998). In the neotropics the only two host records were *Theridion* species: *T. contreras* Levi for an unidentified *Zatypota* species (Jiménez 1987) and *T. evexum* Keyserling for *Z. petronae* Gauld (Barrantes and Weng in press).

The larval behavior of *Zatypota* sp. (Jiménez 1987) differs in some aspects from that of European polysphinctine species (Nielsen 1923, 1932) and *H. argyraphaga* (Eberhard 2000a). The larva of *Zatypota* sp. was said to hold on to the spider by biting the dorsum or sides of the anterior section of the spider's abdomen. This description is likely wrong as detailed descriptions of the behavior of the larva of *Z. albicoxa*

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(Nielsen 1923) and the larva of *H. argyraphaga* (Eberhard 2000a) show that some posterior segments of the larvae lodge ventrally in a "saddle", probably coagulated spider's hemolymph, that adheres tightly to the spider's abdomen (Nielsen 1923, Eberhard 2000a). There is no further information on the biology of larvae of this *Zatypota* species. Here we describe the intensity of parasitism and behavior of the larva of *Z. petronae* and its host *T. evexum*. We describe for the first time how a polysphinctine larva frees itself from the spider's corpse.

#### MATERIALS AND METHODS

Field observations were made from October 2005 to October 2006 in a 250 m<sup>2</sup> plot in the understory of a middle-elevation wet forest patch (9° 54'N, 84° 03'W; elevation 1200 m), the Reserva Biológica Leonel Oviedo on the Universidad de Costa Rica campus, San José Province, Costa Rica. All spiders (or nearly so) from third-fourth instar outside the egg sac to adults were checked for parasites every two weeks; most spiderlings disperse from the mother's web at fourth instar. The small eggs and early instar larvae probably went undetected.

*Theridion evexum* constructs most webs between 0.20 to 1.5 m above the ground (Barrantes and Weng in press), making it possible to find practically all webs. Additionally, we collected seven parasitized spiders and kept them on their webs indoors to observe the behavior of larvae and spiders. In two cases we transplanted the plant on which the parasitized spider had constructed its web indoors, allowing us to observe the larva and host behavior with little disturbance. The complete larval development was not observed in all cases, so sample sizes are not always the same. Behavior and morphological features of more than 10 larvae were observed under a dissecting microscope. Video recordings of behavior were made using a Sony DCR-VX 1000 camcorder with +5 close-up

lenses. Drawings of larval behavior were traced from video recordings. Voucher specimens of wasps and spiders were deposited in the Museo de Zoología of the Universidad de Costa Rica. Wasp species names follow Gauld et al. (1998).

#### RESULTS

*Percentage of parasitism.*—Only juveniles of *T. evexum* were found to be parasitized by *Z. petronae*. A second instar larva was feeding on a juvenile spider, possibly a third instar. However, final instar larvae were found feeding on large immature spiders, possibly juveniles of fourth to fifth instars. The parasitism in *T. evexum* was very low (mean percentage of parasitism/census = 1.39%, SD = 1.80, n = 53 bi-weekly censuses). The reproduction in *T. evexum* is extremely seasonal, and the abundance of immature spiders (4<sup>th</sup> instar or larger) susceptible to attack by *Z. petronae* increased in March and declined drastically through August (Fig. 1). Between September and February the population consists, first, of mature females, and then of very small spiderlings (Fig. 1). Parasitized spiders occurred primarily from March through August.

*Spider web and wasp attack.*—The web of *T. evexum* includes a folded leaf that forms a conical retreat, with a tangle in front of the retreat opening, and long viscid threads extending from the tangle to other leaves (Barrantes and Weng in press). An additional tangle is constructed by the spider inside on the upper side of the retreat.

We witnessed one attack by a female *Z. petronae* wasp. The wasp approached the web and hovered in front of the spider's retreat opening. The wasp then flew inside the retreat. A few seconds later, the spider, with the wasp perched on its dorsum, dropped about 10 cm below the retreat, and hung on its dragline. They struggled for a few seconds and then the wasp flew out of sight. The spider began to climb towards the retreat but after advancing

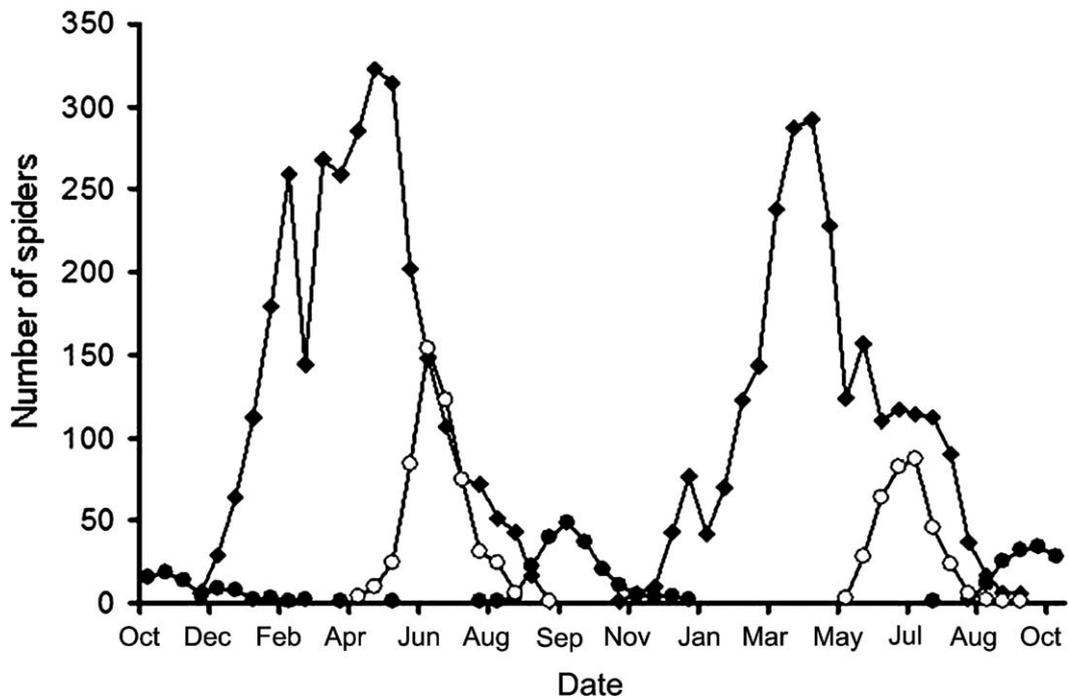


Fig. 1. Temporal changes in the number of immature spiders (black rhombus), males (open circles), and reproductive females (black circles) of *T. evexum*.

about four centimeters, it became paralyzed and fell back, motionless for about 10 min, dangling from its dragline. The spider recovered its motion slowly, and with clumsy movements cleaned some of its legs before ascending to the retreat. When we returned, 30 min later, the spider had fully recovered its mobility. We did not ascertain whether the spider had an egg on its abdomen.

*Larvae.*—We observed one egg of *Z. petronae* glued on the antero-lateral dorsal section of the abdomen of a spider collected in the field, a first instar larva emerged about four hours later. All eleven larvae of different instars checked under the dissecting microscope were attached by their rear end to the cuticle of the antero-lateral surface of the spider's abdomen (Fig. 2A).

The first instar larva ( $n = 3$ ) had its posterior end lodged inside the egg chorion, with its head, thorax, and some abdominal segments protruding; the chorion remained attached to the spider. In the

“second” instar, larvae ( $n = 5$ ) were completely outside the collapsed, flattened egg chorion that was embedded in an apparently rigid, semitransparent layer of brownish material (Fig. 2B) (the “saddle” of Nielsen 1923). The ventral surface of two or three posterior abdominal segments rested on the saddle. In subsequent instars, the cuticles of the previous molts became incorporated into the saddle as they adhered to its upper surface, against the ventral surface of the larva. The egg chorion was near the spider's surface, but not in contact with it. The saddle was attached by a short pedicel to the spider's abdomen (Fig. 2B), and the larva's abdominal segments 7 and 8 secured it to the saddle. Feeding scars were observed on the nearby dorsal and lateral surface of the spider's abdomen (Fig. 2B).

In the final instar, larvae had dorsal, two-lobed, retractable tubercles on eight abdominal segments (1<sup>st</sup> to 8<sup>th</sup>); these structures were absent in previous stages.

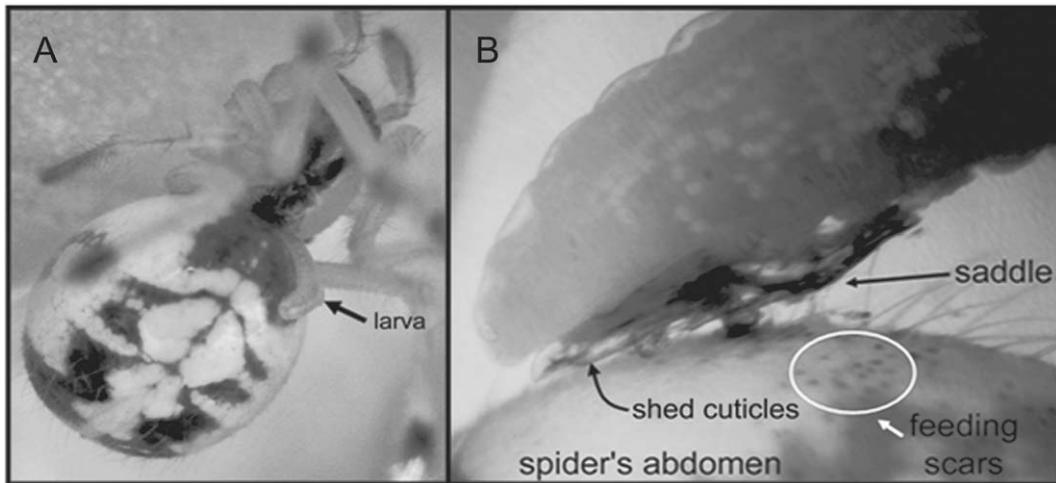


Fig. 2. Larva of *Z. petronae*: A- Second instar larva attached to the antero-lateral surface of the spider's abdomen. B- Penultimate larva with the saddle attached to the spider's abdomen. The shed cuticles of previous molts are visible under the larva. Feeding scars (black dots) are also visible on the surface of the spider's abdomen. (Photo of a specimen in alcohol).

When extended, the tubercles were crowned with a circle of tiny hooks that allowed the larva to grab the threads of the spider's web inside the retreat. The larva could extend or retract independently each lobe of the tubercle, and the tubercles could be retracted rapidly and completely into a pocket. Based on size and morphology, we discerned three instars in the larvae of this wasp. However, the saddle of what we thought was a second instar larva included the chorion and the shed cuticles of two molts. Hence, further observations are needed to confirm the number of instars.

The final instar larva spent about 18 h attached to the spider ( $n = 2$ ), three to six hours after removing the saddle and prior to cocoon construction ( $n = 5$ ), and nearly 18 h constructing the cocoon ( $n = 1$ ). The duration of the larva inside the cocoon before pupation was not recorded. One penultimate instar larva molted during the night and the next morning hung from lines near the roof of the retreat with its dorsal hooks, and fed on the spider for about eight hours. During approximately the first four hours the spider's legs moved slightly, but later we could not detect any

movement. The larva fed first on the spider's abdomen, then on its cephalothorax. When discarded, the spider's carcass was nearly completely empty; even its legs were almost transparent. The larva was thus capable of extracting nearly completely the spider's internal tissues, presumably using capillarity (Eberhard et al. 2006).

*Dislodging the saddle.*—After the larva had finished feeding, it began to free itself from the saddle while hanging inside the spider's retreat. The process, which lasted about two hours, included three types of movement: pressing the spider carcass against the saddle, pushing the spider carcass laterally, and peristaltic movements of the larva's abdominal segments. The pressing and peristaltic movements seemed to be more frequent and intense just before the spider carcass and saddle were completely removed.

**Pressing movements:** The ventral side of the larva's head pushed on the spider's anterior end, steadily pressing the spider's carcass against the saddle until it bent almost completely over the saddle (Fig. 3A, B). The larva then released the pressure completely as it moved its head to

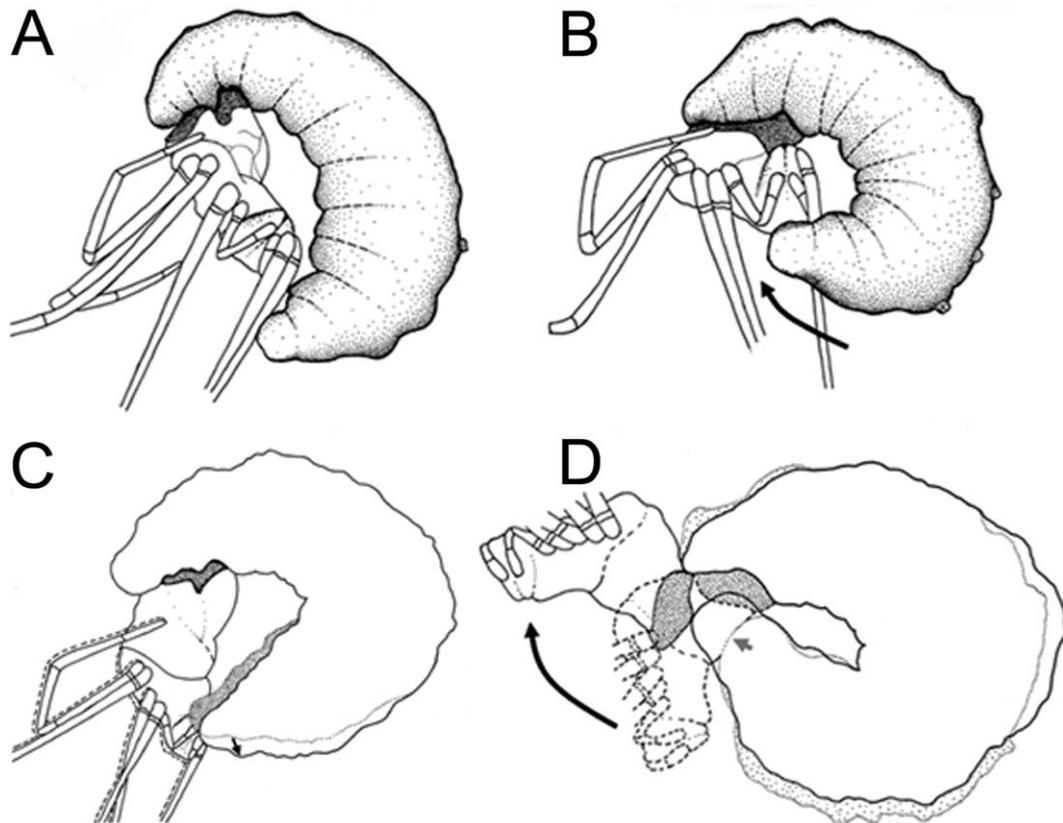


Fig. 3. Movements of the spider to free itself from the saddle (traced from video images). Pressing movement: the larva places its head near the spider's chelicerae (A) and presses the spider carcass against the saddle (B). Lateral pushing: the larva places its head on the anterior tip of the spider carcass and pushes it laterally (C). A backward final push completely dislodges the saddle from the larva (D); the grey arrow shows the position of the larva's head tip before pushing the saddle. Dotted and dashed lines represent the initial positions of the larva and the spider's carcass respectively.

the initial position, and then either made another pressing movement or pushed the spider's carcass laterally (see below).

**Lateral pushing:** The larva bent ventrally until the lateral section of its head contacted the legs and/or cephalothorax of the dead spider, and then pushed laterally (Fig. 3C). Then it moved its head back slightly, maintaining contact with the carcass, and pushed laterally again. The larva pushed repeatedly up to 10 times before reorienting its head; the complete carcass moved visibly with each push by the larva. The larva often placed its head on the opposite side of the spider during successive pushing bouts. During the last three pushing bouts the larva's head was

oriented at about  $30^\circ$  to its longitudinal axis and contacted the saddle, rather than the spider and the force exerted by the pushing movement was toward the rear of the larva's body rather than laterally (Fig. 3D).

**Peristaltic movements:** Peristaltic waves moved posteriorly along the larva's abdominal segments during pressing and pushing movements. The last segment stretched extensively backward as the wave reached it. The peristaltic waves were strongest during the last pressing and lateral movements of the larva.

**Final events:** As soon as the saddle was released the larva rubbed its head against the ventral surface of the segments that

had been connected to the saddle, which were covered with a mucilaginous substance. The small processes on the ventral larval segments that are inserted in the saddle in other polysphinctines (the "taps" of Nielsen 1923, Eberhard 2000a) were not visible in the *Z. petronae* larva at the moment the saddle was released. However, examination of two saddles under the dissecting and compound microscopes showed a wedge-like depression inside the saddle. This depression was likely produced by an abdominal projection that anchored the larva to the saddle.

*Cocoon construction.*—One larva of *Z. petronae* began cocoon construction at about 18:30 h inside the spider's retreat, after resting for nearly two hours. We did not follow cocoon construction in detail, but our incomplete observations indicate that the behavior was quite similar to cocoon construction by *H. argyraphaga* (Eberhard 2000a), except that no suspension line was built. Construction lasted nearly 18 h (N=1). It began with the larva hooked by its dorsal tubercles to the silk threads of the tangle inside the retreat (Fig. 4A).

The larva built the cocoon by attaching a silk line (or lines) produced from its head to the tangle of threads made by the spider, and pulling its head from this point to the next attaching point, which was either another tangle thread or one of its own previously produced lines. Cocoon construction began around the posterior portion of the larva (Fig. 4B) and then gradually extended upward until it enclosed the larva. The first silk lines around the larva formed a loose, fluffy mass (Fig. 4C), but after some hours a much denser wall began to form around the larva (Fig. 4D). The larva frequently paused during the construction for up to 2 min. After 20 h the larva ejected its meconium through the circular hole at the bottom of the cocoon. The recently constructed cocoon had a pale-yellow color that turned to orange-yellow over the next day.

All 57 cocoons found were constructed inside the spiders' retreats, but their attachment varied among retreats: 71% were attached to the threads of the tangle near the retreat's roof, 20% were attached to the threads applied by the spider at the apex of the leaf-cone (Fig. 5A), and 9% were in the middle of the retreat, attached to a thick silk cable formed by several independent threads (Fig. 5B).

*Enemies of the wasp.*—Of the 57 cocoons found, we observed two predation attacks and a possible parasitoid attack on a third cocoon. One pupa was attacked by *Solenopsis* ants inside the spider's retreat. A second pupa or larva inside its cocoon was attacked by a penultimate male of *T. evexum* that fed on the immature wasp through the cocoon silk. The third cocoon had a lateral hole near its bottom that suggested the exit of a parasitoid, as adults of *Z. petronae* exit the cocoon by cutting a circular slit near the cocoon's upper end.

*Host spider behaviour.*—The spiders carrying first and possibly young second instar larvae (N=4) were capable of capturing prey trapped on the long viscid lines of their webs. Their attack behavior was indistinguishable from the attacks of non-parasitized spiders (Barrantes and Eberhard in prep.). However, spiders with a large penultimate instar or a final instar larva did not attack prey that adhered to the sticky threads. The stickiness of the last capture threads produced by a spider with a large penultimate instar larva was notably reduced, as *Drosophila* flies (with their wings cut) walked easily along these threads.

On four occasions we observed that when a larva apparently bit the cuticle of a spider's abdomen, the spider jerked and tried unsuccessfully to reach the larva with its legs I, II and III. This suggests that the spider perceived and was irritated by the wounds produced by the larva. In one case the spider's leg II touched the anterior portion of the larva, and the larva immediately moved its anterior portion toward

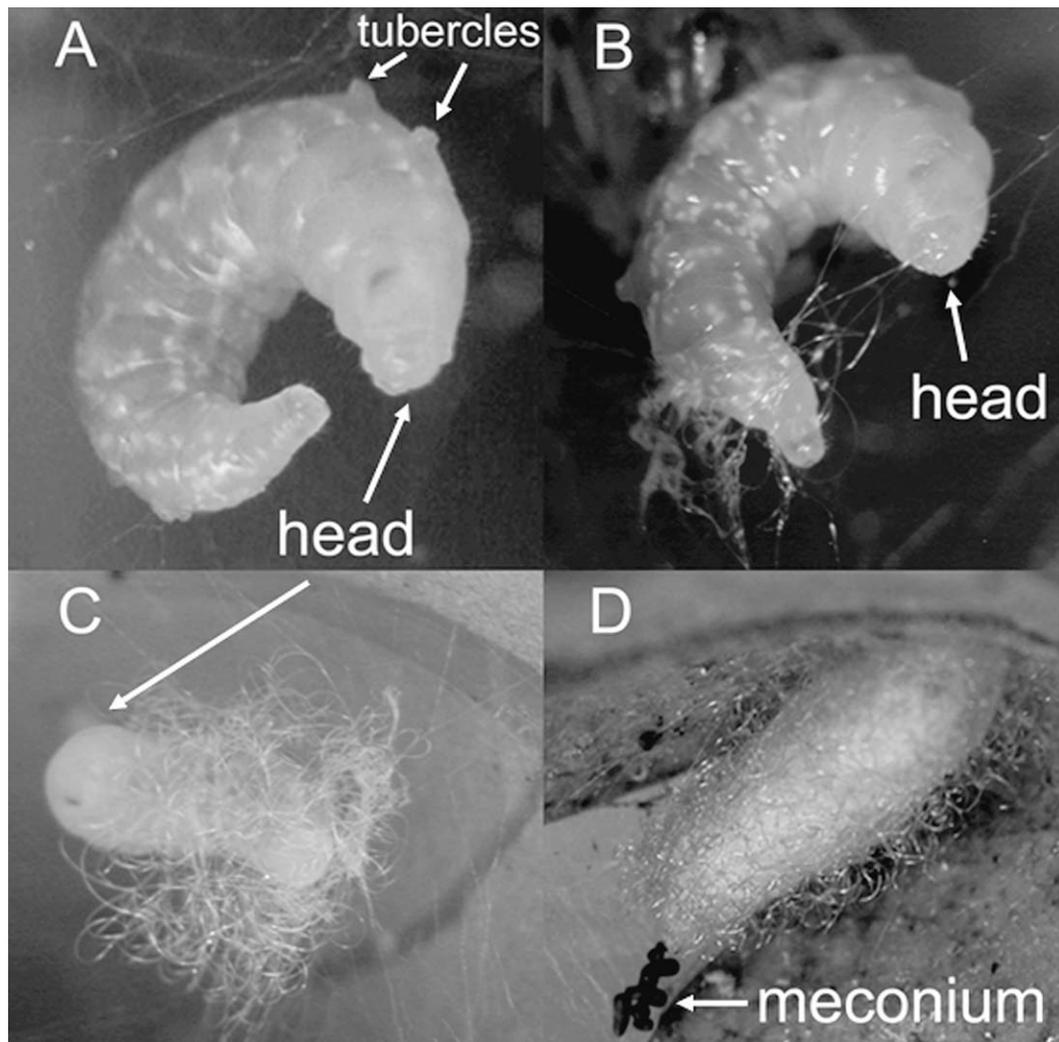


Fig. 4. Sequence of cocoon construction. A) Final instar larva recently freed from the saddle, dorsal tubercles are visible on two abdominal segments. B) Larva about 45 min after cocoon construction began. C) Cocoon construction after approximately 2 h. D) Cocoon after 20 h; note the meconium below the cocoon.

the dorsal-middle section of the spider's abdomen (out of range of the leg) and apparently bit her again. Examination with a hand lens showed that there was a tiny shiny spot, presumably of hemolymph, where the larva had apparently first bitten the spider (documenting that the larva actually bit the host rather than just touched it with its mouthparts is not easy).

The web retreats housing cocoons had additional, non-sticky thick threads either

across the retreat opening (72%,  $n = 57$ ; Fig. 5B), inside, more or less in the middle of the retreat (20%) (Fig. 5B), or both (8%) across the retreat opening and inside it (Fig. 5B). In one case the threads inside the retreat were so dense that they formed a sketchy sheet just below a cocoon (Fig. 5C), which was attached to the tangle threads. A parasitized spider added more threads to the apex of the retreat (Fig. 5A), possibly during the last two nights, before being killed by the larva.

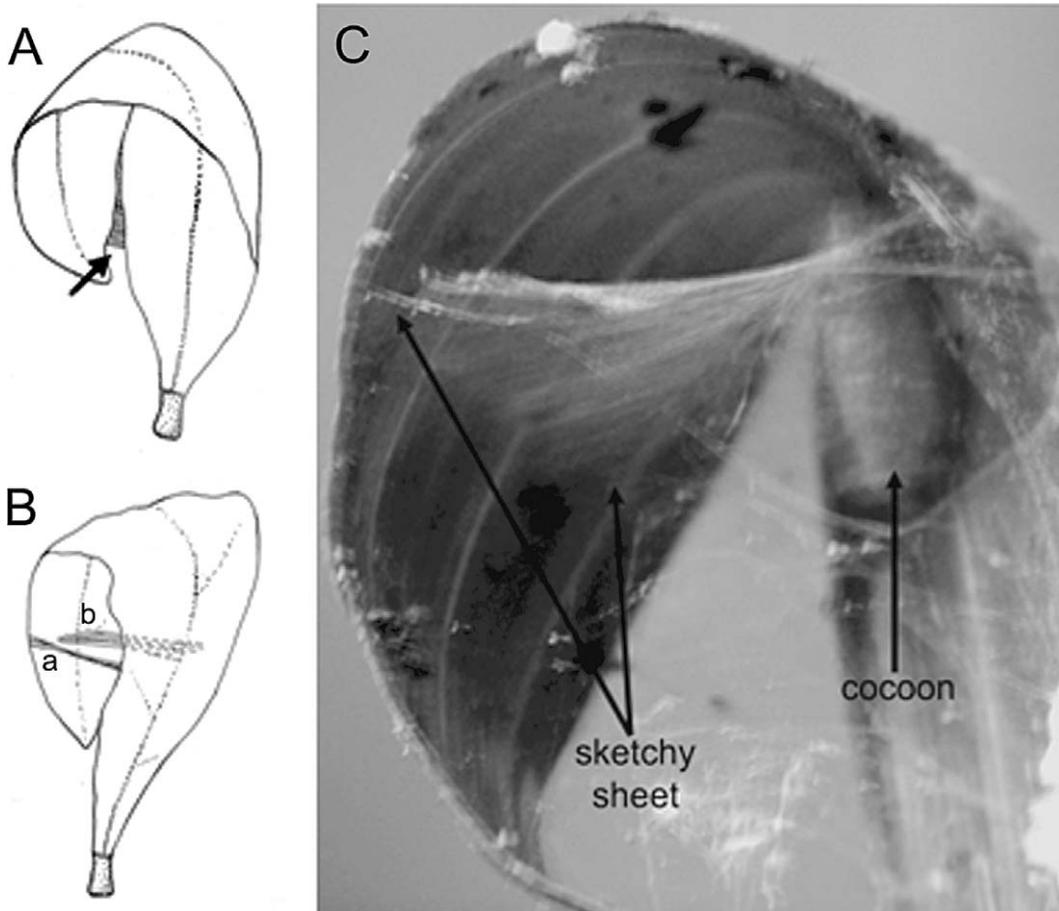


Fig. 5. Retreat constructed by *Theridion evexum*. A) The arrow shows the threads that maintain the leaf-retreat folded. B) Threads added by the spider at the retreat opening (a) and in the middle of the retreat (b). C) Sketchy sheet in the middle of the retreat. Larva of *Zatypota petronae* induces *T. evexum* to produce threads at the retreat opening, inside it and to increase number of threads that maintained the leaf folded (black arrow in A).

#### DISCUSSION

The morphology and behavior of the larva of *Z. petronae* are quite similar to those of larvae of other polysphinctine species (Nielsen 1923, 1932, Fincke 1990, Gauld et al. 1998, Eberhard 2000a). However, they often differ in where and possibly how their cocoons are attached to the host web. The larva of *Z. petronae* attaches its cocoon, which lacks a suspension line, to silk threads inside the retreat of *T. evexum*, *Hymenoepimecis* spp. attach their cocoon to the spider web (e.g. *N. clavipes* and *P. argyra*) with a suspension line (Fincke et al. 1990, Eberhard 2000a,

2001), whereas the larva of *Reclinervellus nielseni* (Roman) [= *Polysphincta nielseni*] (Nielsen 1923, Gauld and Dubois 2006) and *P. gutfreundi* Gauld (Gauld et al. 1998), which also lack suspension lines, attach the cocoons to the threads near, or on the hub of the orbicular web of *Cyclosa conica* (Pallas) (Nielsen 1923) and *Allocyclosa bifurca* (McCook), respectively. These differences are likely determined by the characteristics of the web of each spider species, particularly by the modifications of the web (the "cocoon web" of Eberhard 2001) induced by the parasitoids (e.g. *T. evexum* re-enforcing its retreat).

There are also differences in how larvae adhere to the saddle. Larvae of *Z. petronae* apparently adhere to the saddle using wedge-like projections of one or two segments, rather than taps as in *Z. albicoxa* and *H. argyraphaga*. Differences may also exist in the sensitivity of the host to the wounds caused by the parasitoid. For example, *P. argyra* did not show any reactions to apparent bites of *H. argyraphaga* larvae (Eberhard 2000a). However, *T. evexum* reacted by jerking its body and moving its legs toward the point where the larva was biting the spider's cuticle. This suggests that chemical composition of secretions could vary among parasitoid species. Further research to confirm chemical differences in the saliva of parasitoids and differences in sensitivity of spider hosts to the bites of their parasitoids is needed.

The release of the saddle by final instar larvae is much more complex than simply the muscular movements of the posterior end of the larva as suggested by Nielsen (1923) and Eberhard (2000). Without the powerful pressing and pushing movements of the larva against the saddle, the peristaltic abdominal movements are possibly insufficient to free it from the saddle. More information is needed to examine the possible differences among polysphinctine species.

The larva of *Z. petronae* induces the host spider to add more threads on different sections of the retreat (apex, inside, and across the retreat opening) that make this structure stronger and more durable. Adding threads near the apex of the retreat is apparently a repetition of a subroutine used in the construction of the retreat by an unparasitized spider, since threads applied in similar fashion allows the spider to fold the leaf and maintain the retreat's shape. Similarly, threads across the retreat opening were occasionally present (3 out of 17 webs) when pre-adult female spiders were molting, though these threads were not as abundant as those in retreats of parasitized spiders. However, the thick

cable of silk threads produced inside the retreat was not found in webs of unparasitized spiders. The reinforcement of the retreat with additional silk threads possibly increases the protection of the cocoon, primarily against heavy rains, which is likely important for the wasp's survival. If a retreat opens up, it is unlikely that the thin threads of the tangle inside the retreat, where most cocoons were attached, could survive heavy rains intact.

Our observations suggest that *Z. petronae* is not specialized on a particular species of host. This wasp parasitized intermediate sized spiders (at least 4<sup>th</sup> instar), but the reproduction of *T. evexum* is highly seasonal and large juvenile spiders occur only during five or six months of the year. Thus, it is likely that *Z. petronae* must parasitize at least one other species of spider to maintain its population.

The percentage of parasitism of *T. evexum* (1.39%  $\pm$  1.80) was relatively low when compared with other spider species. Fincke et al. (1990) reported that the annual percentage of parasitism for intermediate-sized juvenile females of *N. clavipes* was 15–30%, and Eberhard (2000) reported that the parasitism on *P. argyra* was higher than 40% for mature females and higher than 3% for mature males. The low parasitism on *T. evexum* also suggests that *Z. petronae* is possibly using other spiders as hosts in the same area.

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#### LITERATURE CITED

- Agnarsson, I. 2004. Morphological phylogeny of cobweb spiders and their relatives (Araneae, Araneoidea, Theridiidae). *Zoological Journal of the Linnean Society* 141: 447–626.
- Barrantes, G. and J. L. Weng. In press. Natural history, courtship, feeding behaviour and parasites of

- Theridion evexum* (Araneae: Theridiidae). *Bulletin of the British Arachnological Society*.
- Eberhard, W. G. 2000a. The natural history and behavior of *Hymenoepimecis argyraphaga* (Hymenoptera: Ichneumonidae) a parasitoid of *Plesiometa argyra* (Araneae: Tetragnathidae). *Journal of Hymenoptera Research* 9: 220–240.
- . 2000b. Spider web manipulation by a wasp larva. *Nature* 406: 255–256.
- . 2001. Under the influence: webs and building behaviour of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepimecis argyrophaga* (Hymenoptera, Ichneumonidae). *Journal of Arachnology* 29: 354–366.
- , G. Barrantes, and J. L. Weng. 2006. The mystery of how spiders extract food without masticating prey. *Bulletin of the British Arachnological Society* 13: 372–376.
- Fincke, O. M., L. Higgins, and E. Rojas. 1990. Parasitism of *Nephila clavipes* (Araneae: Tetragnathidae) by an ichneumonid (Hymenoptera, Polysphinctini) in Panama. *Journal of Arachnology* 18: 321–329.
- Gauld, I. D. and J. Dubois. 2006. Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider ectoparasitoids. *Systematic Entomology* 31: 529–564.
- , J. A. Ugalde G., and P. Hanson. 1998. Guía de los Pimplinae de Costa Rica (Hymenoptera: Ichneumonidae). *Revista Biología Tropical* 46 (Supl. 1): 1–189.
- Hanson, P. and I. D. Gauld. 1995. *The Hymenoptera of Costa Rica*. Oxford University Press, Oxford.
- Jiménez, M. L. 1987. Relaciones entre arañas y avispa. *Folia Entomológica Mexicana* 73: 173–183.
- Nielsen, E. 1923. Contributions to the life history of the pimpline spider parasites (*Polysphincta*, *Zaglyptus*, *Tromatobia*). *Entomologiske Meddelelser* 14: 137–205.
- . 1932. *The Biology of Spiders*. Levin & Munksgaard, Copenhagen.
- Shaw, M. R. 1994. Parasitoid host ranges. Pp. 111–144 in: B. A. Hawkins, and W. Sheehan, eds. *Parasitoid community ecology*. Oxford University Press, Oxford.