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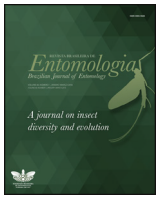

Description of larva, pupa, and genitalia of *Hybosa acutangula* Spaeth, 1913 (Coleoptera: Chrysomelidae: Cassidinae) from the Brazilian Cerrado

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ABSTRACT

Great advances were made in recent years regarding the description of immatures of Cassidinae and their taxonomy as a whole, but many taxa remain undescribed. This study focuses on updating morphological data for *Hybosa acutangula* Spaeth, 1913 (Chrysomelidae: Cassidinae: Ischyrosonychini), a tortoise beetle native of South America, which was collected in *Fridericia florida* (DC.) L. G. Lohmann in the Brazilian Cerrado. We provide descriptions and illustrations of the morphology of the larva, pupa, and genitalia of adults, all of which are novel for this genus. The main discerning features for this species among other Ischyrosonychini are the lack of dark patterns in the dorsum of either the larvae, being limited to the dark scoli and the cranium, or the pupae, and a much reduced anal fork. We also present the first record of parasitism by Chalcididae wasps, *Brachymeria* sp. Westwood, 1832 and *Conura* sp. Spinola, 1837. Morphological comparisons remain limited demanding further studies with other species of Ischyrosonychini, as to better understand the placement of this species within the taxonomy of tortoise beetles.

Introduction

The larvae of Cassidinae are well recorded and well-studied (e.g., *Cassida* Linnaeus, 1758) (Borowiec and Świętojańska, 2003), *Asteriza* Chevrolat (1836), and *Physonota* Boheman (1854) (Świętojańska and Windsor, 2008)), often characterized by well-developed scoli, branched or smooth, variable in length and number, and eighth abdominal segment bearing the anal fork which usually supports the feces or the larval exuviae, while the pupae present a wide well-developed pronotum, with the abdomen usually bearing lateral scoli varying in length and shape (Buzzi, 1996; Chaboo, 2007). The immatures of Cassidinae can be found over and under the leaves they feed upon, often forming aggregations that can be maternally guarded (Jolivet et al., 1988; Costa et al., 1998).

Larvae of Cassidinae have been the subject of taxonomic studies in recent years, with descriptions being added to undescribed larvae of known species, and efforts made to better understand the group's morphological and taxonomic diversity (Borowiec and Świętojańska, 2003; Świętojańska, 2004a, 2004b; Borowiec, 2009). This includes our group of interest, Ischyrosonychini Chapuis (1875), a New World group comprising 66 species in seven genera (Borowiec and Świętojańska, 2022), weakly supported by a head stridulatory file observed in all genera (Schmitt, 1994; Borowiec, 1995). Recent research regarding Ischyrosonychini has mostly focused on immature morphology (Świętojańska, et al., 2018; López-Perez, et al., 2021), and our research aims to add to this information library. Furthermore, the data regarding the morphology of genitalia, equally valuable to accessing diversity and species identification of insects (Konstantinov, 1998; Richmond et al., 2016), is also scarce for Ischyrosonychini, and Neotropical Cassidinae as a whole.

The Neotropical genus *Hybosa* Boheman, 1855, includes 11 described species (Boheman, 1854; Borowiec and Takizawa, 2011), of which eight species are currently known in Brazil. The Brazilian species are known from the states of Amazonas, Espírito Santo, Goiás, Mato Grosso, Pará, Paraná, Rio de Janeiro, Rio Grande do Sul, Rondônia, Santa Catarina, and São Paulo (Borowiec and Świętojańska, 2022; Linzmeier et al., 2024). Currently, all known species of *Hybosa* were described based on the external morphology of adults, with no data for immatures or genitalia (Borowiec and Świętojańska, 2022).

With this in mind, we aim with the present paper to enrich the taxonomical knowledge for Cassidinae, Ischyrosonychini, describing the larva, pupa, and genitalia of male and female, of *Hybosa acutangula* Spaeth (1913), all of which are unknown for the genus so far. We also present illustrations and high-resolution photographs, aiding

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future studies with other species and close genera. Furthermore, we briefly record parasitism by *Brachymeria* Westwood, 1832, and *Conura* Spinola, 1837, wasp (Chalcididae: Hymenoptera), which are known parasitoids of Coleoptera (Gowri et al., 2016).

Material and methods

All individuals, adults and immatures, were collected at the border of the Bosque August Saint Hilaire, a remnant area of Cerrado semideciduous forest (Strassburg et al., 2017) within Universidade Federal de Goiás Campus Samambaia (UFG), in Goiânia, Goiás, Brazil (16°36'12.913"S, 49°15'39.643"W), larvae were collected over and under the leaves of *Fridericia florida* (DC.) L. G. Lohmann, while adults were also collected in the general vicinity. Both adults and larvae were observed feeding on the leaves of *F. florida*. Adults and immatures were observed from late October to mid December, corresponding to the rainy season in the region. When the rain became more intense and frequent in late December, the adults were no longer observed. The larvae were sacrificed in nearly boiling water, which was heated in a microwave for one minute, and preserved in alcohol 80%. The adults were sacrificed by being put in a freezer, at -20ºC between two to three hours, and later pinned.

Two adults, one male and one female (Fig. 1), were dissected for genitalia following protocols described by Smith (1979), which were preserved in glycerin, and stored in a microtube alongside the specimen. A mature larva was also dissected for mouthparts, which were stored in the same microtube of the dissected individual, in alcohol 80%. Adults were pinned, and other immatures were stored in microtubes in alcohol 80%.

Four specimens of adults and six immatures (including the dissected individuals and parasitized pupae) are housed in the Coleoptera collection of Museu de Zoologia da Universidade de São Paulo (MZUSP), and three undissected adult beetles are also housed in the Coleção Zoológica da Universidade Federal de Goiás (ZUFG). The two individuals of parasitic wasps are deposited in the Hymenoptera collection of Museu de Zoologia da Universidade de São Paulo (MZUSP).

Photographs were taken with a DFC550 lens attached to a Leica M205A stereomicroscope, using the LAS (Leica Application Suite) software. The photographs were edited using the software Adobe Photoshop CC 2019. Illustrations were made using the software Adobe Illustrator CC 2015 based on the photographs.

The terminology of immatures follows Świętojańska (2009), Świętojańska et al. (2018) and López-Perez et al. (2021). The terminology of genitalia follows Chaboo (2002) and Simões (2012), while this bibliography does not focus on Ischyrosonychini, it provides good general descriptions for Cassidinae genitalia.

Results

Description of fifth larval instar (Figs. 2-4)

Body length: 10.56 mm (Average for two specimens). Body 2.5 times longer than wider, long, and flattened. Body of early instars wholly brown, older instars and pre-pupa wholly green; sclerotized portions of the body, cranium, legs and scoli, dark brown to black.

Scoli spike-like and slightly curved with small thin setae, varying from two to four setae on each side of the scolus, and one at the tip. Mesonotum, metanotum and abdominal segments I-VII with two rows of small setae disposed horizontally, and groups of small setae present at the base of each scoli, in dorsal view. All thoracic segments with groups of small setae at their outer edges and the median portion, abdominal segments with irregularly distributed setae in ventral view.

Head sclerotized, rounded in frontal view, slightly flattened in lateral view; with six stemmata on each side, four grouped dorsolaterally and two grouped laterally to the antenna. Chaetotaxy of the head symmetrical. On each side of head, frons bearing 12 frontal setae, three small setae near the epicranial suture, three setae near the insertion of the antenna, epicranium bearing three setae. Labrum, posterior margins rounded with slight recess at the median portion, bearing four setae distributed horizontally; with one large and one small seta in the frontal region of the dorsolateral stemmata, one large seta in between the dorsolateral stemmata, two large visible setae behind the stemmata. Antennae two-segmented. Basal segment short and round lacking setae; distal segment cylindrical, with a smaller diameter than the previous segment, bearing two small setae at the apex. Mandibles sclerotized, right mandible with five teeth, left mandible with four teeth; all teeth crenulated. Maxillae and labium connate. Maxillary palps two segmented, each segment bearing two setae at the laterals. Palpiger well-developed, mala conical, with setae at the inner face. Labial palp conical, one segmented. Ligula rounded, divided in the middle. Prementum with two setae. Postmentum with four setae.

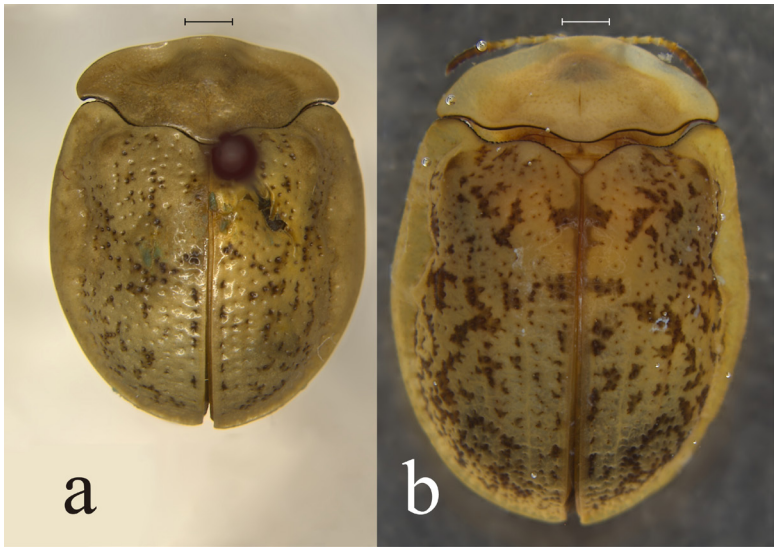

Figure 1 *Hybosa acutangula* Boheman, 1855. Dorsal habitus: **a,** adult female; **b,** adult male. Scale bar = 1 mm.

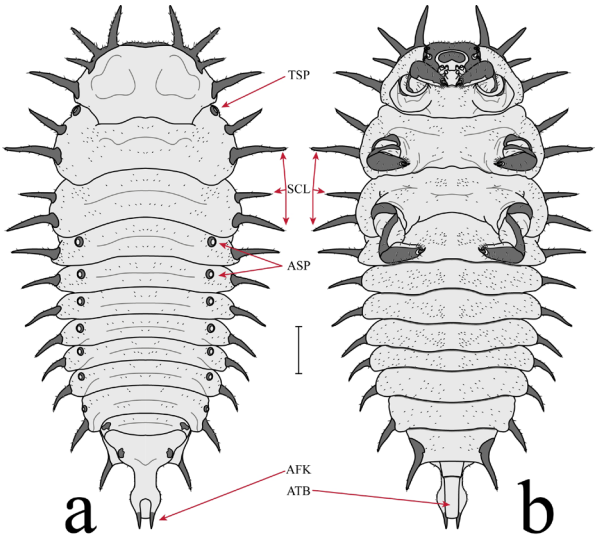

Figure 2 *Hybosa acutangula* Boheman, 1855. Fifth instar larvae: **a,** dorsal view; **b,** ventral view. AFK, anal fork; ASP, abdominal spiracles; ATB, anal tube; SCL, scolus; TSP, thoracic spiracle. Scale bar = 1 mm.

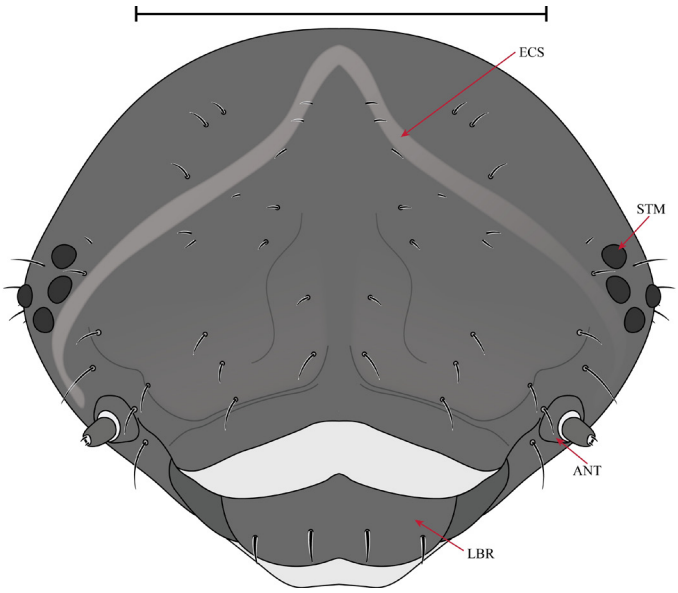

Figure 3 *Hybosa acutangula* Boheman, 1855. Fifth instar larvae, frontal view of the head. ANT, antenna; ECS, epicranial suture; LBR, labrum; STM, stemma. Scale bar = 1 mm.

Thorax wider than the abdomen. Pronotum wide, with two grooved areas in the median portion, with four scoli on each side; three scoli at most frontal region forming a group with a shared black integument insertion, one pointing onwards and two smaller pointing diagonally; one large scolus pointing laterally. Mesonotum and metanotum similar, with two scoli on each side, the anterior scolus smaller than the posterior; each segment also with two rows of small setae dorsally. Mesonotum with one spiracle at the anterior portion, dorsolaterally positioned. All legs similar. Coxa, femur and sclerotized, notably darker on the darker face of the tibia and distal femur; coxa stout and short, femur and tibia subequal in cylindrical, tibia slightly shorter than femur; all segments with irregularly distributed setae, setae of the inner face of femur longer than of the outer face, setae of the outer face of tibia longer than of the inner face, apex of tibia with higher density setae. Tarsungulus sclerotized and hook-like.

Abdominal segments very wide and short, second segment slightly over four times longer than wide; the others similar, gradually narrowing. Segments I-VIII with one lateral scolus on each side; segments I-VII bearing one spiracle on the dorsal portion of each segment near the base of the scolus, gradually decreasing in diameter; anal fork of segment VIII with parallel sides and the apical portion slightly inflated, the anal fork is maintained during all the immature instars; segments VIII and X forming the anal tube.

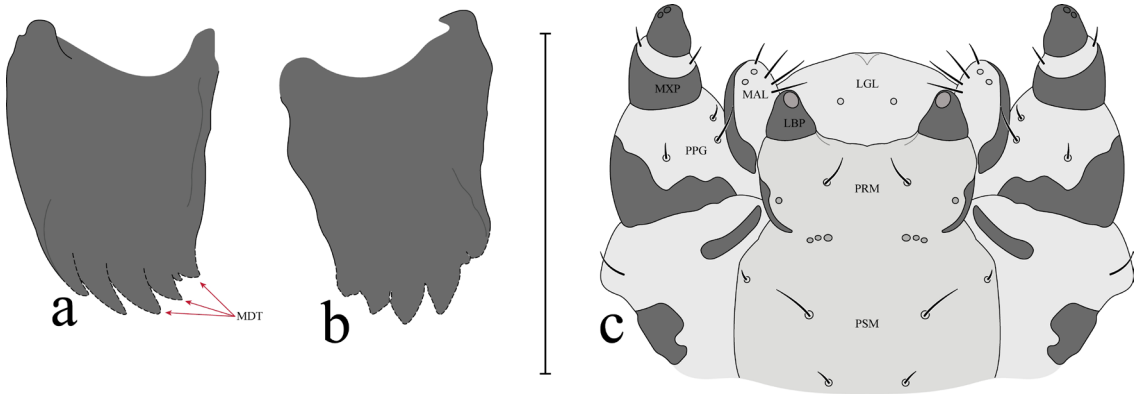

Figure 4 *Hybosa acutangula* Boheman, 1855. Fifth instar larvae, mouthparts: **a,** left mandible, ventral view; **b,** right mandible, ventral view; **c,** labium, ventral view. LBM, labium; LBP, labial palpus; LGL, ligula; MAL, mala; MDT, mandibular teeth; MXP, maxillary palpus; PPG, palpiger. PRM, prementum; PSM, postmentum. Scale bar = 1 mm.

Description of pupa (Fig. 5)

Body length: 9.43 mm (Average for two specimens). Body 1.6 times longer than wide (excluding the scoli), convex shape. Body green and glabrous.

Head 1.4 times longer than wide, with two short, rounded processes at apex; antennae divergent, a portion of the antenna hidden behind anterior leg, apex of the antenna reaching the apex of the femur. Mouthparts visible.

Pronotum wide, over two times wider than long, with a small recess at the front, anterior margin with three short rounded processes bearing very fine small setae on each side; posterior laterals of the pronotum with a rounded process pointing backward; posterior median margin with rounded projection over metanotum. Mesonotum transverse, scutellum noticeable, lateral projections rounded. Metanotum transverse with anterior margin bisinuated, posterior margin transverse, very slightly projected medially. Elytra and wings visible only in ventral view. All legs similar, anterior and middle legs visible, posterior legs covered by the elytra, except the tarsi.

Abdominal segments very wide and short, first segment slightly over 6.5 times wider than long; the others similar, gradually narrowing. Segments I and II with anterior margins slightly bisinuated; segments I-V with one very visible lateral scolus on each side with small setae, reducing in size gradually, scoli in segments I-III larger and curved, scoli on segments IV-V smaller and spike-like, and segment VI with a minute spike-like process; segments I-V bearing one spiracle positioned dorsolaterally of each side, spiracle in segment I larger, spiracles in segment II-IV similar, spiracle in segment V slightly smaller than the previous spiracle; segments I-IV covered by elytra in ventral view. Anal fork reduced, but visible.

Description of female genitalia (Figs. 6a and 6b)

Spermatheca well-sclerotized, vasculum large and curved, with narrow base and rounded distal portion, distal curved portion nearly two times the length of the proximal straight, hook-like; ductus spiral and long. Tignum with base 2.5 times broader than the apex, containing various setae. Laterals slightly less sclerotized. Median section slightly constricted. Apex of the tignum straight, with vertexes slightly pointing forward.

Description of male genitalia (Figs. 6c and 6e)

Median lobe curved, not falciform, tip slightly tapered in lateral view. Tegmen attached to the base of the median lobe, cruciform in ventral view, anterior section rounded, lateral "wing-like" and slightly curved backward, basal section thin.

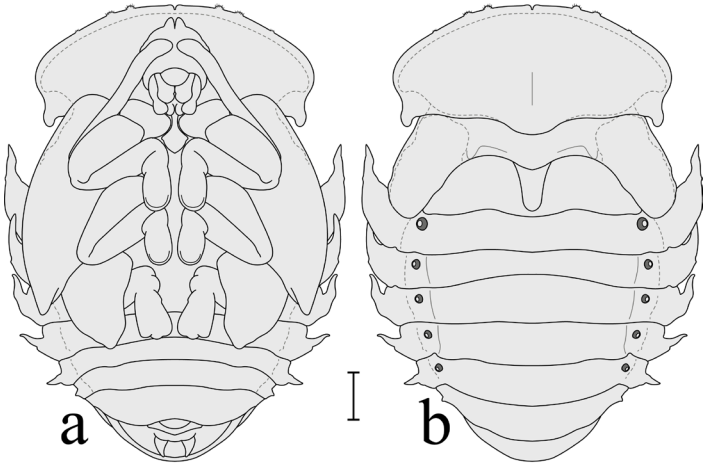

Figure 5 *Hybosa acutangula* Boheman, 1855. Pupa: **a,** dorsal view; **b,** ventral view. Scale bar = 1 mm.

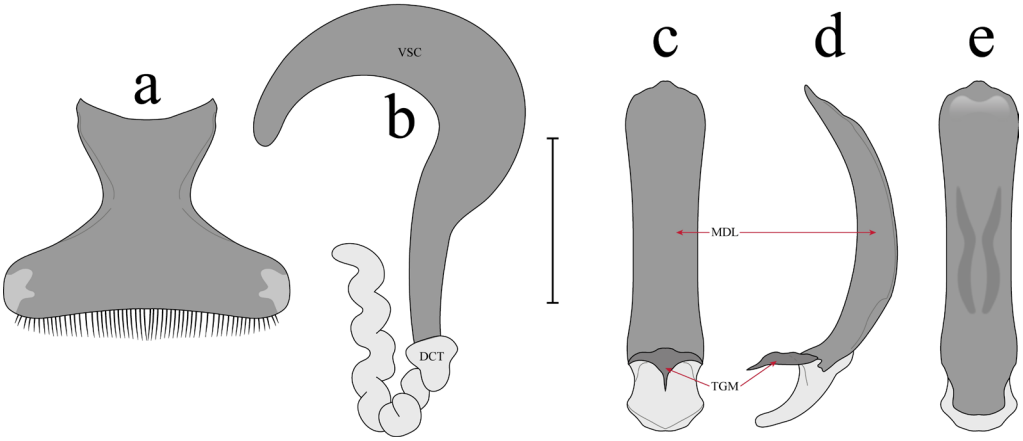

Figure 6 *Hybosa acutangula* Boheman, 1855. Genitalia: **a,** female, tignum, dorsal view; **b,** female, spermatheca, lateral view; **c,** male, aedeagus, ventral view; **d,** male, aedeagus, lateral view; **e,** male, aedeagus, dorsal view. DCT, ductus; MDL, medianl lobe; TGM, tegmen; VSC, vasculum. Scale bar = 500µm.

Material studied

Adult beetles: BRAZIL: Goiás: Goiânia, Bosque August Saint-Hilaire, Manual collection – fence, Begha, B.P., 20.X.2022, 1 dissected male; 31.X.2022, 1 dissected male; 31.X.2022, 2 dissected females (MZUSP); 31.X.2022, three undissected specimens (ZUFG).

Immatures: BRAZIL: Goiás: Goiânia, Bosque August Saint-Hilaire, Manual collection – fence, Begha, B.P., 20.X.2022, 3 larvae; 20.X.2022, larva with dissected mouthparts; 20.X.2022, pupa; 03.XI.2022 parasitized pupa (MZUSP).

Parasitoid wasps: BRAZIL: Goiás: Goiânia, Bosque August Saint-Hilaire, reared in laboratory, Begha, B.P., 03.XI.2022, *Brachymeria* wasp; 06.XII.2022, *Conura* wasp (MZSUP).

Discussion

Record of parasitism by Chalcididae wasps (Hymenoptera)

We attempted to rear *H. acutangula* in the laboratory, trying to form reproductive pairs and properly record all of its development stages, but were unsuccessful. Two of the pupae sampled from *F. florida* leaves were kept in separate plastic recipients with air holes and a humid cotton ball. However, after approximately three days in the lab, we observed that the pupae appeared dry, hollow, and presented an opening corresponding to the exit of the adult parasitic wasp (Fig. 7a). This happened with two of our sampled pupae, first with a specimen of *Brachymeria* Westwood, 1832 (Fig. 7b), and later with a specimen of *Conura*, 1837 Thomson (Fig. 7c), in both cases, the individuals were found dead in the recipient. While Chalcididae wasps are known for targeting pupae of Lepidoptera, Diptera, other Hymenoptera, and Coleoptera (Gowri et al., 2016), with Cassidinae as known targets (Buzzi, 1988; Gómez, 2004), this is the first record of either of these wasps targeting *Hybosa* beetles, and their first record of parasitism for the Brazillian Cerrado.

Distribution pattern

H. acutangula was originally described for Paraguay, Argentina and the state of Espírito Santo (Sintypes located in Manchester Museum, Manchester, England, and Muzeo Zoologico di Storia Naturale, Genova, Italy), and it has been recorded for Brazil in Paraná, Rio Grande do Sul, Santa Catarina and São Paulo (Borowiec and Świętojańska, 2022). This is the first record of *H. acutangula* for the state of Goiás and the Brazilian Cerrado, the only other species of *Hybosa* previously recorded for this state is *H. fornicata* Boheman, 1855 (Borowiec, 2009), but it isn't made clear which environment it was sampled at.

On the morphology of immatures and genitalia

We compared the immatures of *H. acutangula* to the immatures of *Asteriza flavicornis* (Olivier, 1790) and *Physonota alutacea* Boheman, 1854 (Świętojańska and Windsor, 2008), also Neotropical Ischyrosonychini. The immatures of *H. acutangula* follow the general anatomy observed for Ischyrosonychini. The dorsal scoli are disposed as, six in the pronotum, four in the mesothorax (lacking the first scolus near the spiracle present for Cassidini (Borowiec and Świętojańska, 2003)) and metathorax, and two at each abdominal segment. The scoli lack ramifications, presenting various thin setae instead.

Regarding the chaetotaxy of the head for *H. acutangula*, the distribution and high quantity of frontal setae are more similar to *P. alutacea* than to *A. flavicornis*, which the setae are at higher density, and the setae from the labrum and the cranium, especially near the stemmata, are shorter.

The scoli of *P. alutacea* are noticeably curved backward, while the scoli of *H. acutangula* are mostly straight. The scoli of *A. flavicornis* are also mostly straight, but robust. The anal fork of *H. acutangula* is reduced compared to the other species, while the anal fork of *P. alutacea* has half the length of the whole body, and the one for *A. flavicornis* has a length of over four abdominal segments, being very curved.

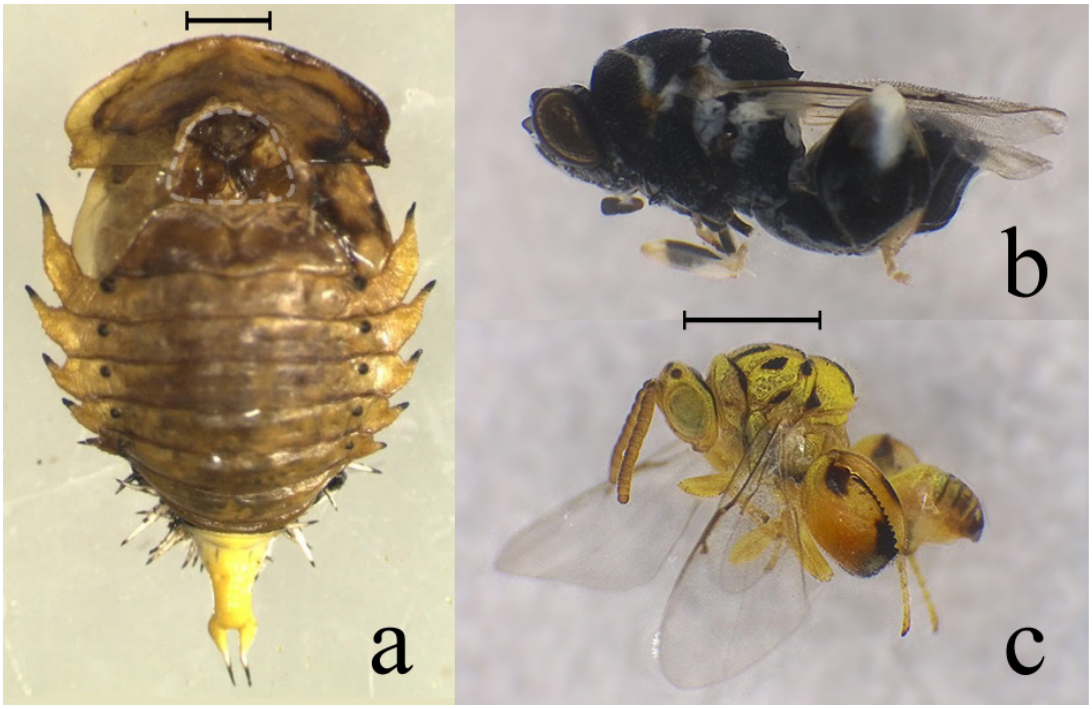

Figure 7 a. Husk of a parasitized pupae of *Hybosa acutangula* Boheman, 1855, opening made by emergence of adult wasp highlighted; Chalcididae which emerged from *H. acutangula* pupae, **b.** *Brachymeria* sp.; **c.** *Conura* sp.

One of the features that stood out when comparing immatures was the coloration. Both, the pupae and the larvae of *A. flavicornis* and *P. alutacea,* present complex dark patterns over their body, with the larvae of *A. flavicornis* presenting horizontal dark lines and no coloration in the scoli, and *P. alutacea* presenting a complex dark pattern and no coloration in the scoli as well. The larvae of *H. acutangula* does not present any dark impressions, being limited to darker scoli, spiracles and head, while the other species have a light colored head. The pupae also don't bear any dark impressions, save for the very tips of the abdominal scoli.

Regarding the genitalia of adults, the shape of the male median lobe is similar to the one of *P. alutacea*, but the apical foramen differs by being bisinuated rather than conical (Sanderson, 1948). The genitalia of the females are also unique, while the spermatheca of other *Physonota* is mostly curved with a shorter and less entangled ductus, *H. acutangula* has a peculiar "question mark shape" spermatheca, with long and very spiralized ductus.

Past literature has placed the genus *Hybosa* as a Cassidini. However, the immatures we observed are much more similar to Ischyrosonychini than to those of Cassidini, and thus we are inclined to characterize *Hybosa*, or at the very least *H. acutangula*, as true Ischyrosonychini.

Regarding biology and behavior of immatures, parental care, common for this family (Flinte et al., 2015), was not observed. We did observe, however, aggregation behavior among younger larvae, while older larvae and pupae were mostly solitary. We attempted to rear adults in the lab, aiming to form reproductive pairs and sample all stages of development, but we were unsuccessful.

We could not find other taxonomic works involving the larvae of *Hybosa*, or records of genera are more closely related to it, so finer comparisons of traits are made difficult, especially concerning the chaetotaxy of the head, often used for specific identifications in Coleoptera (Begha and Santos, 2020). We hope our study sets a basis for future studies involving the immatures of *Hybosa*, which still lack much taxonomy information.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

BPB Conceptualization-Equal, Data curation-Equal, Formal analysis-Equal, Investigation-Equal, Methodology-Equal, Resources-Equal, Visualization-Equal, Writing – original draft-Equal, Writing – review & editing-Equal. SSO Conceptualization-Equal, Project administration-Equal, Supervision-Equal, Visualization-Equal, Writing – original draft-Equal, Writing – review & editing-Equal.

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