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EGG AND FIRST INSTAR OF THE NEOTROPICAL GEOMETRID MOTH *PERO* *OBTUSARIA* PROUT (GEOMETRIDAE: ENNOMINAE: AZELININI)

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ABSTRACT. The external morphology of the egg and first instar of the little-known Neotropical geometrid moth *Pero obtusaria* Prout, 1928 (Lepidoptera: Geometridae: Ennominae: Azelinini) is described and illustrated based on light and scanning electron microscopy. This is the first morphological study dealing with immature stages of a Neotropical species of the highly diverse New World moth genus *Pero*.

Additional key words: Aeropyle, Chaetotaxy, Immature stages, Micropyle, SEM

The highly diverse New World moth genus *Pero* Herrich-Schäffer (Lepidoptera, Geometridae, Ennominae, Azelinini) includes more than 300 described species, most of which are Neotropical (Poole 1987, Pitkin 2002, Ferris 2003, Lévêque 2006, Brown 2007, Vargas 2007). Despite the enormous diversity of this genus in the Neotropics, the natural history of the species has been scarcely documented in this area, and only a few records based on research done on their host plants suggest that their larvae are mainly host specialists (Poole 1987, Barros 2007, Bodner et al. 2010, Vargas 2011). In contrast, the host plants of the Nearctic species are better known, and many cases of polyphagy have been described (Comstock 1963, McGuffin 1963, Poole 1987, Robinson et al. 2010). Detailed morphological descriptions are available for the immature stages of some Nearctic *Pero* (Comstock 1963, McGuffin 1963, Salkeld 1983), but the morphology of the immature stages of the Neotropical species remain unknown.

Pero obtusaria Prout, 1928, is a little-known Neotropical geometrid moth described from Peru. The southern limit of its geographic range extends to the coastal valleys of the Atacama Desert of northern Chile (Vargas & Hausmann 2008), where the native shrub *Pluchea chingoyo* (Asteraceae) is the only host plant recorded so far for its folivorous and mostly nocturnal-feeding larvae (Vargas 2011).

Detailed knowledge of external morphology of immature stages is widely recognized as an important tool in studies dealing with the evolution and systematics of Lepidoptera (Dias et al. 2015, Hernández-Mejía et al. 2015, Salik et al. 2015, Dolinskaya 2016, Neves & Paluch 2016, Nieves-Urbe et al. 2016). Therefore, the objective of this study is to describe and illustrate the external

morphology of the egg and first instar of *P. obtusaria* based on light and scanning electron microscopy, providing the first morphological observations of immature stages of a Neotropical species of *Pero*.

MATERIALS AND METHODS

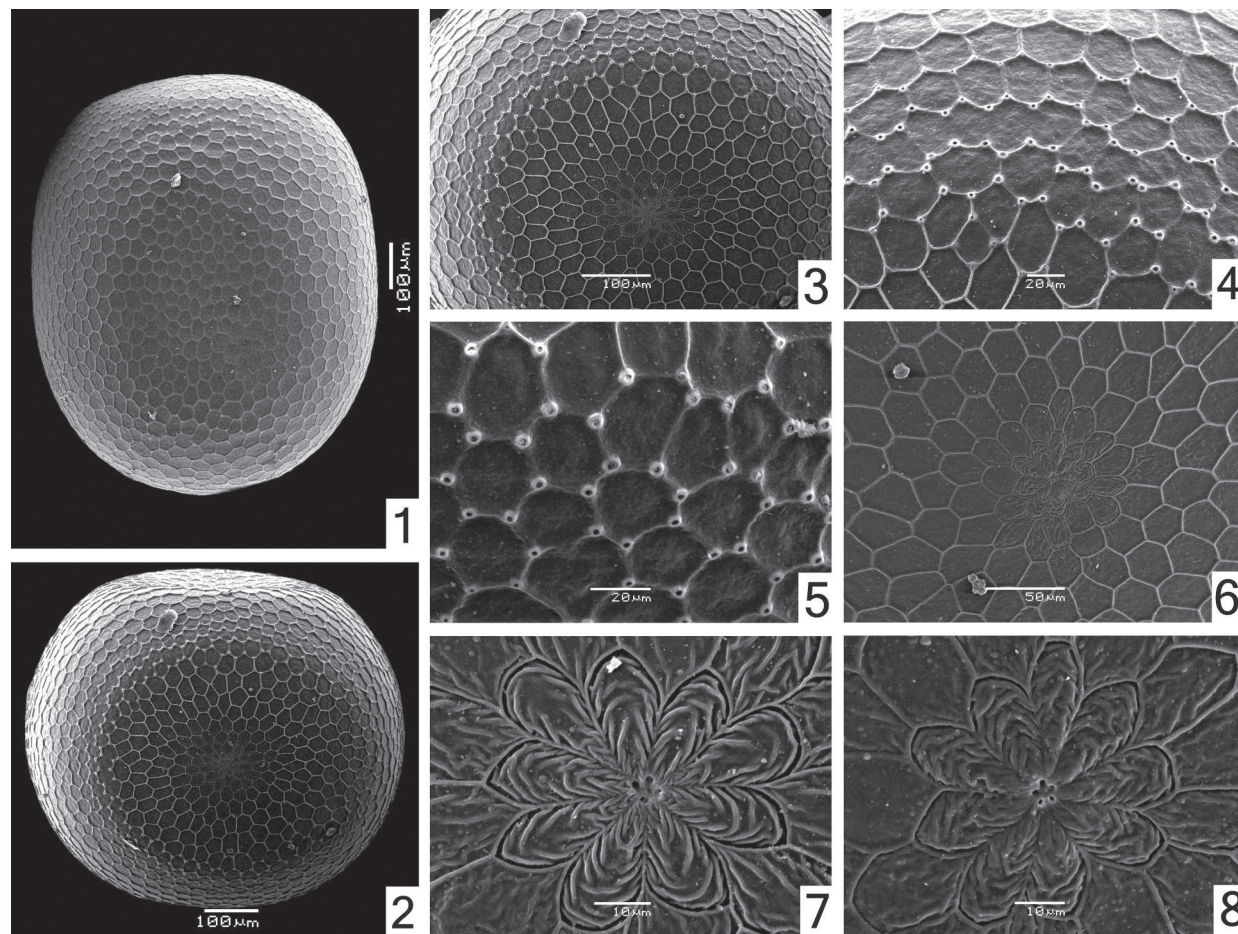
Sampling. One female of *P. obtusaria* was collected at light in the Azapa valley, Arica Province, northern Chile, in November 2006. The individual was transported to the laboratory and kept in a plastic bag until the following day. Twenty-three eggs were deposited by the female, 15 of which were kept in ethanol 70%. The remaining eggs were kept in a plastic vial with paper towel at the bottom and were observed daily until eclosion. The host plant of this species was unknown at the time, so the newly hatched larvae were kept in ethanol 70% for morphological studies.

Morphology. We used the methods described in Vargas et al. (2015) for morphological observations; the nomenclature of Salkeld (1983) for the description of the egg; and the nomenclature of Stehr (1987) for the description of the chaetotaxy of the larva, with the modification proposed by Duarte et al. (2005) for the CD group of the head.

RESULTS

Egg (Fig. 1–8)

Ellipsoid (Fig. 1); micropylar area slightly flattened (Fig. 2); micropylar axis parallel to substrate; surface mostly sculptured by polygonal cells (Fig. 1–6); ridges sometimes poorly differentiated in the cells bearing aeropyles (Fig. 4); 4–5 micropylar openings (Fig. 7–8); micropylar rosette with 7–9 petal-like cells with surface sculptured by transverse grooves (Fig. 7–8); circular



FIGS. 1-8. Egg of *Pero obtusaria*. 1) General view. 2) Micropylar pole. 3) Detail of the micropylar pole, including micropylar rosette and aeropyles. 4) Area of aeropyles showing the poorly differentiated ridges of some cells. 5) Aeropyles. 6) Micropylar area showing the well-defined polygonal cells surrounding the micropylar rosette. 7) Micropylar rosette with four openings. 8) Micropylar rosette with five openings.

aeropyles at vertex of the polygonal cells forming a ring surrounding the micropylar rosette (Fig. 3-6). Silvery grey immediately after laying, yellowish brown after 3-4 days, greyish brown when the larva is ready to emerge.

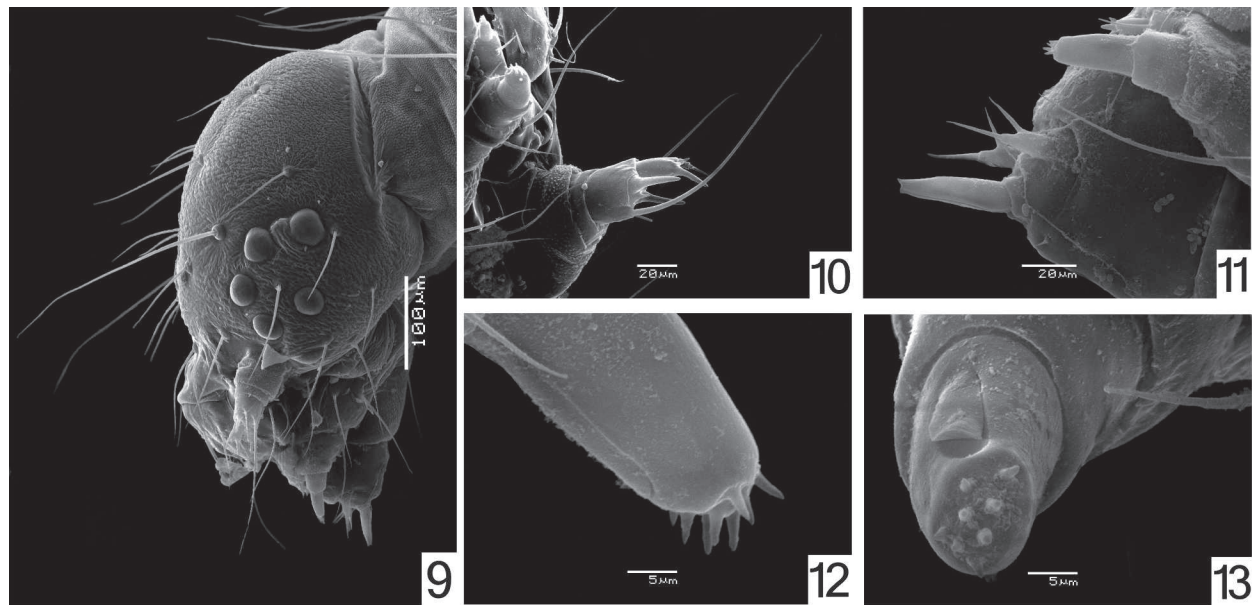
Measurements. Length: 1.54–1.60 mm; width: 1.38–1.42 mm.

First instar (Fig. 9-29)

Head (Fig. 9) hypognathous, integument irregularly reticulated, setae hair-like, seta A3 about twice the length of A2; frontoclypeous triangle-like with ventral margin broadly concave. Six circular stemmata laterally; stemmata 1–5 forming a semicircle, stemma 6 at middle between stemmata 1 and 5. Antenna (Fig. 1-2) short, 3-segmented; first segment annular; second segment cylindrical, about twice the length of the first segment, five sensillae on the distal surface; third segment cylindrical, length about the same and diameter about one half of the second segment, sensillae on the distal surface. Anteclypeous membranous, smooth, as a transverse stripe between the frontoclypeous and the

labrum. Mouthparts (Fig. 9; 11–13; 22–24) of the chewing type. Labrum (Fig. 22–23) bilobed, distal margin sharply cleft at middle; external surface with six pairs of short hair-like setae and three pairs of pores; internal surface (epipharynx) covered with small spines mostly concentrated on the proximal area, three plain teeth on each lobe. Mandible (Fig. 24) with six teeth on the distal margin; two short hair-like setae on the external surface. Maxilla (Fig. 12–13) with well-differentiated galea and palpus; palpus with eight sensillae on distal surface and one on the medial surface. Labium (Fig. 11) with a cylindrical spinneret at apex; a pair of short setae near the base of the spinneret; two bi-segmented palpi, each segment with a sensillum at apex. Chaetotaxy according to Fig. 20–21.

Thorax and abdomen whitish grey; prothoracic dorsal shield, anal shield and legs dark brown; pinnacles and setae dark brown. Integument irregularly reticulated (Fig. 14, 17). Hair-like setae, either with smooth surface and pointed apex, or with longitudinal carinae and multi-



FIGS. 9–13. Head of the first instar of *Pero obtusaria*. **9)** Head in lateral view. **10)** Antenna. **11)** Spinneret and labial palpi. **12)** Distal segment of the maxillary palpus. **13)** Tip of the distal segment of the maxillary palpus.

pointed apex (Fig. 15–16). Circular spiracle (Fig. 14, 17) laterally on the prothorax and A1–8, peritreme elevated. Prothoracic dorsal shield (Fig. 26) rectangle-like, margin sinuous, four pairs of hair-like setae (D and XD groups) and three pairs of pores. Anal shield (Fig. 27) semicircular with a sharp anterior cleft almost touching the posterior margin, four pairs of setae (D and SD groups). Thoracic legs well-developed, tarsal claw (Fig. 18) curved, setae mostly hair-like, but TS3 depressed with distal margin saw-toothed. Prolegs (Fig. 19, 28–29) on A6 and A10, provided with hair-like setae, crochets slightly curved. Chaetotaxy according to Fig. 25–29.

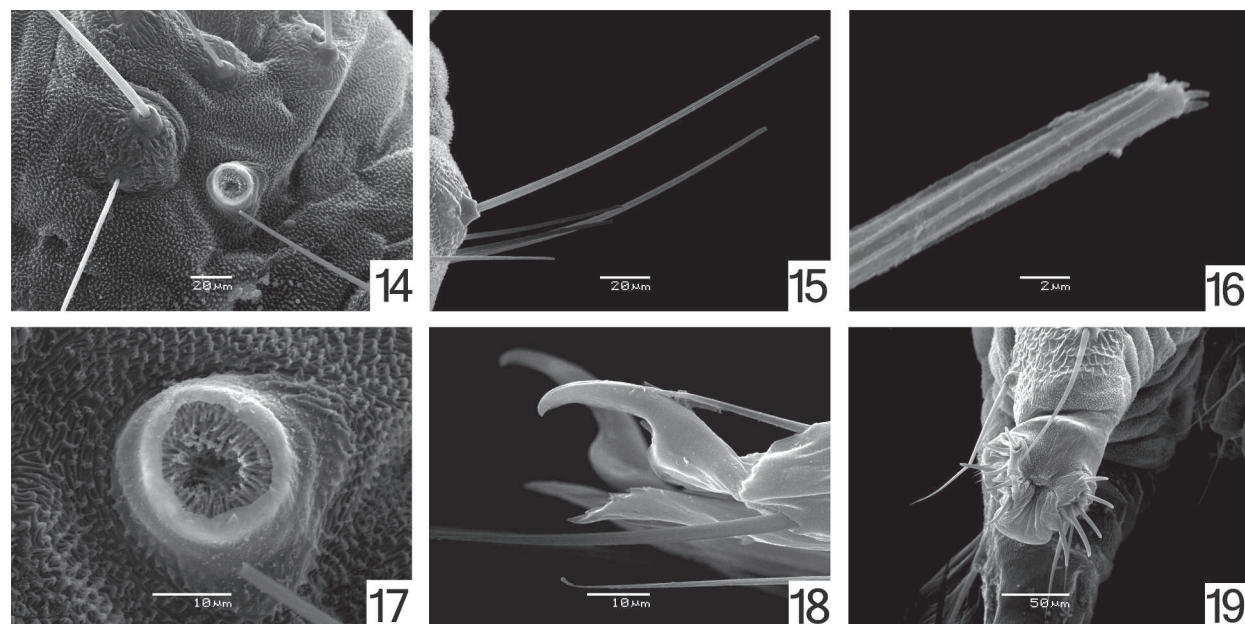
Measurements. Length: 5.0–5.5 mm; head width: 0.48–0.5 mm.

DISCUSSION

The Neotropical Region harbors the highest diversity of Geometridae in the world (Scoble et al. 1995, Brehm et al. 2005). However, although species of Geometridae are important in natural and human-altered environments of the Neotropics either as consumers or as prey, the natural history and morphology of their immature stages remain mostly unknown (Marconato et al. 2008, Bodner et al. 2010, Méndez-Abarca et al. 2012, Nelson et al. 2015, Seifert et al. 2015, Sousa-Lopes et al. 2016, Vargas 2016). For instance, although SEM has been widely recognized as a useful tool for detailed studies of the external morphology of immature stages of

Lepidoptera (Duarte et al. 2005, Brito et al. 2013, Vargas et al. 2015, Dolinskaya 2016), only a few SEM studies have been performed for eggs, larvae or pupae of Neotropical Geometridae (Beéche et al. 1987, Ibarra-Vidal & Parra 1993, Parra & Ibarra-Vidal 2002, Bocaz & Parra 2005, Vargas et al. 2010, King & Parra 2011, Vargas & Parra 2013). Thus, it is not surprising that this is the first morphological study dealing with immature stages of a Neotropical representative of *Pero*. Indeed, it is also the first to include SEM observations of the first instar for a species of this highly diverse New World moth genus.

Although knowledge of the external morphology of the egg of Geometridae is still incomplete at the global level, the detailed studies currently available suggest that the morphology of this life stage can be useful in the systematics of this moth family (Salkeld 1983, Young 2006). Salkeld (1983) described the egg stage of the Nearctic *P. honestaria* (Walker, 1860) based on specimens collected in Canada, indicating that aeropyles occur on a wide band around the shoulders of the anterior and posterior poles. Furthermore, Salkeld (1983) indicated that the eggs of the also Nearctic *P. morrisonaria* (Edwards, 1881) are not distinguishable from those of *P. honestaria*. Accordingly, the morphological pattern enables differentiating the eggs of the two Nearctic species from those of *P. obtusaria*. In addition, the shape and arrangement of the micropylar rosette also separates *P. obtusaria* from these two Nearctic representatives.



FIGS. 14–19. Thorax and abdomen of the first instar of *Pero obtusaria*. **14)** Prothoracic spiracle and pinnacle of the L group in lateral view. **15)** Mesothoracic D2. **16)** Apex of mesothoracic D2. **17)** Detail of the prothoracic spiracle. **18)** Apex of the metathoracic leg showing the tarsal claw and the depressed and sawed TS3. **19)** Proleg of A6.

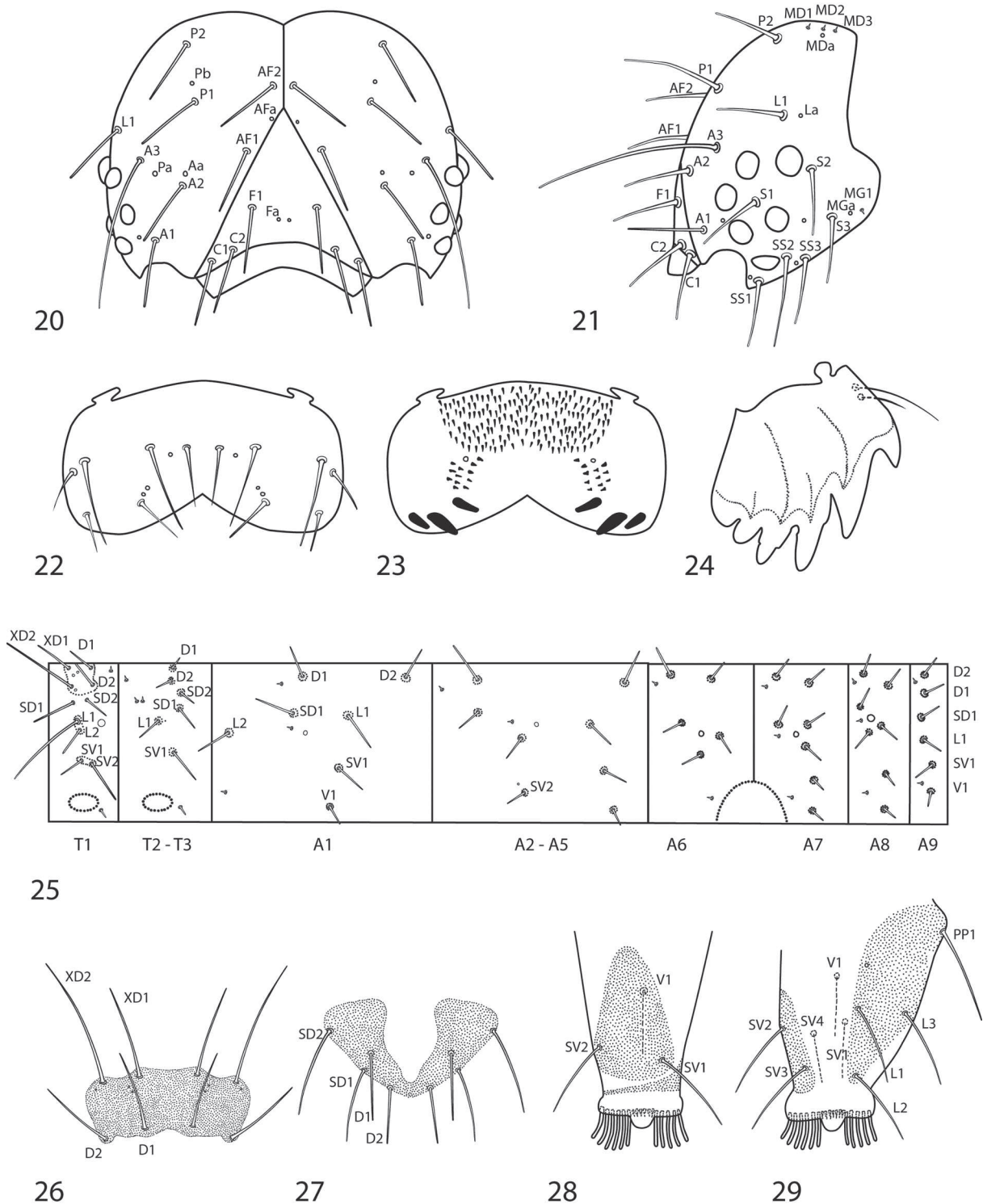
Although the external morphology of the first instar of *P. obtusaria* mostly fits the pattern described for the Nearctic *Pero* (Comstock 1963, McGuffin 1963), a few differences were found in the chaetotaxy of the thorax and abdomen. First, there are six setae pairs on the prothoracic dorsal shield of the Nearctic *Pero*, while four setae pairs are found in *P. obtusaria*. Secondly, the SV group is tri-setose on A2 of the Nearctic *Pero*, while it is bi-setose in *P. obtusaria*. Unfortunately, further comparisons are not possible at the intra-generic level based on the available morphological descriptions (Comstock 1963, McGuffin 1963). Indeed, external morphology of the first instar has been little studied in the family Geometridae (Grehan et al. 1994, Blaik & Malkiewicz 2003, Vargas et al. 2010, Vargas & Parra 2013). However, there are a few characteristics that would be interesting to explore in further comparative studies, such as the relative length of the cephalic setae (A3, Fig. 9) and the morphology of the setae of the thorax and abdomen (Fig. 15–16), including the TS3 (Fig. 18) on the thoracic leg.

Cephalic A3 setae strikingly longer than A2 (Fig. 9), has not been previously described for the first instar of Geometridae. Thoracic and abdominal setae with longitudinal carinae and multi-pointed apex (Fig. 15–16) have been also described for the first instar of other members of Ennominae (Macariini: Blaik & Malkiewicz

2003; Boarmiini: Vargas & Parra 2013) and Larentiinae (Vargas et al. 2010). Depressed tarsal setae TS3 have been already described for the first instar of Larentiinae (Vargas et al. 2013); however, the distal margin saw-toothed, as found here for *P. obtusaria*, has been not previously reported.

The shape of the prothoracic dorsal shield also appears to be variable in Ennominae. It can be rectangle-like, as in *P. obtusaria* (Fig. 26), or as two plates separated by a membranous longitudinal stripe in the middle in Macariini (Blaik & Malkiewicz 2003) and Boarmiini (Vargas & Parra 2013). Furthermore, the number of setae on the prothoracic dorsal shield also appears to be variable in Ennominae: *P. obtusaria* has four pairs (XD and D groups; Fig. 26), while six setae (XD, D and SD groups) are found in Macariini (Blaik & Malkiewicz 2003), Boarmiini (Vargas & Parra 2013) and the Nearctic *Pero* (McGuffin 1963).

The shape of the anal shield of *P. obtusaria*, which is semicircular with a sharp anterior cleft that almost touches the posterior margin (Fig. 27), differs from the previously described patterns described for the first instar of other Geometridae. The anal shield is semicircular without a cleft in Macariini (Blaik & Malkiewicz 2003), while this shield is composed of two triangle-like plates separated by a longitudinal membranous stripe in Boarmiini (Vargas & Parra 2013).



FIGS. 20–29. First instar of *Pero obtusaria*. **20**) Head in frontal view. **21**) Head in lateral view. **22**) Larvum in frontal view. **23**) Internal surface of the larvum. **24**) Mandible. **25**) Chaetotaxy of the thorax and abdomen in lateral view. **26**) Prothoracic dorsal shield in dorsal view. **27**) Anal shield in dorsal view. **28**) Proleg of A6 in lateral view. **29**) Proleg of A10 in lateral view.

An increase in the number of some thoracic and abdominal setae following the first larval molt has been described for several species of Geometridae (e.g.: Parra & Henríquez-Rodríguez 1993, Blaik & Malkiewicz 2003, Vargas et al. 2010, Vargas & Parra 2013). However, this increase is remarkable in the SV group of A6 in the Nearctic species of *Pero*, reaching a few tens of setae on the external surface of the proleg of A6 of the last instars of some species (McGuffin 1963). Unfortunately, as the host plant of *P. obtusaria* was unknown when the specimens of this study were collected, we were unable to assess this character in the subsequent instars of this species. Accordingly, further studies would be required to characterize the external morphology of later instars and pupa of *P. obtusaria*. Meanwhile, we hope the descriptions and illustrations here provided for the egg and first instar of *P. obtusaria* will encourage similar studies involving additional Neotropical representatives of *Pero*, which will help us to reach a better understanding of the systematics of this highly diverse New World moth genus.

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