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Immature stages of *Temenis laothoe meridionalis* Ebert (Lepidoptera, Nymphalidae, Biblidinae)

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ABSTRACT

The external morphology and life cycle of the immature stages of *Temenis laothoe meridionalis* Hübner, [1819] (Lepidoptera, Nymphalidae, Biblidinae), associated with *Serjania laruotteana* (Sapindaceae) in Curitiba, Paraná, Brazil, are described and illustrated with photographs and images from scanning electron microscopy. Special aspects of the external morphology are compared to the immature stages of other species of Biblidinae.

Introduction

Temenis laothoe (Cramer, 1777) is a widely distributed species in the Neotropical region. Adults of the seven subspecies of *T. laothoe* (Lamas, 2004) have a striking variation in the presence of markings, the coloration and the shape of the wings, besides differences in the genitalia, including the *hypandrium* (L.M.G. Salik, personal observation). They are found mainly in the canopy (DeVries and Walla, 2001; Ribeiro and Freitas, 2012), being restricted to forest environments from sea level until 1500 m, and their larvae are associated to species of a number of genera of Sapindaceae, such as *Allophylus*, *Cardiospermum*, *Paullinia*, *Serjania* and *Urvillea* (Santin, 2004; Beccaloni et al., 2008).

Although immature stages and adults are common and easily found (Muyshondt, 1974b), there are few studies that contain detailed information on the external morphology of the immatures of species of *Temenis* Hûbner, [1819] (Müller, 1886; Muyshondt, 1974b; D'Almeida, 1922; Muyshondt, 2005; Teshirogi, 2007) and related genera, such as *Nica* Hübner, [1826] (Teshirogi, 2007) and *Epiphile* Doubleday, [1845] (Müller, 1886; Muyshondt, 1973a; Jenkins, 1986; Muyshondt, 2005). However, from the last decades, detailed morphological studies in other Biblidinae have presented potentially informative characters for the classification and systematics of the subfamily (e.g., Muyshondt, 2005; Teshirogi, 2007; Barbosa et al., 2010; Dias et al., 2012, 2014; Leite et al., 2012a,b, 2014; Salik et al., 2015; Nieves-Uribe et al., 2015, 2016).

Due to the importance of the study of immature stages and the morphological characters to better understand the phylogenetic relationships and taxonomy in Biblidinae, the current study describes and illustrates in detail the external morphology of the immatures of *T. laothoe meridionalis* Ebert, 1965 (Figs. 1, 2).

Material and methods

Eggs of *T. laothoe meridionalis*, found on the hostplant *Serjania laruotteana* (Sapindaceae) (Fig. 3), were collected in the Parque Municipal Barigui (25°25′ S, 49°18′ W, 910 m), Curitiba, Paraná, Brazil. Immatures were reared and studied in the laboratory. To slow the foliar dehydration process, the extremities of the host plant branches were covered with moist cotton, and the eggs were kept in Petri dishes placed in plastic containers with paper towels. Humidity was maintained daily and the leaves were changed every time there were signs of dehydration. After the eclosion, the larvae were transferred to rearing cages where they were periodically observed in search for behavioral changes and exuviae.

Scanning electron microscopy of the eggs, larvae and pupa was performed on a Jeol®model JSM – 6360LV Scanning Electron Microscope in the Centro de Microscopia Eletrônica of the Universidade Federal do Paraná (CEM-UFPR). Furthermore, the eggs and first instars were photographed with a digital camera set on a stereoscopic microscope, and further instars and pupa were recorded only with a DSLR Nikon d3100 digital camera. Larvae were killed through

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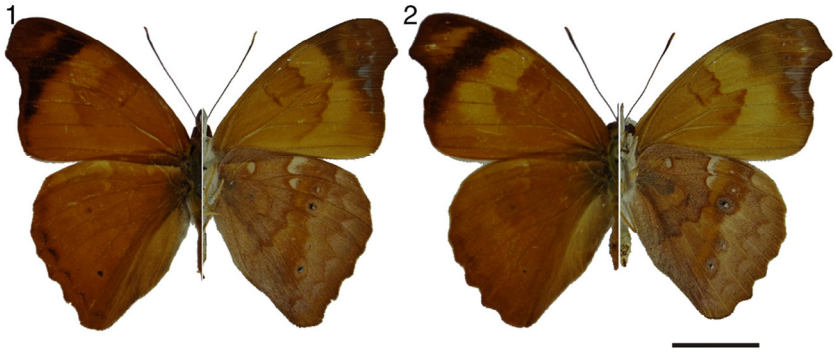


Figs. 1–2. *Temenis laothoe meridionalis*. Adult: 1, dorsal and ventral view of the male; 2, dorsal and ventral view of the female. Brasil, Paraná, Jaguariaíva, Parque Estadual do Cerrado, 800 m, 22.XI.2009. O. Mielke, E. Carneiro, F. Maia, A. Ribeiro & D. Dolibaina *leg.* DZ26760 (Male) and DZ26776 (Female). Scale = 1 cm.

immersion in hot water, fixed in Kahle-Dietrich 10% for 72 h, posteriorly preserved in ethanol 70% and then deposited in the Coleção de Imaturos de Lepidoptera da Coleção Entomológica Pe. Jesus Santiago Moure, Universidade Federal do Paraná, under batch number DZUPIL 0083.

The study of the chaetotaxy of the head capsule, thorax and abdomen of the 1st instar, the measurements and drawings of the head capsule of all instars were performed with the aid of a camera lucida set on a stereoscopic microscope with an ocular micrometer. Head capsules and pupal exuviae were kept dry and placed in glass tubes. Terminology followed Scoble (1992) for eggs, Hinton (1946), Peterson (1962), Stehr (1987), Dias (2006), and Huertas-Dionisio (2006) for larvae, and Mosher (1916), and Dias (2006) for pupae.

Host plants were identified by Dr. Jorge Bizarro (Reserva Ecológica de Guapiaçu – REGUA, Guapimirim, Rio de Janeiro State, Brazil). Immature and adult specimens were deposited in the Coleção Entomológica Pe. Jesus Santiago Moure, Universidade Federal do Paraná.

Results

Temenis laothoe meridionalis Ebert, 1965
(Figs. 1, 2, 4–41)

Egg (Figs. 4, 5, 21, 22, 25, 26)

Yellowish green, barrel-shaped, with nine vertical carinae, which are more expanded dorsally, and with dorsal aeropiles. Micropilar area concave, surrounded by a circular dorsally projected carina ("bottleneck" sensu Nieves-Uribe et al., 2015, 2016), connected to the nine vertical carinae.

Eggs oviposited singly on the underside of the folioles of *S. laruotellana*.

Average duration: 4 days (n = 33). Average diameter – 1.5 mm; average height – 1 mm.

Larva
First instar (Figs. 6–8, 23, 27–33)

Head capsule dark brown, round, smooth, with dark brown setae. Labrum bilobed. Mandibles strongly sclerotized. Stemmata in groups of six, located on the latero-inferior region of the epicranium, isometric, being 1–4 and 6 arranged in a semicircle and 5 positioned ventrally and close to the antennal base.

Body greenish brown with dorsal and subdorsal white markings; prothoracic plate trapezoidal; anal plate irregularly sclerotized; both dark brown. Thoracic and abdominal legs dark brown.

Larvae initially feed on the chorion, and then they moved to the apical portion of the leaves where they feed on the leaf blade. When inactive, the larvae rest on a perch constituted of silk and fecal pellets attached to the end of the midrib of a leaflet, occasionally on secondary veins. Often the pellets are adhered by the larvae to the larval body.

Average duration: 6 days (n = 23). Head capsule: average diameter – 1.05 mm; average height – 1.05 mm.

Head chaetotaxy (Figs. 23, 27–30)

21 pairs of primary setae on the head capsule:

Clypeal group (C): C1 smaller in relation to C2, positioned close to the latero-ventral region of the clypeus; C2 positioned close to the sagittal midline of the head.

Frontal group (F): F1 positioned close to the epicranial suture, the only one present in the frons; pore Fa latero-ventral to F1, close to the sagittal midline of the head.

Adfrontal group (AF): AF1 and AF2 of equal size, positioned in the adfrontal region, AF1 ventral to AF2; pore AFa at a small distance from AF2.

Anterior group (A): A1 longer than other setae, positioned on the latero-inferior region of the epicranium; between stemmata 4 and 5 and the clypeus; A2 dorso-lateral to A1, at level with stemma 3; A3 latero-dorsal to the stemmata region; pore Aa latero-ventral to A3.

Stemmatal group (S): S1 lateral to stemmata 2 and 3; S2 dorsal to stemma 1; S3 lateral to the semicircle of stemmata; pore Sa lateral to stemma 4 and Sb latero-dorsal to S3.

Substemmatal group (SS): SS1 lateral to SS2 and close to mouthparts; SS2 located between SS1 and SS3, and ventral to pore SSb; SS3 ventral to stemma 6 and smaller than the others.

Lateral group (L): L1 dorsal to S2; pore La latero-ventral to L1.

Postero-dorsal group (P): P1 between AF2 and P2; P2 latero-dorsal to P1 and of equal length; pore Pa close and lateral to A3; pore Pb ventral to P2.

Microdorsal group (MD): MD1 dorsal to MD2; MD2 dorsal to MD3; MD3 latero-dorsal to L1; pore MDa at the same level and between MD2 and MD1.

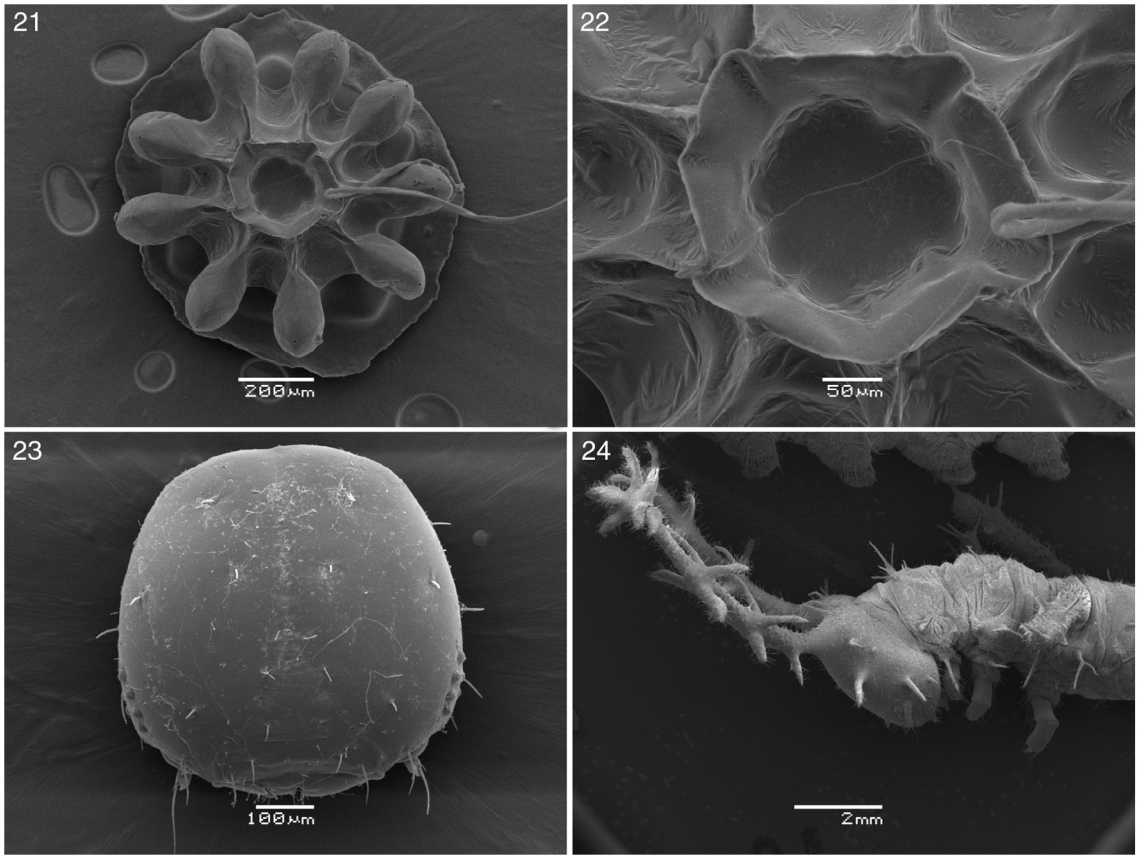
Microgenal group (MG): MG1 very small, close to postero-inferior margin of the epicranium and the occipital foramen; pore MGa latero-ventral to MG1 and latero-dorsal to S3.

Thorax chaetotaxy (Figs. 31, 32)

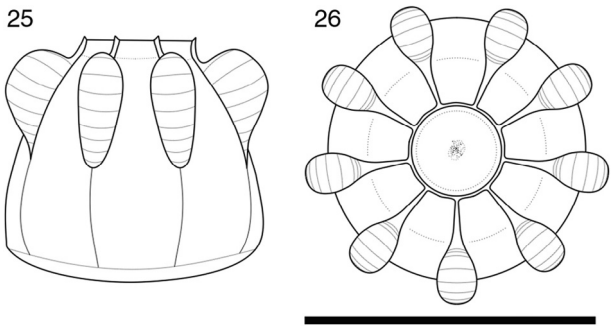
Prothorax: Ten pairs of acutely tipped setae. Dorsal Group (D): D1 and D2 positioned on the antero-dorsal margin of the pronotal

Figs. 3–16. *Temenis laothoe meridionalis.* 3, Host plant; 4–5, egg: 4, dorsal view; 5, lateral view; 6–16, immatures: 6, lateral view of 1st instar on perch; 7, dorsal view of 1st instar; 8, lateral view of 1st instar; 9, dorsal view of 2nd instar; 10, lateral view of 2nd instar; 11, dorsal view of 3rd instar; 12, lateral view of 3rd instar; 13, dorsal view of 4th instar; 14, lateral view of 4th instar; 15, dorsal view of 5th instar; 16, lateral view of 5th instar. Scale: egg – 0.25 cm, 1st to 5th instar – 1 cm.

Figs. 17–20. *Temenis laothoe meridionalis.* Pre-pupa: 17, dorsal view; pupa: 18, lateral view; 19, dorsal view; 20, ventral view. Scale: pre-pupa and pupa – 1 cm.



Figs. 21–24. *Temenis laothoe meridionalis*. 21–22, egg: 21, dorsal view; 22, micropilar area; 23–24, head capsule: 23, 1st instar, dorsal view; 24, 5th instar, lateral view.

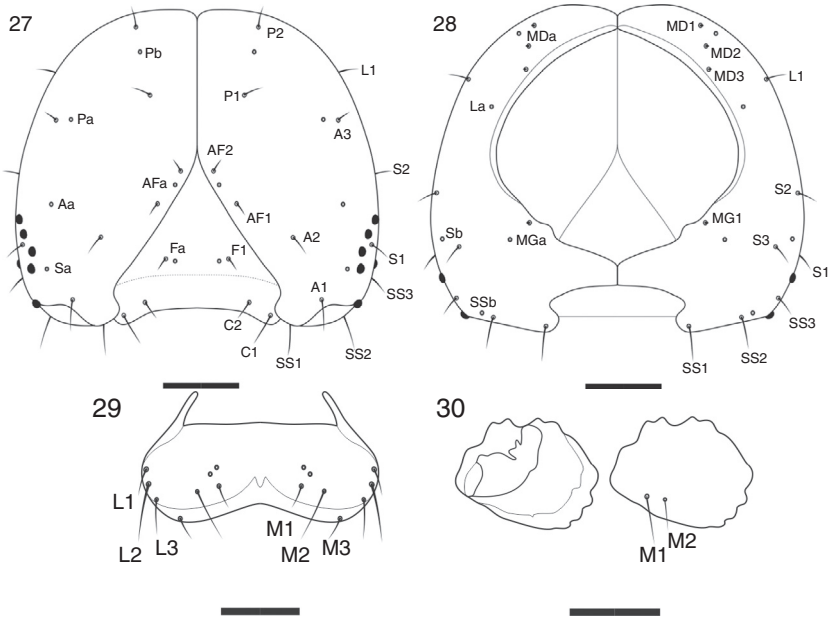


Figs. 25–26. *Temenis laothoe meridionalis.* Egg: 25, lateral view; 26, dorsal view. Scale = 1 mm.

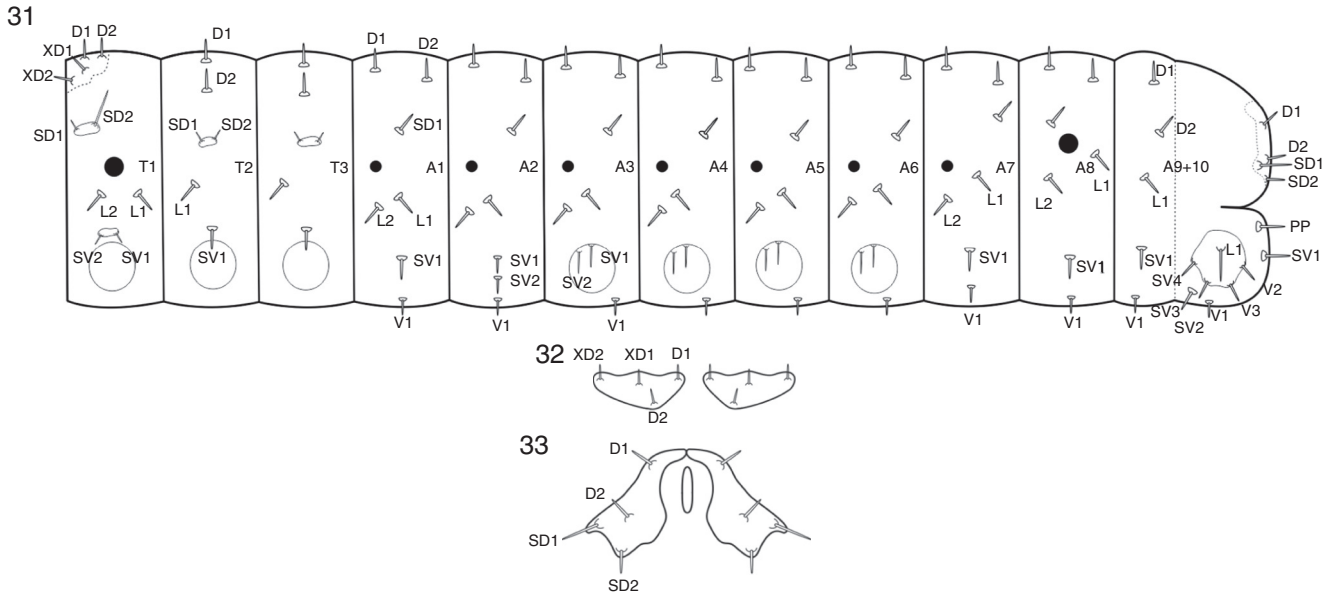
plate; D1 positioned on the antero-lateral region to D2; XD1 ventral to D1, and XD2 latero-ventral to XD1. Subdorsal Group (SD) ventral to pronotal plate, positioned on a wart, SD1 very small and antero-lateral to SD2. Lateral Group (L) ventral to Subdorsal Group, L1 postero-lateral to L2. Supraventral Group (SV) dorsal to prothoracic leg, positioned on a wart, SV1 smaller and postero-lateral to SV2.

Meso and Methatorax: Six pairs of acutely tipped setae. Dorsal Group (D): D1 dorsal to D2. Subdorsal Group (SD) ventral to D2, positioned on a wart, SD1 antero-lateral to SD2. Lateral Group (L): L1 antero-ventral to Subdorsal Group. Supraventral Group (SV): SV1 ventral to Lateral Group.

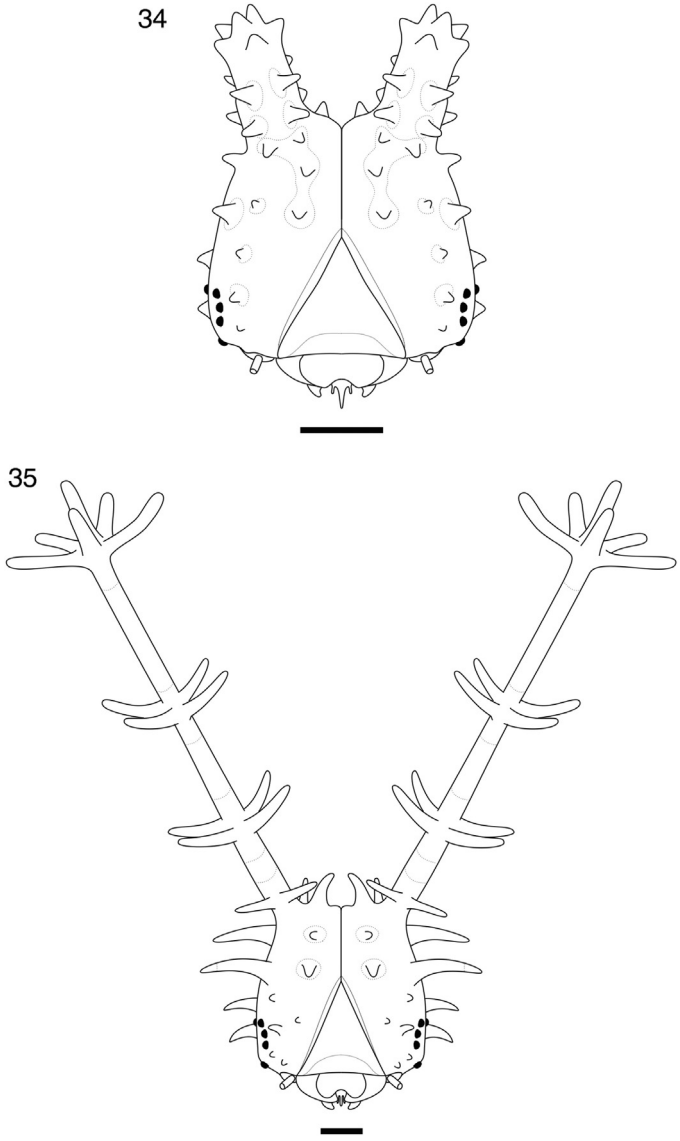
Abdomen chaetotaxy (Fig. 31, 33)



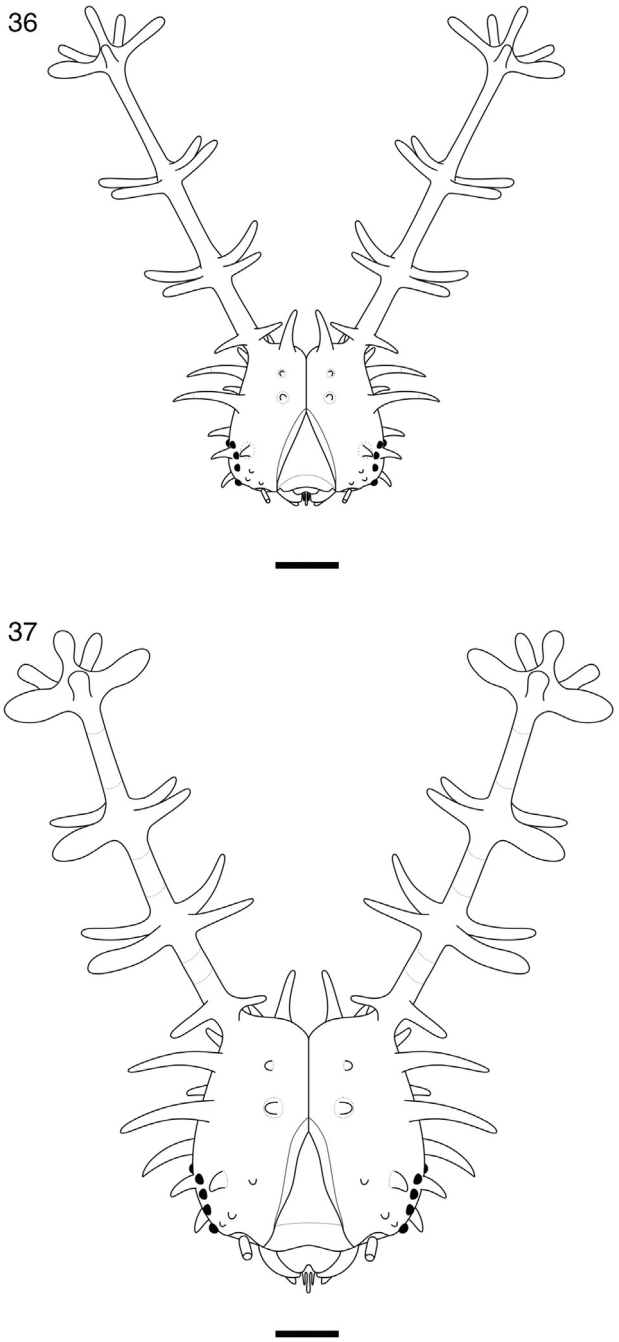
Figs. 27–30. *Temenis laothoe meridionalis.* 1st instar, head chaetotaxy: 27, frontal view; 28, posterior view; 29, labrum; 30, mandible. Scale = 0.05 mm.



Figs. 31–33. *Temenis laothoe meridionalisi* 1⁺ instar: 31, thorax and abdomen chaetotaxy; 32, pronotal plate chaetotaxy; 33, anal plate chaetotaxy.



Figs. 34–35. *Temenis laothoe meridionalis*, 2nd and 3rd instar: head capsule: 34, frontal view of the 2nd instar; 35, frontal view of the 3rd instar. Scale = 0.25 mm.



Figs. 36–37. *Temenis laothoe meridionalis*, 4th and 5th instar: head capsules: 36, frontal view of the 4th instar; 37, frontal view of the 5th instar. Scale = 0.5 mm.

A1: Seven pairs of setae. D1 antero-dorsal to D2. SD1 ventral to Dorsal Group. L1 postero-ventral to spiracle; L2 antero-ventral to L1. SV1 ventral to Lateral Group. V1 ventral to SV1.

A2: Eight pairs of setae, with similar positions to A1, except for the presence of SV2 ventral to SV1; V1 ventral to SV2 and with similar size.

A3–A6: Eight pairs of setae, with similar positions to A2, except for the position of subventral setae.

A7: Seven pairs of setae, with positions similar to A1, except for the closer proximity of L1 and L2 to the spiracle.

A8: Seven pairs of setae, with disposition similar to A7, except for the closer proximity of L1 and L2 to the dorsally displaced spiracle, SD1 ventral to D1, and V1 with position similar to A3–A6.

A9 + A10: Eighteen pairs of setae. On the A9 region: D1 antero-dorsal to D2; L1 antero-ventral to D2; SV1 ventral to L1; V1 antero-ventral to SV1. On the A10 region: D1 dorsal to D2; SD1 antero-ventral to D2 and closer to the anterior margin of the anal plate in comparison to the remaining setae; SD2 postero-ventral to SD1. On the A10 postero-ventral region, PP dorsal to SV1; L1, L2, L3, SV2 and SV3 positioned on the ocrea, being L1 dorsal to the remaining setae; L2 and L3 on the posterior region, and SV2 and SV4 on the anterior margin of the wart; SV3 ventral to SV4 and V1 ventral to SV1.

Second instar (Figs. 9, 10, 34)

Head capsule dark brown, region of the stemmata, labrum and mandible pale brown, with a pair of truncated scoli. Position of the stemmata similar to first instar. Body dark brown, with small whitish brown scoli. In this instar the larvae remain on a 'perch' that may be built on the same foliole where the larva is feeding or in another foliole of the same leaf.

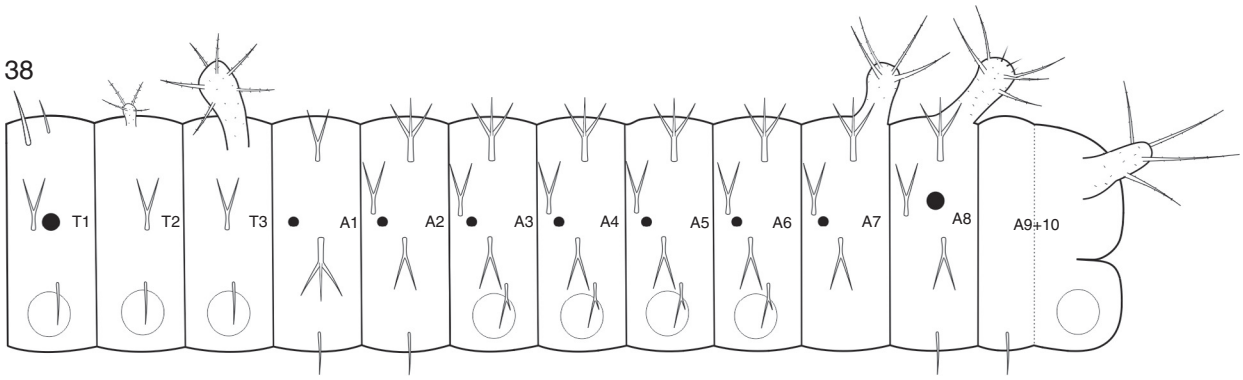
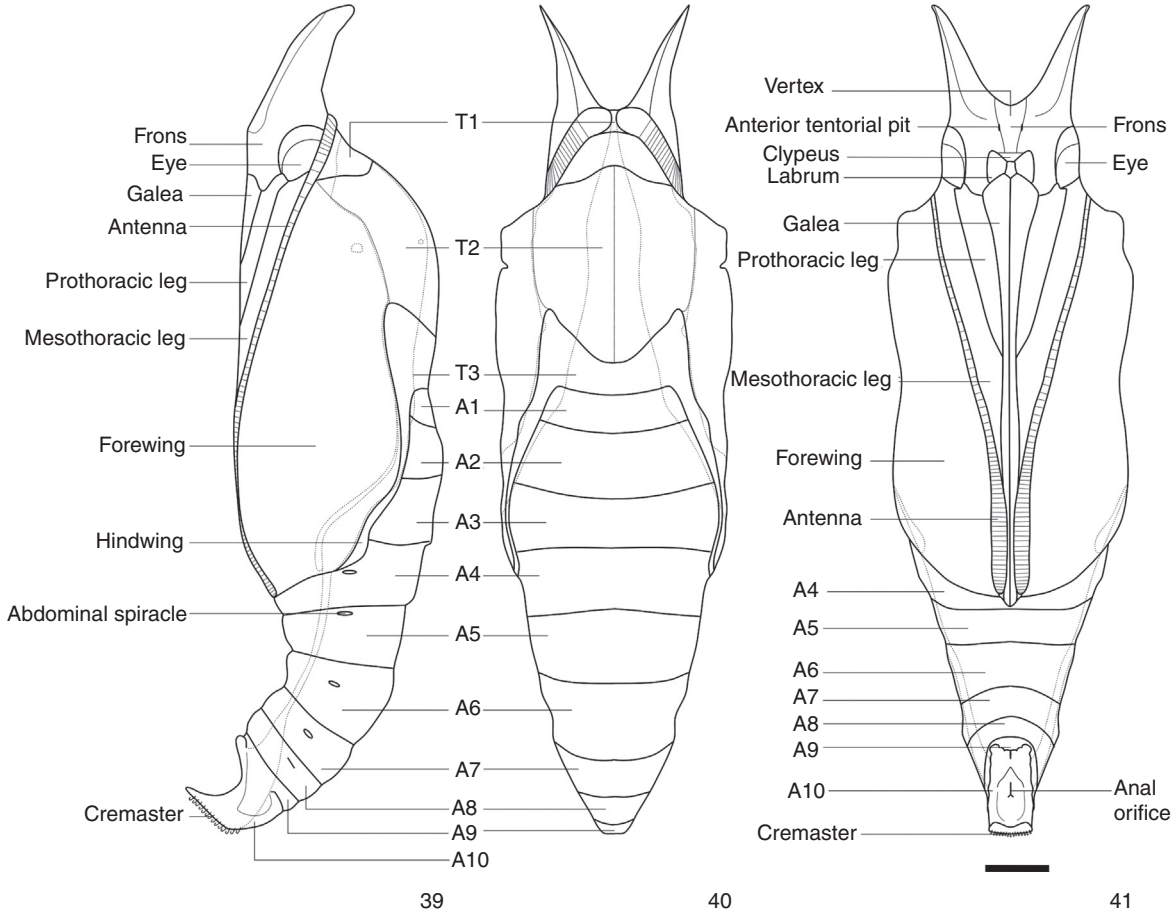


Fig. 38. *Temenis laothoe meridionalis*, 5th instar: thorax and abdomen.



Figs. 39–41. *Temenis laothoe meridionalis*. Pupa: 39, lateral view; 40, dorsal view; 41, ventral view. Scale = 1 cm.

Average duration: 6 days (n = 23). Head capsule: average diameter – 1.4 mm; average height – 1.45 mm.

Third instar (Figs 11, 12, 35)

Head capsule dark brown, with developed scoli around 2.5 times the height of the head, with 4 series of 2, 4, 4 and 5 spines from base to apex; body alternating between dark and pale brown on the thoracic and abdominal segments; scoli large, with metallic blue base on the subdorsal and dorsal scoli of T2, T3, A3, A5, A7 and A8; scoli with clavate projections on T3, A7 and A8.

Average duration: 7 days (n = 24). Head capsule: average diameter – 2.05 mm; average height – 2.3 mm.

Fourth instar (Figs 13, 14, 36)

Similar to the third, but larger. Color pattern similar to the previous instar, but more clearly noticeable.

Average duration: 7 days (n = 25). Head capsule: average diameter – 3.1 mm; average height – 3.4 mm.

Fifth instar (Figs 15, 16, 24, 37, 38)

Head capsule similar to the fourth instar. Body dark brown, dorsal and subdorsal areas mostly green with dark brown markings in T1–T2, T3, A3, A5, A7 and A8; lateral area with pale green diagonal

stripes; body with subdorsal and lateral scoli; T3 subdorsal scoli clavate and projected, around half the length of the scoli from the head capsule; A7 and A8 single dorsal scoli clavate and projected, around 2/3 the length of the clavate projections of T3; A9 subdorsal scoli large, yellowish brown.

From the third to the fifth instar, when resting, the head capsule stays parallel to the substrate.

Average duration: 14 days ($n = 22$). Head capsule: average diameter – 4 mm; average height – 4.3 mm.

Pupa (Figs. 17–20, 39–41)

Pupa mainly pale green, encircled by a pale yellowish green stripe from the vertex until A3, with whitish markings throughout the dorsum; the dark green lateral band on the prothorax, mesothorax, and metathorax narrows between A4–A8 and A9 + 10; hind wings dark green; irregular whitish markings mixed with pale and dark brown on T1, on the margin of the anterior wings and laterally on A4–A8; thoracic and abdominal spiracles dark brown.

Ventral region mainly pale green, with reticulate dark green markings; with three whitish markings surrounded by pale brown on the frons; prothoracic leg with an anterior whitish spot and medial dark brown spot; antenna pale yellow; A4–A8 with a narrow pale brown stripe surrounded by yellowish green medially, and two dark brown spots in each segment; dark green on A9 + 10, and dark green and brown on the cremaster.

Vertex with a pair of projections, being half the extension of the prothoracic leg; frons smooth; eye oval and positioned laterally on head; tentorial fovea visible; mandible trapezoidal, labium pentagonal and between the mandibles; galea extending from distal margin of the mandibles and labium until the proximal margin of A4.

Prothorax subtriangular with proximal region rounded; mesothorax projected dorsally, concave on mesonotum; meso and metathoracic spiracles visible dorsally and laterally; prothoracic legs 2/3 of the length of the mesothoracic ones; metathorax with strongly convex antero-medial margin.

Abdomen conical, moving laterally when disturbed; abdominal spiracles ellipsoidal; spiracle A1 not visible, A2 and A3 dorsal, A4–A8 lateral.

Cremaster bilobed, directed ventrally, with bifid hooks on its extremity.

Average duration: 13 days ($n = 19$). Average length from the vertex to the cremaster – 17.5 mm.

Discussion

Species of *Temenis* Hübner, [1819], *Nica* Hübner, [1826], *Epiphile* E. Doubleday, [1845], *Pyrrhogyra* Hübner, [1819], *Haematera* Doubleday, 1849, *Diaethria* Billberg, 1820 and *Callicore* Hübner, [1819] are chiefly associated to some genera of Sapindaceae, the most common being *Serjania*, *Allophylus*, *Cardiospermun*, *Paullinia*, and *Urvillea*. Associations with other plant families have also been reported, but less frequently and/or in need of confirmation, such as species of *Stimaphyllon* (Malpighiaceae) (Beccaloni et al., 2008), *Celtis* and *Trema* (Ulmaceae) (Brown, 1992; Pastrana, 2004; Santin, 2004; Beccaloni et al., 2008; Barbosa et al., 2010).

The borders of the study area have a great number of *Serjania* and *Allophylus* species in different stages of development, where there often are eggs and larvae of species of *Temenis*, *Epiphile*, *Callicore* and *Diaethria*. However, *T. laothoe meridionalis* was found only on *Serjania laruotteana* in the area, while the remaining species of Biblidinae used other Sapindaceae as hostplants. Based on the hostplant choices observed, and current literature, it is possible to infer that these hostplant preference patterns depend on local factors, probably related to the presence of different hostplant species as well as the presence and interactions with other species of Biblidinae that might use the same food resource (Beccaloni et al., 2008). Literature further reports *Serjania meridionalis*, *Serjania multiflora* and *Paullinia seminuda* as hostplants of *Temenis laothoe* throughout its wide geographical distribution (Beccaloni et al., 2008).

Eggs of Biblidinae are characterized by three distinct shapes: acicular, globose and with carinae (Nieves-Uribe et al., 2015, 2016). Acicular eggs are shared with species of Biblidini: Biblidina and Eurytelina (Bauzá, 2000; Teshirogi, 2007; Freitas and Brown, 2008; Nieves-Uribe et al., 2015); globose eggs are characteristic of species of Biblidini and Ageroniina of the genera *Hamadryas* Hübner, [1806] and *Ectima* E. Doubleday, [1848] (Young, 1974; Muyshondt and Muyshondt, 1975a,b; Leite et al., 2012b, 2014; Nieves-Uribe et al., 2015, 2016; Salik et al., 2015), which were also called "eggs with irregular aspect" for not bearing distinct carinae on the chorium (Leite et al., 2012b, 2014; Nieves-Uribe et al., 2015, 2016); eggs with carinae are found on the remaining Biblidinae species (Nieves-Uribe et al., 2015, 2016). Although the eggs of the species of Biblidini: Epiphilina present a "truncated cone" shape (Muyshondt, 1973a,b; Muyshondt, 1974a,b), it has been observed that this character varies between the species of the genera of this subtribe and also intraspecifically (Müller, 1886; Freitas and Oliveira, 1992; Otero, 1994; Teshirogi, 2007; Dias et al., 2014). Furthermore, eggs of *Temenis* species are more flattened when compared to other Epiphilina and the number of carinae varies in the subtribe (Nieves-Uribe et al., 2015, 2016). *Temenis laothoe hondurensis* Fruhstorfer, 1907, *Nica flavilla* (Godart, [1824]) and *Asterope degandii* (Hewitson, 1858) share a "bottleneck" in the region next to the micropilar area of the chorium, being an exclusive character of this genera (Nieves-Uribe et al., 2015, 2016) and also found in *T. laothoe meridionalis*. These morphological data are potentially informative in taxonomic and phylogenetic studies.

The head capsule presents scoli starting from the second instar, a character widely distributed in Biblidinae (Freitas et al., 1997; Freitas and Brown, 2004), although absent in *Sea sophoronia* (Godart, [1824]) (Otero, 1994) and some species of *Dynamine* Hübner, [1819] (Müller, 1886; Leite et al., 2014). The spines of the head capsule scoli are not acutely tipped as most Biblidinae, including *Nica* species, but somewhat bulbous. *Nica*, considered the sister genus of *Temenis* (Freitas and Brown, 2004), shares with *T. laothoe* the presence of similar clavate and projected subdorsal scoli on T3, and color pattern (Müller, 1886; Muyshondt, 1973b, 1974b; Teshirogi, 2007). Those lateral clavate and projected subdorsal scoli can be viewed as a putative synapomorphy for that clade, given that in other genera of Biblidinae, the scoli in this segment are neither laterally projected nor clavate, or entirely absent (Teshirogi, 2007; Leite et al., 2012a,b, 2014; Dias et al., 2012, 2014; Janzen and Hallwachs, 2015).

According to Leite et al. (2012b), the vertical projections present different sizes and shapes in pupae of Biblidinae. *Ectima ericinoides* C. Felder and R. Felder, 1867 (Janzen and Hallwachs, 2015) and some species of *Hamadryas* (Young, 1974; Muyshondt and Muyshondt, 1975a,b,c; Teshirogi, 2007; Leite et al., 2012b; Salik et al., 2015), for example, have very long and leaf-shaped projections. In contrast, these projections are similarly laterally flattened, but not leaf-shaped and reduced in *T. laothoe meridionalis*, and even more in *Nica* species (Muyshondt, 1973b) and the remaining species of Epiphilina.

It is noteworthy that the similarity between the morphological characters found in the current study and those in *Nica* give further support to the current phylogenetic hypotheses involving these two taxa (Freitas and Brown, 2004; Wahlberg et al., 2009). Thus, studies on immature stages, involving morphological and natural history aspects, are paramount for elucidating the taxonomy and phylogeny of Biblidinae as a whole.

Conflicts of interest

The authors declare no conflicts of interest.

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