

External morphology of the immature stages of Neotropical heliconians: *X. Heliconius sara apseudes* (Lepidoptera, Nymphalidae, Heliconiinae)

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ABSTRACT. The biology and morphology of the immature stages of *Heliconius sara apseudes* (Hübner, [1813]) are still little known. External features of the egg, larvae and pupa of *H. sara apseudes* are described and illustrated, based upon light and scanning electron microscopy. Eggs with smooth carina, first instar larva with scaly setae, and body of second to fifth instars covered with scattered pinnacles distinguish *H. sara apseudes* from other heliconiine species.

KEYWORDS. Passion vine butterflies, egg, larva, pupa, tegumentary ultrastructure.

RESUMO. Morfologia externa dos estágios imaturos de heliconíneos Neotropicais: *X. Heliconius sara apseudes* (Lepidoptera, Nymphalidae, Heliconiinae). A biologia e morfologia de estágios imaturos de *Heliconius sara apseudes* (Hübner, [1813]) ainda são pouco conhecidos. Características da morfologia externa dos ovos, larvas e pupa de *H. sara apseudes* são descritas e ilustradas, utilizando-se microscopias óptica e eletrônica de varredura. Ovos com carenas lisas, larvas de primeiro instar com cerdas contendo projeções escamiformes e larvas do segundo ao quinto instares com corpo apresentando pináculos dispersas são características distintivas de *H. sara apseudes* em relação às demais espécies de heliconíneos.

PALAVRAS-CHAVE. borboletas do maracujá, ovo, larva, pupa, ultraestrutura tegumentar.

Within the genera *Heliconius* Kluk, 1780, *Eueides* Hübner, 1816 and *Neruda* Turner, 1976, *Heliconius sara* (Fabricius, 1793) is one of the few species that has bluish-black fore- and hind wings with either yellow or white stripes. This species is included within a clade commonly called *sara/sapho*, which is comprised of 12 species (BELTRÁN *et al.*, 2007; KOZAK *et al.*, 2015). Most species within this clade show the same wing color patterning (HOLZINGER & HOLZINGER, 1994), except *H. demeter* Staudinger, 1897. They have gregarious larval habits (exceptions are *H. demeter*, *H. charitonia* (Linnaeus, 1767), and *H. peruvianus* C. Felder & R. Felder, 1859; BELTRÁN *et al.*, 2007) and all show pupal-mating behavior (BELTRÁN *et al.*, 2007).

With a broad distribution in Central and South America, most subspecies of *H. sara* occur in the Amazonian region (ROSSER *et al.*, 2012). *Heliconius sara apseudes* (Hübner, [1813]) is the only subspecies found throughout the South American Atlantic Forest (EMSLEY, 1963; HOLZINGER & HOLZINGER, 1994; ROSSER *et al.*, 2012). Recently, CARDOSO (2005) and ISERHARD *et al.* (2010) recorded new localities of *H. sara apseudes*, extending its northern and southern distributions to states of Bahia and Rio Grande do Sul, respectively.

Heliconius sara lays egg clusters at the branch apex of passion-vines (Passifloraceae) and larvae are semi-gregarious. Five species of host plants are recorded for *H. sara apseudes* in southeastern Brazil (BECCALONI *et al.*, 2008): *Passiflora edulis* Sims, *P. pentagona* Masters, *P. rhamnifolia* Masters [= *P. sidaefolia* (M. Roemer)], and *P.*

ovalis Vell. ex M. Roem. Additional field observations made by us indicate that this subspecies also uses *P. truncata* Regel and *P. haematostigma* Masters in Santa Catarina and Paraná states, respectively.

Even though resources exist to enable species identification at early stages and to elucidate taxonomic and phylogenetic relationships (e.g. PENZ, 1999), the natural history and external tegumentary ultrastructure of immature stages of species of the *sara/sapho* clade are still poorly documented. Descriptions of immature stages of *H. sara* are available only at the gross-morphology level for the subspecies occurring in Trinidad and Tobago (BEEBE *et al.*, 1960; FLEMING, 1960), and few species diagnostic characteristics have been supplied by BROWN (1981).

As part of a series of articles focusing on the external morphology of the immature stages of Neotropical heliconiines (ANTUNES *et al.*, 2002; KAMINSKI *et al.*, 2002, 2008; TAVARES *et al.*, 2002; PAIM *et al.*, 2004; SILVA *et al.*, 2006, 2008; BARÃO & MOREIRA, 2010; VARGAS *et al.*, 2014), here we describe and illustrate in detail the early stages of *H. sara apseudes* based on light and scanning electron microscopy.

MATERIAL AND METHODS

Eggs and larvae were collected from leaves of *P. haematostigma* in Quatro Barras municipality, Paraná, Brazil. Eggs were placed on moist filter paper in Petri dishes for incubation. After hatching, larvae were grown on branches of *P. haematostigma*, kept in plastic bottles

equipped with a wire support. The bottles were filled with tap water, covered with a fine mesh, and kept under laboratory conditions ($25 \pm 1^\circ\text{C}$, 14 h light/day). Head capsules were collected after each ecdysis, fixed in Dietrich's fluid, and preserved in 70% ethanol. Additional eggs, larvae and pupae were also similarly fixed and preserved. The examined material was deposited in the collection of the Laboratório de Morfologia e Comportamento de Insetos (LMCI), of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil, under accession numbers 132 and 136.

Aspects of general morphology of immature stages were analyzed from material preserved in ethanol 70%. Head capsules were rehydrated, cleared in a 10% potassium hydroxide solution (KOH), and slide-mounted in glycerin jelly. Drawings were made from preserved specimens, using a reticulated ocular attached to a Leica® M125. The tegumentary ultrastructure was studied at the Centro de Microscopia Eletrônica (CME) of UFRGS. Specimens were dehydrated in a Bal-Tec® CPD 030 critical-point dryer, mounted with double-sided tape on metal stubs, coated with gold in a Bal-Tec® SCD 050 sputter coater, and analyzed on a JEOL® JSM-6060 scanning electron microscope.

To distinguish larval instars, the greatest width of the head capsule in frontal view was measured with a micrometer scale mounted in the ocular of the stereomicroscope. By using the least-squares method, the resulting data were adjusted to the power function $y = ae^{bx}$ (SNEDECOR & COCHRAN, 1980).

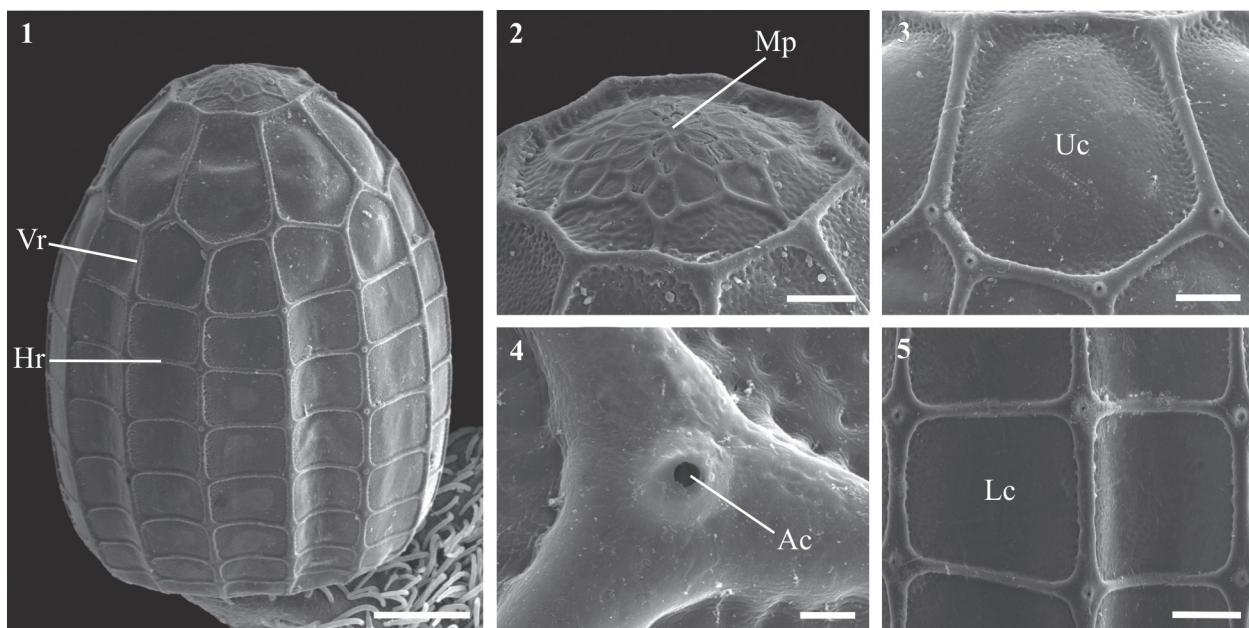
The nomenclature of DELL'ERBA *et al.* (2005) is used for eggs, PETERSON (1962) for larval body regions, STEHR

(1987) for primary chaetotaxy and crochet of prolegs, KITCHING (1984) for the setae of the paraproct and of the anal prolegs, BEEBE *et al.* (1960) for the scoli, and MOSHER (1916) for the pupa.

RESULTS AND DISCUSSION

Egg (Figs 1-5, 32). Barrel-shaped, base flat, apex narrower than base (Fig. 1). Dimensions (mean \pm standard error; $n = 10$): diameter = 0.779 ± 0.010 mm; height = 1.004 ± 0.017 mm. These heights are slightly larger than those found by BEEBE *et al.* (1960) and BROWN (1981). Yellow (Fig. 32) throughout embryonic development, differing from BEEBE *et al.* (1960), who observed varying shades of pale yellow and orangish-yellow.

Chorion ornamented with vertical (Vr) and horizontal (Hr) smooth carinae (Fig. 1), differing from *H. erato phyllis* (Fabricius, 1775), *H. ethilla narcaea* Godart, 1819, and *H. besckei* Ménétrés, 1857, which have rugose carinae (DELL'ERBA *et al.*, 2005). Vertical carinae twice as thick as horizontal ones (Fig. 5). Carina number varying from 13 to 14 Vr and from 8 to 10 Hr. BEEBE *et al.* (1960) and BROWN (1981) found slightly lower values for Hr (6-8 and 7, respectively). Intersections of vertical and horizontal carinae delimit cells; surface of cells rugose close to carinae and smooth medially (Fig. 3). Lower cells (Lc; Fig. 5) tetragonal; upper cells (Uc; Fig. 3) varying from tetragonal to hexagonal. Apical Hr border the micropylar region (Fig. 2), but do not project over it as found in *H. erato phyllis* (KAMINSKI *et al.*, 2002). Micropylar region ornamented with polygonal, rugose cells peripherally, forming the annulus, and smooth cells centrally, forming the rosette, in the center of which are the micropyles (Mp; Fig. 2).



Figs 1–5. Scanning electron micrographs of the egg of *Heliconius sara apseudes* (Hübner, [1813]). 1, lateral view; 2, micropylar region; 3, upper cells; 4, aeropyle; 5, lower cells (Ac, aeropyle; Hr, horizontal carina; Lc, lower cell; Mp, micropyles; Uc, upper cell; Vr, vertical carina). Bars = 200, 100, 100, 10, and 100 mm, respectively.

Fig. 2; DOWNEY & ALLYN, 1981). Circular aeropyles with slightly elevated peritreme at carina intersections (Ac; Fig. 4), similar to all *Heliconius* species cited above.

Eggs of *H. sara apseudes* can be distinguished from those of *Agraulis* Boisduval & Le Conte, [1835], *Dione* Hübner, [1819], *Dryadula* Michener, 1942, *Dryas* Hübner, [1807], *Eueides* and *Philaethria* Billberg, 1820 studied by DELL'ERBA *et al.* (2005) by having the surface of cells rugose close to the carinae and smooth medially. From *Heliconius* species also studied by these authors, *H. sara apseudes* is distinguishable by its smooth carinae.

First-instar larva (Figs 6-9, 11-20, 34). Head dark-brown with blackish stripe basally, stemmatal region blackish. Thorax and abdomen yellow; prothoracic dorsal shield, anal shield, pinnacles, legs, and lateral plates of prolegs brown; setae black, unpigmented apically (Fig. 34). Coloration of first-instar larva of *Heliconius sara apseudes* differs markedly from other species of *Heliconius*: light-colored head, plates, and pinnacles have been recorded in *H. besckei*, *H. erato phyllis* and *H. ethilla narcaea*.

Head and mouthparts (Figs 6-7, 13) of first-instar larva following morphological pattern of Lepidoptera (STEHR, 1987). Frons and clypeus fused, frontoclypeal suture inconspicuous (Fig. 6); anteclypeus as a straight transverse stripe between frontoclypeus and labrum; labrum ample, bilobed, partially covering mandibles in frontal view; antennal pocket smooth, lateral to mouthparts; antennae trisegmented (Fig. 12); six stemmata dorsal to antennal pocket, stemmata 1-5 in semicircle, stemma 6 slightly posterior to imaginary line from stemmata 1 to 5, stemmata 1 to 4 equidistant from each other (Figs 7, 11).

Setae of chalaza type, clavate apically; setae covered with scale-like projections (Figs 15, 16). On head (Figs 6, 7), pairs of setae, microsetae, and pores are clustered in ten groups. Adfrontal (AF) group: setae AF1 and AF2 close to superior angle of frontoclypeus; AFa pore between AF setae, closer to AF2. Anterior (A) group: A1 anterior to stemma 1; A2 and A3 dorsal to stemmata; Aa pore close to stemmata 2 and 3. Clypeal (C) group: bisetose; setae near ventral margin of frontoclypeus; C2 closer to adfrontal suture. Cephalo-dorsal (CD) group: CD1, CD2 and CD3 microsetae posterior to P2; CDa pore between CD1 and CD2. Frontal (F) group: F1 slightly displaced from midline toward ventral half of frontoclypeus; Fa pore dorsally to F1. Lateral (L) group: L1 about midway between P2 and S2; La pore posterior to L1. Microgenal (MG) group: MG1 a microseta; MGa anterodorsal to MG1. Posterodorsal (P) group: P2 dorsal to P1, both same size; Pa pore ventral to P1 and Pb pore between P1 and P2. Stemmatal (S) group: S1 in center of stemmatal area; S2 and S3 posterior to stemmata; Sa pore posterior to stemmata; Sb pore anterior to stemma 4. Substemmatal (SS) group: SS2 level with stemma 5; SS1 and SS3 ventral to stemmata; SSa pore ventral to stemma 6; SSb pore ventral to stemma 5.

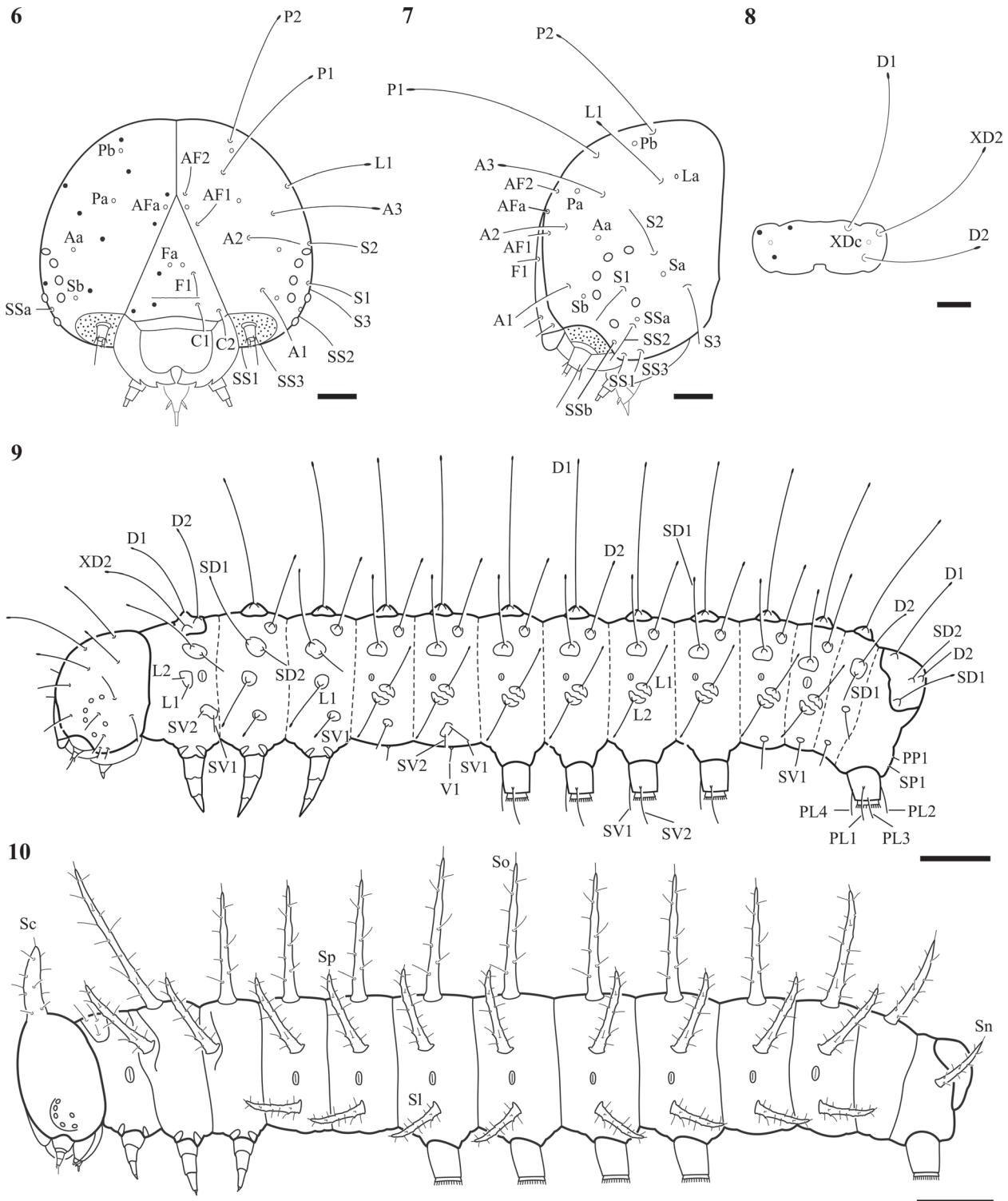
Thorax and abdomen bear ten groups of setae (Figs 8, 9): prothoracic shield (XD), dorsal (D), subdorsal (SD), lateral (L), subventral (SV), ventral (V), paraproctal (PP),

subparaproctal (SP), tarsal (Ts) and proleg of tenth segment (PL). Pores and microsetae occur scattered over tegument (KITCHING, 1984), but are omitted here. On prothorax, prothoracic shield setae D1, D2, and XD2 (Fig. 8), D1 and D2 dorsally to XD2. SD setae on same pinnacle, SD1 anterior to and longer than SD2. L1 and L2 of same size, both on same pinnacle, anterior to spiracle; L1 at least half size of SD2, contrary to description by BEEBE *et al.* (1960) for another subspecies of *H. sara*, where L1 is considerably larger than SD2. SV on same pinnacle; SV1 larger than and ventral to SV2. On mesothorax (T2), D1 and D2 setae on separate pinnacles; D1 larger than and dorsal to D2. SD1 and SD2 on same pinnacle; SD1 larger than and anterior to SD2. Groups L and S each with one seta; L1 as long as SD1; SV1 shorter than SD2. Legs with tibial and tarsal setae Ts1, Ts2, Ts3, and Ts4 (*sensu* MILLER, 1991); Ts4 longer than remaining tarsal setae, resembling tarsal claw (Ga, Fig. 14). Metathoracic (T3) setae similar in composition and arrangement to setae of T2.

Dorsal, subdorsal and lateral setae similar from first to eighth abdominal segments. D1 dorsal and anterior to D2. SD1 ventral to D2 and aligned with D1. Lateral group with two setae, each on semicircular pinnacle; L1 posterodorsal to L2. SV1 on AB1-9 and SV2 on AB2-6; on AB2, SV1 and SV2 share same pinnacle; on AB3-6, SV1 and SV2 on proleg shield. Small and slender V1 on AB2. On AB9, pinnacles of D1 fused, visible in dorsal view; D2 and SD1 sharing same pinnacle, D2 dorsal to SD1; L1 as long as SD1. On AB10, dorsal and subdorsal setae on anal shield; D1 longer than and dorsal to D2, SD1 and SD2; paraproctal seta, PP1 (*sensu* STEHR, 1987), and subparaproctal seta, SP1 (*sensu* KITCHING, 1984), are shorter and sharp, in relation to remaining setae on AB10; proleg with five setae, PL1 to PL4 visible in lateral view, PL5 visible only in ventral view, PL1 and PL2 longer than remaining PL setae.

Integument mostly smooth; ventral area, legs, prolegs, and paraproct provided with conical microtrichia (Fig. 17); those on paraproct elongate (Fig. 18). Spiracle round, peritreme elevated, present on prothorax and from AB1 to AB8 (Fig. 19). On base of prolegs, uniordinal and uniserical crochets arranged in circles (Fig. 20).

The gross morphology and chaetotaxy of the first instar of *H. sara apseudes* fit the general pattern described for Heliconiini (ANTUNES *et al.*, 2002; KAMINSKI *et al.*, 2002, 2008; TAVARES *et al.*, 2002; PAIM *et al.*, 2004; SILVA *et al.*, 2006, 2008; BARÃO & MOREIRA, 2010; VARGAS *et al.*, 2014). Several characteristics make it feasible to identify the first-instar larva of *H. sara apseudes*. First, the smooth antennal pocket is similar to the antennal pocket of *H. erato phyllis* (KAMINSKI *et al.*, 2002), but differs from the rugose antennal pocket found in other species (TAVARES *et al.*, 2002; PAIM *et al.*, 2004; SILVA *et al.*, 2006, 2008; KAMINSKI *et al.*, 2008). Second, the scale-like projections along the setae of *H. sara apseudes* are unique among the heliconiine species studied (e.g., KAMINSKI *et al.*, 2002, 2008; SILVA *et al.*, 2006, 2008; BARÃO & MOREIRA, 2010;



Figs 6–10. Head capsule, prothoracic plate and first and fifth instar larvae of *Heliconius sara apseudes* (Hübner, [1813]). 6, frontal view of first instar head capsule; 7, lateral view of first instar head capsule; 8, dorsal view of first instar prothoracic plate; 9, lateral view of first instar; 10, lateral view of fifth instar (A, anterior seta; Aa, anterior pore; AF, adfrontal seta; AFa, adfrontal pore; C, clypeal seta; D, dorsal seta; F, frontal seta; Fa, frontal pore; L, lateral seta; P, posterodorsal seta; Pa and Pb, posterodorsal pores; PL, seta of proleg on cylindrical section of tenth abdominal segment; PP, paraproctal seta; S, stemmatal seta; Sa and Sb, stemmatal pores; Sc, cephalic scolus; SD, subdorsal seta; S1, subspiracular scolus; Sn, anal scolus; So, dorsal scolus; SP, subparaproctal seta; Sp, supraspiracular scolus; SS, substemmatal seta; SSa and SSb, substemmatal pores; SV, subventral seta; V, ventral seta; XD, prothoracic seta; XDc, prothoracic pore). Bars = 100, 100, 100, 250, and 2500 μ m, respectively.

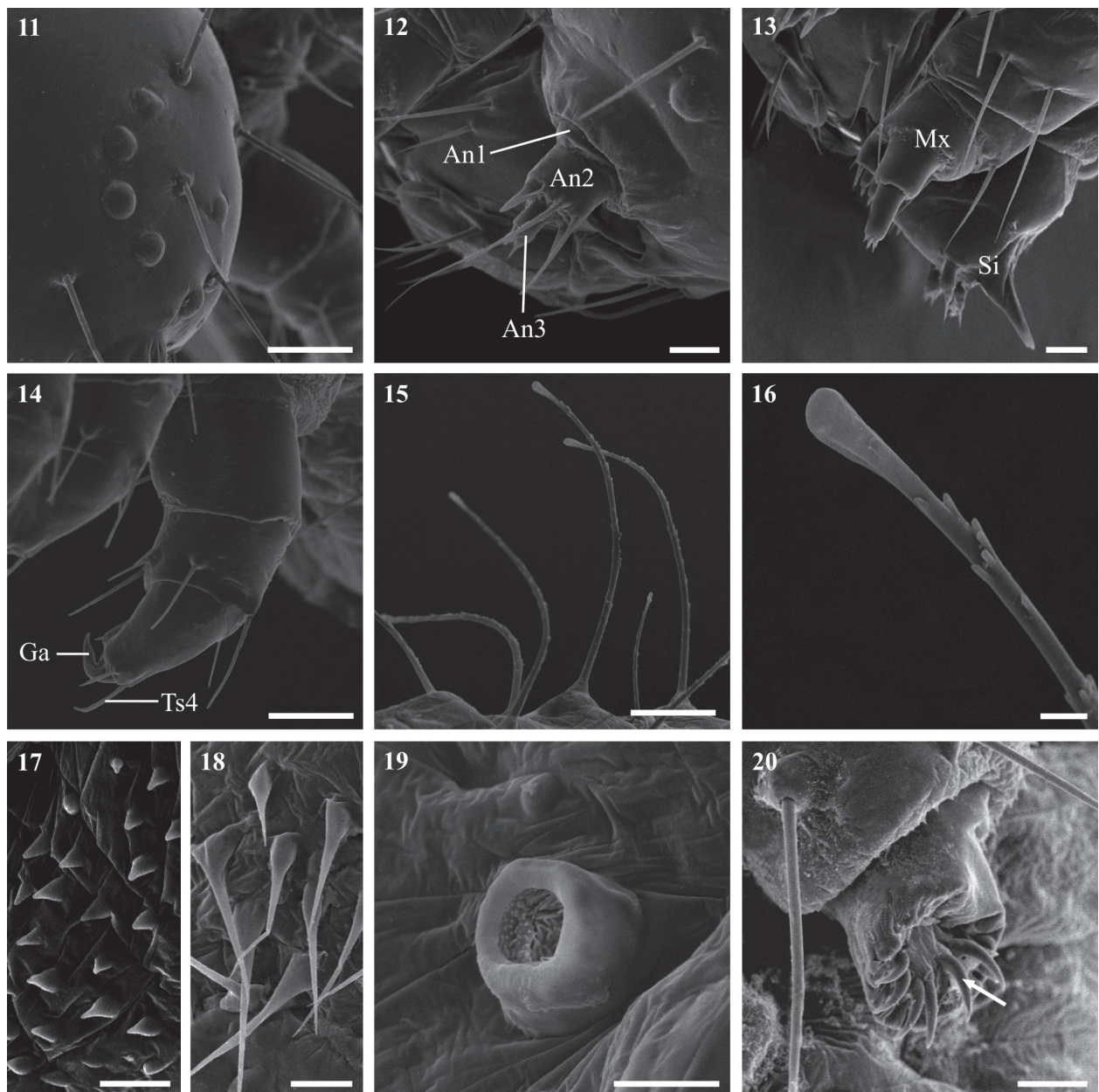
VARGAS *et al.*, 2014). Last, although lepidopteran AB9 chaetotaxy is very diverse (e.g., NAKANISHI, 1988; PALUCH *et al.*, 1999, 2001; MURRAY, 2001; CASAGRANDE, 2002; SAKAMAKI & HAYAKAWA, 2004; BALLMER & WRIGHT, 2008), the clustering of D2 and SD1 setae on the same pinnacle is not reported for any other species of Heliconiini (ex: FLEMING, 1960; ANTUNES *et al.*, 2002; TAVARES *et al.*, 2002; KAMINSKI *et al.*, 2002, 2008; PAIM *et al.*, 2004; SILVA *et al.*, 2006, 2008; BARÃO & MOREIRA, 2010; VARGAS *et al.*, 2014).

Remaining instars (Figs 10, 21-31, 35). From the second instar on, both the chaetotaxy and coloration change (Fig. 35): secondary setae, scoli and verrucae arise,

and the head becomes black; the general thoracic and abdominal color is grayish-yellow; and the prothoracic and anal shields, legs, prolegs, scoli, verrucae, and spiracles turn black.

Head with pair of short, stout cephalic scoli (Sc), measuring approximately half height of head; apex of cephalic scoli rounded, with more than one apical seta (Fig. 24). Mouthparts and stemmata (Fig. 21) as in first instar.

Thoracic and abdominal scoli with one apical seta (Figs 23, 25). Prothorax lacking scoli (T1; Figs 10, 35); prothoracic shield bearing setae D1, D2, and XD2 and several secondary setae (Fig. 22); primary setae at same



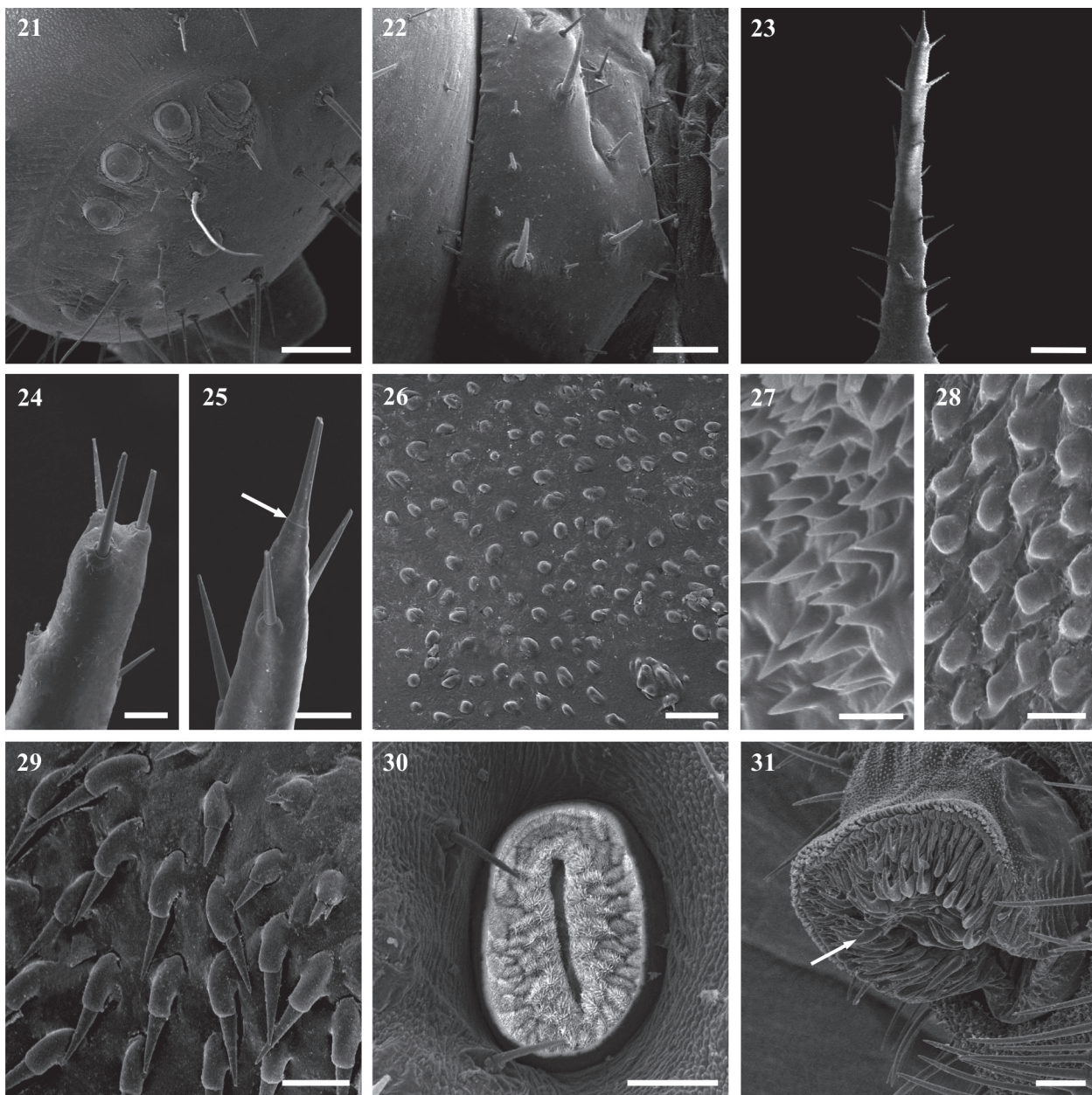
Figs 11–20. Scanning electron micrographs of the first instar larva of *Heliconius sara apseudes* (Hübner, [1813]). 11, stemmatal region, frontal view; 12, antenna, lateral view; 13, maxilla and spinneret, lateral view; 14, metathoracic leg, antero-lateral view; 15, chalazae, lateral view; 16, apex of dorsal seta; 17, microtrichia of ventral region of body; 18, microtrichia of anal plate; 19, spiracle of second abdominal segment; 20, base of proleg with exposed crochets (arrow) (An, antennomere; Ga, tarsal claw; Mx, maxilla; Si, spinneret; Ts, tarsal seta). Bars = 50, 20, 20, 50, 100, 10, 10, 10, 10 and 20 mm, respectively.

position as in first instar. Pair of dorsal (So) and supra-spiracular (Sp) scoli on meso- (T2) and metathorax (T3); So on T2 projected anteriorly, 1.5x longer than So on remaining AB; Sp on T2-3 shifted anteriorly and pointing toward head (Fig. 10).

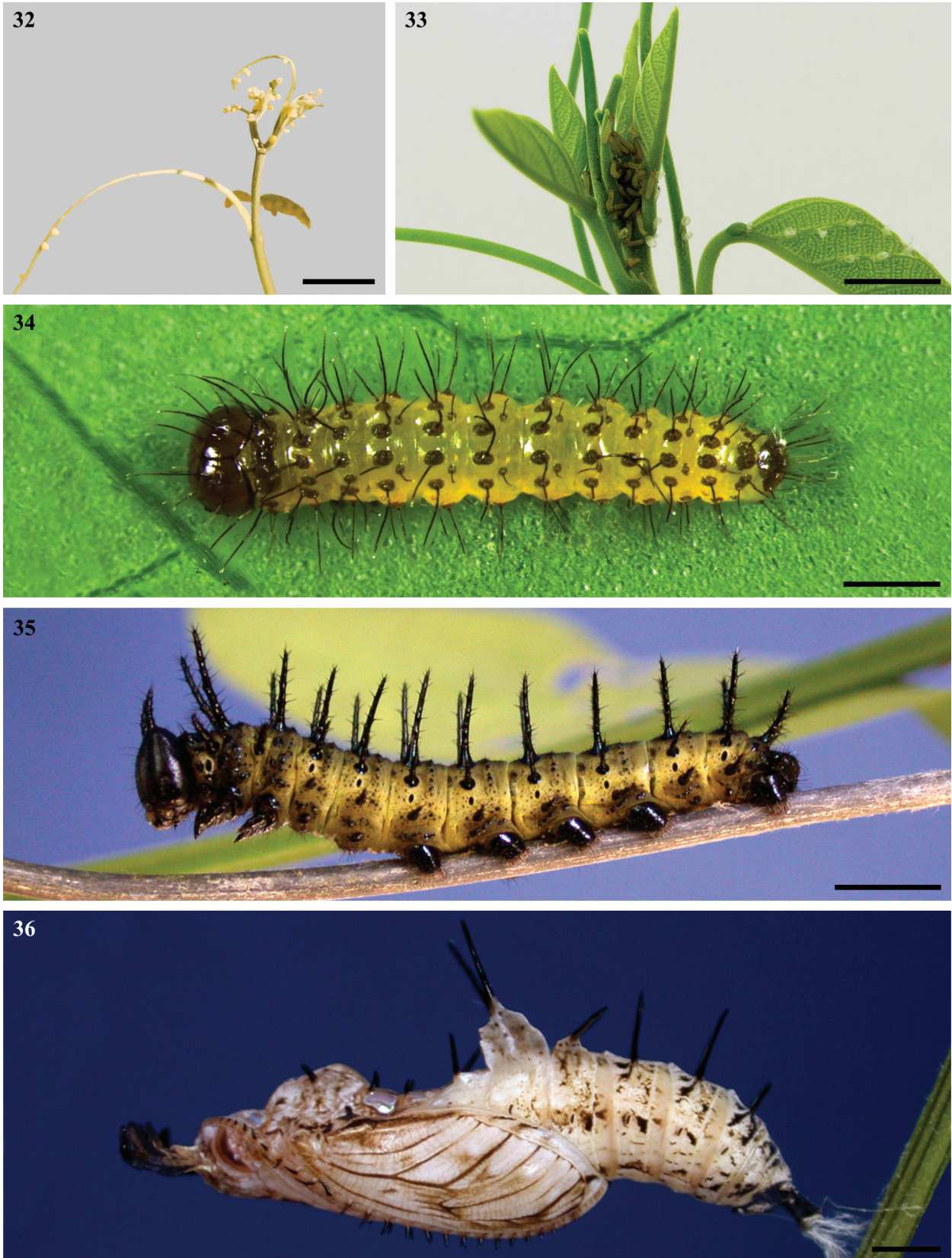
Three pairs of scoli from AB1 to AB8, one dorsal (So) pair, one supra-spiracular (Sp) pair, and one sub-spiracular (Sl) pair, following the *Heliconiini* pattern (e.g., KAMINSKI *et al.*, 2008; SILVA *et al.*, 2008; BARÃO & MOREIRA, 2010). Abdominal scoli shorter than height of abdominal segments, as in *H. erato phyllis* (KAMINSKI *et al.*, 2002). On AB9 only So is present. One pair of anal scoli (Sn) on AB10.

Several minute, black pinnacles and verrucae scattered over integument; on each abdominal segment, posterior to scoli, pinnacles organized in transverse band (Fig. 35). Primary setae SV1 replaced by verruca on AB1, AB2, AB7, and AB8.

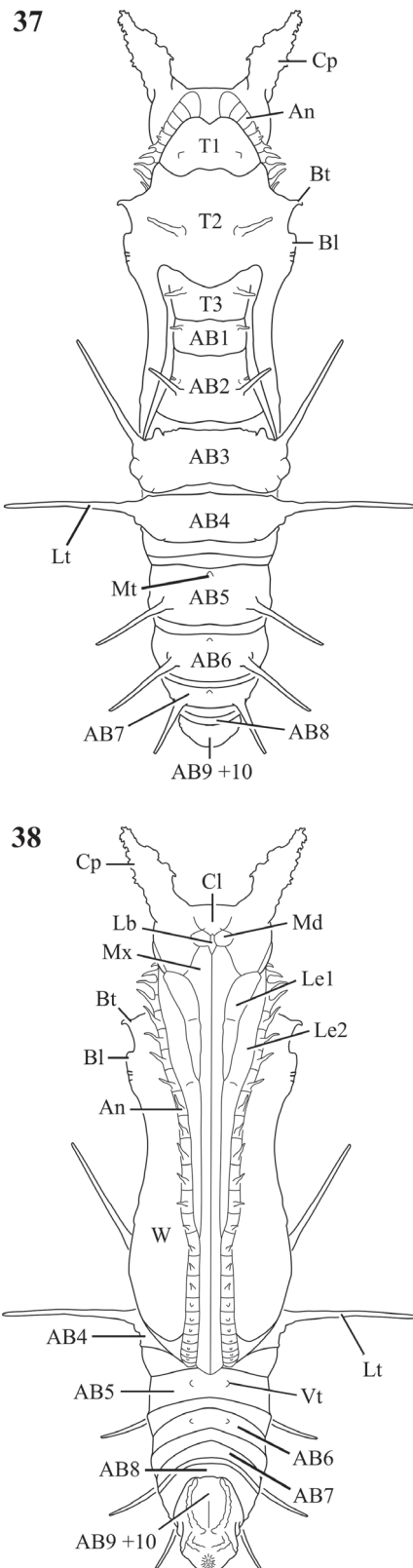
Head integument with striated, semi-spherical sculpturing (Fig. 26). Ventral body surface and base of prolegs provided with conical, smooth and apically acute microtrichia (Fig. 27); laterally and dorsally, larval body bearing smooth, semi-spherical microtrichia (Fig. 28). Conical sensilla on anal shield (Fig. 29). Elliptic spiracles (Fig. 30). Triordinal and uniserial crochets (Fig. 31) on base of prolegs.



Figs 21–31. Scanning electron micrographs of the fifth instar larva of *Heliconius sara apseudes* (Hübner, [1813]). 21, stemmatal region, lateral view; 22, prothoracic plate, dorsolateral view; 23, dorsal scolus; 24, apex of cephalic scolus; 25, apex of dorsal scolus with one seta (arrow); 26, ornament of head integument; 27, microtrichia of proleg; 28, microtrichia of lateral region of body; 29, epiproct sensilla; 30, spiracle of first abdominal segment; 31, base of proleg with exposed crochets (arrow). Bars = 200, 200, 500, 100, 100, 20, 10, 10, 50, 100 and 100 mm, respectively.



Figs 32–36. Coloration pattern of *Heliconius sara apseudes* (Hübner, [1813]) egg, larval and pupal stages. 32, eggs laid on *Passiflora truncata*; 33, hatchlings on *Passiflora haematostigma*; 34, first-instar larva, dorsal view; 35, fifth-instar larva, lateral view; 36, pupa, lateral view. Bars = 20, 10, 0.5, 5, 2.5 mm, respectively.



Figs 37, 38. Pupa of *Heliconius sara apseudes* (Hübner, [1813]). 37, dorsal view; 38, ventral view (AB, abdominal segment; An, antenna; Bl, longitudinal crest; Bt, basilar tubercle; Cl, clypeus; Cp, cephalic projection; Lb, labium; Le1, prothoracic leg; Le2, mesothoracic leg; Lt, laterodorsal tubercle; Md, mandible; Mt, mediodorsal tubercle; Mx, maxilla; T, thoracic segment; Vt, ventral tubercle; W, wing). Bars = 2.5 mm.

As in the first instar, the gross morphology and chaetotaxy of the subsequent instars of *H. sara apseudes* fit the general pattern described for Heliconiini (BEEBE *et al.*, 1960; ANTUNES *et al.*, 2002; KAMINSKI *et al.*, 2002, 2008; TAVARES *et al.*, 2002; PAIM *et al.*, 2004; SILVA *et al.*, 2006, 2008; BARÃO & MOREIRA, 2010; VARGAS *et al.*, 2014). From the second to fifth instars, the coloration of *H. sara apseudes* differs from those of *H. besckei*, *H. erato phyllis* and *H. ethilla narcaea*, which have the head in varying shades of yellow and the body white with black spots. The multiple setae on apex of Sc and So on T2 projected anteriorly and larger than any other scoli are also unique features of *H. sara apseudes* larvae (BEEBE *et al.*, 1960; TAVARES *et al.*, 2002; KAMINSKI *et al.*, 2002, 2008; PAIM *et al.*, 2004; SILVA *et al.*, 2006, 2008; BARÃO & MOREIRA, 2010; VARGAS *et al.*, 2014).

Instar identification. Besides the striking differences in chaetotaxy and coloration between the first and remaining instars, there are insufficient morphological or coloration changes to precisely identify instars of *H. sara apseudes*. However, they can be accurately distinguished by the size of their head capsule, because head widths of successive instars do not overlap (Tab. I). The following exponential growth equation was adjusted for the five instars: $y = 0.0289e^{0.533x}$; $n = 59$; $r = 0.98$; $p = 0.0001$. The mean growth ratio among instars was 1.67, similar to ratios previously reported for other Neotropical heliconiines (ANTUNES *et al.*, 2002; KAMINSKI *et al.*, 2002, 2008; TAVARES *et al.*, 2002; PAIM *et al.*, 2004; SILVA *et al.*, 2006, 2008; BARÃO & MOREIRA, 2010; VARGAS *et al.*, 2014).

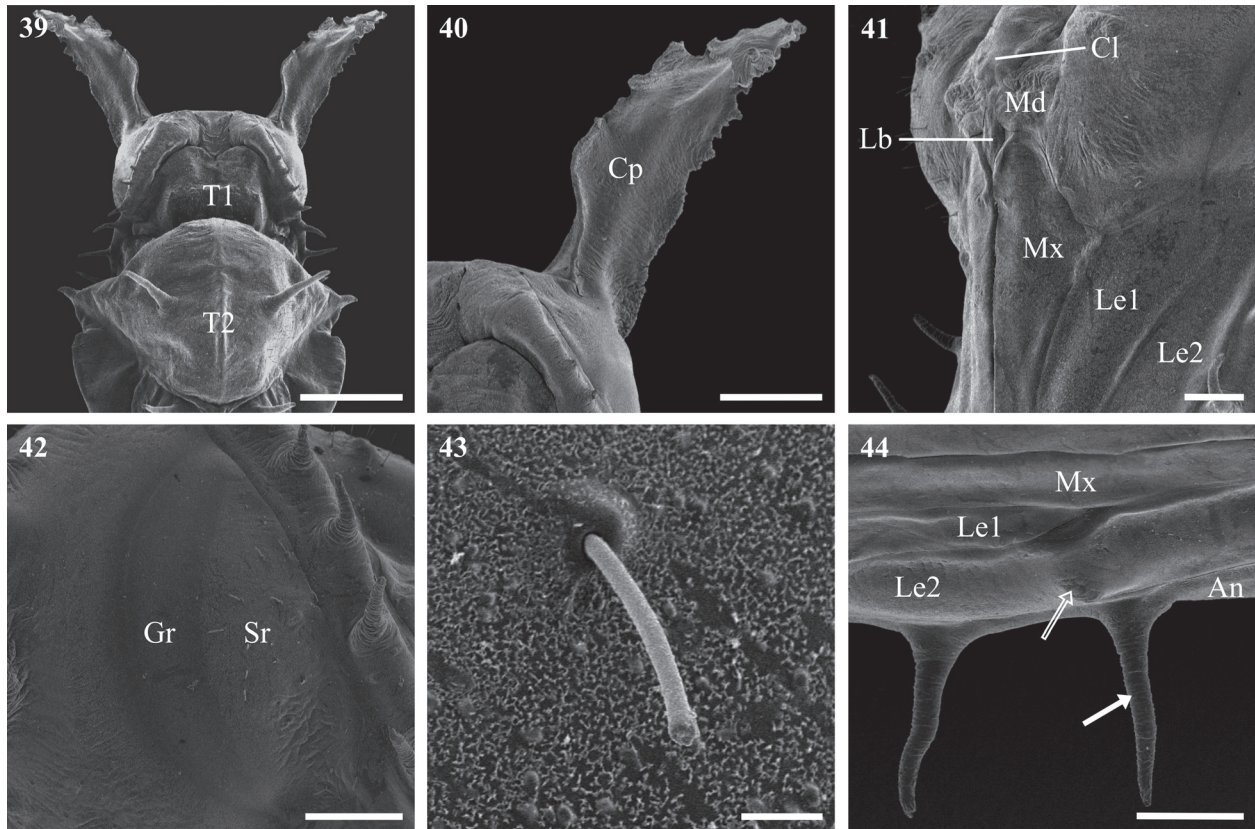
Pupa (Figs 36-53). Yellowish with scattered black markings (Fig. 36); dorsal pairs of golden spots on T1, T3, AB1, and AB2. BEEBE *et al.* (1960) noted the absence of dorsal golden spots on T2 for populations of *H. sara* in Trinidad.

Head with pair of long, compressed cephalic projections (Figs 37-40); edges of cephalic projections irregular. Ventrally, mandibles (Md; Figs 38, 41) surrounding labrum (Lb); clypeus (Cl; Fig. 38, 41) anterior to Lb; maxillae (Mx) posterior to Lb, extending to distal portion of antennae (Fig. 38); eyes (Fig. 42) with sculptured region near antennae, bearing few, short, apically clavate setae (Fig. 43), and another smooth region near frons. Antennae (An) arising dorsally on head (Fig. 37), projected ventrally between maxillae and legs, reaching apex of maxillae; each antennomere with an elongated, spike-like tubercle (Fig. 44).

Pro- (Le1) and mesothoracic (Le2) legs (Fig. 38) visible in ventral view; metathoracic leg concealed by antennae; Le1 and Le2 each with tibial tubercles (Fig. 44), similar to *H. erato phyllis* (KAMINSKI *et al.*, 2002). Each forewing with sharp basilar tubercle (Bt) and longitudinal crest (Bl) basally (Fig. 45); three slightly pronounced submarginal tubercles (St). Mesothorax (T2) with median meso-dorsal crest (Fig. 45). Medio-dorsal (Mt) tubercle on anterior margin of each AB5-7 (Fig. 46). Spike-like latero-dorsal (Lt) tubercles from T2 to AB7 (Figs 36-38, 47);

Tab. I. Mean and standard error (SE), interval of variation (IV), and growth rates (GR) of the head-capsule width in larval instars of *Heliconius sara apseudes* (Hübner, [1813]), reared on *Passiflora haematostigma* Masters.

Instar	N	Head-capsule width (mm)		
		Mean \pm SE	IV	GR
I	13	0.456 \pm 0.007	0.388 – 0.493	-
II	16	0.860 \pm 0.007	0.810 – 0.907	1.884
III	16	1.557 \pm 0.021	1.367 – 1.651	1.812
IV	9	2.460 \pm 0.035	2.210 – 2.543	1.579
V	5	3.496 \pm 0.047	3.349 – 3.594	1.421



Figs 39–44. Scanning electron micrographs of the pupa of *Heliconius sara apseudes* (Hübner, [1813]). 39, head and thorax, dorsal view; 40, cephalic projection, dorsal view; 41, mouthparts, ventrolateral view; 42, eye, lateral view; 43, seta from sculptured region of eye; 44, tubercles on antenna (solid white arrow) and mesothoracic leg (hollow white arrow) (An, antenna; Cl, clypeus; Cp, cephalic projection; Gr, glazed stripe of eye; Lb, labium; Le1, prothoracic leg; Le2, mesothoracic leg; Md, mandible; Mx, proximal portion of maxilla; Sr, sculptured region of eye; T, thoracic segment). Bars = 2000, 1000, 500, 500, 25 and 500 nm, respectively.

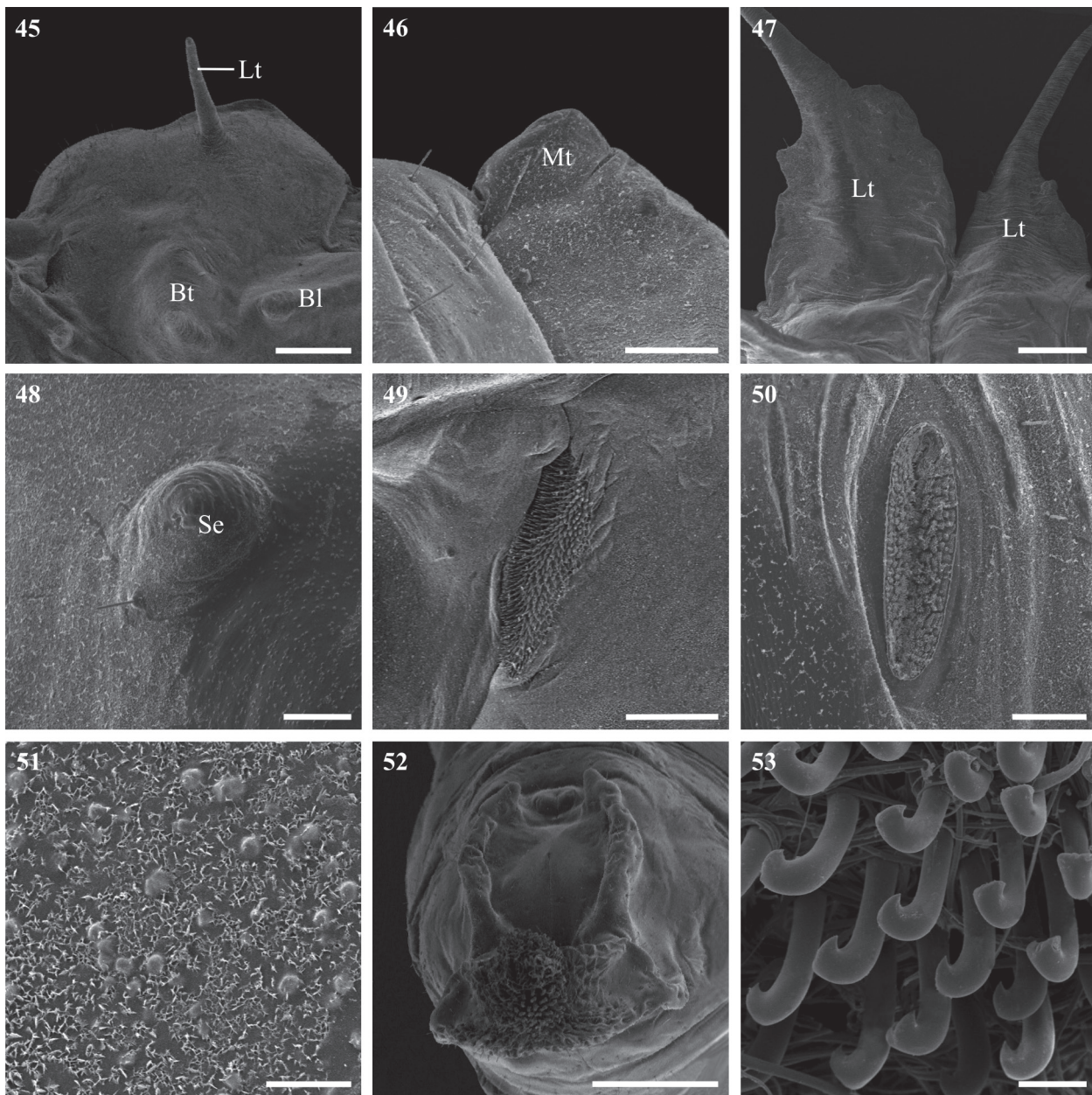
base of each Lt on AB3 and AB4 expanded longitudinally, forming flanges (Fig. 47); flanges not fused with each other; spike-like projections of Lt on AB3 and AB4 directed anteriorly and laterally, respectively. Supra-spiracular (Se) tubercles present from AB2 to AB4 (Fig. 48). Pairs of sub-median, ventral (Vt) tubercles present on AB6 and AB7 (Fig. 38).

Mesothoracic spiracle (Fig. 49) cleft-shaped; abdominal spiracles elliptic (Fig. 50), visible from AB2 to AB7. Integument bearing scattered semi-spherical protuberances and covered with waxy layer (Fig. 51). Cremaster subquadrate (Fig. 52), with hooks; each hook with recurved apex directed toward its base (Fig. 53).

The gross morphology of the pupa of *H. sara apseudes* is similar to other Heliconiini, following the pattern proposed by MOSHER (1916) for Nymphalidae. The

presence of spike-like tubercles on the antennae, thorax and abdomen, well-developed latero-dorsal flanges at least on AB3, and three to five pairs of dorsal golden spots fits the morphological pattern of “Group C” proposed by BEEBE *et al.* (1960). Other species of *Heliconius* (e.g. *H. erato*, *H. wallacei* and *H. melpomene*) also fit this pattern (BEEBE *et al.*, 1960).

The cephalic projections of *H. sara apseudes* are similar to those of *H. erato phyllis* and *Dryadula phaetusa* (Linnaeus, 1758) (KAMINSKI *et al.*, 2002; SILVA *et al.*, 2008) and differ from the short and palmate cephalic projections of *Dryas iulia alcionea* (Cramer, 1779), *Agraulis vanillae maculosa* (Stichel, [1908]) and *Philaethria wernickei* (Röber, 1906) (PAIM *et al.*, 2004; SILVA *et al.*, 2006; BARÃO & MOREIRA, 2010). Thoracic and abdominal tubercles of *H. sara apseudes* are similar to those of *H. erato phyllis*



Figs 45–53. Scanning electron micrographs of the pupa of *Heliconius sara apseudes* (Hübner, [1813]). 45, mesothoracic segment, lateral view; 46, mediadorsal tubercle, lateral view; 47, third and fourth laterodorsal tubercles, internal dorsal view; 48, supraspiracular tubercle, lateral view; 49, mesothoracic spiracle; 50, spiracle of eighth abdominal segment; 51, detail of tegumentary sculpture, 52, cremaster, posteroventral view; 53, cremaster hooks (Bl, longitudinal crest; Bt, basilar tubercle; Lt, laterodorsal tubercle; Mt, mediadorsal tubercle; Se, supraspiracular tubercle). Bars = 1000, 200, 1000, 100, 250, 100, 20, 1000 and 50 mm, respectively.

(KAMINSKI *et al.*, 2002); the Lt differs from *H. erato phyllis* in not having the flanges of thoracic Lt tubercles fused (KAMINSKI *et al.*, 2002).

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