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Morphological description of immature stages of *Trichophoromyia brachipyga* (Mangabeira) (Diptera: Psychodidae: Phlebotominae)

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Abstract

Trichophoromyia brachipyga (Mangabeira) (Diptera: Psychodidae: Phlebotominae) is a widely distributed species that has been recently regarded as a putative vector of *Leishmania* (*Viannia*) *lainsoni* Silveira, Shaw, Braga & Ishikawa in the Brazilian Amazon region. Currently, no immature stages of the genus *Trichophoromyia* Barretto have been morphologically described. The present study provided, for the first time, using a light microscopy morpho-taxonomical approach, the description of the egg, fourth-instar larva, and pupa of *Th. brachipyga*, with comparison of their morphological traits with other phlebotomine species, particularly those of the subtribe Psychodopygina, which is closely related.

Key words: Chaetotaxy, pre-imaginal, putative vector, cutaneous leishmaniasis

Introduction

Trichophoromyia Barretto comprises a group of phlebotomines (Diptera: Psychodidae: Phlebotominae), originally established as a subgenus of *Lutzomyia* França by Barretto (1962) and later upgraded to the genus level, according to Galati (2003). This genus belongs to the tribe Phlebotomini Rondani, subtribe Psychodopygina Galati (Galati 2003). Its type-species is *Th. ubiquitalis* (Mangabeira). Forty-four species of *Trichophoromyia* have been reported in the New World (Vasconcelos dos Santos *et al*. 2019), most of which are geographically restricted to the Amazon Basin. Several females of *Trichophoromyia* are isomorphic; therefore, identification is usually based on external characteristics of the males (Young and Duncan 1994). Presently, no immature stages from this genus have been morphologically described (Alencar *et al*. 2018; Galati 2018).

The medical importance of *Trichophoromyia* was obscured for many years, although beginning in the 1990s, some species began garnering attention, since they were spatiotemporally associated with American cutaneous leishmaniasis *foci* and/or harboring *Leishmania* Ross parasites, based on microscopic observations and/or DNA analyses.

Currently, five species of *Trichophoromyia* are suggested to have vector importance, and among them, *Th. brachipyga* (Mangabeira) has been included in the list of suspected vectors of *Leishmania* (*Viannia*) *lainsoni* Silveira, Shaw, Braga & Ishikawa, which are naturally infected by this parasite in Pará State, Brazil (Sánchez-Uzcátegui *et al*. 2020; Vasconcelos dos Santos & Silveira 2020). *Th. brachipyga* was described from male specimens captured with horse-bait and in animal burrows in Aurá (Belém municipality) and Piratuba (Abaetetuba municipality), Pará State, Brazil (Mangabeira 1942).

To advance the understanding of Phlebotominae ecology and systematics, it is critical to obtain knowledge of taxonomically informative features of immature developmental stages (Hanson 1968; Ward & Ready 1975; Ward

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1976a; Arrivillaga *et al*. 1999; Montes de Oca-Aguilar *et al*. 2014; Alencar *et al*. 2018). Therefore, due to the lack of information of immature stages of *Trichophoromyia* species, the present work aimed to describe, for the first time, using a light microscopy morpho-taxonomical approach, the egg, fourth instar larva, and pupa of *Th. brachipyga*, and compare their morphological traits with those of other species within Phlebotominae, particularly those of Psychodopygina.

Material and methods

Sampling

Adult phlebotomines were captured in an urban park in Belém (1°25′48.2″ S; 48°27′24.9″ W), Pará State, Brazil, with CDC light traps installed at 1.5m above ground, from 6:00 pm to 6:00 am, from December 2020–February 2021. Captured specimens were fed on a previously anesthetized hamster. Females engorged from the field or the lab were individually separated for egg laying and morphologically identified after death, according to the method of Galati (2018). After egg hatching, larvae were reared in the Insectarium of the Ralph Lainson Leishmaniases Lab (Instituto Evandro Chagas, Brazil), according to the method described by Killick-Kendrick and Killick-Kendrick (1991).

Ethical/environmental aspects

Capturing and processing invertebrate fauna (phlebotomines) were authorized by the *Sistema de Autorização e Informação em Biodiversidade* - SISBIO (Biodiversity Authorization and Information System), under protocol No. 70142.

Procedures involving vertebrate animals (hamsters) were reviewed and approved by the *Comissão de Ética no Uso de Animais* - CEUA (Committee on Ethical Use of Animals) of the Instituto Evandro Chagas, under protocol CEUA/IEC No. 44/2019.

Processing

The specimens were analyzed using optical microscopy, the larvae and pupa exuviae under bright-field microscopy, and eggs under phase-contrast microscopy. The mature larvae had been previously killed using hot water (50 °C). All specimens were fixed in 70% ethanol until mounting. They were clarified with 10% KOH for 12 h, neutralized with 10% acetic acid for 30 min, dehydrated in an ethanol series (80%, 90%, 95%, 100%) for 30 min each, slide-mounted using Canada balsam (Reagen) diluted with xylene (Neon) (3:1), and ring-sealed with Glyceel (BDH). Cephalic capsules of the exuviae of fourth-instar larvae were clarified in 10% KOH solution for 24 h, and their mouthparts were dissected for slide-mounting as described above. Larvae were mounted in the dorsal, ventral, and lateral positions. Pupae exuviae were entirely mounted in the lateral position, with the abdomen and head out in the dorsal and frontal views, respectively.

Measurements were obtained using the software AxioVision version 4.7, coupled with a previously calibrated microscope, camera, and computer system equipped with phase contrast (Zeiss Axiostar plus; Camera AxioCam HRC, Germany). Morphological structures were outlined for drawing using a camera lucida (Zeiss, Germany) coupled with a microscope (Zeiss Axiostar plus, Germany). The mean measurements were given in micrometers (μm), followed by, in brackets, the measurement range, and the number of specimens examined for each structure. Setae shorter than 10 μm were not measured.

Analysis

Egg morphology followed the categorization of exochorionic sculpture patterns and terminology (Feliciangeli *et al*. 1993; Almeida *et al*. 2004). Numerical chaetotaxy and terminology of the larvae and pupae followed the proposal suggested by Montes de Oca-Aguilar *et al*. (2014), with the principles of chaetotaxy nomenclature originally adopted for Culicidae (Harbach & Knight 1980), sensilla classification of Zacharuck & Shields (1991), terminology of the mouthparts from the larvae provided by Arrivillaga *et al*. (1999) with modifications of Montes de Oca-Aguilar *et al*. (2014), and labium definition using the terminology of Satchell (1953). Voucher material was deposited in the *Coleção de Flebotomíneos do Instituto Evandro Chagas*, Brazil (ColFleb IEC).

Results

Material examined

The specimens used were from BRAZIL, Pará State, Belém, December 2020, Sánchez-Uzcátegui YdV col. Founder: 1♀; offspring: 1♂, 2♀♀; breeding specimens for analysis: pupa exuviae: 1♂, 2♀♀; fourth-instar larvae: 3; fourth-instar larva exuviae 3; eggs: 6.

Egg

Elongated, elliptical, with one side slightly flattened, length 460.8 (440–480; n = 6) maximal width 128.1 (120–136; n = 6); rounded poles; exochorion dark brown; entirely covered with ridges arranged in a polygonal cell pattern (predominantly hexagonal); with approximately 8 (7–9; n = 6) and 19 (18–21; n = 6) rowed polygonal cells on their transversal and longitudinal extensions, respectively (Fig. 1).

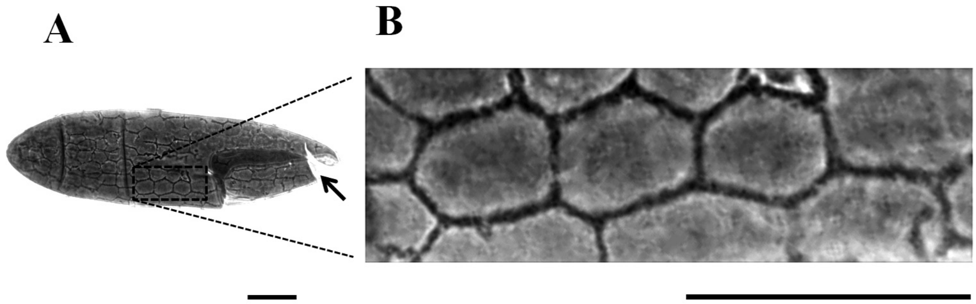

FIGURE 1. Optical microscopy of the egg of *Trichophoromyia brachipyga*. A: general view of a hatched egg showing the hatching slit (arrow), bar = 50μm; B: eggshell exochorionic ornamentation showing detail of the ridges with polygonal, predominantly hexagonal pattern, bar = 50μm.

Fourth-instar larva

General appearance: caterpillar-like with chaetotaxic ornamentation, amphipneustic respiratory system, body integument predominantly yellowish, slightly more pigmented in the ventral side, with an oval, well-sclerotized, hypognathous, non-retractile head; abdominal segments VIII-IX predominantly brownish; body length (excluding caudal setae): 3,843 (3,799–3,870; n=3), maximal width, at the metathorax: 655.3 (640–676; n=3).

Head (Fig. 2): Measuring 552.6 (547–559; n=5) length, 371.3 (366–378; n=5) maximal width; antenna 105.6 (102–109; n=5) in length; cephalic chaetotaxy as described in Table 1; antennae hyaline bearing two closely appressed segments, inserted on the vertex of a conic tubercle which do not extend beyond the head capsule in the dorsal view; basal segment 20 (18–22; n=5) and distal 85.6 (80–89; n=5) in length, cylindrical, bearing a basiconic and a campaniform sensillum on its apex and middle third, respectively. Postgena and frontoclypeal apotomes densely covered with homogeneously distributed spiculae. Head bearing seven setae with the following distribution: cephalic apotome (2), gena (3), postgena (1), and subgena (1). Chaetotaxy of the cephalic capsule is shown in Table 1.

Mouthparts (Fig. 3): labrum with two setae; epipharynx hyaline, covered with parallel rows of digitiform setae combs, bearing a peg-like apical and four-tipped middle teeth, and apical, basal and U-sclerite setae; mandible strongly sclerotized and dark brown; 153.8 (146–160; n=5) length, 63.8 (60–69; n=5) maximum width, three sensilla present on the dorsal and external lateral surfaces, spinose area with four spines, lateral internal surface bearing prostheca and a mandibular comb, incisor lobe with three sharp teeth, molar lobe discontinuously aligned; maxilla length: 126.2 (122–130; n=5), maximal width: 92.6 (90–98; n= 5); lacinia poorly differentiated, maxillary brush on dorso-lateral border. In the dorso-lateral view, maxillary ventral teeth can be seen; in the ventral view, the maxillary plate appears sclerotized. Postmentum is strongly sclerotized and dark brown, bearing four teeth, which progressively decrease in size from the median to the lateral.

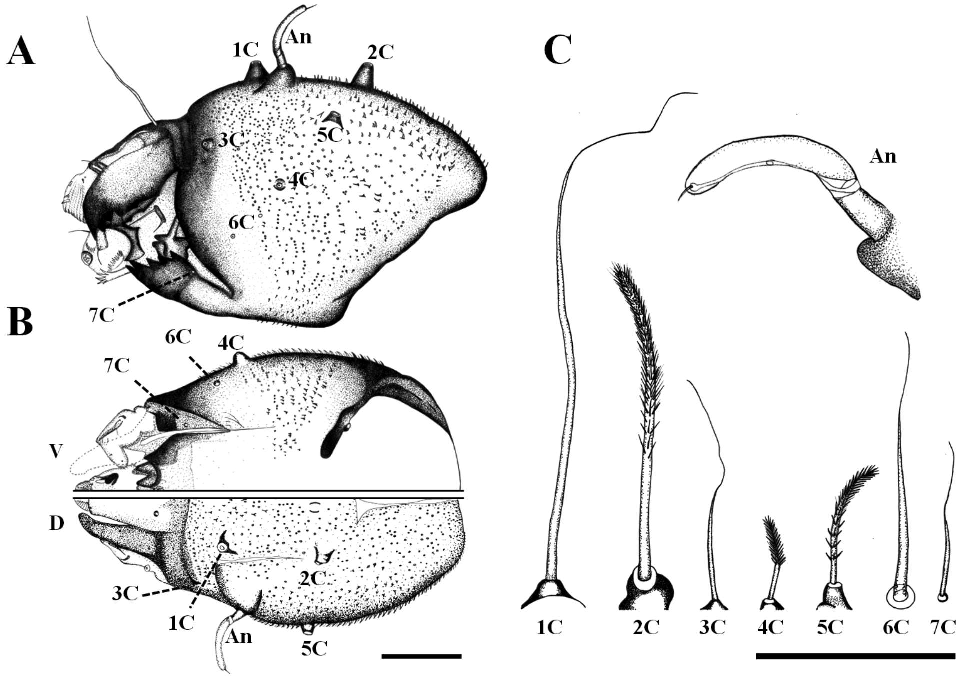

FIGURE 2. Morphology and chaetotaxy of cephalic capsule of *Trichophoromyia brachipyga* fourth-instar larva. A: cephalic capsule in lateral view; B: cephalic capsule in ventral and dorsal view; C: setae and antenna of the cephalic capsule; An: antenna; bar = 50μm.

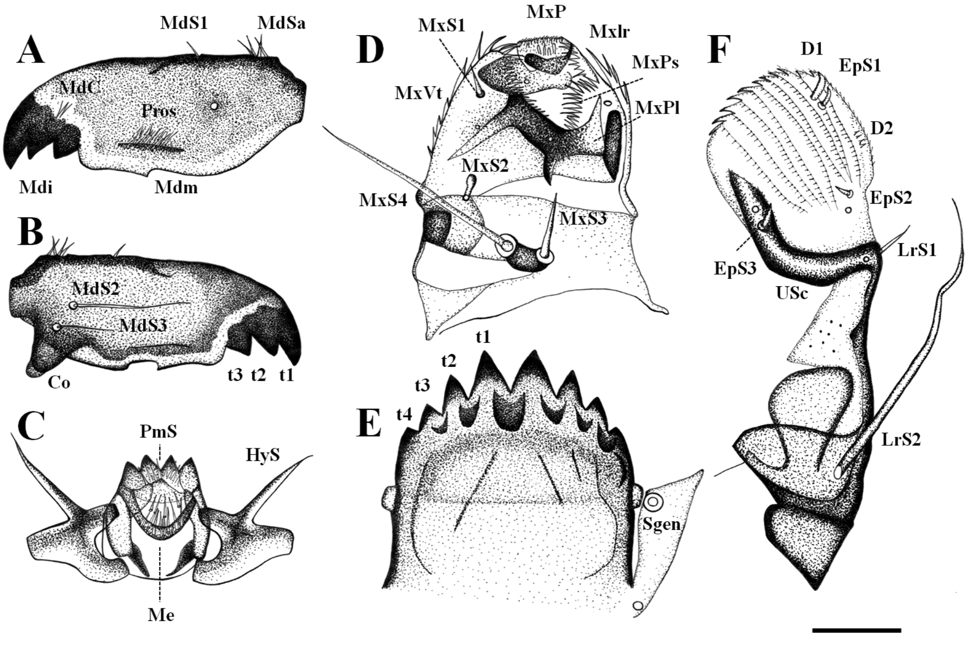

FIGURE 3. Morphology of mouthparts of *Trichophoromyia brachipyga* fourth-instar larva. A and B: mandible in lateral internal and lateral external view, respectively; C: labium in ventral view; D: maxilla in lateral external view; E: postmentum and subgena in ventral view; F: labrum-epypharynx in lateral view; Co: condyle; D1: apical tooth; D2: Middle tooth; Ep: epypharynx; EpS1: epypharynx apical seta; EpS2: epypharynx basal seta; EpS3: U-sclerite seta; HyS: suspensorium of hypopharynx; Lr: labrum; LrS1: distal labrum seta; MdC: mandible comb-like; Mdi: incisor lobe; Mdm: molar lobe; MdSa: spiny area; MdS1-MdS3: mandible setae; Me: mentum; Mxlr: lacinia maxillary rake; MxS1-MxS4: maxilla setae; MxP: maxillary palpus; MxPl: maxillary plate; MxPs: maxillary palpus microtrichia; MxVt: ventral tooth; Pros: prostheca; PmS: premental sclerite; Sgen: subgena; t1-t3: teeth of incisor lobe; t1-t4: teeth of the postmentum; USc: U-sclerite; bar = 30μm.

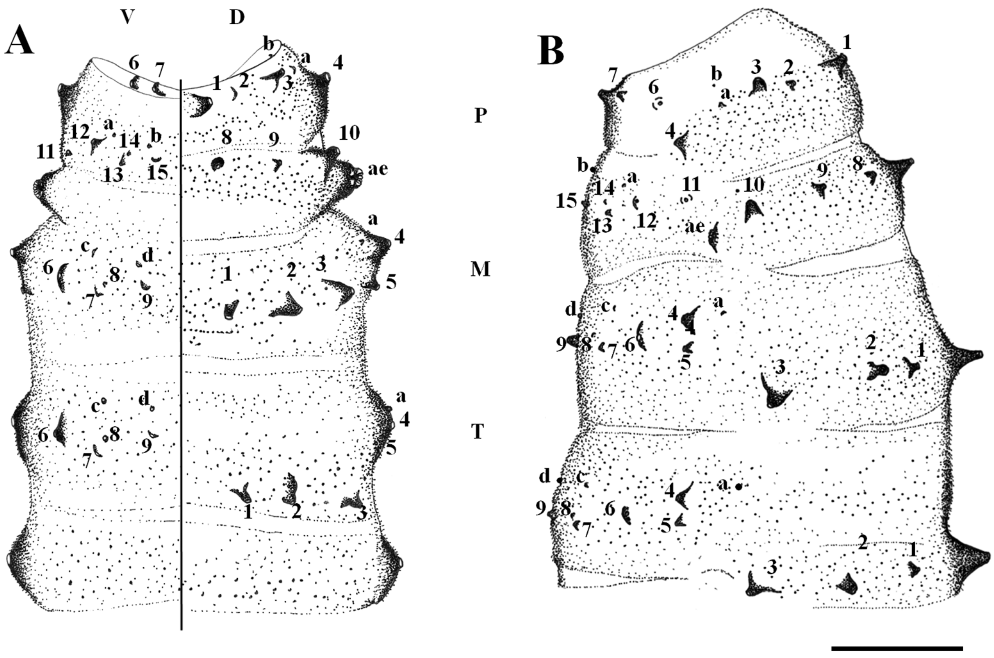

FIGURE 4. Morphology of the thorax of *Trichophoromyia brachipyga* fourth-instar larva, with distributional pattern of the setae. A: ventral and dorsal view; B: lateral view; M: mesothorax; P: prothorax; ae: anterior spiracle; T: metathorax; bar = 100μm.

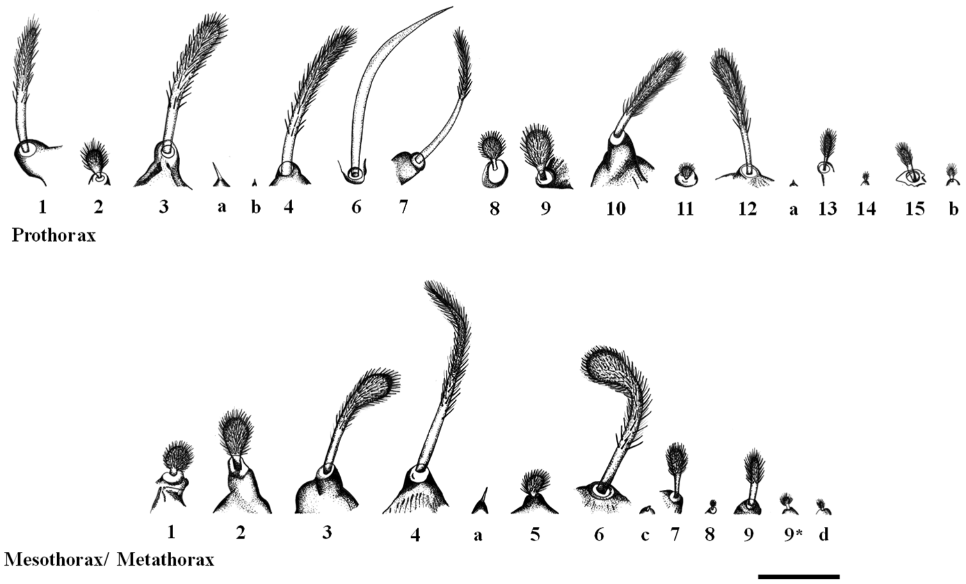

FIGURE 5. Setae types of thorax of *Trichophoromyia brachipyga* fourth-instar larva. Seta 9 with asterisk is in the metathorax; bar = 50μm.

Thorax (Figs. 4, 5) with the following segmental division and setae numbering: anterior prothorax (9), posterior prothorax (9), and meso- and metathorax (12) with homologous chaetotaxy, with the exception of seta 9, which was four times longer in the mesothorax. Crater-like thoracic spiracles, placed dorsolaterally on the prothorax II, measuring 22.3 (22–23; n=3) in diameter and bearing 10 papillae (9–10; n=3). Chaetotaxy of the thorax is shown in Table 2.

Abdomen (Figs. 6, 7): nine visible segments; segments I–VII with homologous chaetotaxy, each with nine setae, large prolegs on the sternites bearing two pairs of placoid sensilla; segment VIII with nine setae and tergal surface brownish pigmented, except on its anterior margin and on the bare spots of muscle plaques, which comprise five evenly distributed pairs, with the posterior ones placed very close to the posterior margin; a pair of campaniform sensilla are present; segment IX with nine setae, with the following distribution: caudal lobe (4), anal lobe (5), with the former ending in two prominent tubercles, each of which bear the caudal filaments 2A and 2B; crater-like abdominal spiracles, inserted dorsolaterally on the posterior half of abdominal segment VIII with a 38.3 (37–39; n=3) diameter and bearing 14 (14–15; n=3) papillae. Chaetotaxy of the abdomen is presented in Table 3.

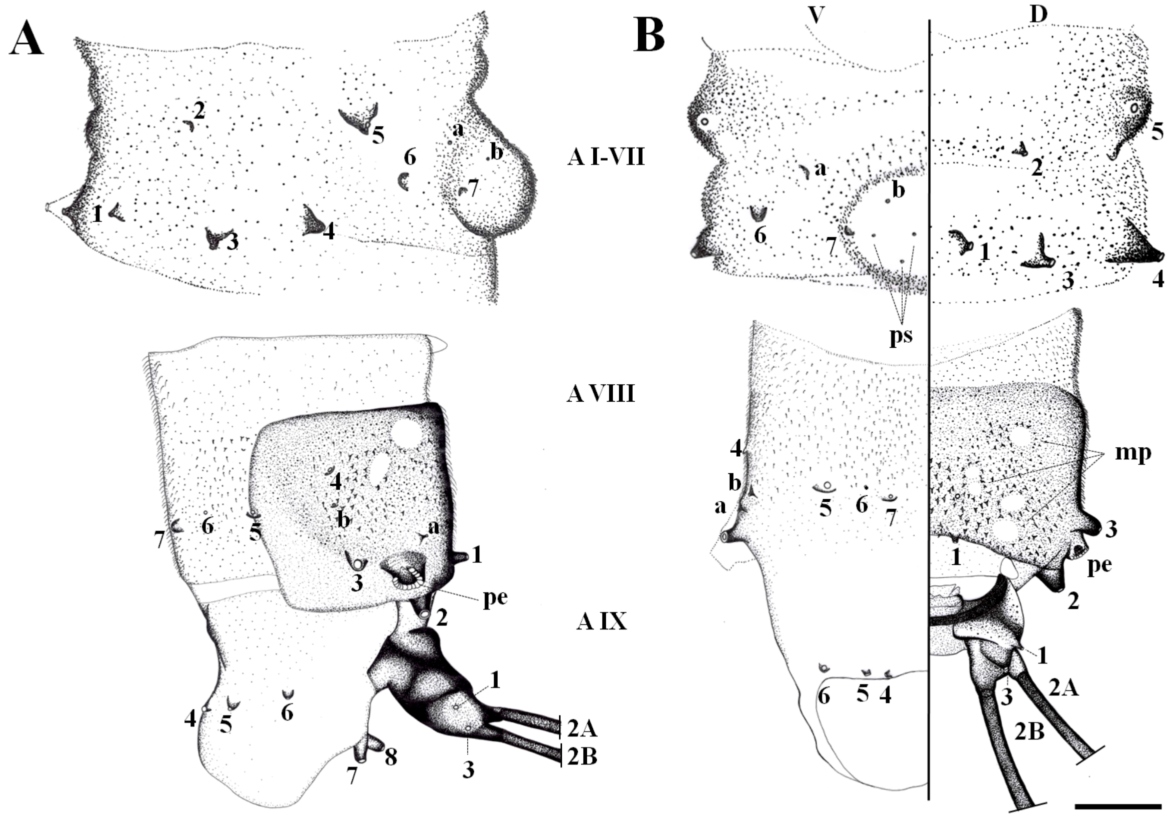

FIGURE 6. Morphology of the abdomen of *Trichophoromyia brachipyga* fourth-instar larva, with distributional pattern of the setae. A: lateral view; B: ventral and dorsal view; AI-VII: abdominal segments I-VII; AVIII: abdominal segment VIII; AIX: abdominal segment IX; mp: muscle plaques; pe: posterior spiracle; ps: placoid sensilla; bar = 100μm.

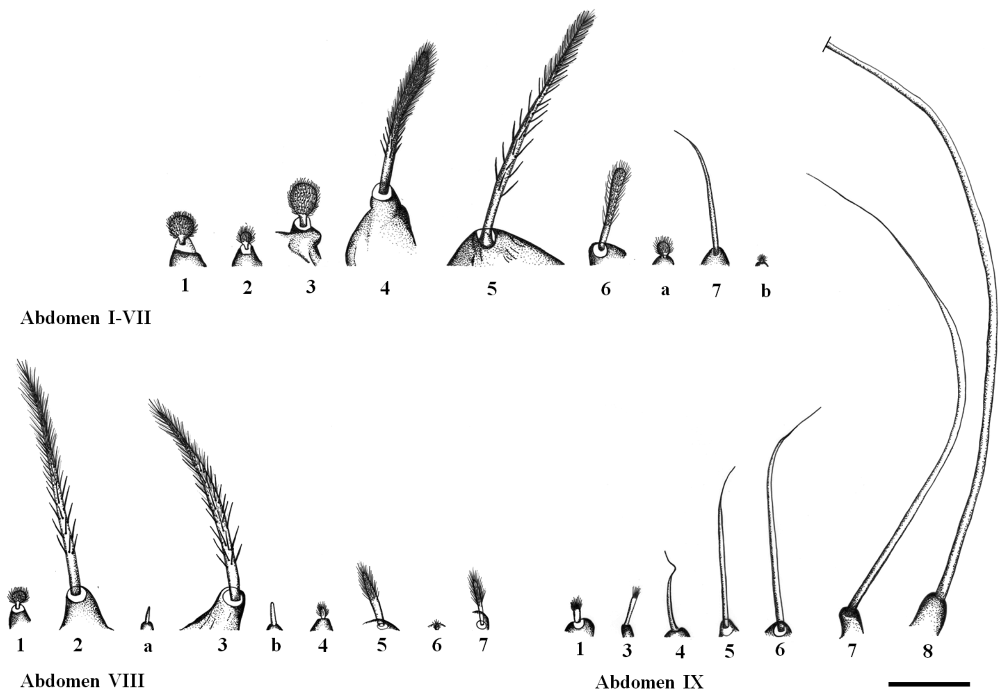

FIGURE 7. Setae types of abdomen of *Trichophoromyia brachipyga* fourth-instar larva; bar = 50μm.

TABLE 1. Chaetotaxy (with morphology and morphometrics) of cephalic capsule (mouthparts and head) of *Trichophoromyia brachipyga* fourth-instar larva.

	Terminology	Abbreviation	Type	Length (range; n)
Mouthparts	Epipharynx spiniform seta 1	EpS1	B. spiniform	16.3 (14-19; n=3)
	Epipharynx spiniform seta 2	EpS2	B. spiniform	110 (102-118; n=3)
	Epipharynx spiniform seta 3	EpS3	B. spiniform	18.1 (14-20; n=3)
	Distal labrum seta	LrS1	Simple	22.6 (17-26; n=4)
	Basal labrum seta	LrS2	Simple	176 (170-182; n=4)
	Mandible seta 1	MdS1	Simple	N.m.
	Mandible seta 2	MdS2	Simple	60.3 (58-63; n=3)
	Mandible seta 3	MdS3	Simple	35.3 (35-37; n=3)
	Apical maxillary seta	MxS1	Simple	22.3 (20-26; n=3)
	Basal maxillary setae 3	MxS3	Simple	24.8 (22-28; n=4)
	Basal maxillary setae 4	MxS4	Simple	67 (63-72; n=4)
	Medial maxillary seta	MxS2	Spatulate	N.m.
Head	Frontoclypeal anterior	1C	Simple	349 (340-355; n=3)
	Frontoclypeal posterior	2C	Barbed	145.3 (130-162; n=3)
	Genal anterior	3C	Simple	237.6 (233-245; n=3)
	Genal medial	4C	Barbed	83.3 (81-86; n=3)
	Genal posterior	5C	Barbed	141.6 (139-145; n=3)
	Postgenal	6C	Simple	156 (152-161; n=3)
	Subgena	7C	Simple	74.3 (72-76; n=3)

Abbreviations: B. basiconic; n: number of specimens measured for that structure; N.m.: not measured. Length in micrometers.

TABLE 2. Chaetotaxy (with morphology and morphometrics) of thorax (anterior and posterior prothorax, and mesothorax/ metathorax) of *Trichophoromyia brachipyga* fourth-instar larva.

	Terminology	Number	Type	Length (range; n)
Anterior prothorax	D. internal	1	Barbed	91.5 (87-96; n=3)
	D. intermediate	2	Barbed	27 (24-32; n=3)
	D. external	3	Barbed	100 (98-102; n=3)
	D. accessory a	a	Spiniform	N.m.
	D. accessory b	b	Spiniform	N.m.
	Lateroventral anterior	4	Barbed	123 (120-126; n=3)
	V. a. intermediate	6	Simple	105 (102-108; n=3)
	V. a. internal	7	Barbed	118 (114-122; n=3)
Posterior prothorax	D. p. internal	8	Barbed	24 (22-28; n=3)
	D. p. intermediate	9	Barbed	29.5 (26-33; n=3)
	D. p. external	10	Barbed	68.5 (62-75; n=3)
	Latero-ventral	11	Barbed	N.m.
	V. p. external	12	Barbed	85 (82-88; n=3)
	V. p. e. accessory a	a	Spiniform	N.m.
	V. p. intermediate	13	Barbed	24.5 (22-27; n=3)
	V. p. intermediate basal	14	Barbed	N.m.
	V. p. internal	15	Barbed	21 (18-24; n=3)
	V. p. accessory b	b	Barbed	N.m.
Mesothorax/ metathorax	D. p. internal	1	Barbed	25 (22-27; n=3)
	D. p. intermediate	2	Barbed	34.6 (33-37; n=3)

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TABLE 2. (continued)

	Terminology	Number	Type	Length (range; n)
	D. p. external	3	Barbed	88.3 (85-92; n=3)
	Lateral superior accessory	a	Spiniform	11 (10-12; n=3)
	Lateroanterior	4	Barbed	151.6 (147-155; n=3)
	Laterobasal	5	Spiniform	21 (18-24; n=3)
	V. external	6	Barbed	98 (95-101; n=3)
	V. external accessory	c	Spiniform	N.m.
	Ventral intermediate	7	Barbed	35.3 (33-37; n=3)
	V. p. intermediate basal	8	Barbed	N.m.
	V. internal	9	Barbed	38.6 (36-41; n=3)*
	V. internal accessory	d	Barbed	N.m
	D. p. internal	1	Barbed	25 (22-27; n=3)

Abbreviations: a.: anterior; e.: external; D.: Dorsal; p.: posterior; V: Ventral; * Corresponding to the morphometrics of the mesothorax (not measured on the metathorax). Length in micrometers.

TABLE 3. Chaetotaxy (with morphology and morphometrics) of abdomen of *Trichophoromyia brachipyga* fourth-instar larva.

Segment	Terminology	Number	Setae type	Lenght
I-VII	D. internal	1	Barbed	21.3 (20-22; n=3)
	D. a. intermediate	2	Barbed	12.3 (10-15; n=3)
	D. intermediate	3	Barbed	28.6 (26-31;n=3)
	D. external	4	Barbed	104.3 (100-108; n=3)
	Lateroanterior	5	Barbed	138 (102-157; n=3)
	Lateroposterior	6	Barbed	46 (44-48; n=3)
	Lateroventral accessory a	a	Barbed	N.m.
	Ventral	7	Simple	90.6 (88-94; n=3)
	V. accessory b	b	Barbed	N.m.
VIII	D. internal	1	Barbed	14 (13-15; n=3)
	D. intermediate	2	Barbed	157.3 (155-160; n=3)
	Lateral accessory a	a	Spiniform	N.m.
	Laterodorsal	3	Barbed	144.6 (140-153; n=3)
	Lateral accessory b	b	Spiniform	N.m.
	Lateroventral	4	Barbed	N.m.
	V. external	5	Barbed	34.3 (33-35; n=3)
	V. intermediate	6	Barbed	N.m.
	V. internal	7	Barbed	37 (36-38; n=3)
IX - Caudal lobe	Lobe external	1	Barbed	N.m.
	Caudal external	2a	Chaetic	2673.6 (2666-2680; n=3)
	Caudal internal	2b	Chaetic	2673.3 (2663-2680; n=3)
	Lobe posterior	3	Barbed	20 (18-22; n=3)
IX - Anal lobe	Post-anal internal	4	Chaetic	N.m.
	Post-anal external	5	Chaetic	107 (100-109; n=3)
	Lateral-anal	6	Chaetic	167 (165-170; n=3)
	Pre-anal external	7	Chaetic	320 (312-328; n=3)
	Pre-anal internal	8	Chaetic	747 (740-753; n=3)

Abbreviations: a.: anterior; D.: Dorsal; V: Ventral; N.m.: not measured. Length in micrometers.

Pupa

General appearance: Claviform, divided into cephalothorax and abdomen; measuring (excluding larval exuviae) 2,934 (2,910–2,952; n=3) in length. A sheath outlines the structures of the pre-imaginal stage.

Head (Fig. 8): post-ocular lobe laterally situated, non-divided, cone-shaped. Mouthparts sheath smooth; clypeal sheath slightly conspicuous; sexual dimorphism is present, maxillary sheath shorter than the labrum-epipharynx and hypopharynx sheaths in males; head bearing ten pairs of setae (Fig. 9), with the postocular external larger 76 (74–78; n=3); head chaetotaxy as presented in Table 4.

Thorax (Figs. 8, 9): 13 pairs of setae (s) and four pairs of alveoli (a), with the following distribution: prothorax (s=3, a=2), mesothorax (s=5, a=1), and metathorax (s=5, a=1). Mesothorax bearing two branch-tipped pre-alars 267.3 (262–271; n=3) in length, originated on pronounced conic tubercles; mesonotal tubercle blunt and moderately developed; the ventral side of the thorax bears the legs and wing sheaths with outlined venation; chaetotaxy of the thorax in shown in Table 5.

TABLE 4. Chaetotaxy of head of *Trichophoromyia brachipyga* pupa.

Terminology	Number	Type
Clypeal inferior	1C	Basiconic
Palpal seta	2C	Basiconic
Clypeal superior	3C	Basiconic
Frontal inferior	4C	Basiconic
Frontal superior	9C	Basiconic
Vertical	10C	Basiconic
Frontal medial	8C	Basiconic
Postocular medial	5C	Basiconic
Postocular internal	6C	Basiconic
Postocular external	7C	Basiconic

TABLE 5. Chaetotaxy of thorax of *Trichophoromyia brachipyga* pupa.

Segment	Terminology	Number	Type
Prothorax	Prothoracic superior	1P	Styloconic
	Prothoracic medial	2P	Styloconic
	Prothoracic inferior	3P	Basiconic
Mesothorax	Mesothoracic inferior	1M	Styloconic
	Mesothoracic medial	2M	Styloconic
	Mesothoracic superior	3M	Styloconic
	Pre-alar	4aM, 4bM	Chaetic
Metathorax	Metathoracic internal	1T	Styloconic
	Metathoracic medial	2T	Styloconic
	Metathoracic external	3T	Styloconic
	Pre-halter	4aT, 4bT	Basiconic

Abdomen (Figs. 8, 9): with nine segments, diminishing regularly in size towards the distal region; abdominal segments I-III: tergum with four pairs of sensilla, pleura, and sternum partially or totally covered with the thoracic appendage sheaths (chaetotaxy not presented); abdominal segments IV-VII: each tergum with four pairs of sensilla homologous in form and location as the segments I-III; each sternum with four pairs of sensilla; abdominal segment VIII: with a pair of sensilla (chaetotaxy presented for the segments IV-VII and VIII in Fig. 9); abdominal segment IX: without sensilla; sexual dimorphism can be observed: males bear two lobes at each side, one simple and the other markedly divided, while females bear two simple and short lobes at each side. Chaetotaxy of the abdomen is presented in Table 6.

TABLE 6. Chaetotaxy of abdomen of *Trichophoromyia brachipyga* pupa.

Segment	Terminology	Number	Type
IV–VII	Dorsal anterior	1	Styloconic
	Dorsal posterior internal	2	Styloconic
	Dorsal posterior external	3	Styloconic
	Laterodorsal	4	Basiconic
	Lateral anterior	5	Styloconic
	Lateral posterior	5’	Basiconic
	Ventral posterior external	6	Styloconic
	Ventral posterior internal	8	Styloconic
VIII	Dorsal inferior	2	Basiconic
	Lateral	3	Basiconic

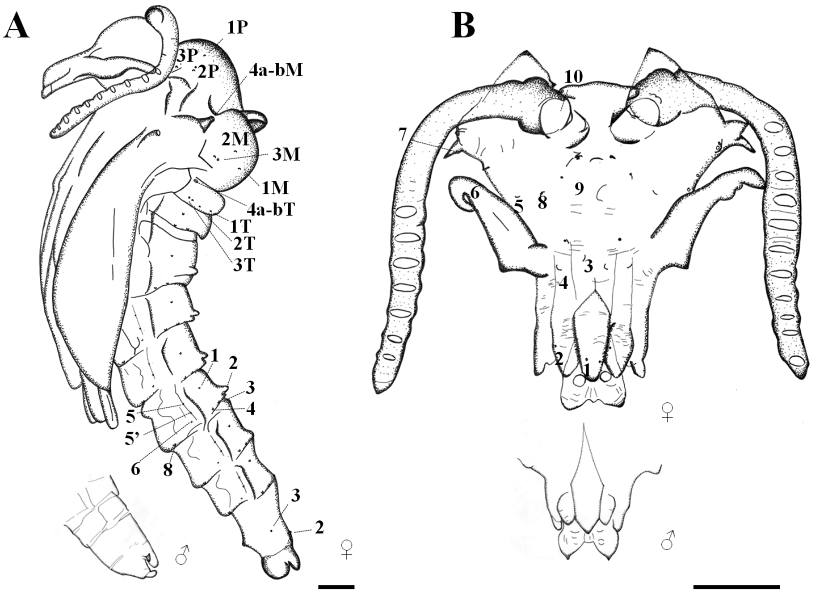

FIGURE 8. Morphology of *Trichophoromyia brachipyga* pupa, with distributional pattern of the setae. A: general appearance of the body in lateral view; B: head, in frontal view; bar = 100µm.

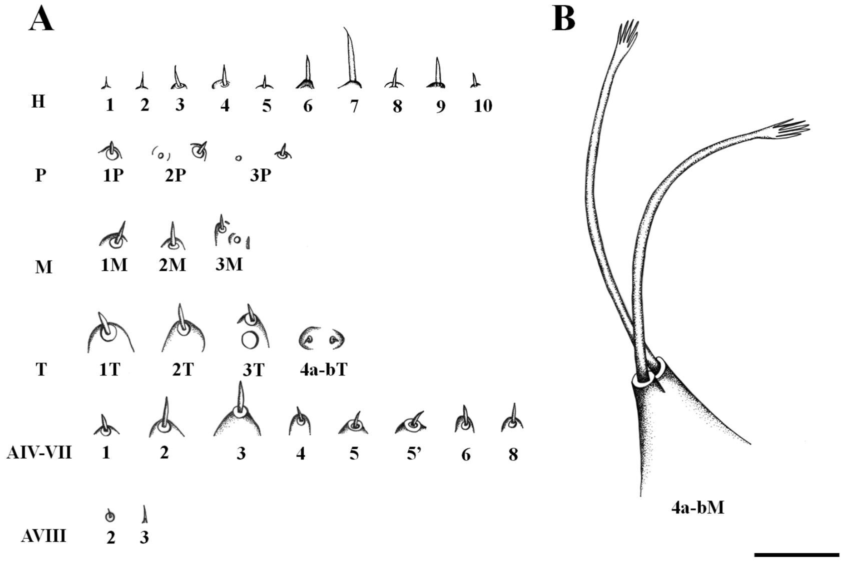

FIGURE 9. Setae types of *Trichophoromyia brachipyga* pupa. A: setae of head, thorax and abdomen; B: pre-alars setae; AIV-VII: setae of the abodominal segments AIV-VII; VIII: setae of the abdominal segment VIII; H: setae of the head; M: setae of the mesothorax, P: setae of the prothorax; T: setae of the metathorax; bar = 50µm.

Discussion

The polygonal pattern found in the egg exocorionic sculpture of *Th. brachipyga* allocates this species to group A, as proposed by Feliciangeli *et al*. (1993), and corroborated by Almeida *et al*. (2004). This pattern is the most frequent among Neotropical phlebotomines and found in other genera of Psychodopygina (some species of *Nyssomyia* Barretto and *Psathyromyia* Barretto), as well as Lutzomyiina Abonnenc & Lèger (*Evandromyia* Mangabeira*, Lutzomyia,* and *Pintomyia* Costa Lima) and Sergentomiina Galati (*Micropygomyia* Barretto) (Almeida *et al*. 2004). This feature could be phylogenetically significant since the classification by sculpture patterns is generally convergent with the adult-based systematic position. However, it is not well understood if exochorion patterns differ more according to the environmental conditions of the breeding sites (Ward & Ready 1975) or reflect the structure of the follicular cells in the ovariole, which is a manifestation of an adult character (Van Emden 1957; Ghosh & Mukhopadhyay 1996). Based on the appearance of the antennae, the fourth-instar larvae of New World phlebotomines can be classified into four categories. *Th. brachipyga* belongs to Group 2, defined by: antennal tubercle in the form of a truncated cone, basal segment short, distal segment cylindrical (Leite & Williams 1997). Until now, all known larvae of Psychodopygina are also classified into this group (Barretto 1941; Hanson 1968; Ward 1976b; Alencar *et al*. 2018).

Hanson (1968) highlighted the arrangement of the cephalic spicules of Phlebotominae but considered it a limited taxonomic character, due to the lack of precise differences between the studied *taxa*. These spicules were homogeneously distributed in *Th. brachipyga*, similar to that of *Ny. antunesi* (Coutinho) and *Ny. umbratilis* (Ward & Fraiha) (Ward 1976b; Alencar *et al*. 2018), but distinct from those of *Ny. intermedia* (Lutz & Neiva), *Ny. trapidoi* (Fairchild & Hertig), and *Ny. ylephiletor* (Fairchild & Hertig) (Barretto 1941; Hanson 1968), which are irregularly arranged and form semicircles and/or irregular polygons.

Mouthparts of Phlebotominae have been subjected to taxonomic attention only in the last twenty years (Arrivillaga *et al*. 1999; Mukhopadhyay & Ghosh 2000; Pessoa *et al*. 2008), thus, detailed studies and comparisons are still limited to a few *taxa*. Setae distribution of *Th. brachipyga* mandibula (tree setae in the dorsal view) is similar to the known mandibulae of *Lutzomyia, Dampfomyia* Addis, *Micropygomyia* Barretto, and *Nyssomyia* Barretto (Arrivillaga *et al*. 1999; Arrivillaga & Feliciangeli 2000; Montes de Oca-Aguilar *et al*. 2014, 2016; Alencar *et al*. 2018), and clearly distinct from that of *Evandromyia* spp., which have three additional setae in ventral view (Pessoa *et al*. 2008). The incisor lobe with three equally sharp teeth in *Th. brachipyga* differs from that of *Da. beltrani* (Vargas & Díaz-Nájera) and *Mi. chiapanensis* (Dampf), which have bilobate, discontinuously bordered proximal teeth (Montes de Oca-Aguilar *et al*. 2014, 2016). The middle and proximal teeth also appeared blunt in *Ny. umbratilis* and *Lu. longipalpis* (Lutz & Neiva) (Alencar *et al*. 2018; Arrivillaga & Feliciangeli 2000) and sharp in *Th. brachipyga*. Marialva *et al*. (2020) registered the median tooth of *Migonemyia migonei* (França) as double-paired, visible only through scanning electron microscopy. However, under light microscopy, it is still believed that the incisor lobe of *Th. brachipyga* is likely to be as described. *Th. brachipyga* can also be differentiated from *Ny. umbratilis, Da. beltrani* and *Mi. chiapanensis* by the arrangement of teeth and setae on the epipharynx (Alencar *et al*. 2018; Montes de Oca-Aguilar *et al*. 2014, 2016).

Larva of *Th. brachipyga* can be mainly differentiated from those of the other genera within Psychodopygina by the following: dorsal intermediate seta (2) of prothorax I are present, whereas these are absent in *Nyssomyia*; the ovoid head and antennal tubercles, do not extending beyond the head capsule on dorsal view, differing from those of *Psychodopygus* Mangabeira and *Bichromomyia* Galati, which have round-ovoid heads, frequently angular in outline, and large antennal tubercles extending beyond the margin of the head capsule on dorsal view (Ward *et al*. 1976b); the presence of a tiny lateroventral seta (4) of prothorax I and lack of size homology on ventral internal setae (9) of meso and metathorax distinguishes it from *Viannamyia*, which lack this seta of prothorax I and possess homology between all setae of the meso- and metathorax (Hanson 1968); presence of antenna with curved, cylindrical distal segment, differs from *Martinsmyia* Galati, in which this segment is cylindrical and likely straight (Barretto 1941); presence of antenna with the proximal segment under 25% the length of the distal, and with the apex anterior-ward faced, differs it from *Psathyromyia* of the Shannoni complex, in which this proportion of these segments are likely equal or sub equal, and the apex is posterior-ward faced (Hanson 1968).

Apart from studies by Montes de Oca-Aguiar *et al*. (2016a, 2016b), little taxonomic attention is given to the arrangement of muscle plaques on the tergal surface of abdominal segment VIII; bare circular patches indicating previous attachment of muscle sets are likely used to emerge from the cuticular structure encasing the new instar (Finalyson 1975; Stoffolano *et al*. 1988; Borkent 2012). The arrangement found in *Th. brachipyga* comprises five pairs of muscle plaques that are evenly distributed, differing from those of *Da. beltrani* (two pairs), *Mi. chiapanensis* (four pairs), and *Lu. cruciata* (Coquillett) (four pairs), with the posterior ones not being near the tergal margin (Montes de Oca-Aguilar *et al*. 2014, 2016a, 2016b).

Pupae chaetotaxy of *Th. brachipyga* differs from that of *Ny. umbratilis* (see Alencar *et al*. 2018), mainly for the number and distribution pattern of sensilla in the abdominal segments. In the segments I-VII, *Th. brachipyga* presents lateral posterior sensilla, which are described and mentioned by Forattini (1973), even though they are not present in the chaeotaxy nomenclature system proposed by Montes de Oca-Aguilar *et al*. (2014). Thus, the authors of the present paper referred these sensilla as 5' to avoid confusion with these previous systems. Moreover, in *Th. brachipyga*, the abdominal segment VIII bears two setae, lateral (3) and dorsal inferior (2), while in *Ny. umbratilis,* only seta dorsal superior (1) is present. Pupae chaetotaxy of *Th. brachipyga* is also clearly distinct from that of *Lu. longipalpis*, *Da. beltrani*, *Mi. chiapanensis* and *Lu. cruciata* (Leite *et al*. 1991; Montes de Oca-Aguilar *et al*. 2014; Montes de Oca-Aguilar *et al*. 2016a, 2016b), which have more setae and a distinct distribution pattern. The mesonotal tubercle in *Th. brachipyga* is blunt and moderately developed, similar to that of the Psychodopygina species *Pa. shannoni* (Dyar) and *Ny. umbratilis* (Hanson 1968; Alencar *et al*. 2018), but distinct from that of *Ny. trapidoi*, which is blunt and well-developed (prominent), and *Vi. furcata* (Mangabeira), which is sharp and developed, greater in length than width, and sometimes posteriorly curved (Hanson 1968). Prealar setae in *Th. brachipyga* are chaetic, large, and branch-tipped, differing from those of *Pa. shannoni* and *Bi. flaviscutellata* (Mangabeira), which are shorter, with the former being clavate or bud-like (Hanson 1968; Ward 1976b), and from those of *Ny. umbratilis*, which are equally large but simple-tipped (Alencar *et al*. 2018).

In the metathorax, the type of prehaltere setae of *Th. brachipyga* is characterized by a set of two (sometimes three) inconspicuous alveoli and styloconic setae, which are morphologically similar to those of *Lu. longipalpis*, *Da. beltrani*, and *Mi. chiapanensis* (Leite *et al*. 1991; Montes de Oca-Aguilar *et al*. 2014; Montes de Oca-Aguilar *et al*. 2016a), slightly different from those of *Mi. migonei*, which are basiconic (Marialva *et al*. 2020), and markedly distinct from those of *Lu. cruciata*, which are chaetic (Montes de Oca-Aguilar *et al*. 2016b).

Alveoli-like structures were observed near the prothoracic 3P, mesothoracic 3M, metathoracic 3T and 4a-bT, sensillum 5 of abdominal segments I-VII, and between sensilla 1, 2, and 3 of abdominal segments I-VII. Montes de Oca-Aguilar *et al*. (2016b) stated that placoid sensilla could be included in the numerical system of Abonnenc (1956), also referred to as superficial sensorial organs by Forattini (1973).

The present study provided a first step in understanding the morpho-taxonomy of *Trichophoromyia* immatures. Further studies are needed to determine the significance of the characters described in the Phlebotominae adult-based phylogeny.

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References

Abonnenc, E. (1956) L'œuf et les formes pré-imaginales de deux Phlébotomes africains: *P. antennatus var. occidentalis* Theodor, 1933 et *P. dubius* Parrot, Mornet et Cadenat, 1945. *Archives de l'Institut Pasteur d'Algérie*, 34, 518–539.

Alencar, R.B., Justiniano, S.C.B. & Scarpassa, V.M. (2018) Morphological description of the immature stages of *Nyssomyia umbratilis* (Ward & Frahia) (Diptera: Psychodidae: Phlebotominae), the main vector of *Leishmania guyanensis* Floch (Kinetoplastida: Trypanosomatidae) in the Brazilian Amazon Region. *Neotropical Entomology*, 47 (5), 668–680. https://doi.org/10.1007/s13744-018-0587-6.

Almeida, D.N., Oliveira, R.S., Brazil, B.G. & Soares, M.J. (2004) Patterns of exochorion ornaments on eggs of seven South American species of *Lutzomyia* sand flies (Diptera: Psychodidae). *Entomological Society of America*, 41, 819–825. https://doi.org/10.1603/0022-2585-41.5.819

Arrivillaga, J. & Feliciangeli, M.D. (2000) Diferenciación morfológica larval entre poblaciones de *Lutzomyia longipalpis* (Lutz & Neiva 1912) en Venezuela (Diptera: Psychodidae). *Boletín de Entomología Venezolana*, 15 (2), 229–234.

Arrivillaga, J., Navarro, J.C. & Feliciangeli, M.D. (1999) Morfología y quetotaxia del tagma cefálico larval de *Lutzomyia* França, 1924 (Diptera: Psychodidae): Proposición de un sistema de nomenclatura. *Boletín de Entomología Venezolana*, 14 (1), 1–13.

Barretto, M.P. (1941) Morfologia dos ovos, larvas e pupas de alguns Flebotomus de São Paulo. *Anais da Faculdade de Medicina da Universidade de São Paulo*, 17, 357–427.

Barretto, M.P. (1962) Novos subgêneros de *Lutzomyia* França, 1924 (Psychodidae, subfamília Phlebotominae). *Revista do Instituto de Medicina Tropical de São Paulo*, 4, 91–100.

Borkent, A. (2012) The pupae of Culicomorpha-morphology and a new phylogenetic tree. *Zootaxa*, 3396 (1), 1–98. https://doi.org/10.11646/zootaxa.3396.1

Feliciangeli, M.D., Castejon, O.C. & Limongi, J. (1993) Egg surface ultrastructure of eight New World phlebotomine sand fly species (Diptera: Psychodidae). *Journal of Medical Entomology*, 30 (4), 651–656. https://doi.org/10.1093/jmedent/30.4.651

Finlayson, L.H. (1975) Development and degeneration. *In*: Usherwood, P.N.R. (Eds.), *Insect muscle*. Academic Press, London and New York, pp. 75–149.

Forattini, O.P. (1973) *Entomologia Médica: 4, Psychodidae. Phlebotominae. Leishmanioses. Bartonelose.* Edgard Blucher/ EDUSP, São Paulo, 658 pp.

Galati, E.A.B. (2003) Morfologia, terminologia de adultos e identificação dos táxons da América. *In*: Rangel, E.F. & Lainson, R. (Eds.), *Flebotomíneos do Brasil*. Fiocruz, Rio de Janeiro, pp. 53–175.

Galati, E.A.B. (2018) Phlebotominae (Diptera, Psychodidae): Classification, morphology and terminology of adults and identification of American taxa. *In*: Rangel, E.F. & Shaw, J. (Eds.), *Brazilian sand flies*. Springer, Cham, pp. 9–212. https://doi.org/10.1007/978-3-319-75544-1_2

Ghosh, K.N. & Mukhopadhyay, J. (1996) A comparison of chorionic sculpturing of four Indian phlebotomine sandflies (Diptera: Psychodidae) by scanning electron microscopy. *Parasite*, 3(1), 61–67. https://doi.org/10.1051/parasite/1996031061

Hanson, W.J. (1968) *The immature stages of the subfamily phlebotominae in Panama (Diptera: Psychodidae)*. Ph. D. Thesis, University of Kansas, University Microfilms Inc., Ann Arbor, Michigan, pp.1–67.

Harbach, R.E. & Knight, K.L. (1980) *Taxonomists' Glossary of Mosquito Anatomy*. Plexus Publishing, Inc. Marlton, New Jersey, 415 pp.

Killick-Kendrick, M. & Killick-Kendrick, R. (1991) The initial establishment of sandfly colonies. *Parassitologia*, 33, 315–320. [PMID: 1841223]

Leite, A.C.R. & Williams, P. (1997) The first instar larva of *Lutzomyia longipalpis* (Diptera: Phlebotomidae). *Memórias do Instituto Oswaldo Cruz*, 92, 197–203. https://doi.org/10.1590/S0074-02761997000200011.

Leite, A.C., Williams, P. & dos Santos, M.C. (1991) The pupa of *Lutzomyia longipalpis* (Diptera: Psychodidae—Phlebotominae). *Parassitologia*, 33, 477–484. [PMID: 1841247]

Mangabeira Filho, O. (1942) 7ª Contribuição ao estudo dos Flebotomus (Diptera: Psychodidae): descrição dos machos de 24 novas espécies. *Memórias do Instituto Oswaldo Cruz*, 37 (2), 111–218. https://doi.org/10.1590/S0074-02761942000200003

Marialva, E.F., Secundino, N.F., Fernandes, F.F., Araújo, H.R., Ríos-Velásquez, C.M., Pimenta, P.F. & Pessoa, F.A. (2020) Morphological aspects of immature stages of *Migonemyia migonei* (Diptera: Psychodidae, Phlebotominae), an important vector of Leishmaniosis in South America, described by scanning electron microscopy. *Plos One*, 15(11), e0242163. https://doi.org/10.1371/journal.pone.0242163

Montes de Oca-Aguilar, A.C., Rebollar-Téllez, E.A. & Ibáñez-Bernal, S. (2016a) The immature stages of *Micropygomyia (Coquillettimyia) chiapanensis* (Dampf) (Diptera: Psychodidae, \Phlebotominae). *Zootaxa*, 4105 (5), 455–482. https://doi.org/10.11646/zootaxa.4105.5.3

Montes de Oca-Aguilar, A.C., Rebollar-Téllez, E.A., Piermarini, P.M. & Ibáñez-Bernal, S. (2016b) Descriptions of the immature stages of *Lutzomyia (Tricholateralis) cruciata* (Coquillett) (Diptera: Psychodidae, Phlebotominae). *Neotropical Entomology*, 46 (1), 66–85. https://doi.org/10.1007/s13744-016-0439-1

Montes de Oca-Aguilar, A.C., Rebollar-Téllez, E. & Ibáñez-Bernal, S. (2014) Descriptions of the immature stages of *Dampfomyia (Coromyia) beltrani* (Vargas & Díaz-Nájera) (Diptera: Psychodidae), with notes on morphology and chaetotaxy nomenclature. *Zootaxa*, 3887 (3), 251–297. https://doi.org/10.11646/zootaxa.3887.3.1

Mukhopadhyay, J. & Ghosh, K. (2000) Morphology of larval antennae and mouthparts of four Indian sand flies (Diptera: Psy-

chodidae) by scanning electron microscopy. *Journal of Medical Entomology*, 37 (4), 575–580.
https://doi.org/10.1603/0022-2585-37.4.575

Pessoa, F.A.C., Feitosa, M.A.C., Castellón-Bermúdez, E.G., Ríos-Velásquez, C.M. & Ward, R.D. (2008) Immature stages of two species of *Evandromyia (Aldamyia)* and the systematic importance of larval mouthparts within Psychodidae (Diptera, Phlebotominae, Psychodinae). *Zootaxa*, 1740 (1), 1–14.
https://doi.org/10.11646/zootaxa.1740.1.1

Sánchez Uzcátegui, Y.D., Vasconcelos dos Santos, T., Silveira, F.T., Ramos, P.K., dos Santos, E.J.M. & Póvoa, M.M. (2020) Phlebotomines (Diptera: Psychodidae) from an urban park of Belém, Pará State, northern Brazil and potential implications in the transmission of American cutaneous leishmaniasis. *Journal of Medical Entomology*, 57 (1), 281–288.
https://doi.org/10.1093/jme/tjz153

Satchell, G.H. (1953) On the early stages of *Bruchomyia argentina* Alexander (Diptera: Psychodidae). *Physiological entomology*, 28 (1–3), 1–12.
https://doi.org/10.1111/j.1365-3032.1953.tb00719.x

Stoffolano, J.G., Woodley, N.E., Borkent, A. & Yin, L.R. (1988) Ultrastructural studies of the abdominal plaques of some Diptera. *Annals of the Entomological Society of America*, 81 (3), 503–510.
https://doi.org/10.1093/aesa/81.3.503

Van Emden, F.L. (1957) The taxonomic significance of the characters of immature insects. *Annual Review of Entomology*, 2 (1), 91–106.
https://doi.org/10.1146/annurev.en.02.010157.000515

Vasconcelos dos Santos, T., Santos Neto, N.F., Sánchez Uzcátegui, Y.D.V. & Galardo, A.K.R. (2019) *Trichophoromyia iorlandobaratai* (Diptera: Psychodidae), a new phlebotomine species from the Brazilian Amazonia. *Journal of Medical Entomology*, 56 (2), 416–420.
https://doi.org/10.1093/jme/tjy194

Vasconcelos dos Santos, T. & Silveira, F.T. (2020) Increasing putative vector importance of *Trichophoromyia* phlebotomines (Diptera: Psychodidae). *Memórias do Instituto Oswaldo Cruz*, 115, e190284.
https://doi.org/10.1590/0074-02760190284

Ward, R.D. (1976a) A revised numerical chaetotaxy for neotropical phlebotomine sandfly larvae (Diptera: Psychodidae). *Systematic Entomology*, 1 (1), 89–94.
https://doi.org/10.1111/j.1365-3113.1976.tb00035.x

Ward, R.D. (1976b) The immature stages of some phlebotomine sandflies from Brazil (Diptera: Psychodidae). *Systematic Entomology*, 1 (3), 227–240.
https://doi.org/10.1111/j.1365-3113.1976.tb00043.x

Ward, R.D. & Ready, P.A. (1975) Chorionic sculpturing in some sandfly eggs (Diptera, Psychodidae) 1. *Journal of Entomology Series A, General Entomology*, 50 (2), 127–134.
https://doi.org/10.1111/j.1365-3032.1975.tb00101.x

Young, D.G. & Duncan, M.A. (1994) *Guide to the identification and geographic distribution of Lutzomyia sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Memoirs of the American Entomological Institute 54*. Associated Publishers, American Entomological Institute, Gainesville, Florida, 881 pp.
https://doi.org/10.21236/ADA285737

Zacharuk, R.Y. & Shields, V.D. (1991) Sensilla of immature insects. *Annual Review of Entomology*, 36, 331–354.
https://doi.org/10.1146/annurev.en.36.010191.001555