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A new species of *Alipumilio* Shannon (Diptera, Syrphidae) found in association with the exudate resin of *Schinus terebinthifolius* Raddi (Anacardiaceae)

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Abstract

The adult stage of a new species of flower fly, *Alipumilio athesphatus* Thompson (Diptera, Syrphidae) is described. The gross morphology and external integumentary features of the egg, third-instar larva and pupa are also presented. All immature stages were found in association with the exudate resin of *Schinus terebinthifolius* Raddi (Anacardiaceae) in Porto Alegre, Rio Grande do Sul State, Brazil.

Key words. insect taxonomy, insect morphology, insect fine structure, neotropics, flower flies, brazilian peppertree, tree resins

Introduction

The genus *Alipumilio* Shannon, 1927 (Diptera, Syrphidae) is restricted to the Neotropical region, and was described on the basis of a single female collected by H. W. Bates on Amazon, which was named as *Alipumilio femoratus* Shannon (1927: 12).

Vockeroth (1964) revised the genus and described three new species based on single females from Peru and Mexico. Thompson (1972) redescribed the genus and described the first male, placing *Alipumilio* in the tribe Eumerini with *Nausigaster* Williston, 1883 and *Psilota* Meigen, 1822. Rotheray *et al.* (2000) described the larval stage of the type species and suggested based on cladistic analysis that *Alipumilio* is closely related to *Eumerus*, but distant of *Nausigaster.* He also pointed out the presence of a hook-bearing cephalo-pharyngeal skeleton, also found in the larvae of some species of *Cheilosia* Meigen, 1822. Later a more comprehensive analysis was made (Ståhls *et al.* 2003) where the relationship of *Alipumilio* to other syrphids varied greatly depending on the character set used (adult, larval, or DNA), but the combined analysis always included *Alipumilio* in the tribe Eumerini (Ståhls *et al.* 2003).

Alipumilio comprises six recognized species, including the new species described herein. The immature stages live in association with trees that produce resin. Some *Alipumilio* larvae were reared from pine trees in southern Brazil (Thompson 1972); larvae of *A. femoratus* were reared from the sap of *Psychotria* sp. (Rubiaceae) in Ecuador (Rotheray *et al.* 2000); and Plowden *et al.* (2004) found an *Alipumilio* species in the exuded resin of *Protium* species (Burseraceae) in the Eastern Brazilian Amazon.

Alipumilio athesphatus **sp. n.** eggs are laid on small crevices of *Schinus terebinthifolius* Raddi (Anacardiaceae) stem surface. Soon after eclosion, the first instar larva digs a gallery, burying itself into the

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stem up to the resin vessels from which it feeds upon. The larva leaves the gallery for pupation, the pupa being found outside, covered by the corresponding exudate resin on the stem surface. The larval feeding mechanism of *A. athesphatus* is described in detail elsewhere (Massardo 2009). In this study, in addition to the species description, we characterize and illustrate the integumentary morphology of the immature stages based upon light and scanning electron microscopy.

Schinus terebinthifolius Raddi (Anacardiaceae), the Brazilian peppertree, is considered an invasive (noxious) weed in southern Florida. So, if *Alipumilio* stresses and or reduces the vigor of the plant by its mining, then the fly may be useful in the biological control of this noxious weed.

Material and methods

All stages of *Alipumilio athesphatus* were collected from *Schinus terebinthifolius* plants found in Parque Natural Morro do Osso, Porto Alegre, Rio Grande do Sul State, Brazil (30°07'S 51°14'W).

Eggs were collected directly from the stem surface by slicing the stem cork. Larvae were removed from the plant by gently digging them from their galleries with the aid of a sharp knife. Pupae were collected by scraping the outside exudate resin from the larval feeding galleries. The adults were trapped upon emergence by enclosing the outside exudate resin with a small bag (9 by 12 cm) made of a fine cloth. The bag was partially cut to encircle the stem, and closely tied on the upper side of the stem using a rubber band.

To clear away the resin, the larvae were immersed in turpentine essence for 48 hours, cleared in a 10% KOH solution for 48 hours, neutralized with glacial acetic acid, washed with 70% alcohol, washed with distilled water, and ultimately fixed and preserved in Dietrich´s fluid.

The gross morphology of the immatures and adults was studied with the aid of a stereo microscope Leica® MZ 75. Light micrographs were obtained with a Leica® DFC 500 digital camera attached to a stereo microscope Leica® MZ 16. Images were captured by using the IM 50 (Image Manager) software, and then manipulation was performed by using the Automontage® (Syncroscopy) software.

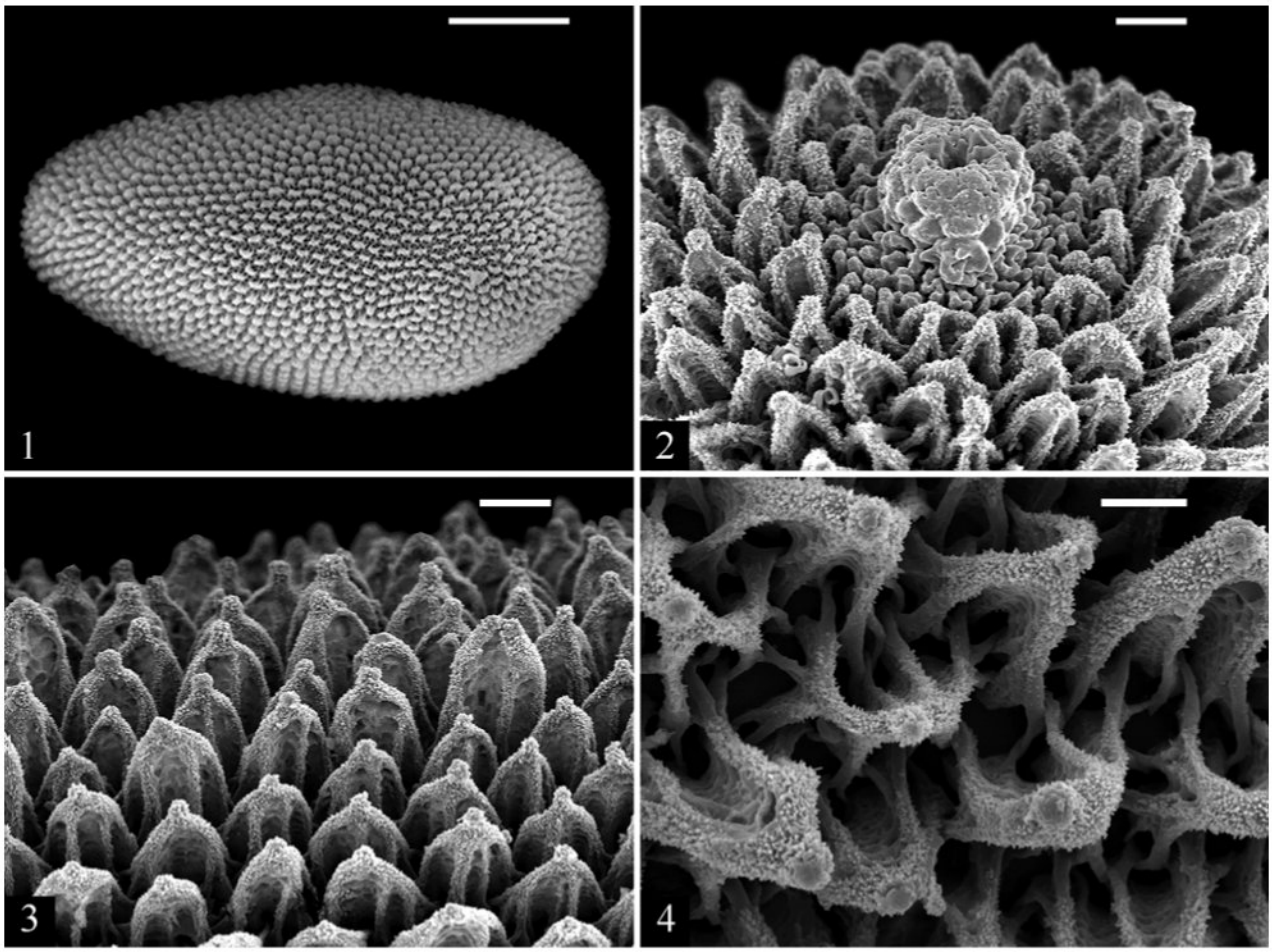
The male and female genitalia were drawn with the aid of a camera lucida attached to a Zeiss® Standard 20 compound microscope. The genitalia was cleared in a 10% KOH solution for 36 hours, neutralized with glacial acetic acid, washed with 70% alcohol, washed with distilled water and preserved in liquid glycerin.

For the identification of larval instars, preserved larvae were re-hydrated, immersed in liquid glycerin, and mounted in glycerin jelly. The length of the respiratory process was measured in dorsal view by using an ocular grid attached to the stereo microscope. Data were adjusted to the exponential function through the least square method (Snedecor & Cochran 1980).

For the scanning electron microscopy (SEM), preserved specimens were immersed overnight in acetone and then critical-point-dried (Bal-tec® - CPD030). They were then mounted on aluminum stubs and coated with gold, using a Bal-tec® - SCD050 sputter coater, and observed and photographed in a JEOL® 5800 scanning electron microscope.

The terminology for larvae follows Roberts (1970) and Rotheray & Gilbert (1999), and for adults follows Thompson (1999).

The following institutional acronyms are used: (CAS)—California Academy of Sciences, San Francisco, USA; (CBFC)—Coleccion Boliviana de Fauna, La Paz, Bolivia; (DZRS)—Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Porto Alegre, Rio Grande do Sul, Brazil; (DZUP)—Coleção Entomológica Padre Jesus Santiago Moure, Departamento de Zoologia da Universidade Federal do Paraná, Curitiba, Paraná, Brazil; (USNM)—National Museum of Natural History, Smithsonian Institution, Washington DC, USA.



FIGURES 1–4. *Alipumilio athesphatus* **sp. n.**, egg (SEM). (1) Laterodorsal view. (2) Detail of micropyle. (3–4) Detail of chorionic surface. Scale bar: Fig. (1): 200 µm; Figs (2–3): 20 µm; Fig. (4): 10 µm.

Results

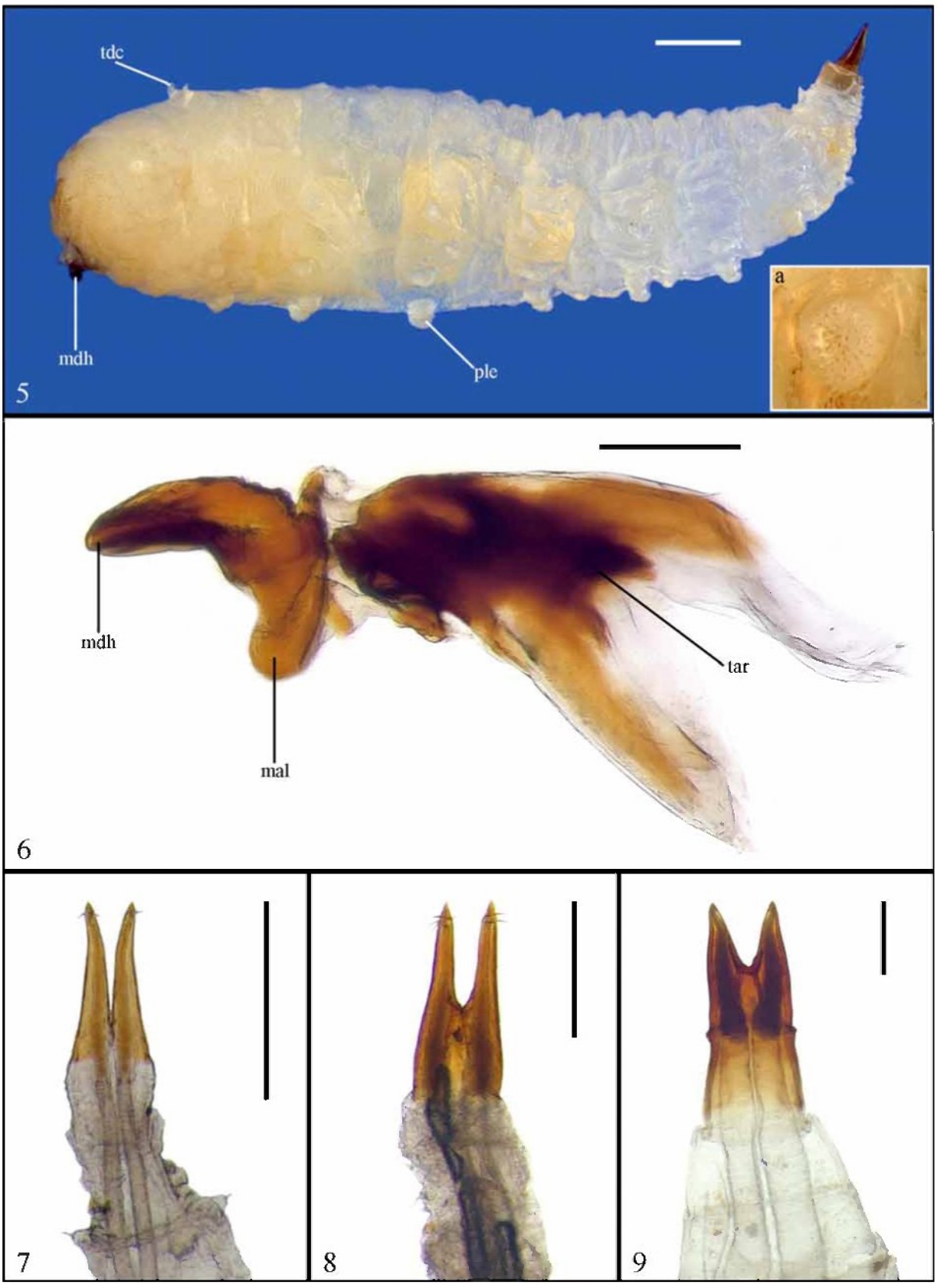
Alipumilio athesphatus sp. n. Thompson

Alipumilio species 1. Thompson 1972: 127 (male genitalia).

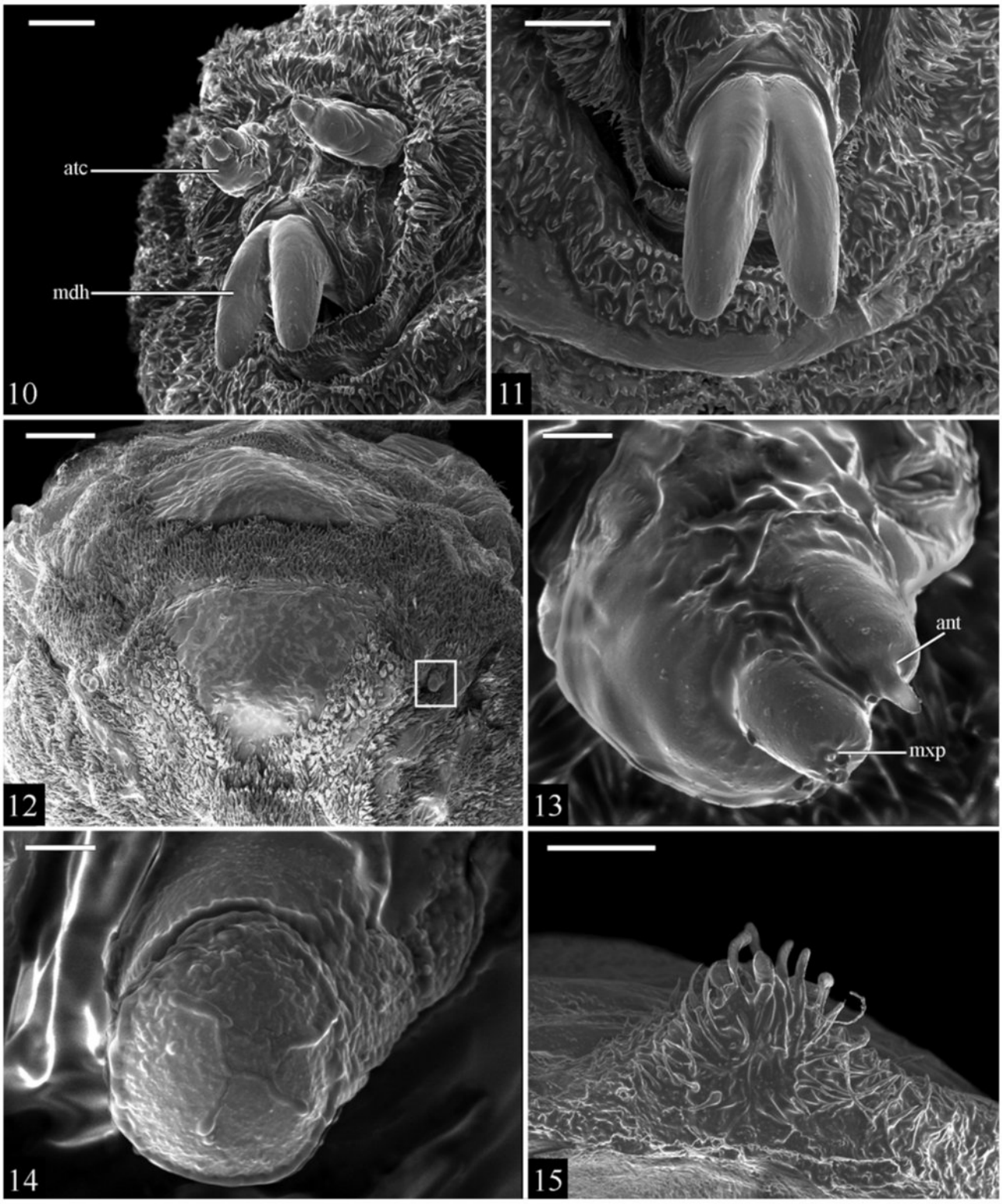
Description. Egg (Figs 1–4)**.** Length 1.2–1.26 mm (n = 8), maximum width 0.61 mm. White in color throughout the embryonic development. Elongated-oval in shape, rounded at both ends and slightly tapering towards the anterior pole, and laid with the micropylar axis parallel to the substrate (Fig. 1). The micropyle appears as a conspicuous black crown-like on the anterior pole (Fig. 2). The chorionic surface sculpturing is delicate: a plastron meshwork (Hinton 1981) composed of longitudinally ridged cuneiform projections (Figs 3–4).

Alipumilio athesphatus eggs are laid isolated into small bark crevices of *S. terebinthifolius* stems. Additional laboratory observation showed that soon after hatching**,** the larva digs a small hole and buries itself into the plant tissues below. Remains of the chorion is frequently found attached to resin exudate on *S. terebinthifolius* plants.

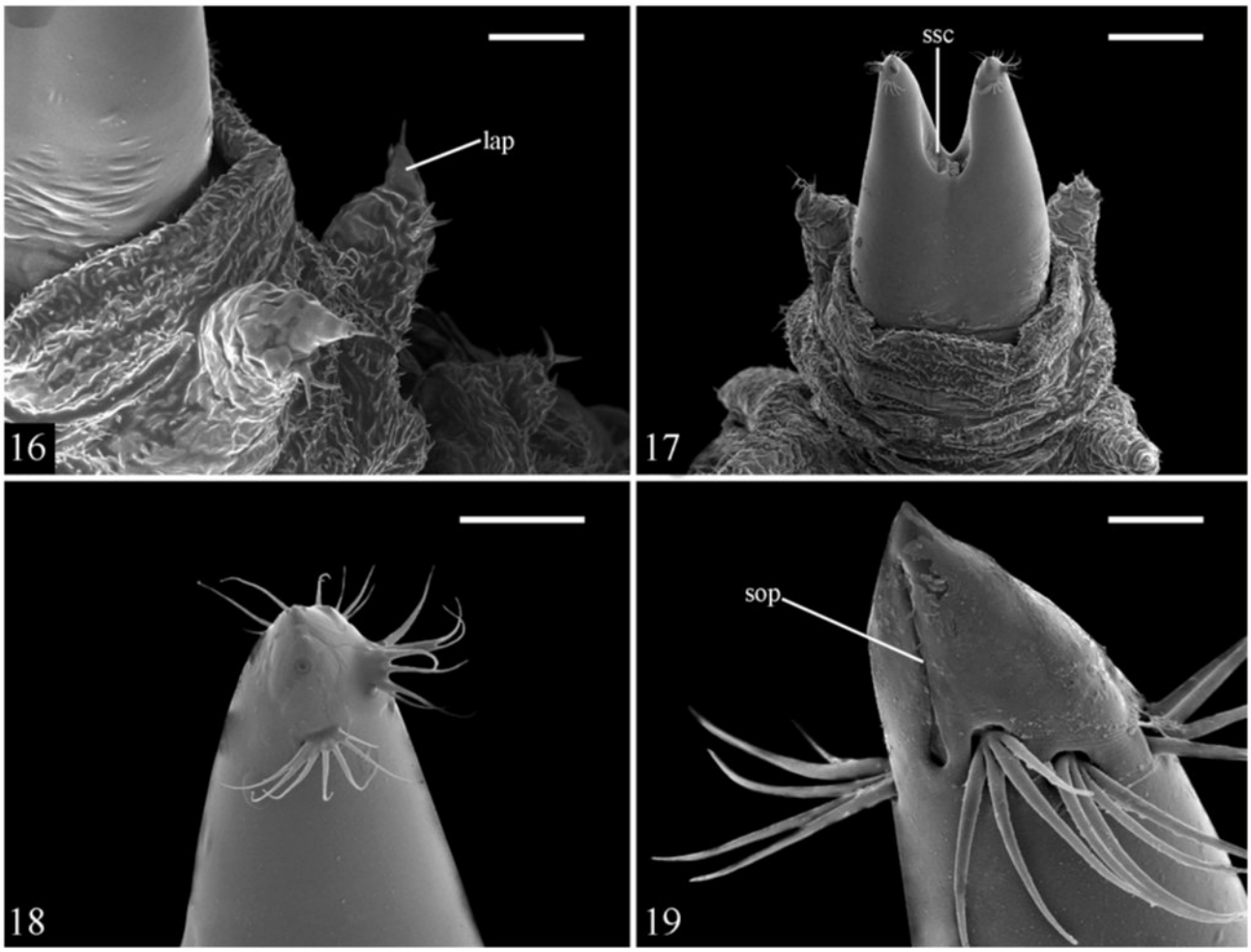
Third instar larva (Figs 5–19)**. Shape and dimensions**. Length 8–16 mm (n = 10); maximum width 3mm; larva pale in color; cylindrical in cross-section, truncate anteriorly and tapering posteriorly (Fig. 5). Dorsal body surface coated in fine and sub-developed pubescence, patchily distributed. Anterior fold with longitudinal bands of short spicules. Sensilla accompanied by two or more setae; pattern of sensilla similar to other Syrphidae (Rotheray & Gilbert 1999).



FIGURES 5–9. *Alipumilio athesphatus* **sp. n.**, immature stages. Third instar larvae: (5) lateral view: (a) detail of proleg. (6) Mandible, lateral view. Posterior respiratory process: (7) First instar larvae. (8) Second instar larvae. (9) Third instar larvae. Scale bar: Fig. (5): 1 mm; Fig. (6): 0.5 mm; Figs (7–9): 0.25 mm. (mal) mandibular apodeme fused to mandibular lobe. (mdh) mandibular hook. (ple) proleg. (tar) tentorial arm. (tdc) tubercle of differentiated cuticle.



FIGURES 10–15. *Alipumilio athesphatus* **sp. n.**, third instar larvae (SEM). (10) Prothorax, anteroventral view. (11) Mandibular hook, anterior view. (12) Prothorax, dorsal view (anterior spiracle released with a square). (13) Antennomaxillary complex, anterodorsal view. (14) Anterior spiracle, anterodorsal view. (15) Tubercle of differentiated cuticle, first abdominal segment, anterior view. Scale bar: Figs (10, 11, 15): 100 µm; Fig. (12): 200 µm; Fig. (13): 20 µm; Fig. (14): 10 µm. (ant) antenna. (atc) antennomaxillary complex. (mdh) mandibular hook. (mxp) maxillary palp.



FIGURES 16–19. *Alipumilio athesphatus* **sp. n.**, posterior respiratory process, third instar larvae. (16) Anterobasal view. (17) Anterior view. (18–19) Apical tip. Scale bar: Fig. (16): 100 µm; Fig. (17): 200 µm; Fig. (18): 50 µm; Fig. (19): 10 µm. (lap) lappet. (sop) spiracular opening. (ssc) spiracular scar.

Prothorax. *Ventroanterior region.* Antennomaxillary complex well developed (Figs 10, 13). Dorsal lip smooth, lacking setae medially (Figs 10, 11). Lateral lips little developed and covered with conspicuous setae. Cephalo-pharyngeal skeleton with a huge, black and heavily sclerotized pair of mandibular hooks (Figs 5, 6, 10, 11); mandibular lobes are fused on mandibular apodeme (Fig. 6). *Lateroanterior region.* Anterior spiracles sclerotized and strongly reduced (Figs 12, 14). *Dorsal region.* Surface with a triangular heavily sclerotized plate, without spicules, bearing sensilla 1–3 (Fig. 12).

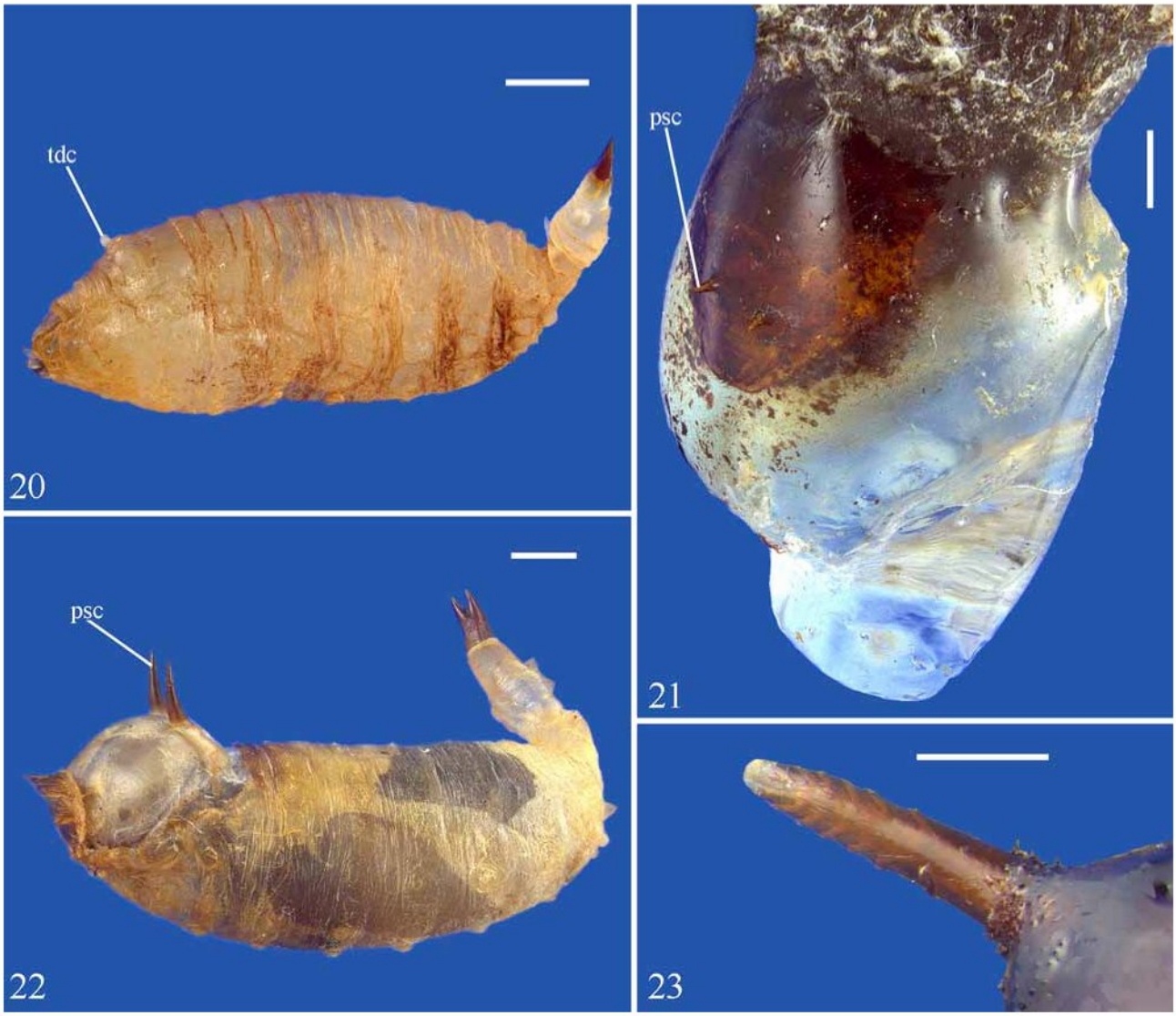
Mesothorax. Dorsal surface with a less sclerotised region than dorsal surface of prothorax, without spicules (Fig. 12).

Abdomen. Prolegs on segments 1–7 with bearing sparse distributed crochets and lacking a planta (Fig. 5a). Lateral region of abdominal segments 1–8 with two pseudopodium, vertically distributed, coated by delicate setae. Dorsal surface of first segment with two tubercle of differentiated cuticle forming the opening through which the pupal spiracle will be thrust (Fig. 5, 15, 20). Lappets on eighth segment have one sensillum at the tip, a second lower down and a third bifurcated towards the base (Fig. 16). Posterior respiratory process dark-brown, lustrous, heavily sclerotized, bifurcated and retractile (Figs 5, 7–9,17); each apical tip bearing longitudinal spiracular openings (Fig. 19), with four spiracular groups of setae, one anterior, one posterior and two lateroexternal (Fig. 18, 19); base of the bifurcation, from the second and third larval instars, bearing two outer scars of the preceding spiracles (Fig. 17).

Instar identification. *Alipumilio athesphatus* larvae pass through three larval instars, which can be

identified by differences existing in the size of their respiratory processes. In the first instar, the base of the respiratory process is shorter than the arms, and to the contrary in the last (= third) instar, where the base is much greater in length. These portions of the respiratory process are similar in length in the intermediate (= second) instar (Figs 7–9).

Data resulting from measurement of the total length of the respiratory process for the different larval instars significantly adjusted to the equation ln y = 0.548x – 2.192; r = 0,978; n = 30; p < 0,001. There was no overlap on such values among instars (Table 1), and thus they can also be identified by measuring that structure. The respiratory process grew geometrically at an average rate of 1.73 among instars, thus following the Brooks-Dyar rule found for several insects (Daly 1985).



FIGURES 20–23. *Alipumilio athesphatus* **sp. n.**, pupae. (20) Pupa, initial stage of development. (21) Pupa into a drop of exudate resin. (22) Pupa, final stage of development (note the adult emerging). (23) Detail of pupal spiracle. Scale bar: Figs (20–22): 1 mm; Fig. (23): 0.25 mm. (psc) pupal spiracle. (tdc) tubercle of differentiated cuticle.

Pupa (Figs 20–24). Cylindrical in cross section. Anterior end truncate, tapered posteriorly (Figs 20, 21). *Pupal spiracles*. Dark brown in color; projecting from middle of upper part of operculum, separated by distance similar to the length of one spiracle (Figs 21–24). These processes are horn-like structures, approximately 1.2mm in length and tapering with spiracular openings clustered at lateral edges (Figs 23, 24). Each tubercle has from 4 to 5 oval openings (Fig. 24a). Entire surface smooth, except lightly reticulated in region which is internal in the tegument.

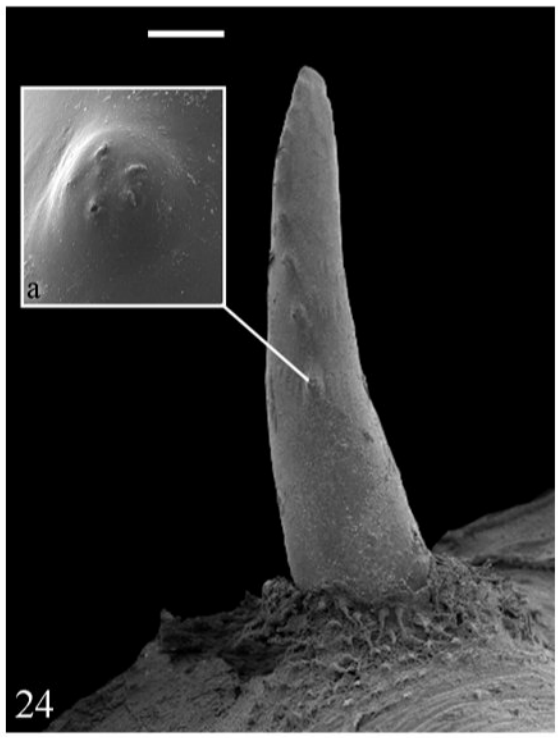
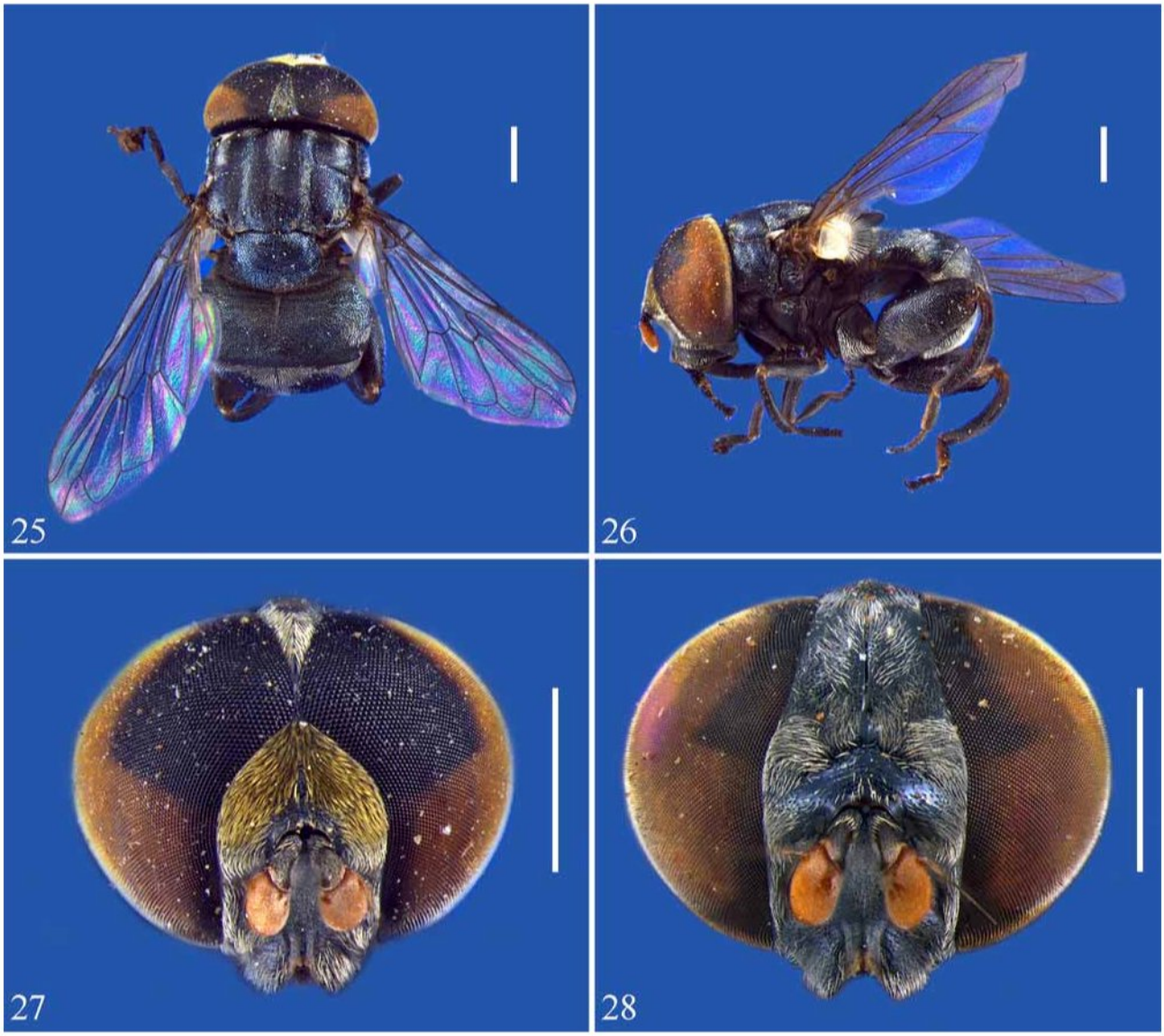


FIGURE 24. *Alipumilio athesphatus* **sp. n.**, pupal spiracle: (a) detail of circular-shaped tubercle, with five spiracular openings (which are covered by resin). Scale bar: 100 µm.

TABLE I. Arithmetic Mean (± Standard Error) and interval of variation (IV) for the size of the respiratory process among larval instars of *Alipumilio athesphatus* **sp. n**. Thompson reared on *Schinus terebinthifolius*. N = 30 (10 larvae per instar).

	Length (mm)	
Instar	Mean ± SE	IV
I	0.189 ± 0.0054	0.168 – 0.210
II	0.353 ± 0.0168	0.315 – 0.399
III	0.567 ± 0,0207	0.504 – 0.672

Adult (Figs 25–32). **Male.** *Length***:** body, 6.5–7.4 mm; wing, 5.1–6.3 mm (n = 10) (Figs 25, 26). *Head.* Black; face and gena white pilose, narrowly white pollinose ventrad to antenna, punctate on ventrolateral 1/3; frontal triangle punctate, golden pilose; vertical triangle golden pilose (Fig. 27); occiput yellow pilose; antenna brownish orange to black, yellow and black pilose; arista black; eye yellow and brown pilose, with brown pile narrowly along anterior edge, broadly on area of enlarged ommatidia on anterodorsal 1/2 and broadly extending to posterior edge dorsally *Thorax.* Black; scutum punctate, short black pilose except with yellow pilose vittae, with medial, submedial and lateral pilose vittae; scutellum black pilose except for yellow submedial pilose vitta continuous from scutum to apex of scutellum; katepisternum punctate, yellow pilose; plumula black; calypter white; halter brown. *Legs.* Black except metafemur dark reddish brown in holotype; coxae, trochanters and femora yellow pilose; tibiae reddish-brown pilose; tarsi reddish-brown pilose dorsally, yellow pilose ventrally. *Wing.* Brownish, with stigma brown, entirely microtrichose. *Abdomen.* Black except sterna reddish brown; first tergum short black pilose except yellow apicolaterally; second tergum yellow pilose apicolaterally and medially on basal 1/3, short black pilose elsewhere; third tergum similar to second but also with yellow pilose apicomedially; fourth tergum yellow pilose; sterna yellow pilose. *Genitalia.* Sparsely grayish pollinose, yellow pilose. Cercus trapezoidal in lateral view (Fig. 29). Surstylus broad, with a medial indentation in lateral view (Fig. 29). Hypandrium dilated, ctenidion absent (Fig. 30).



FIGURES 25–28. *Alipumilio athesphatus* **sp. n.**, adult. Male: (25) Dorsal view. (26) Lateral view. (27) Head, anterior view. Female: (28) Head, anterior view. Scale bar: 1 mm.

Female. Similar to the male, except for normal sexual dimorphism and: dichoptic (Fig. 28); frons white pilose and pollinose (Fig. 28); wing hyaline. *Genitalia.* Three spermathecae drop-like in shape (Fig. 31). Tergum VIII slightly sclerotinized (Fig. 32)**.**

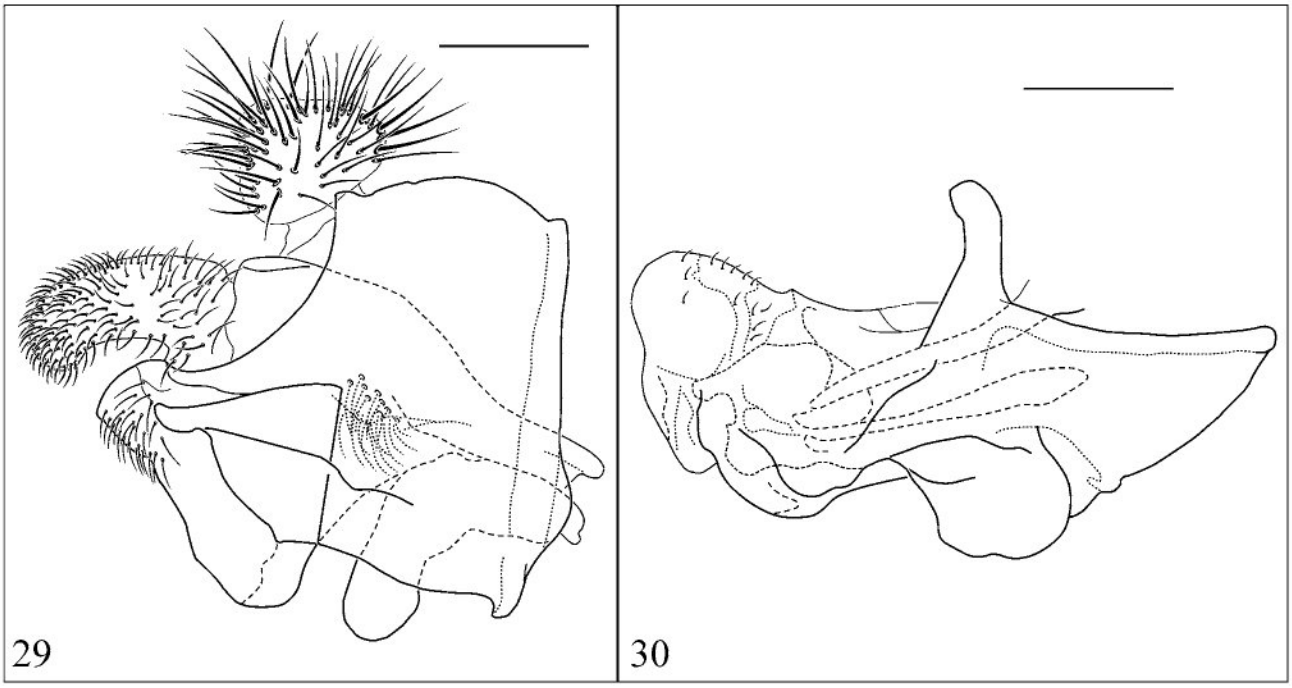
Variation: The specimens from Bolivia are much darker, black instead of brownish black; their antennae are entirely black, whereas the type and other Argentinean specimen have the basoflagellomere orange. As the male genitalia are the same in the Argentinean and Bolivian specimens, we have not recognized this color difference as significant.

Remarks. *Alipumilio athesphatus* is easily recognized as it is the only species in the genus with a dark halter. Also, the alternating vittae of pale and dark pile on the scutum is distinctive.

Type Material. Holotype (Male). **ARGENTINA**. **Entre Rios**: Pronunciamento, December 1966, F. Walz, from the personal collection of F. C. Thompson, to be deposited in USNM, Washington.

Paratypes (11): **ARGENTINA**. **Cordoba**: Dean Funes, 24 km S of, 8 February 1951, Ross & Michelbacher (1 male, CAS). **BOLIVIA**. **Cochabamba**: Cochabamba, 17°23'3"S 66°07'N, 2610 m, 25 March 2001, A. Freidberg (5 males, 3 females CBFC, USNM). **BRAZIL**. **Rio Grande do Sul**: Porto Alegre, Parque Natural Morro do Osso, 18.xii.2007, D. Massardo reared from *Schinus terebinthinfolius* stems (1 male,

2 females, DZUP). **Santa Catarina**: Imbituba, 14 km north of, 27.00620°S 48.58206°W,10 July 2008, Wheeler & McKay, reared from *Schinus terebinthinfolius* stems (1 male, 1 female, USNM).



FIGURES 29–30. *Alipumilio athesphatus* **sp. n.**, male genitalia. (29) Epandrium, cercus and surstylus, lateral view. (30) Hypandrium and associated structures, lateral view. Scale bar: 0.25 mm.

Additional examined material. BRAZIL. Rio Grande do Sul. Porto Alegre, UFRGS-Campus do Vale, D. Massardo, 7.xii.2007 (8 larvae, DZUP); 18.xii.2007 (4 larvae, DZUP); i.2008 (16 larvae, DZUP); 14.i.2008 (female, DZUP). Parque Natural Morro do Osso, D. Massardo, 27.xi.2008 (27 larvae, DZRS); 18.xii.2007 (4 males and 2 females, DZUP); xii.2007 (2 males and 2 females, DZUP).

Distribution: Brazil, Bolivia and Argentina.

Derivation of specific epithet: The epithet is derived from the Greek, *athesphatos*, meaning inexpressible or marvelously great (Brown 1956: 110) and is used as an adjective.

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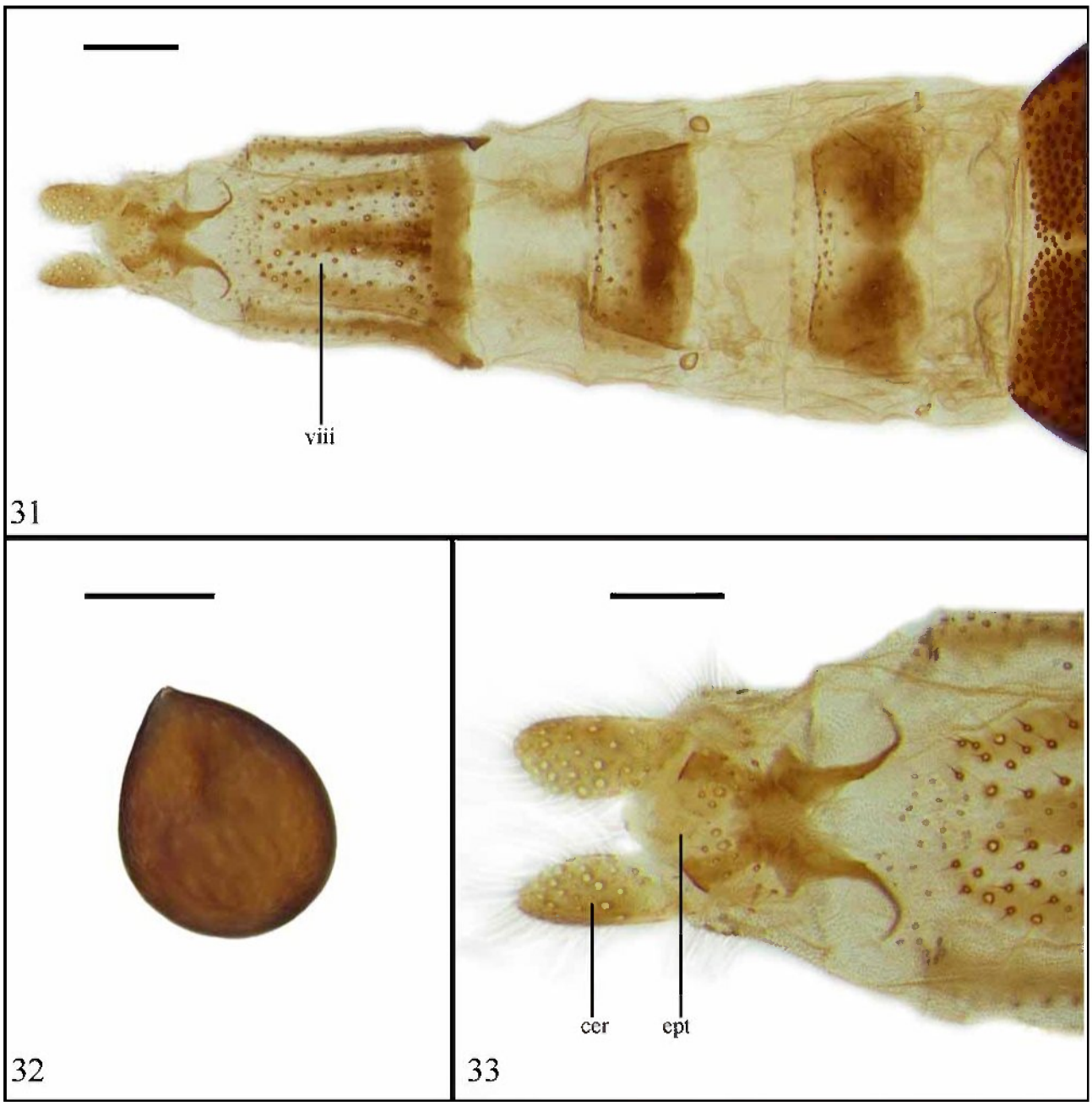
Although most specimens used for this study are in the collections of our own institutions, others were borrowed. To our colleagues and their institutions who loaned specimens, we express our sincere thanks: CAS (Paul H. Arnaud, Jr.) and CBFC (Alvaro Garitano-Zavala) as without their cooperation this study could not have been as comprehensive.



FIGURES 31–33. *Alipumilio athesphatus* **sp. n.**, female genitalia. (31) Dorsal view. (32) Spermatheca. (33) Detail of epiproct and cercus. Scale bar: Fig. (31): 0.25 mm. Figs (32–33): 0.15 mm. (cer) cercus. (ept) epiproct. (viii) = tergum VIII.

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