

Morphology of immature stages of *Paraegidium costalimai* (Coleoptera: Scarabaeidae: Orphninae) and remarks on egg-busters in Scarabaeidae first-instar larvae

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Abstract. Studies about the immature stages of Orphninae (Coleoptera: Scarabaeidae) species are scarce. The subfamily includes 214 species, but only 5 have the immature stages described: *Chaetonyx robustus liguricus* Mariani, 1946, *Hybalus benoiti* Tournier, 1864, *H. rotrovi* Petrovitz, 1964 and *Triodontus nitidulus* (Guérin, 1844) from Old World; and *Aegidium cribratum* Bates, 1887 from the New World. The Neotropical genus *Paraegidium* Vulcano, Pereira & Martinez, 1966 encompass five species, mainly recorded from Brazil. Herein, the immature stages of *P. costalimai* Vulcano, Pereira & Martinez, 1966 are described and illustrated, along with remarks on the presence of egg-buster in Scarabaeidae first-instar larvae. A key to the third-instar larvae of known Orphninae and a comparative study of chaetotaxy are also provided.

Key-Words. Aegidiini; Lamellicornia; Pleurosticti; Pupa; White grub.

INTRODUCTION

Orphninae is a subfamily of Scarabaeidae (Coleoptera) and includes 214 named species (and 1 known species not yet named) distributed in two tribes: Aegidiini and Orphnini. The subfamily occurs in tropical and subtropical regions of the World, except Australia, and is classified in 16 genera (Frolov, 2012; Frolov & Vaz-de-Mello, 2015; Frolov *et al.*, 2016, 2017a, b; Rojkoff & Frolov, 2016). The tribe Aegidiini comprises the genera *Aegidiellus* Paulian, 1984 (Brazil, monotypic), *Aegidinus* Arrow, 1904 (South America, 10 spp.), *Aegidium* Westwood, 1845 (South and Central America, Caribbean region, 23 spp.), *Onorius* Frolov & Vaz-de-Mello, 2015 (Ecuador, monotypic but with an unnamed known species, Frolov *et al.*, 2019), *Paraegidium* Vulcano, Pereira, et Martínez, 1966 (South America, 6 spp.), and *Stenosternus* Karsch, 1881 (Gulf of Guinea: São Tomé Island, monotypic); and the tribe Orphnini includes the genera *Chaetonyx* Schaum, 1862 (Mediterranean region, 3 spp.), *Craniorphnus* Kolbe, 1895 (Afrotropical region, monotypic), *Goniorphnus* Arrow, 1911 (Afrotropical region, monotypic), *Hybaloides* Quedenfeldt, 1884 (Afrotropical

region, monotypic), *Hybalus* Dejean, 1833 (Mediterranean region, 35 spp.), *Madecorphnus* Paulian, 1992 (Madagascar, 19 spp.), *Orphnus* Macleay, 1819 (Afrotropical and Indo-Malayan regions, 92 spp.), *Renorphnus* Frolov & Montreuil, 2009 (Madagascar, monotypic), *Pseudorphnus* Benderitter, 1913 (Madagascar, 4 spp.), and *Triodontus* Westwood, 1846 (Madagascar, 15 spp.).

The subfamily is distinguished from the other Scarabaeidae by having (Frolov, 2012; Frolov & Vaz-de-Mello, 2015): mandibles and labrum exposed in dorsal view (hidden by clypeus in *Onorius*), antennae with 10 antennomeres and 3 lamellae, protibiae of males without spur, anterior area of procoxae with a transversal furrow, dorsal surface of metacoxae with stridulatory striae, spurs of metatibiae separated by tarsal insertion, lateral areas of abdominal sternite II (hidden by metacoxae in ventral view) with a conspicuous sclerotized and pigmented area (plectrum). Sexual dimorphism is usually evident, with males' head and pronotum bearing horns or other ornamentation.

The Neotropical genus *Paraegidium* was described by Vulcano, Pereira & Martinez (1966) to include a single species, *P. costalimai*, from Brazil.

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Recently, Frolov *et al.* (2017a) revised the genus and described five species mainly recorded in Brazil, with the exception of *P. howdeni* Frolov, Akhmetova & Vaz-de-Mello, 2017, from the subandean region of Peru.

Studies about immatures of Orphniinae are scarce and only five species have been described so far: *Hybalus benoiti* Tournier, 1864 and *H. rotroui* Petrovitz, 1964 (third-instar larva described by Paulian & Lumaret, 1982), *Aegidium cribratum* Bates, 1887 (third-instar larva and pupa described by Morón, 1991), *Chaetonyx robustus liguricus* Mariani, 1946 (third-instar larva described by Barbero & Palestrini, 1993) and *Triodontus nitidulus* (Guérin, 1844) (third-instar larva described by Randriamanantsoa *et al.*, 2011).

Gradinarov & Petrova (2012), Gradinarov (2014) and Gradinarov *et al.* (2015) studied the biology of Orphniinae from Bulgaria and provided notes on the habitat preferences and life cycle of *Chaetonyx robustus* Schaum, 1862 and included some measurements and photographs of immatures of this species. Even though 3 subspecies for *C. robustus* are known (the nominotypical subspecies, *H. robustus italicus* Mariani, 1946, and *H. robustus liguricus* Mariani, 1946), the above-mentioned authors did not use the subspecific classification and noted that it is doubtful.

The purpose of the present study is I) to describe the immature stages of *Paraegidium costalimai*, II) to discuss the egg-busters of first-instar larvae, and III) clarify the classification of immature stages of Orphniinae.

MATERIAL AND METHODS

Larvae of *P. costalimai* were collected at two urban parks in São Paulo (state of São Paulo, SP): Instituto Biológico de São Paulo (IBSP) and Parque da Independência, both with a secondary woodland cover (Fig. 1). Larvae were found at 10 cm depth in the soil.

The reared adult was identified in comparison with paratypes of *P. costalimai* (MZSP) and following Frolov *et al.* (2017a).

Immatures were killed in boiling water and preserved in 70% alcohol. Observations and drawings were carried out in a Zeiss Stemi SV6 stereomicroscope or Zeiss Axioscop microscope, both with a coupled light camera. Detached larval structures (*e.g.*, mouthparts and legs) were provisionally mounted on glycerin jelly slides (see Burrows, 1965; Johansen, 1940; Perina & Camacho, 2016; Widden, 2001).

Photographs were taken with a Canon EOS 80D DSLR Camera with a Canon MP-E 65 mm f/2.8 lens and an LED illumination system (Kawada & Buffington, 2016). For small dissected a Lucky Zoom USB eyepiece camera attached to a microscope was used. Images were processed using Zerene stacker. The Adobe Photoshop CC 2018 software was used for image processing. Measurements were taken in millimeters using a micrometer.

The terminology used followed Böving (1936) and Lawrence (1991) with modifications by Sousa *et al.* (2018). Head chaetotaxy followed Ritcher (1966) and

Sawada (1991), as summarized by Sousa *et al.* (2018). Lobes of thorax and abdomen followed Rodrigues *et al.* (2018). The hair-like setae were separated into the two well-defined groups: minute setae and short-long setae (modification of Šípek *et al.*, 2008; see Rodrigues *et al.*, 2018). The term phobae was used by Böving (1936) to the fleshy projections, even though in *P. costalimai* it is stiff and slightly sclerotized.

RESULTS

Paraegidium costalimai Vulcano, Pereira & Martinez, 1966 (Figs. 2-74)

Material examined: Brazil. **Pará:** Cachimbo, x.1955, Padre Pereira (collector), 1 ♀ adult, x.1959, M. Alvarenga (collector), 3 ♀ and 3 ♂ adults (paratypes). **Rio de Janeiro:** Itatiaia, 1 ♂ adult (paratype), xii.1949, W. Zikán (collector), 2 ♀ and 1 ♂ adults (paratypes), 07.xii.1951, W. Zikán (collector), 1 ♀ adult (paratype), Rio de Janeiro, Corcovado, xi.1959, M. Alvarenga (collector), 1 ♂ adult (paratype). **São Paulo:** São Paulo, Cantareira, ii.1937, 1 ♀ adult (paratype), Ipiranga, xii.1993, Sérgio Ide (collector), 1 ♀ adult,

Table 1. Chaetotaxy of the known third instars of Orphniinae.

	Sp.	<i>A. cribratum</i>	<i>H. benoiti</i>	<i>C. r. liguricus</i>	<i>H. rotroui</i>	<i>P. costalimai</i>	<i>T. nitidulus</i>
parietals	des	2	3	2	4	2	3
	pes	1	2	2-3	2	2-3	—
	aes	1	1	1	1	1	—
	ees	3	4	4	4	4-5	5
frons	pfs	0	0	0	0	0	—
	efs	1	1	0	1	1	—
	aas	0	1	1	1	1	1
clypeus	afs	1	—	0	—	1	—
	acs	1	1	1	1	1	1
labrum	ecs	2	2	1	1	2	1
	pls	2	4	2	4	2	2
	lls	2	3	2	3	3	2
	mls	2	2	2	2	2	2
raster	als	2	1	1	1	2	2
	tg	18	—	—	—	78-82	36
	pr	8	—	—	—	24-31	12
	pa	19-23	—	—	—	—	20
	al	0	0	0	0	0	0

The chaetotaxy is given for one side of the structure, except for ventral anal lobe (**al**). ~ = about; — = setae not quantified or figured by studies.

Raster and anal lobe: only hamate setae quantified, when hamate setae absent the number of hair-like setae is given between square brackets.

Preseptular setae (pr): tegillar setae anterior to palidia-septula. **u** = unapplied (*i.e.*, when palidia-septula are absent, it is impossible define the preseptular setae).

Setae group: **aas** = anterofrontal angle setae; **acs** = anterooclypeal setae; **aes** = anteroepicranial setae; **afs** = anterofrontal setae; **al** = ventral anal lobe setae; **als** = anterolabral setae; **des** = dorsoepicranial setae; **lls** = laterolabral setae; **mls** = mediolabral setae; **ecs** = externoclypeal setae; **ees** = externoepicranial setae; **efs** = externofrontal setae; **pa** = palidium setae (pali); **pes** = posteroepicranial setae; **pfs** = posterofrontal setae; **pls** = posterolabral setae; **pr** = tegillar preseptular setae; **tg** = tegillar setae (including **pr**).

22.i.1997, S. Ide (collector), 1 ♀ adult, x.2001-i.2002, 2 ♀ and 2 ♂ adults, Parque do Instituto Biológico, 02.x.2011, J. Fuhrmann & D.C. Bená (collectors), 1 ♂ pupa (MZSP 10366); 09.x.2011, J. Fuhrmann (collector), 1 ♀ pupa (MZSP 10367), Parque da Independência, 25.vii.2018, J. Fuhrmann, R. Sousa, B. Zilberman & L. Migliori (collectors), 1 first-instar larva, 6 third-instar larvae, 1 ♀ reared to adult (MZSP 10364).

Third-instar larva (Figs. 5-55, 73-74): Body posteriorly enlarged (Fig. 5), white, head and respiratory plates yellow, mandibles darker and body setae yellowish brown. **Head** (Figs. 6-7, setation in Table 1) width about 2.2 mm, epicranial and epistomal sutures distinct, stemmata absent, antennifer somewhat cylindrical and with 2 punctures, cranium with homogeneously distributed punctures, and each side of clypeus with 5 punctures. Each



Figures 1-3. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. (1) Collect site at the Parque da Independência (São Paulo, Brazil); (2) Male, adult; (3) Female, adult. Scale = 1 mm.

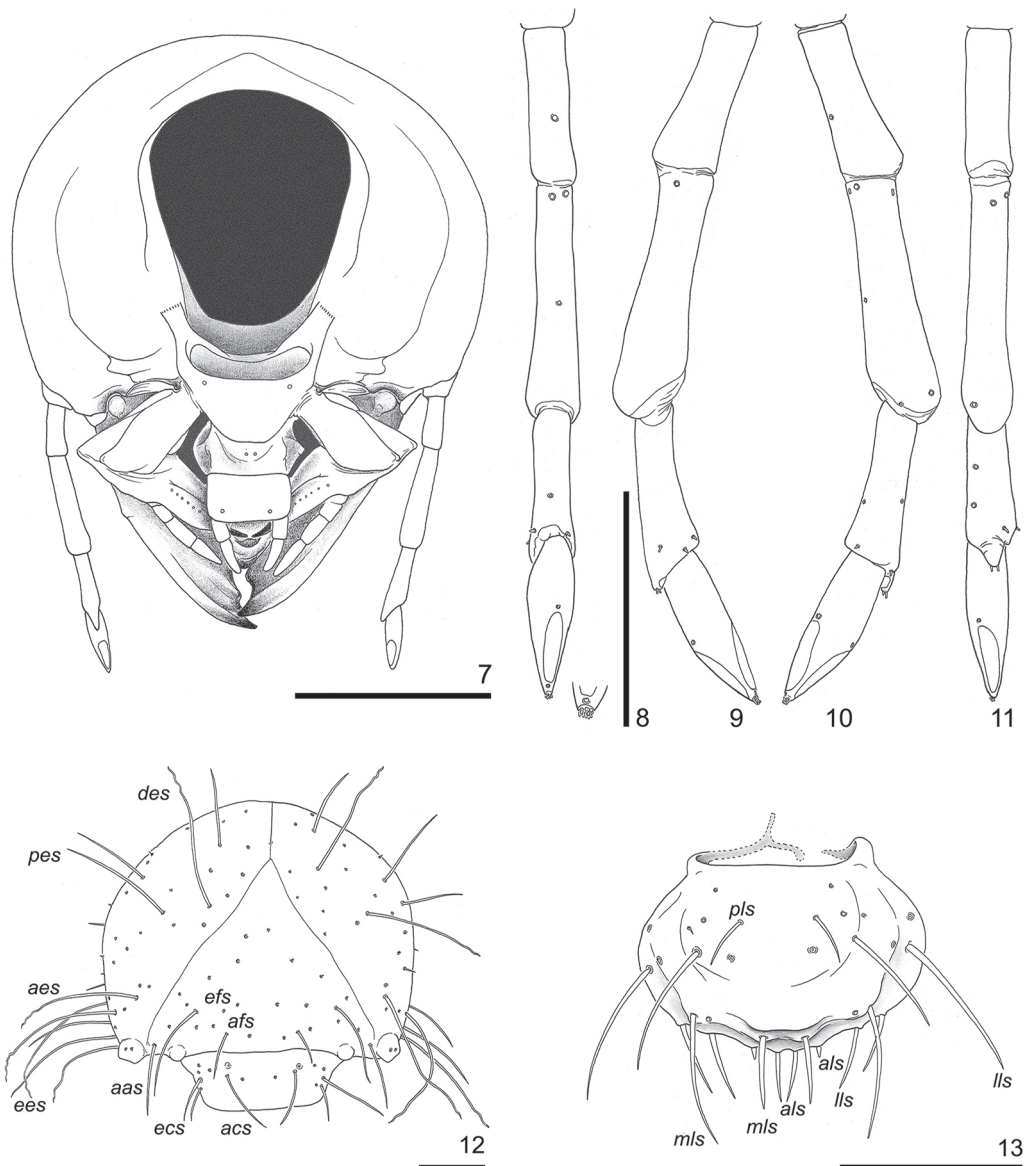


Figures 4-5. Larvae of *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Habitus lateral: (4) First instar. (5) Third instar. Scale = 1 mm.

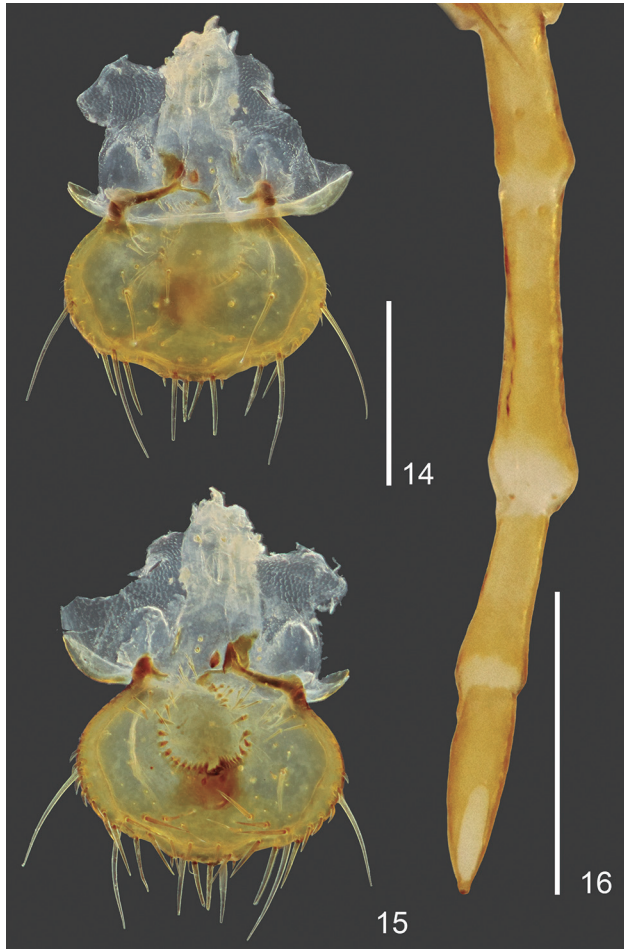


Figure 6. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: head, dorsal. Scale = 1mm.

side of cranium and clypeus with (Figs. 6, 12): 2 dorsoepicranial setae (*des*), 2-3 posteroepicranial setae (*pes*), 1 anteroepicranial seta (*aes*), 4-5 exteroepicranial setae (*ees*), posterofrontal setae (*pfs*) absent, 1 externofrontal seta (*efs*), 1 anterofrontal angle seta (*aas*), 1 anterofrontal seta (*afs*), 2 externoclypeal setae (*ecs*), and 1 antero-clypeal seta (*acs*). Each side of **labrum** (Figs. 13-14) with 7 sensilla (3 posterior, 2 lateral, 1 anterior), 2 long posterolabral setae (*pls*), 2 mediolabral setae (*mls*), 3 laterolabral setae (*lls*), and 1 long and 1 short anterolabral setae (*als*). **Antenna** (Figs. 8-11, 16) with 4 antennomeres: I with 1 dorsal sensillum; II with 7 sensilla (3 dorsal, 1 inner, 1 outer, 2 ventral); III with 7 sensilla (3 dorsal, 2 inner, 2 ventral), ventrodistal process bearing a dorsal sensorial spot and 3 sensilla (2 distal, 1 proximal); IV with 3 sensilla (2 dorsal, 1 ventral), 1 dorsal and 1 ventral sensorial spots, and a distal sensorial area bearing about 8 minute sensilla.



Figures 7-13. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: (7) Head, ventral; (8-11) Left antenna (dorsal, inner, outer, ventral); (12) Cranium and clypeus; (13) Labrum. **aas** = anterofrontal angle setae; **acs** = antero-clypeal setae; **aes** = anteroepicranial setae; **afs** = anterofrontal setae; **als** = anterolabral setae; **des** = dorsoepicranial setae; **lls** = laterolabral setae; **mls** = mediolabral setae; **ecs** = externoclypeal setae; **ees** = exteroepicranial setae; **efs** = externofrontal setae; **pes** = posteroepicranial setae; **pfs** = posterofrontal setae; **pls** = posterolabral setae. Scale: Fig. 7 = 1 mm, Figs. 8-13 = 0.5 mm (antennal details with magnification two times bigger than antennae).



Figures 14-16. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: (14) Labrum; (15) Epipharynx; (16) Antenna. Scale = 0.5 mm.

Table 2. Thorax and abdomen chaetotaxy of *Paraegidium costalimai*.

	sgs	t1	t2	t3	a1-2	a3	a4-5	a6	a7	a8	a9
	tl*	78-84	u	u	u	u	u	u	62-64	54-56	42-58
	tal	u	4-5	=	6-10	12-16	2-5 [15]	=	u	u	u
Dorsal Lobes	tml	u	29-34	=	16-30	41-44	21-26 [28-29]	=	u	u	u
	tpl	u	7-8	=	14-20	11-15 [11-14]	2-4 [25-26]	19-20	u	u	u
	ts	#	u	u	u	u	u	u	u	u	u
Dorsolateral Lobes	pl*	1-2	3	u	1-4	=	=	=	=	=	6-9
	pal	u	u	1	u	u	u	u	u	u	u
	ppl	u	u	1	u	u	u	u	u	u	u
	esl	u	u	u	3-5	=	=	=	=	1-2	u
	til	u	u	u	u	u	1-2	=	0-1	u	u
Ventral Lobes	vl*	6-8	u	u	u	u	u	u	u	4	=
	val	u	7-8	=	8-10	=	4-6	=	=	u	u
	vml	u	u	u	u	u	u	u	u	u	u
	vpl	u	0	=	=	=	=	=	=	u	u

The chaetotaxy is given for the entire lobe, main interval related to thin setae, stout setae between square brackets.

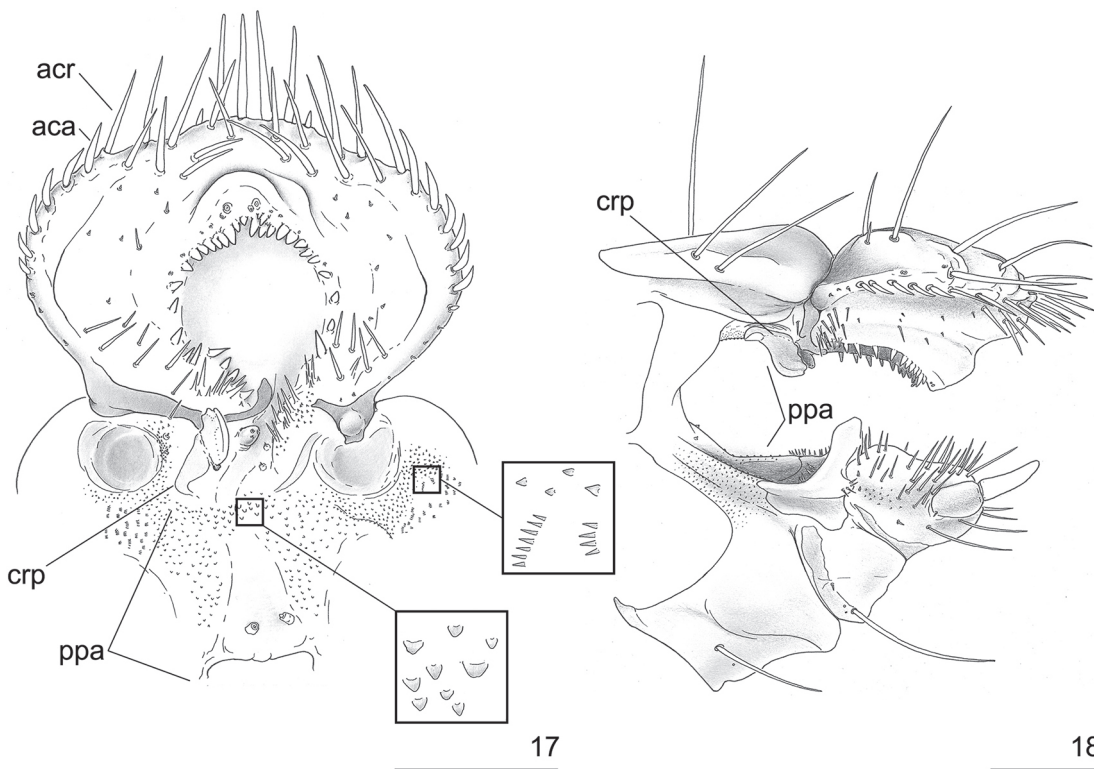
* = When segment lobes (sgs) are undivided, the general lobe (tl, pl, vl) chaetotaxy is given. = = similar to immediately anterior segment; u = unapplied (when lobe is indistinct); # = thoracic tergal sclerite is barely distinct in *P. costalimai* and setae of the region are quantified as part of tergal lobe (tl).

Segments: t1-3 = pro-, meso- and metathorax; a1-9 = abdominal segment I-IX.

Dorsal lobes: tal = tergal anterior lobe; tl = tergal lobe; tml = tergal medial lobe; tpl = tergal posterior lobe; ts = tergal sclerite of prothorax.

Dorsolateral lobes: esl = spiracular lobe; pla = pleural anterior lobe; ppl = pleural posterior lobe; til = tergal lateral lobe.

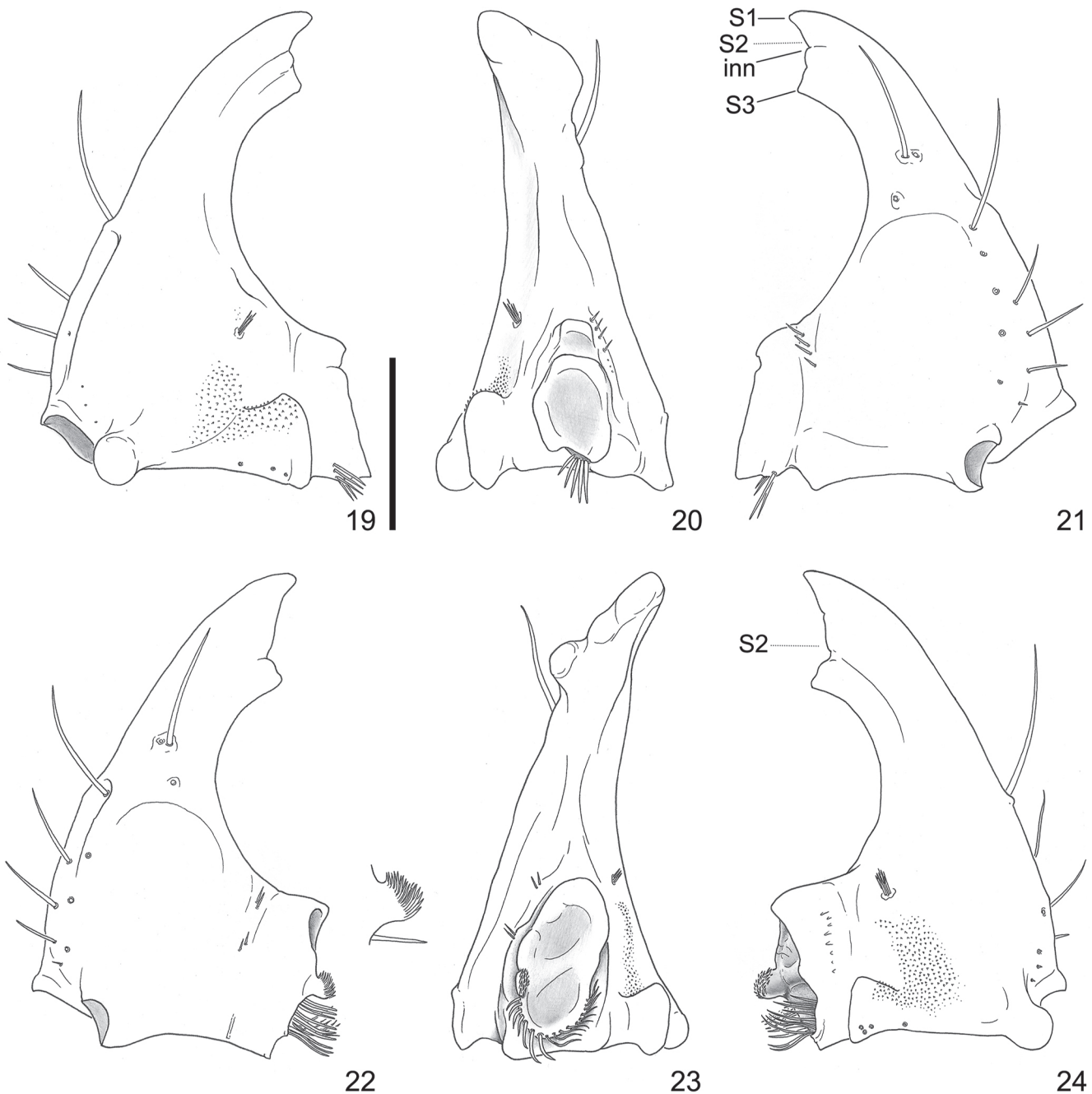
Ventral lobes: val = ventral anterior lobe; vl = ventral lobe; vml = ventral medial lobe; vpl = ventral posterior lobe.



Figures 17-18. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: (17) Epipharynx; (18) Cibarium, lateral. aca = acanthoparia; acr = acroparia; crp = crepis; ppa = posterior preoral area. Scale = 0.3 mm (asperites details with magnification eight times bigger than the epipharynx and posterior preoral area).

Epipharynx (Figs. 15, 17-18). **Corypha** with epizygom and clithra indistinct. **Haptomerum** prominent, sclerotized, with 2 sensilla on each side, zygom indistinct. **Paria** with acroparia evidently separated from chaetoparia, each side of acroparia with 8 setae, right and left acanthoparia with 6-7 anterior setae and 3-4 minute posterior setae, gymnoparia wide, right and left chaetoparia with about 7 minute or short setae and 5-7 posterior long setae. Dexiotorma four times longer than laeotorma, left pterotorma rounded, apotorma indistinct, epitorma three times longer than laeotorma and contiguous to dexiotorma. Plegmatia and proplegmatia absent, and right and left spiny phobae present. **Pedium** circular and surrounded by about 30 heli and 8 minute sensilla.

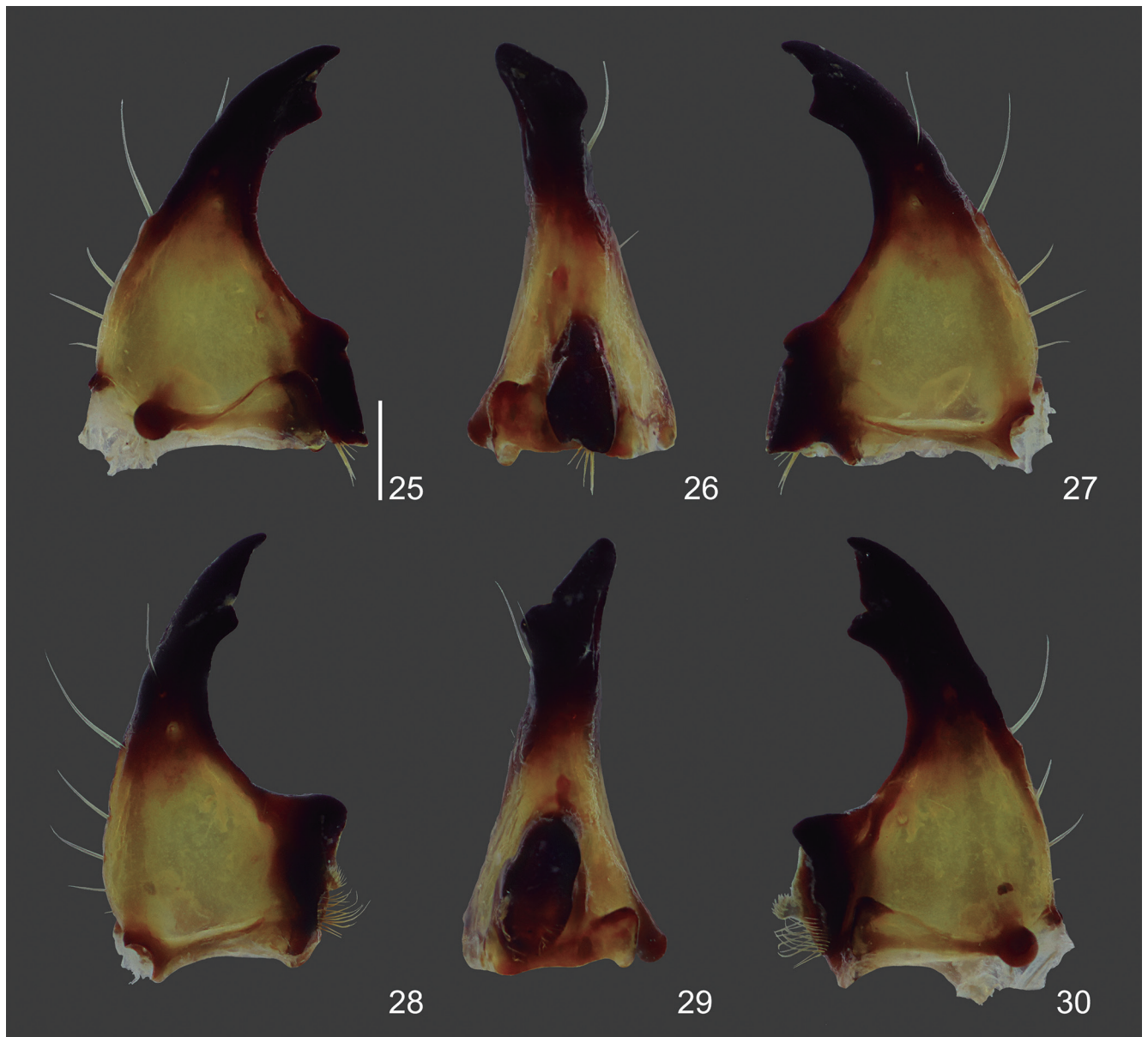
Haptolachus with right area bearing a seta and 2 sensilla, left area bearing 2 sensilla; nesium internum (sensorial cone) tubercle-like and with 3 sensilla; nesium externum (sclerotized plate) prominent and blade-like; crepis lateral area conspicuous and medial area indistinct. **Mandible** (Figs. 19-30). Incisor with 2 well-defined teeth (S2 inconspicuous), dorsoproximal area with 2 punctures and a seta. Ventral area with posterior asperites. Scrobe (outer proximal area) with about 4 setae and 4-5 sensilla. Ventral processes with 3-4 ventroproximal sensilla. Each molar with about 4 dorsoproximal setae in a row and about 6 ventroproximal setae in a tuft; right brustia 5 stout setae and left brustia with 7 dorsal stout setae and 10 ventral thin setae; right molar with 2 chisel-like teeth and calx



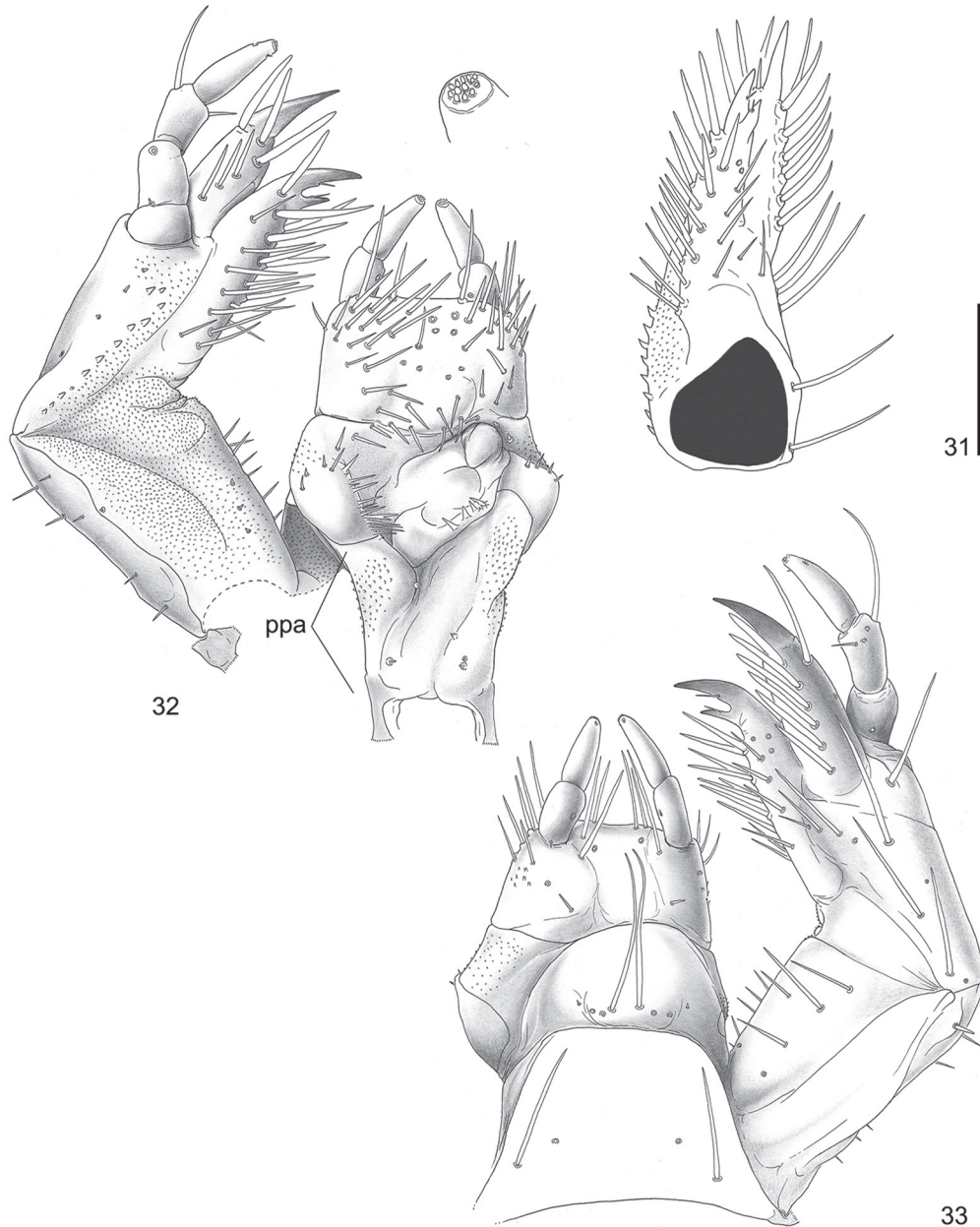
Figures 19-24. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: (19-21) right mandible (ventral, inner, dorsal); (22-24) left mandibles (dorsal, inner ventral). **S1-3** = distal, medial and proximal teeth of incisor, medial tooth barely distinct; **inn** = incisor notch (between S2 and S3). Scale = 0.5 mm (acia details with magnification two times bigger than the mandible).

slightly sinuous; left molar with an anterior chisel-like tooth, acia with apex obliquely truncate and bearing a dense group of thin spines (Fig. 22 detail), calyx semicircular. **Maxilla** (Figs. 31-35). Galea and lacinia separate; galea with an uncus and a row of 6 ventral setae; lacinia with 2 unci, each uncus with a proximal seta. Stipes with stridulatory area bearing 12-15 acute teeth. Palpus with 4 palpomeres: I small; II with 3 sensilla (2 dorsal, 1 ventral); III with an outer seta, a ventral seta, and 2 ventral sensilla; IV with 2 sensilla (1 inner, 1 outer), distal sensorial area bearing about 13 sensilla. **Hypopharynx** (Figs. 18, 32, 34) with asymmetrical sclerite, each lateral lobe with 4-6 setae; left lateral lobe with a spiny phoba bearing about 13 projections, some projections bifurcate; right area with a prominent anterior tooth and 4-6 posterior heli. **Posterior preoral area** (Fig. 18): each side of dorsal area (posterior to epipharynx, Fig. 17) with a sensillum and an anterior concavity; each side of the ventral area (posterior

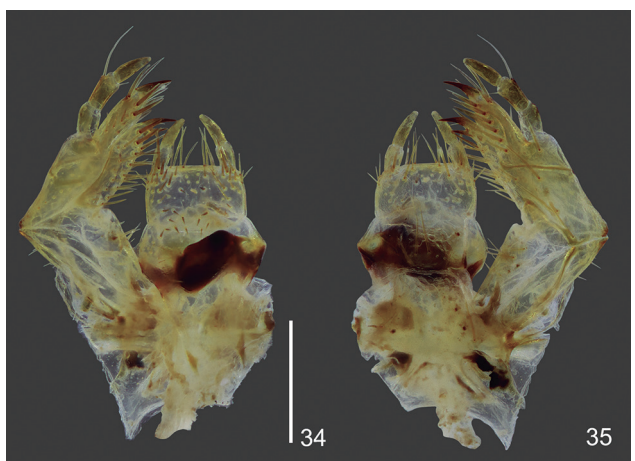
or to hypopharynx, Fig. 32) with 2-3 sensilla. **Labium** (Figs. 18, 32-33). Submentum with an inconspicuous sclerite, each side with a seta and a sensillum. Mentum with a transversal sclerite, each side with a seta and 3 sensilla. Prementum with a transversal sclerite, each side with a posteromedial short seta, 3 distal setae, and 2 sensilla (1 medial, 1 distal); ligula (Figs. 18, 32, 34) rectangular, each side with about 24 setae (9 posterolateral, 15 anterolateral) and 4 sensilla (2 anterior, 2 medial), posterior area without asperites. Palp with 2 palpomeres: I with a minute ventroproximal seta and an inner sensillum; II with a ventrodistal sensillum, distal sensorial area with about 13 sensilla (Fig. 32 detail). **Thorax** (Fig. 5, for setation refer to Table 2) with tergal sclerite thin and barely distinct. Prothorax with tergal lobe bearing 78-84 setae, pleural lobe with 1-2 setae, and ventral lobe with 6-8 setae. Mesothorax with tergal anterior lobe bearing 4-5 setae, tergal medial lobe with 29-34 setae, tergal posterior



Figures 25-30. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: (25-27) right mandible (ventral, inner, dorsal); (28-30) left mandibles (dorsal, inner ventral). Scale = 0.25 mm.



Figures 31-33. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: (31) Left maxilla, inner (cardo omitted); (32) Maxilla, hypopharynx and ligula, dorsal; (33) Maxilla and labium, ventral. **ppa** = posterior preoral area. Scale = 0.3 mm (palp details with magnification eight times bigger than the maxillolabial complex).

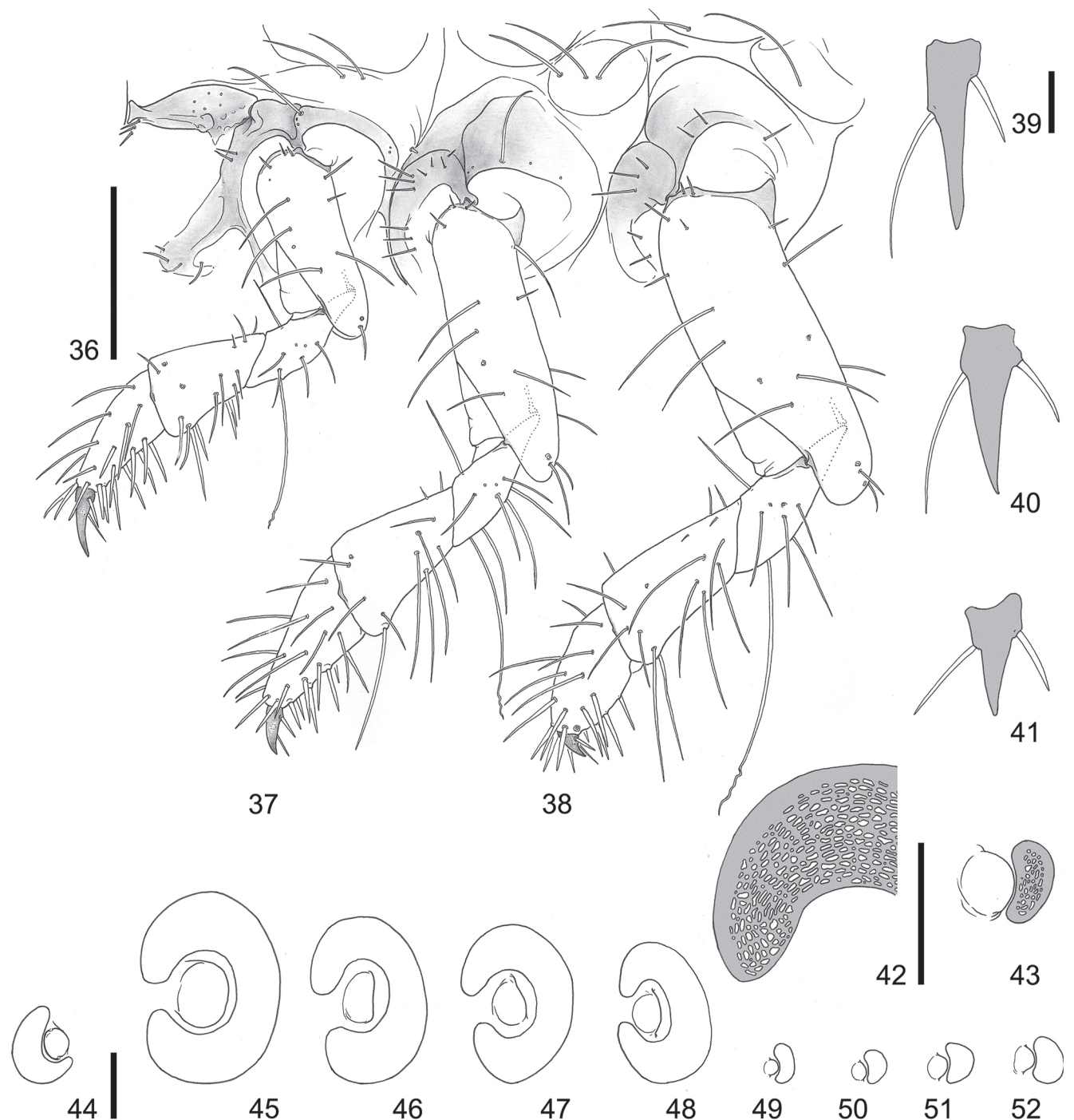


Figures 34-35. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: (34) Maxilla, hypopharynx and ligula, dorsal; (35) Maxilla and labium, ventral. Scale = 0.5mm.

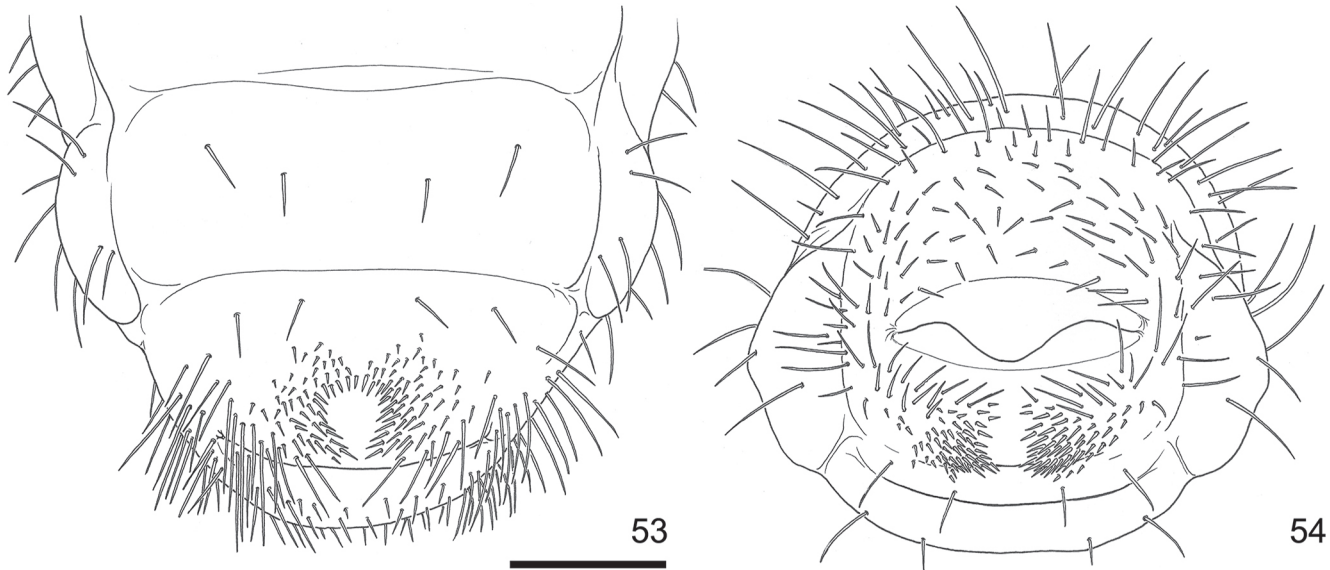
lobe with 7-8 setae, pleural lobe with 3 setae, ventral anteromedial lobe with 7-8 setae, and ventral posterior lobe bare. Metathorax with pleural anterior and posterior lobes bearing a seta each, other lobes as mesothorax. **Legs** (Figs. 36-38) with trochanters bearing an anterior erect seta and a long posterior seta; pretarsus (Figs. 39-41) with 2 lateroventral setae and an acuminate apex, propretarsus longer than meso- and meso- longer than metapretarsus. **Thoracic spiracle** (Figs. 42-44, 74) with respiratory plate bearing oblong perforations, with 8 perforations in longitudinal radius and 4 perforations in ventral radius, and dorsal arm indistinct. **Abdomen** (Fig. 5, setation summarized in Table 2). Segment I-II with tergal anterior lobe bearing 6-10 setae, tergal medial lobe with 16-30 setae, tergal posterior lobe with 14-20 setae, pleural lobe with 1-4 setae, spiracular lobe with 3-5 setae, ventral anterior lobe with 8-10 setae, and ventral posterior lobe bare. Segment III with tergal anterior lobe bear-

ing 12-16 setae, tergal medial lobe with 41-44 setae, tergal posterior lobe with 11-15 thin setae and 11-14 stout setae, other lobes as segments I-II. Segments IV-V with tergal anterior lobe bearing 2-5 thin setae and 15 stout setae, tergal medial lobe with 21-26 thin setae and 28-29 stout setae, tergal posterior lobe with 2-4 thin setae and 25-26 stout setae, tergal lateral lobe with 1-2 setae, ventral anterior lobe with 4-6 setae, and other lobes as segments I-II. Segment VI with tergal posterior lobe bearing 19-20 thin setae, and other lobes as segments IV-V. Segment VII with tergal lobe bearing 62-64 setae, tergal

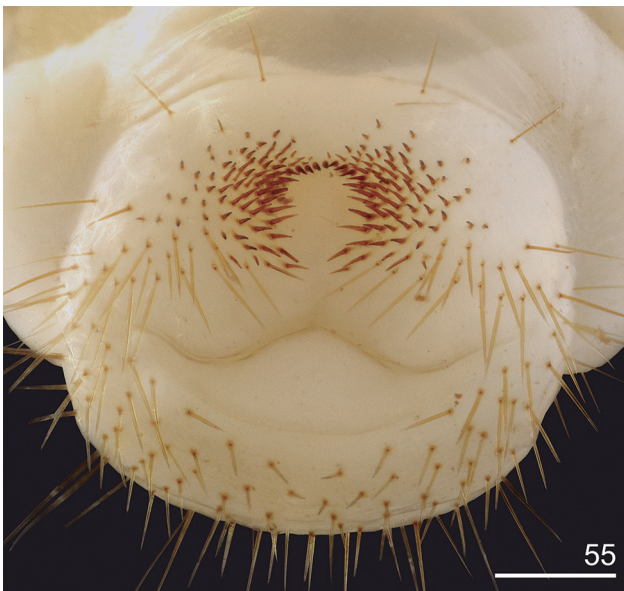
lateral lobe with 0-1 seta, and dorsolateral and ventral lobes as segments IV-V. Segment VIII with a tergal lobe bearing 54-56 setae, pleural lobe with 1-4 setae, spiracular lobe with 1-2 setae, and ventral lobe with 4 setae. Segment IX with tergal lobe bearing 42-58 setae, pleural lobe with 6-9 setae, and ventral lobe with 4 setae. Segment X (Figs. 54-55) with a sinuous anal opening and, and tergite with 98-110 thin setae. **Spiracles** (Figs. 45-48, 49-52) I-IV larger than V-VIII and C-shaped; respiratory plate I with 15 perforations in dorsal radius (DR), 13 perforations in longitudinal radius (LR), and 18



Figures 36-52. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: (36-38) Left legs and pleurites (anterior, medial, posterior); (39-41) Left pro-, meso-, and metapretarsus, dorsal; (42) detail of dorsal arm of abdominal spiracle I; (43) Abdominal spiracle V; (44) Mesothoracic spiracle; (45-52) abdominal spiracle I-VIII. Scales: Figs. 36-38 = 0.5 mm, Figs. 39-52 = 0.1 mm.



Figures 53-54. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: (53) Raster, ventral; (54) Abdominal segment X, posterior. Scale = 1 mm.



Figures 55. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Third-instar larva: abdominal segment X, posteroventral. Scale = 1 mm.

perforations in ventral radius (VR); respiratory plates II-VI with 10-12 perforations in DR, 9-12 perforations in LR, and 11-15 perforations in VR; bulla I-IV slightly wider than the distances between respiratory plate arms; respiratory plates V-VIII with respiratory plate coma-like and with about 40 perforations. **Raster** (Figs. 53-55, setation in Table 1) with palidia not distinguished from tegilla, septula rounded; each side of tegillar area with 13-14 posterolateral thin setae, and 65-68 stout setae, of which 24-31 are preseptular setae; each side of campus with 2 setae. **Anal lobes:** dorsal lobe parabolic and bare, ventral lobes slightly defined, transverse, and bare.

Remarks: Including present contribution, six species of Orphninae have their third-instar larvae described, and all of them have a distinct epipharynx: pedium surrounded by heli, but Morón (1991) described *A. cribratum* larvae with pedium surrounded by pine-like setae. Some

confusion and discordant interpretations were done regarding heli (fixed spine) and spine-like setae (see discussion in Albertoni *et al.*, 2014) and the revision of material of *A. cribratum* is here emphasized to check the nature of the ornamentation that surround the pedium.

Other characters shared by Orphninae larvae are: antennomere IV with a dorsal and a ventral sensorial spot; ligula somewhat rectangular; raster with septula oval and palidia and tegillar setae barely differentiated from each other.

Larvae of *P. costalimai* are distinguished from other orphnine larvae by lacinia with 2 unci (Figs. 31-35) and abdominal spiracles I-IV distinctly larger than V-VIII (Figs. 45-52).

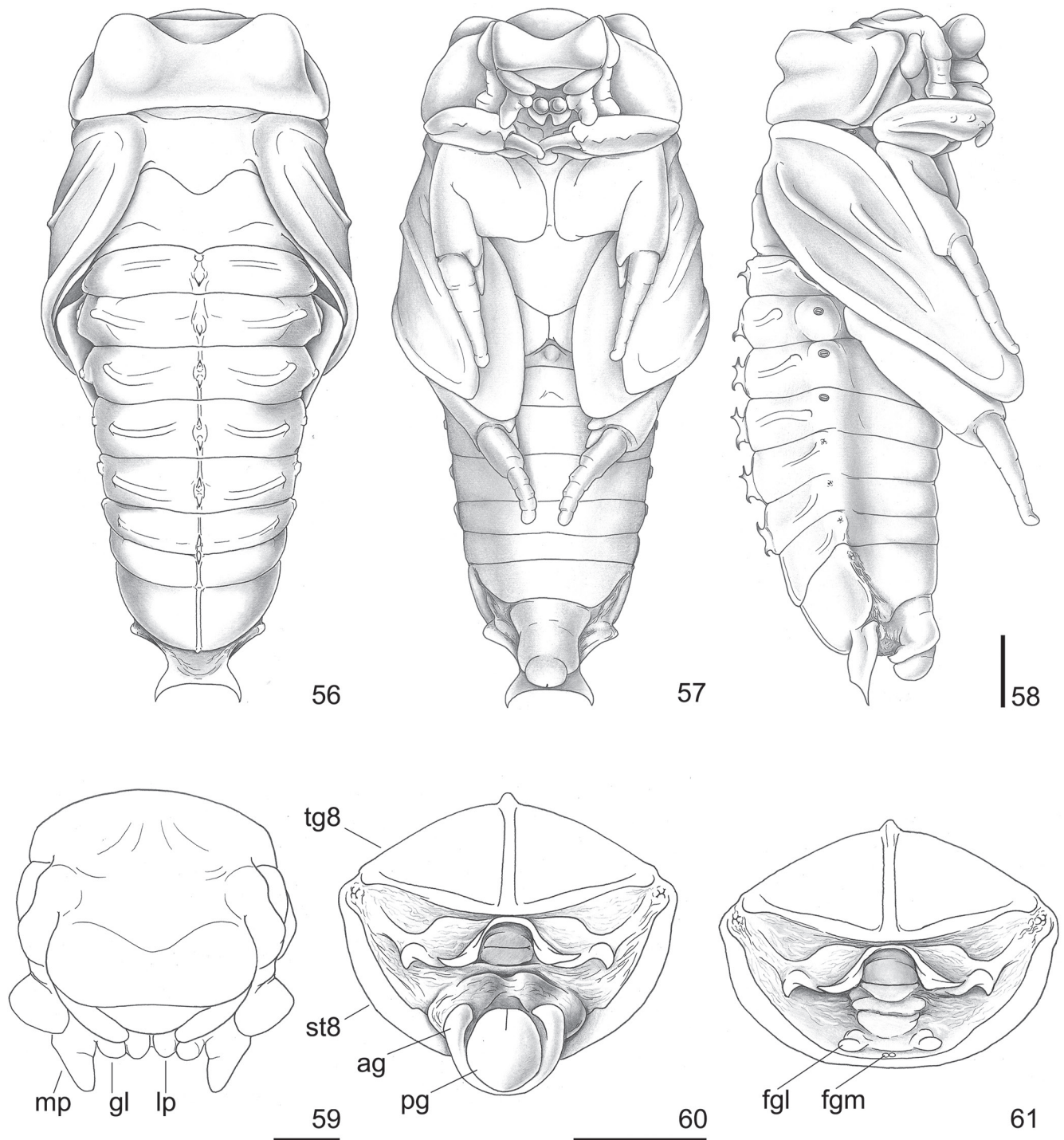
First-instar larva (Figs. 4, 62-72): like that of third instar above described, with following relative differences. **Head** (Figs. 4, 62-65) width about 0.7 mm. **Antennae** (Fig. 64) with same sensilla pattern than the third instar, but segments relatively shortened. **Epipharynx** (Fig. 62) with right and left acanthoparia with 4-5 anterior setae and 5-6 minute posterior setae. Left molar bearing an acia with truncate apex and bearing a dense group of thin spines (Fig. 65). **Hypopharynx** (Fig. 63) with sclerite bearing about five right heli and about 7 left heli; phobae absent. **Thorax.** Metathoracic tergal lobe with a dorsolateral egg-buster on each side, egg-buster with a long and a minute backward directed spines, two conic upward-directed projections, a long seta, and a separated sclerite bearing projections (Fig. 71). **Thoracic and abdominal spiracles** (Figs. 66-70, 72) without bulla, abdominal respiratory plates I-IV (Figs. 67-68, 72) with anterior emargination, abdominal respiratory plates V-VIII (Figs. 69-70) with dorsal area smooth.

Remarks: The first and third-instar larvae are quite similar to each other as noted to other scarab beetles (*e.g.*, Böving, 1936). However, the presence of egg-busters on the first instar can be useful to distinguish it from other instars as mentioned in some studies (*e.g.*, Emden,

1941; Šípek *et al.*, 2008). Orphninae first-instar larvae are known only for *Paraegidium costalimai* (here described) and *Chaetonyx robustus* (illustrated by Gradinarov *et al.*, 2015).

Pupa (Figs. 56-61): **Body** (Figs. 56-58) length about 9.1 mm; thorax width about 3.5 mm; white, integument macroscopically smooth and glabrous but covered by a thin and short microscopic pubescence, which gives a

velvety appearance to the surface (magnification of 30x). **Head** (Fig. 59). Vertex is almost hidden under pronotum in dorsal view (Fig. 56). Epistomal suture and canthus indistinct. Clypeus trapezoid, male with lateral areas prominent (Fig. 59). **Labrum** transverse and triangular (Figs. 57-59). Maxillary palps prominent and somewhat triangular. **Labium** with a deep medial groove (Fig. 57). **Antenna** with three defined regions: scape-pedicel, funicle and clava. **Thorax:** Pronotum wider than long, great-

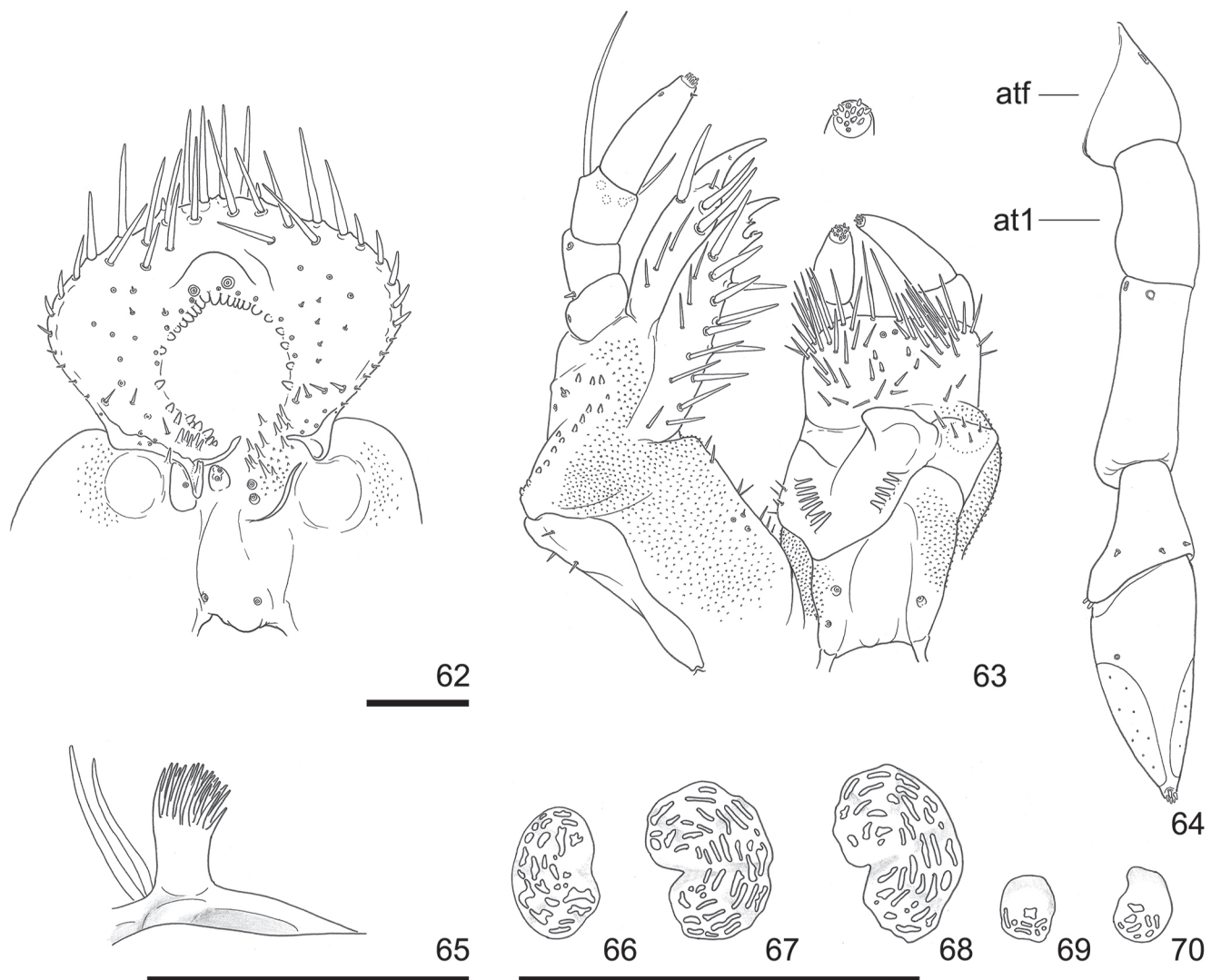


Figures 56-61. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Pupae: (56-58) Male (dorsal, ventral, lateral); (59) Head, male; (60) Male terminalia, posterior; (61) Female terminalia, posterior. **ag** = male anterior genital ampulla; **fgl** = female genital ampulla, posterolateral process; **fgm** = female genital ampulla, anteromedial tubercle; **gl** = galea; **lp** = labial palp; **mp** = maxillary palp; **pg** = male posterior genital ampulla; **st8** = abdominal sternite VIII; **tg8** = abdominal tergite VIII. Scale = 1 mm.

est width at posterior margin, lateral margins rounded, males with a pair of large semispherical tubercles. Prosternum with posterior process rounded. Pronotum seeming longer than mesonotum, and mesonotum longer than metanotum in dorsal view (Fig. 56). Elytra curved ventrally around the body and with raised longitudinal carinae. Pro-, meso- and metacoxa contiguous; femur-tibia slightly exposed in prothorax and exposed in metathorax; protibia with three outer tubercle-like teeth; meso- and metatibia with 2 tubercle-like spurs, posterior spur at least twice longer than anterior spur; metatarsus with tarsomere I as long as other tarsomeres combined, or almost so. **Mesothoracic spiracle** present in a cavity between the pronotum, elytron and anterior and medial legs. **Abdomen:** Gin traps (dioneiform organs *sensu* Costa *et al.*, 1988) absent, tergites I-II with a dorsal spine, III-V with three spines (anterior, dorsal, posterior), VI with two spines (anterior, posterior); VII-VIII with a longitudinal carina; I-VII with laterodorsally transverse carina. **Abdominal spiracles** I-IV with peritreme, I hidden under the wings, V-VIII as cuticular invagination; urogomphi

present. **Male terminalia** (Fig. 60). Proximal genital ampulla divided in a cylindrical anterior area (phallobasis) and a posterior area bearing two dorsal spines (parameres); posterior ampulla semispherical and with a distal impressed line; sternite X hidden in ventral view. **Female terminalia** (Fig. 61). Sternite IX with genital ampulla formed by two parts, an anteromedial pair of small tubercles, and a posterolateral pair of prominent processes (see remarks); tergite X ventrally exposed as a membranous lobe.

Remarks: The pupae are known to Orphniinae species: *A. cribratum* (described by Morón, 1991), *C. robustus* (figured and measured by Gradinarov *et al.*, 2015), and *P. costalimai* (here described). All three species have urogomphi, abdominal tergites I-VI with medial projection bearing hook-like acute spines, abdominal tergites VII-VIII with longitudinal carina, and abdomen without gin traps. Each medial projection of abdominal tergite I-VI has one spine in *C. robustus*, two spines in *A. cribratum*, and one spine (abdominal segments I-II), two (VI), or

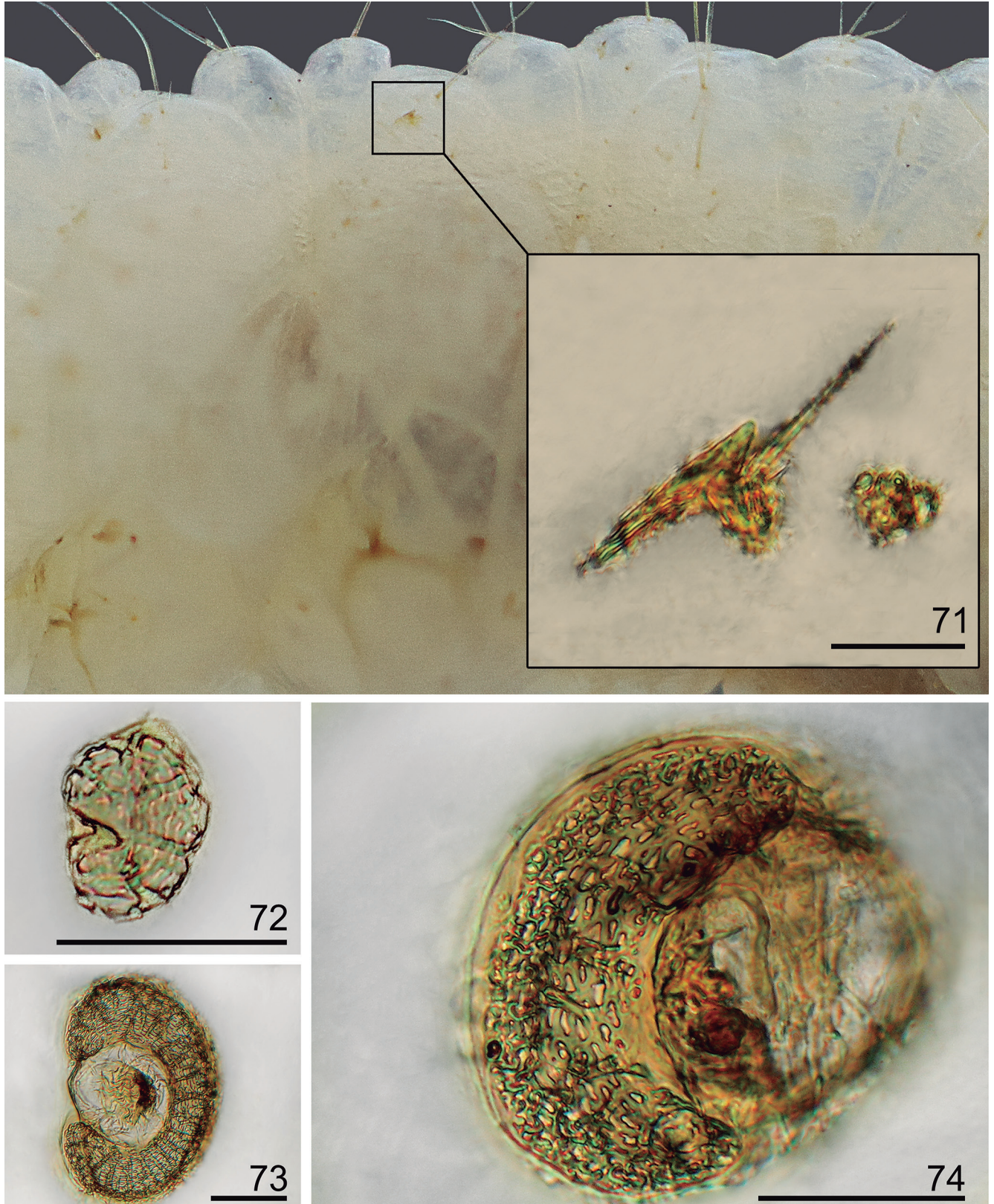


Figures 62-70. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. First-instar larva: (62) Epipharynx; (63) Maxilla, hypopharynx and ligula, dorsal; (64) Left antenna, inner; (65) Acia of right mandible, ventral; (66) Mesothoracic spiracle; (67-70) Abdominal spiracles I-II, and V-VI. **atf** = antennifer; **at1** = antennomere I. Scale = 0.1 mm (palp details with magnification eight times bigger than the maxillolabial complex).

three spines (III-V) in *P. costalimai*. Other diagnostic characters to *P. costalimai* are (opposition to *A. cribratum* in parentheses): abdomen without prominent pleural lobes (tergites II-VII with pleural lobes prominent and covered with conspicuous minute pubescence), longitudinal ca-

rina of abdominal tergite VII smooth (carina serrate). The description of pupa of *C. robustus* is needed to clarify its morphology.

Female pupae of *A. cribratum* and *P. costalimai* have genital ampullae with two parts: an anteromedial pair



Figures 71-74. *Paraegidium costalimai* Vulcano, Pereira & Martinez, 1966. Larvae: (71-72) First instar; (73-74) Third instar; (71) Egg-buster, laterodorsal; (72-73) Abdominal spiracle I; (74) Mesothoracic spiracle. Scales: Fig. 71 = 0.01 mm, Figs. 72-74 = 0.1 mm.

of small tubercles and a posterolateral pair of prominent finger-like processes. The small tubercles are found in other Scarabaeidae pupae, but the finger-like processes are only observed in Orphninae (see Morón, 1991, 1993; Sousa *et al.*, 2018). Morón (1991) showed that these finger-like processes are related to the gonostyli of adult females.

Key to third instar of Orphninae known larvae

- 1 Epipharynx without plegmatia and corypha not prominent; lacinia with 1 or 2 unci (never with 3 tips); posterior area of ligula with thin setae; anal slit sinuate (*Aegidiini*) 2
- Epipharynx with plegmatia and corypha anteriorly prominent; lacinia with 3 unci (or 1 tridentate uncus); posterior area of ligula with stout setae, these setae are distinctly wider than anterior setae; anal slit Y-shaped (*Orphnini*) 3
- 2 Lacinia with 1 uncus; abdominal spiracles decreasing progressively in diameter posteriorly; metapretarsus with rounded and reduced apex (Mexico) *Aegidium cribratum* Bates, 1887
- Lacinia with 2 unci (Figs. 31-35); abdominal spiracles I-IV distinctly larger than V-VIII (Figs. 45-52); metapretarsus with well-developed and acuminate apex (Figs. 38, 41) (Brazil)
..... *Paraegidium costalimai* Vulcano *et al.*, 1966
- 3 Surface of labrum without transverse carinae; stridulatory area of stipes with 14-15 acute teeth 4
- Surface of labrum with two transverse carinae; stridulatory area of stipes with 20 acute teeth (*Hybalus*) 5
- 4 Surface of labrum not trilobate and with small tubercles irregularly distributed (Italy) *Chaetonyx robustus liguricus* Mariani, 1946
- Surface of labrum trilobate and without irregular distributed tubercles (Madagascar) *Triodontus nitidulus* (Guérin, 1844)
- 5 Clypeus with two externoclypeal setae; apex of galea with 2-3 setae distinctly larger than outer setae; hypopharyngeal sclerome tridentate (Italy) *H. benoiti* Tournier, 1864
- Clypeus with one externoclypeal seta; apex of galea with similar shaped setae; hypopharyngeal sclerome bidentate (Morocco)
..... *H. rotrouni* Petrovitz, 1964

DISCUSSION

Egg-buster or egg-hatching structure of the first-instar larvae

The embryo of many insects has spines which aid the rupture of the chorion during hatching. These have been called hatching spines, oviruptors, or egg-busters (Hayes, 1929). With the aid of muscular contractions, the egg-busters assist the embryo to break the chorion during the hatching process (Vitner & Král, 2009). Besides the function of egg-busters, some authors showed that the structure is also useful to identify some first-instar Coleoptera larvae (*e.g.*, Scarabaeidae, Emden, 1941; Šípek *et al.*, 2008).

Egg-hatching structure was first described in Scarabaeoidea by Rittershaus (1925: figs. 1-4; 1927: figs. 133-143) in two European species of Rutelinae (*Anomala aenea* Della Beffa, 1910; *Phyllopertha horticola*

(Linnaeus, 1758)). This author described the structure as a triangular and sclerotized spine present on each dorso-lateral side of metathorax. Afterwards, Gray *et al.* (1947) studied the embryo and the hatching process of *P. horticola*, and noted the egg-busters.

Hayes (1929) did not find egg-busters in the first-instar larvae of several Scarabaeidae species, and indicated that some stiff dorsal setae probably aid the insect somehow to burst the egg chorion. The author added that the mandibles possibly also aid the hatching process.

Some studies have been reporting the presence of egg-busters with variable shapes in some Scarabaeidae species. Gardner (1935) found egg-busters in Rutelinae (*Anomala polita* Blanchard, 1851) and Cetoniinae (*Heterorrhina elegans* (Fabricius, 1781) and *Oxycetonia albopunctata* (Fabricius, 1798)), but Fidler (1936) did not find this structure in Melolonthinae (*Amphimallon solstitialis* (Linnaeus, 1758), *Melolontha melolontha* (Linnaeus, 1758), and *Serica brunnea* (Linnaeus, 1758)).

Vitner & Král (2009) in a study of immatures of *Synopsis yunnanus* Arrow, 1933 (Scarabaeinae) suggested that egg-busters may not always be responsible for the initial fracture of chorion and that these sclerites possibly have a supportive function. The authors noted that the egg-busters probably serve as mechanoreceptors, since the sclerotized insertions of setae of the egg-busters keep them in a constant position relative to the chorion.

The shape and the organization of egg-busters in scarab beetles are similar to each other, consisting of long and sclerotized projection backward directed (eventually described as spine-like projection, conical projection or peg-like projection), with a long seta near to median area. This shape was reported in several works, mainly on Cetoniinae first-instar larvae: *Neoscelis dohrni* (Westwood, 1855) (described by Nogueira *et al.*, 2004), *Dicronocephalus wallichii bourgoini* Pouillaude, 1914 (described by Šípek *et al.*, 2008), and *Homothyrea inornatipennis* Gahan, 1903 (described by Šípek *et al.*, 2012); but also to melolonthine *Propomacrus bimucronatus* (Pallas, 1781) and *P. cypriacus* Alexis & Markis, 2002 (described by Šípek *et al.*, 2011).

The shape of egg busters in other scarab beetles can be different from the above-mentioned. The egg-buster of the dung beetle *Synopsis yunnanus* (Scarabaeinae, described by Vitner & Král, 2009) has three conical projections backward directed, each projection bearing one robust subdistal seta. However, the egg-busters of *P. costalimai* are composed of a backward directed spine and other two small conic upward-directed projection, and a separated sclerite bearing projections. The presence of a separated sclerite was not reported in any of the works cited above.

Studies on the egg-busters and their function are very scarce in most families of Scarabaeoidea. Additional data on the functional morphology of this structure can help us to understand better the behavior and biology of these animals. Also, it can provide important information for taxonomy, systematics and cladistics.

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