

Description of the larva and pupa of *Apion brevicorne* Gerstaecker, 1854 (Coleoptera: Brentidae: Apioninae) with biological information

Rosana Maria de Lima^{1,3}; Sandreli Berenice de Oliveira Santos^{1,4}; Stephanie Vaz²;
Ana Laura Soares Gomes^{1,5} & Wesley Oliveira de Sousa^{1,6}

¹ Universidade Federal de Rondonópolis (UFR), Departamento de Biologia. Rondonópolis, MT, Brasil.

² Universidade Federal do Rio de Janeiro (UFRJ), Instituto de Biologia (IB), Departamento de Ecologia. Rio de Janeiro, RJ, Brasil.
ORCID: <http://orcid.org/0000-0002-2616-640X>. E-mail: anievaz@gmail.com

³ ORCID: <http://orcid.org/0000-0002-0076-6909>. E-mail: rosana.lima0203@gmail.com

⁴ ORCID: <http://orcid.org/0000-0001-6788-8209>. E-mail: sandy.bio2016@gmail.com

⁵ ORCID: <http://orcid.org/0000-0002-8554-5522>. E-mail: sg.analaura@gmail.com

⁶ ORCID: <http://orcid.org/0000-0002-5564-1759>. E-mail: entomoi@hotmail.com

Abstract. *Apion brevicorne* Gerstaecker, 1854 (Coleoptera: Brentidae: Apioninae) is an ecologically and economically important weevil that feeds on seeds and tissues of trees in the genus *Copaifera* L. (Fabaceae). Although the genus *Apion* comprises 16 species restricted to the Palearctic region, the Neotropical species *A. brevicorne* is still considered as *incertae sedis* due to the absence of a systematic study about it. The first descriptions and illustrations of *Apion brevicorne* are provided here. Diagnostic characters of larva and pupa are included and compared with 13 species from other biogeographic regions. Details of immature Apioninae species associated with host plants from the Neotropical region are described for the first time.

Key-Words. Apionini; Cerrado; *Copaifera malmei*; South America; Trichapiina.

INTRODUCTION

The subfamily Apioninae (Brentidae) (Kuschel, 1995; Wanat, 2001; Marvaldi *et al.*, 2002; Alonso-Zarazaga & Wanat, 2014) comprises often very small beetles (0.75-13.00 mm long; more than 95% known species is less than 3 mm). Adults tend to feed on the foliage of the larval host plant. Larvae feed on a variety of plant parts, including roots, inflorescences, seeds, and other tissues where they are endophagous and often form galls (Alonso-Zarazaga & Wanat, 2014; De Sousa *et al.*, 2019). The most diverse group of apionines (supertribe Apionitae) feed on at least 23 families (15 orders) of angiosperms, including the widespread plant family Fabaceae (Anderson & Kissinger, 2002; Badenes-Perez & Jhonson, 2007; Lima *et al.*, 2008; Maia, 2012; Alonso-Zarazaga & Wanat, 2014). With a total of around 2,200 described species (Alonso-Zarazaga & Wanat, 2014), they are reasonably well-known elsewhere, while in the Neotropical Region the Apioninae and their host associations are poorly known (Alonso-Zarazaga & Wanat, 2014).

Apionine species can be found from high altitudes to sea level wherever they occur (Anderson & Kissinger, 2002; Alonso-Zarazaga, 2004; Oberprieler *et al.*, 2007; Alonso-Zarazaga & Wanat, 2014). The Apioninae includes about 205 genera and subgenera (Alonso-Zarazaga & Lyal, 1999; Alonso-Zarazaga & Wanat, 2014), seven supertribes and nine tribes (Alonso-Zarazaga, 1990; Bouchard *et al.*, 2011; De Sousa *et al.*, 2019). The supertribes Antliarhinitae, Cybebitae, Mecolenitae, Myrmacelitae and Tanaitae comprise less than 60 species in Africa and the Australo-Pacific region. The Rhadinocybitae (> 400 species in *ca.* 40 genera) are found throughout the Australo-Pacific region. The Apionitae is the largest supertribe (with five tribes) and includes *ca.* 90% of all Apioninae species. The Apionini is the most diverse tribe with 15 subtribes and *ca.* 1,350 species (Kuschel, 1995; Wanat, 2001; Bouchard *et al.*, 2011; Alonso-Zarazaga & Wanat, 2014; Winter *et al.*, 2017; De Sousa *et al.*, 2019).

In the Neotropical region, all 400 species (190 in South America) belong in the Apionitae with 22 genera (19 in South American) and three

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tribes. The Apionini includes 10 genera (seven from South America) and the monotypic South American Chilapiini and Noterapiini. Ten genera remain *incertae sedis* within the Apionitae (Kissinger, 1968, 2002, 2003, 2005a, 2005b; Wibmer & O'Brien, 1986; Alonso-Zarazaga, 2004; De Sousa et al., 2019). A recent, updated, classification of the Apioninae in Brazil includes 10 genera, two new species, and a new record, for a current total of 89 species (De Sousa & Ribeiro-Costa, 2018; De Sousa, et al., 2019). Fifty-three species remain *incertae sedis*, including *Apion brevicorne* Gerstaecker, 1854 (see De Sousa et al., 2019). We assume that this species most likely does not belong in *Apion*, which is monophyletic and includes 16 species restricted to the Palearctic region (Alonso-Zarazaga, 1990). However, in the absence of a systematic study for the species, we are not sure which genus to assign it to. Thus, for the purpose of the immatures descriptions, this species is kept being regarded as *Apion brevicorne*.

Adult of Apioninae is reasonably well described, while the immature stages remain poorly known in much of the world (Emden, 1938; Wang et al., 2013) but are completely unknown in the South America region. Some small amount of information on larvae is available for ca. 40 species outside of the Neotropical Region, much of which includes only simple descriptions for larvae identification in keys. Of the 35 species in the Palearctic, 23 are in one key for larvae identification (Emden, 1938), while larvae of 12 other species have been described in detail: *Malvapiion malvae* (Fabricius, 1775) (Malvapiina), *Pirapion immune* (Kirby, 1808) (Oxystomatina) (Williams, 1968), six *Exapion* Bedel species (Exapiina) (May, 1994; Sanz Benito & Gurrea Sanz, 1999) also with description of pupae, and only larvae are described in *Apion frumentarium* (Linnaeus, 1758) (Apionina), *Pseudaplemonus aeneicollis* (Gerstaecker, 1854) (Aplemonina) and *Stenopteropion margelanicum* (Wagner, 1912) (Oxystomatina) (Nikulina, 2016), and both larvae and pupae of *Squamapion elongatum* (Germar, 1817) (Kalcapiina) (Letowski et al., 2015). Larvae and pupae in the Palearctic and Oriental *Pseudaspidapion botanicum* Alonso Zarazaga & Wang, 2011 (Aspidapiina) are also described in detail (Wang et al., 2013). The Nearctic region includes five species in three genera that were briefly treated in a single key for larval identification: *Coelocephalapion segnipes* (Say, 1831), *C. subornatum* (Fall, 1898) (Oxystomatina), *Fallapion ellipticum* (Smith, 1884) (Piezotrachelina), *Trichapion griseum* (Smith, 1884) and *T. rostrum* (Say, 1826) (Trichapiina) (Emden, 1938).

Because *Apion brevicorne* is an important fruit and seed predator of *Copaifera* spp., here we describe and illustrate in detail the external morphology of their last larval instar and their pupa. This is the first description of immature stages in the Neotropical Apioninae, and this information is fundamental for future studies of the biology, ecology, taxonomy and systematics of weevils in the Neotropical region, and for providing foundations for a future re-assessment of the Neotropical species currently classified in *Apion*.

MATERIAL AND METHODS

The larvae and pupa were collected individually by hand, from April through September 2018, from seed pods of *Copaifera malmei* Harms, in the Cerrado (Brazilian savanna) at the Universidade Federal de Rondonópolis (UFR, 16°46'S, 54°58'W). Seed pods were dissected to find larvae and pupae in the Water Analysis and Applied Ecology Laboratory (LAHEA/UFR). About 50 larvae and 50 pupae were given in hot water and then set in 70% alcohol and deposited in the Entomological Reference Collection of the Department of Biology (DBFR) for morphological study.

Larvae, their mouthparts, and pupae were drawn using a Zeiss Stemi 200-C stereomicroscope (50×) and Nikon Eclipse E200 microscope coupled to a camera lucida (40×). Larvae were decapitated, and the head was cleared in a 10% potassium hydroxide (KOH) solution and then rinsed in water. The head capsule and all mouthparts were first examined using a Zeiss Stemi 200-C stereomicroscope, then mounted on slides or placed in depression slides containing glycerin (May, 1994), and subsequently examined under optical Nikon Eclipse E200 microscope coupled to a camera lucida. Illustrations were made by a line drawing CorelDRAW X5 program. Specimens were later examined using stereo- and compound light microscopes and scanning electron microscopy (SEM). For the latter, specimens were first dehydrated in a series of progressively increasing concentrations of ethanol (70–100%) and then critical-point dried. Specimens were then mounted on stubs, sputter coated with 35 nm of gold and examined using a JEOL JSM-6390LV scanning electron microscope. The material was photographed at the Optical and Scanning Microscopy Image Laboratory at the Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.

Morphological characters were measured by using a BEL Photonics stereomicroscope with Optical Microscope Imaging Software. Larval measurements (reported in mm) included body length (with head, as distance between apical margin of head capsule and dorsal margin of the tenth abdominal segment) in lateral view (N = 20 individuals); body width (at metathorax, N = 10); width of head capsule in front view (N = 10). Pupal (10 individuals) measurements included length (lateral view) and width (dorsal view). The number of setae and bilateral structures were counted on one side of the body. Terminology, abbreviations and chaetotaxy of both larvae and pupae followed Alonso-Zarazaga & Wanat (2014) and Oberprieler et al. (2014). Suprageneric classification follows Bouchard et al. (2011).

Larval and pupal chaetotaxy of *Apion brevicorne* were compared with 13 species in the Apioninae: *Malvapiion malvae* (Malvapiina), *Pirapion immune* (Oxystomatina) (Williams, 1968), six *Exapion* species (Exapiina) (May, 1994; Sanz Benito & Gurrea Sanz, 1999), *Apion frumentarium* (Apionina), *Pseudaplemonus aeneicollis* (Aplemonina) and *Stenopteropion margelanicum* (Oxystomatina) (Nikulina, 2016), *Squamapion elongatum* (Kalcapiina) (Letowski et al., 2015) and *Pseudaspidapion botanicum* (Aspidapiina) (Wang et al., 2013).

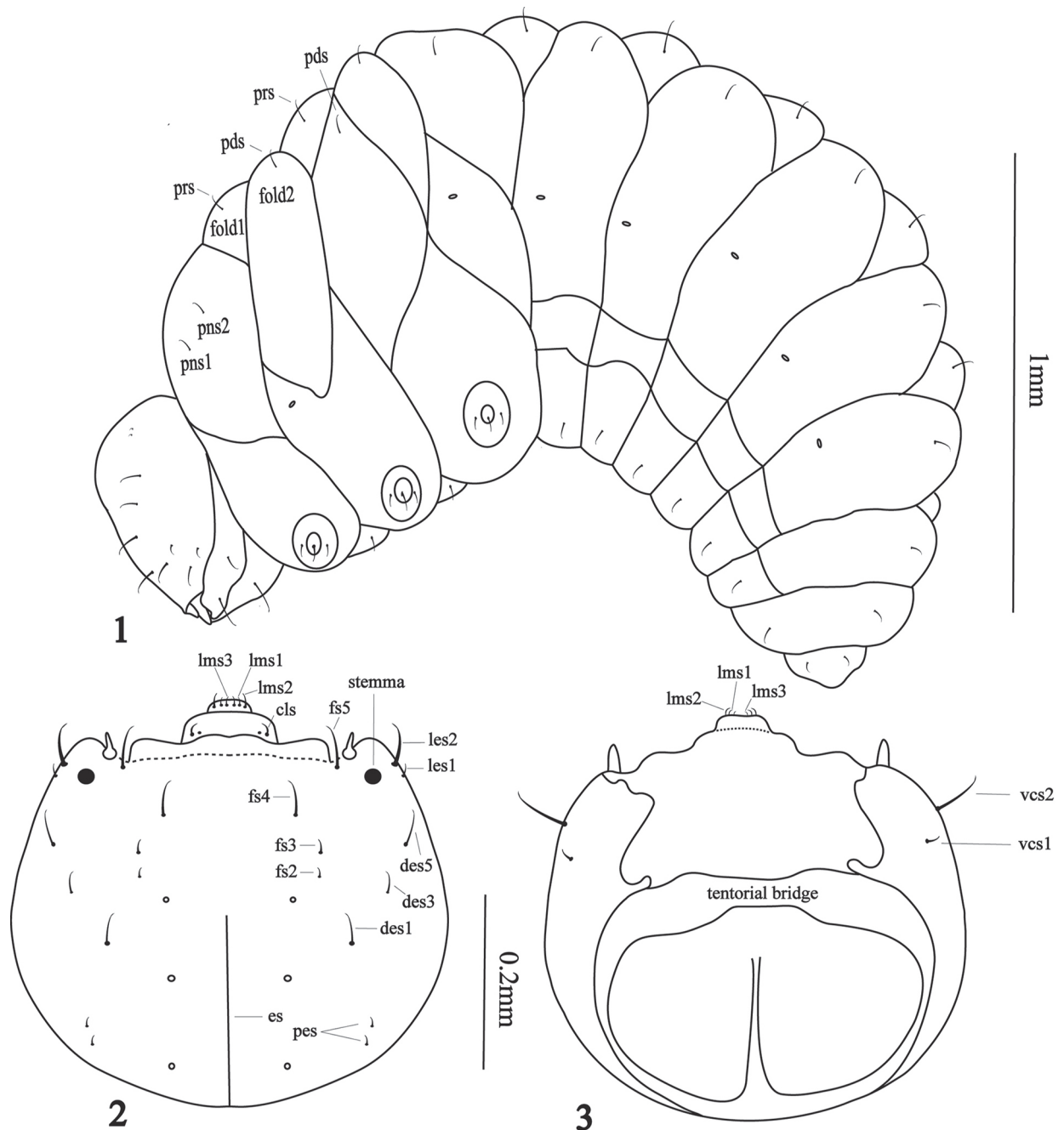
The identification of adults in this study was confirmed by comparison with a specimen of *Apion brevicorne* identified by David G. Kissinger (label data: 11.308; Esc. Nac. Agr., Brasil Central: IX-1945, C. de Araujo; 98; *Apion brevicorne* Gerst. det. Kissinger) on loan to us by the Museu de Zoologia, Universidade de São Paulo, São Paulo (MZSP). Adult male and female images and drawings of the male genitalia allowed the identification of *A. brevicorne*. We provided five adult specimens of each sex to the following Brazilian institutions: Coleção Entomológica Pe. J.S. Moure, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba (DZUP); Coleção Entomológica do Instituto de Biociências, Universidade Federal de Mato Grosso, Cuiabá (UFMT); Instituto Nacional de Pesquisas da Amazônia, Manaus (INPA);

Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro (MNRJ); Museu de Zoologia, Universidade de São Paulo, São Paulo (MZSP).

RESULTS

Apion brevicorne Gerstaecker, 1854 (Figs. 1-22; 29-35)

Material examined: Brazil. Mato Grosso: Rondonópolis, Campus da Universidade Federal de Rondonópolis (UFR), April to September 2018, host plant (*Copaifera malmei* Harms), 50 larvae (10 dissected, 9 metalized for SEM); 50 pupae, (9 metalized for SEM) (DBFR).



Figures 1-3. *Apion brevicorne* Gerstaecker, last instar larva: (1) habitus, lateral; (2) head capsule, frontal; (3) head capsule, ventral. Abbreviations: [seta (ae)-s.]: *des* = dorsal epicranial s.; *es* = epicranial suture; *fs* = frontal epicranial s.; *les* = lateral epicranial s.; *pes* = posterior epicranial s.; *pns* = pronotal s.; *prs* = prodorsal s.; *vcs* = ventral epicranial s.

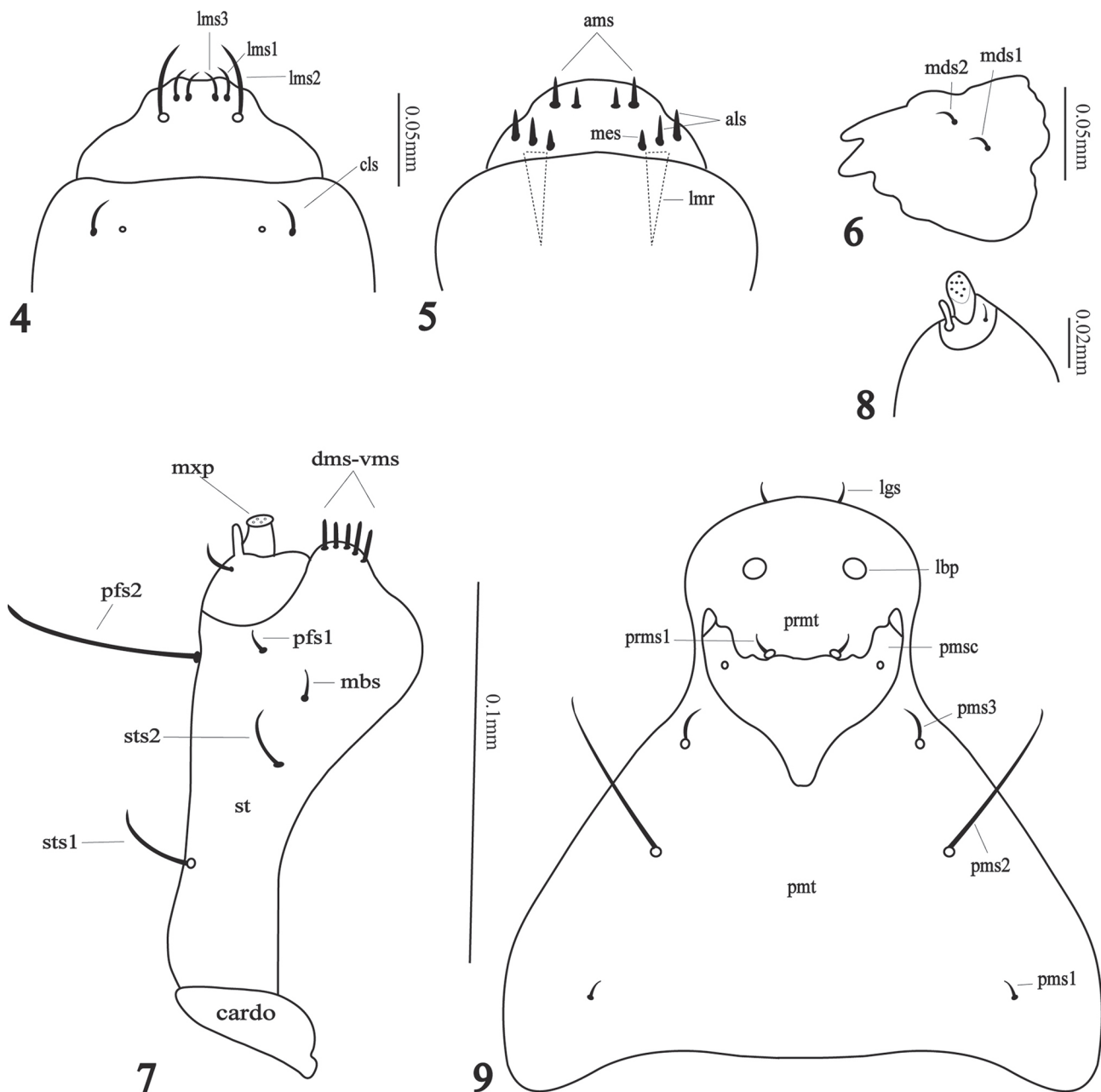
Mature larva description (Figs. 1, 10): Measurements (in mm): body length: 2.11-2.82, body width (metathorax): 1.30-1.50, head width: 0.66-0.76.

General: Body robust, sub-circular in cross-section, strongly dorso-ventrally curved (C-shaped); cuticle minutely spiculate, without visible pigmentation, areas sclerotized and devoid of roughness.

Colouration: Body distinctly white with copious white fat within; head capsule yellowish to pale brown.

Head (Figs. 2-3, 11): Slightly sclerotized; clypeus slightly darkened at the base, darkened labrum; epicranial line

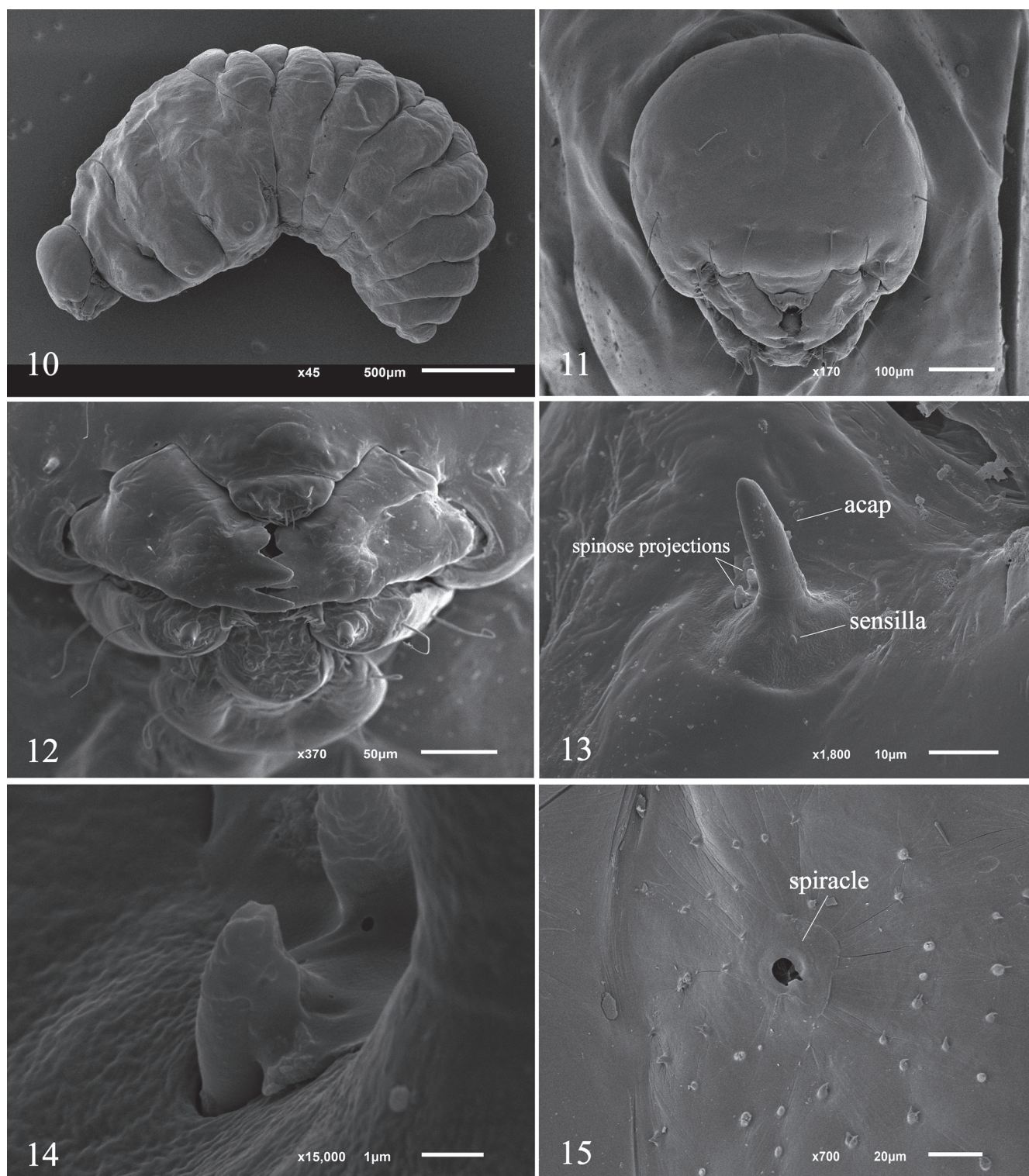
narrow with endocarina as an extension; frontal lines indistinct. Epicranium with 2 lateral setae (*les*), *les1* shorter than *les2*. Dorsal epicranium with 3 setae (*des 1, 3* and *5*). Posterior epicranium with 2 tiny setae (*pes*). Frons with 4 setae (*fs*), *fs2* and *fs3* very short, *fs4* medial and *fs5* laterally positioned close to antennae. Ventral epicranium with 2 ventral setae (*vcs*), near *les*. Postoccipital condyles absent; tentorial bridge without anterior and posterior projections; hypopharyngeal bracon absent. Clypeus transverse, bearing 1 seta (*cls*), inner side of *cls* bearing 1 sensilla. Antenna (Figs. 13-14) one-segmented, with rod-like accessory sensory appendage (*acap*) more than 3x as long as wide, with 2 spinose projections and 1 sensillum. Stemma (ocular spots) at the base of the antenna.



Figures 4-9. *Apion brevicorne* Gerstaecker, mouthparts: (4) clypeus and labrum, dorsal; (5) epipharynx; (6) mandible, dorsal; (7) maxilla; (8) maxillary palp; (9) labium. Abbreviations: [seta (ae)-s.]: *ams* = anteromedian epipharyngeal s.; *als* = anterolateral epipharyngeal s.; *cls* = clypeal s.; *dms* = dorsal malar s.; *lbp* = labial palp; *lmr* = labral rods; *lgs* = ligular s.; *lms* = labral s.; *mbs* = basioventral seta s.; *mes* = median epipharyngeal s.; *mds* = mandibular s.; *mxp* = maxillary palp; *pmt* = postmentum; *pms* = postmental s.; *prmt* = prementum; *prms* = premental s.; *pmsc* = premental sclerite; *pfs* = palpiferal s.; *st* = stipes; *sts* = stipital s.; *vms* = ventral malar s.

Mouthparts (Figs. 4-9, 12): Labrum (Fig. 4) subtrapezoidal with lateral margins sinuose, with 3 setae (*lms*), *lms*2 three times longer than two remaining setae. Epipharynx (Fig. 5) with 2 long, stout lateral rods (ImR), 2 anterolateral setae (*als*), 2 anteromedian setae (*ams*), and 1 median seta (*mes*); epipharyngeal setae stout, short and apically rounded, lacking epipharyngeal sensory pores between pairs of ImR. Mandibles (Fig. 6) symmetrical, apically bidentate, length and width approximately equal,

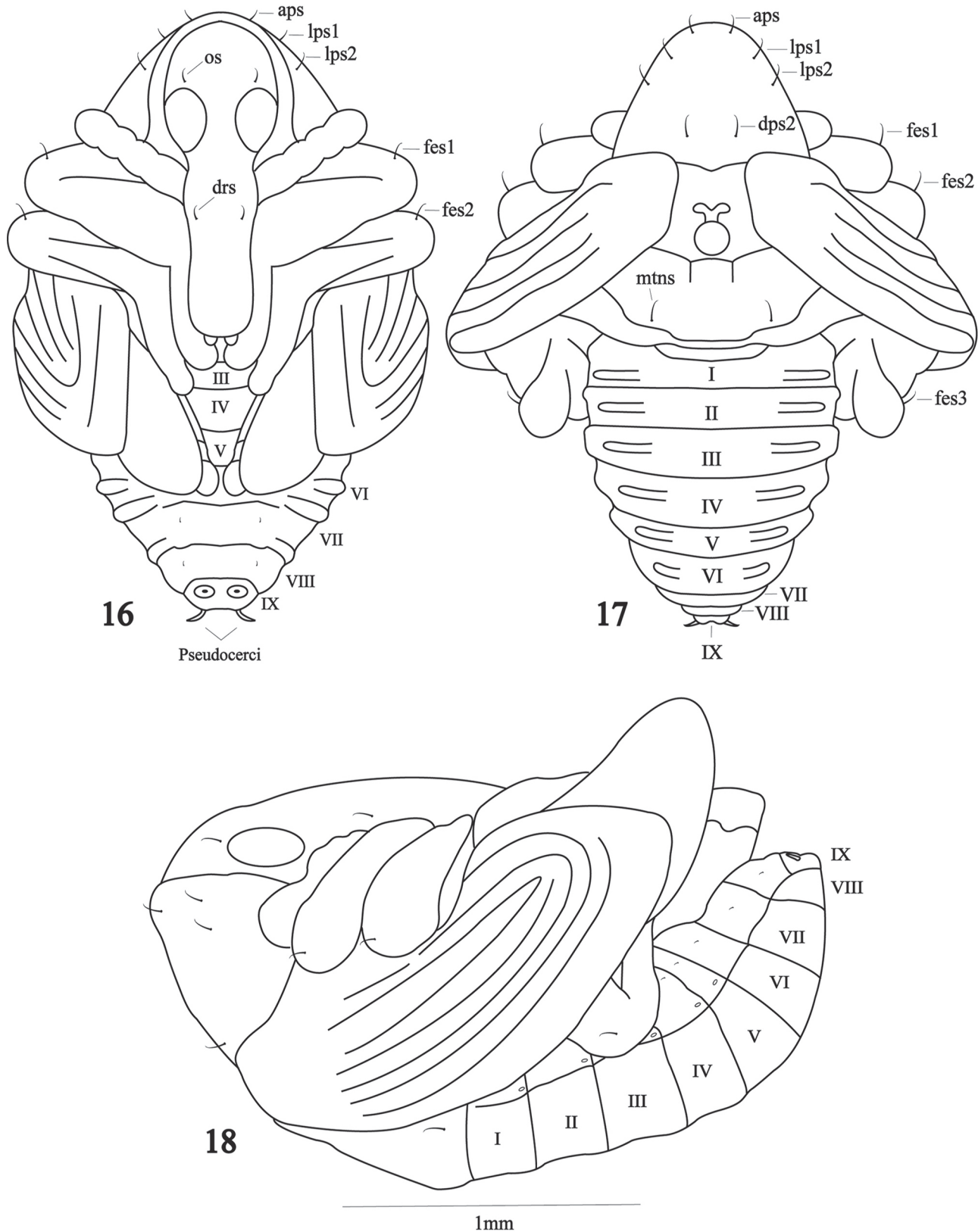
cutting edge with 1 small, but evident, rounded tooth; laterodorsal surface with 2 mandibular setae (*mds* 1-2), without sensilla. Maxilla (Fig. 7): palpifer indistinct; maxillary palpi (MxP, Figs. 7-8) with 2 segments, basal segment with 1 long, basal accessory process, 1 short seta, apical segment cylindrical, apically flattened with dense crenulate setae; mala with 5 stout, short, indistinguishable setae *dms-vms*; stipes (*st*) with 2 stipital setae (*sts* 1-2), 1 basioventral seta (*mbs*) and 2 palpiferal setae (*pfs* 1-2),



Figures 10-15. *Apion brevicorne* Gerstaecker, SEM micrographs larva: (10) habitus, lateral; (11) head, frontal; (12) mouthparts, frontal; (13-14) antenna; (15) spiracle. Legend: Acap = accessory sensory appendage.

cardo completely separated from stipes. Labium (Fig. 9) subconical, preapically constricted and apically rounded, almost membranous except in sclerotized area (pmsc); labial palpus (lbP) vestigial, lobe-like. Premental sclerite (pmsc) distinctly dilated, "Y" shaped, with 1 pair of sensil-

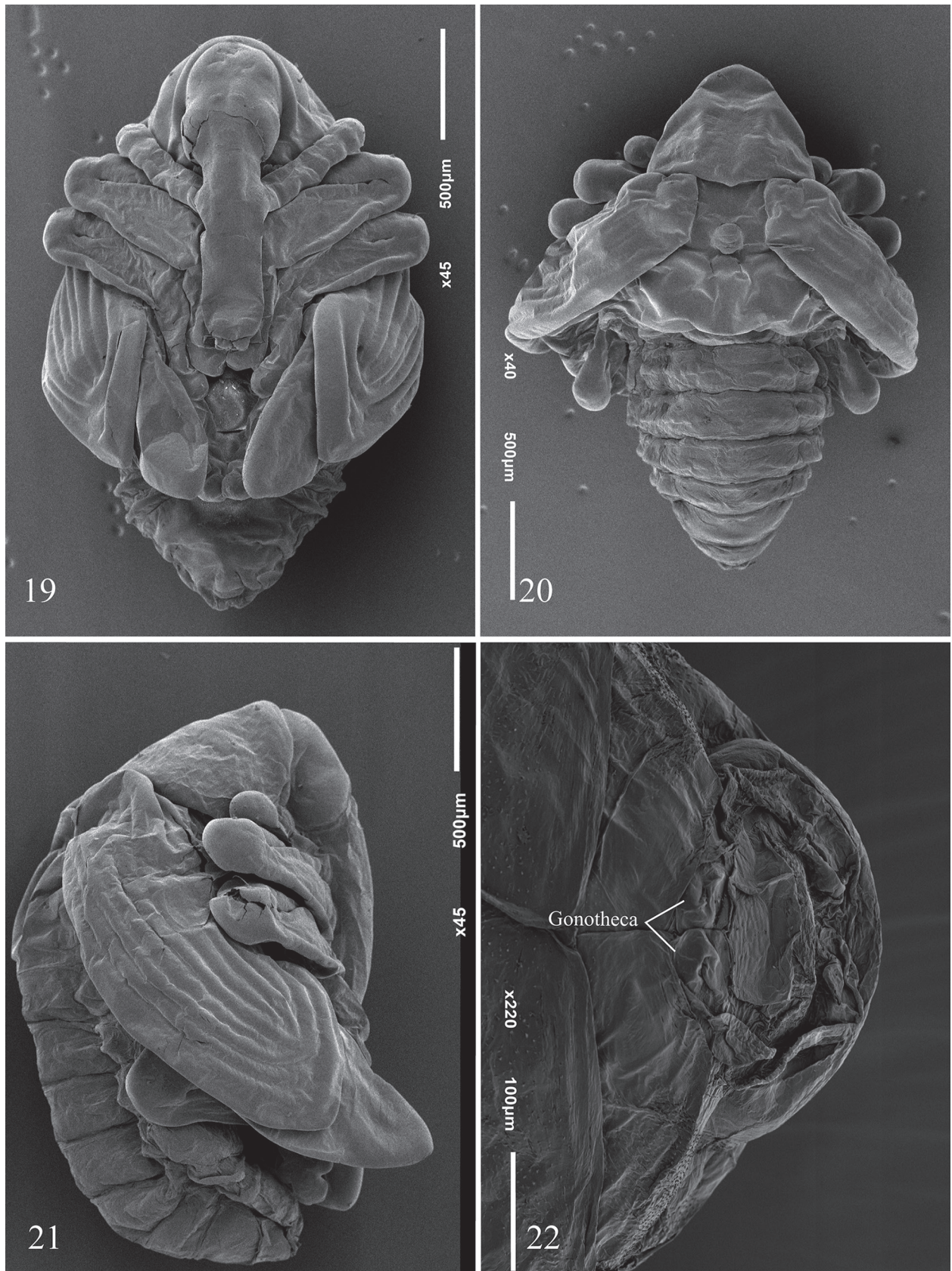
la. Ligulate area with 1 tiny seta (*lgs*). Prementum (Prmt) with 1 seta (*prms*); postmentum (Pmt) with 3 setae (*pms*) laterally, *pms2* (located medially) much longer than *pms3* (located apically) which is somewhat longer than *pms1* (located basally).



Figures 16-18. *Apion brevicorne* Gerstaecker, female pupal habitus: (16) ventral; (17) dorsal; (18) lateral. Abbreviations: [seta (ae)-s]: *aps* = apical pronotal s.; *dps* = discal pronotal s.; *drs* = distirostral s.; *dps* = discal pronotal s.; *fes* = femoral s.; *lps* = lateral pronotal s.; *mtns* = metanotal s.; *os* = orbital s.

Thorax (Fig. 1): Pronotal shield simple, without fold, unsclerotized; meso- and metanotum each with 2 folds (prodorsum and postdorsum). Spiracle (Fig. 15) laterally

intersegmental between pro- and mesothorax, bicameral. Prothoracic epipleura indistinct, meso- and metathoracic epipleura distinct, not centrally tuberculate, without



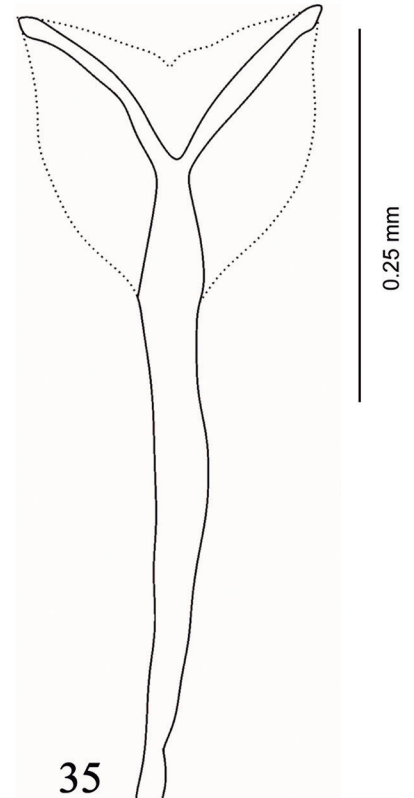
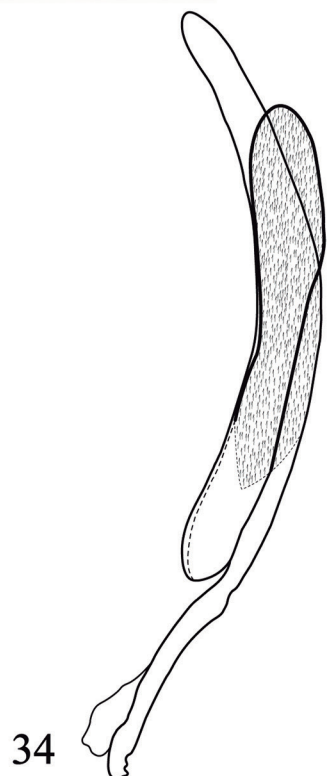
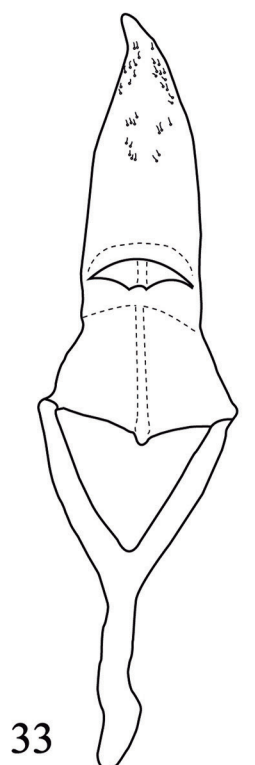
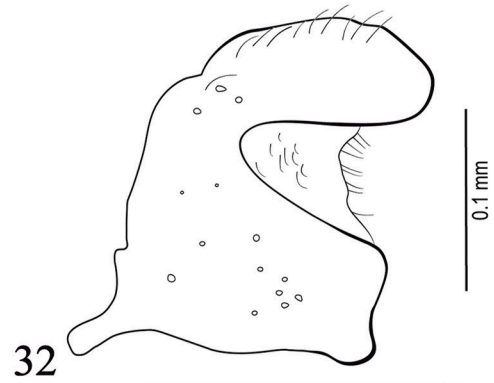
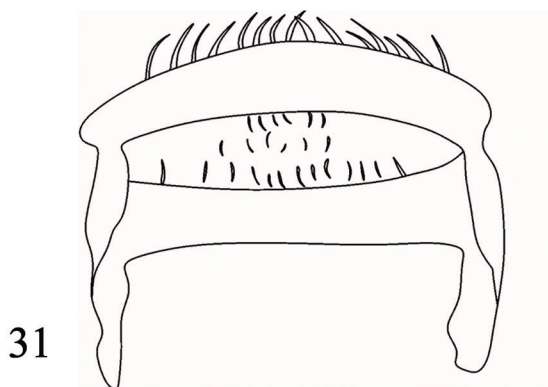
Figures 19-22. *Apion brevicorne* Gerstaecker, SEM micrographs pupa: (19-21) habitus: (19) ventral; (20) dorsal; (21) lateral; (22) female, abdominal segments VII-IX, ventral.



Figures 23-28. *Copaifera malmei* host plant of the *Apion brevicorne*: (23) host plant habitus; (24) fruit in intermediate stages of development; (25) mature fruit in dehiscence stage; (26) *A. brevicorne* feeding on floral nectar; (27) attacked fruit and (28) healthy fruit.

setae. Pedal area defined, pedal lobe (papillae) present, 2-segmented. Pronotum with 2 setae (*pns* 1-2), transversally aligned near to middle area; meso- and metanotum

each with an 1 prodorsum (*prs*) and 1 postdorsum (*pds*) setae, epipleurum without setae; pedal area with 3 setae (*pda*); sternum with 1 tiny seta.



Figures 29-35. *Apion brevicorne* Gerstaecker: (29-30) habitus lateral, male, female; (31, 32) male pygidium, dorsal, lateral; (33) tegmen dorsal; (34) penis lateral; (35) spiculum gastrale.

Table 1. Larval chaetotaxy (of one side of the body) compared among Neotropical *Apion brevicorne* and Palearctic species of Apioninae. Question marks indicate that setae were either not mentioned, not illustrated or doubtful in descriptions and figures. "Ind" indicates that the setae could not be counted because they are densely packed.

Body Part	Character	Species													
		<i>A. brevicorne</i>	<i>A. frumentarius</i>	<i>E. ulicis</i>	<i>E. fuscirostre</i>	<i>E. elongatissimum</i>	<i>E. laufferi</i>	<i>E. putoni</i>	<i>E. compactum</i>	<i>M. malvae</i>	<i>P. immune</i>	<i>P. aeneicollis</i>	<i>P. botanicum</i>	<i>S. elongatum</i>	<i>S. margelanicum</i>
Head	posterior epicranial setae (<i>pes</i>)	2	0	?	4	4	4	4	4	0	0	0	6	4	0
	dorsal epicranial setae (<i>des</i>)	3	5	4	4	4	4	4	4	4	5	3	5	5	3
	lateral epicranial setae (<i>les</i>)	2	1	1	1	1	1	1	1	2	2	1	2	2	2
	frontal setae (<i>fs</i>)	4	3	2	2	2	2	2	2	?	3	3	4	5	3
	ventral cranial setae (<i>vcs</i>)	2	0	0	1	1	1	1	1	?	2	0	2	?	0
	clypeal setae (<i>cls</i>)	1	1	1	1	1	1	1	1	2	1	1	1	1	1
Mouthparts	labral setae (<i>lms</i>)	3	3	1	1	1	1	1	1	1	?	3	3	3	3
	anteromedian setae epipharynx (<i>ams</i>)	2	3	2	2	2	2	2	2	3	2	2	2	3	3
	anterolateral setae epipharynx (<i>als</i>)	2	4	3	2	2	2	2	2	2	3	2	2	3	3
	median epipharyngeal setae (<i>mes</i>)	1	0	1	1	1	1	1	1	1	1	2	1	1	2
	mandibular setae (<i>mds</i>)	2	1	2	2	2	2	2	2	2	2	1	1	2	0
	dorsal malar setae (<i>dms</i>)	Ind	5	5	5	5	5	5	5	5	4	4	5	8	5
	ventral malar setae (<i>vms</i>)	Ind	3	?	3	4	4	4	4	1	1	3	4	?	2
	stipetal setae (<i>sts</i>)	2	3	1	1	1	1	1	1	2	2	1	1	1	3
	basioventral setae (<i>mbs</i>)	1	1	1	1	1	1	1	1	0	0	1	0	?	0
	palpifer setae (<i>pfs</i>)	2	0	2	2	2	2	2	2	2	2	0	2	2	0
	ligular setae (<i>lgs</i>)	1	1	0	1	1	1	1	1	2	2	2	1	2	1
	premental setae (<i>prms</i>)	1	0	1	1	1	1	1	1	1	1	1	1	1	1
	postmental setae (<i>pms</i>)	3	3	3	3	3	3	3	3	3	2	3	3	2	3
	Thorax	pronotal setae (<i>pns</i>)	2	6	6	6	6	6	6	6	12	12	6	6	5
prodorsal setae (<i>prs</i>)		1	1	1	1	1	1	1	1	1	1	2	1	1	1
postdorsal setae (<i>pds</i>)		0	3	2	1	1	1	1	1	4	4	4	4	5	4
alar setae (<i>as</i>)		0	1	0	3	3	3	3	3	1	1	1	0	0	1
spiracular setae (<i>ss</i>)		0	0	2	?	?	?	?	?	3	3	0	0	0	0
epipleural setae (<i>eps</i>)		0	2-3	?	1	1	1	1	1	1	1	2-3	4	4	1-3
pleural setae (<i>ps</i>)		0	1	?	1	1	1	1	1	1	1	2	0	1	1
pedal setae (<i>pdas</i>)		3	4	2	2	2	2	2	2	2	2	6	3-4	2	3
sternal setae (<i>sts</i>)		1	2	1	1	1	1	1	1	1	1	2	1	1	1
Abdomen		prodorsal setae (<i>prs</i>)	1	1	1	1	1	1	1	1	1	2	1	1	1
	postdorsal setae (<i>pds</i>)	1	2	2	2	2	2	2	2	4	4	5	6	5	3
	setae of spiracular area (<i>ss</i>)	0	1	1	1	1	1	1	1	1	1	1	0	0	0
	epipleural setae (<i>eps</i>)	0	1	1	1	1	1	1	1	1	1	2	0	1	1
	pleural setae (<i>ps</i>)	0	1	1	?	?	?	?	?	0	0	2	0	1	1
	sternal setae (<i>sts</i>)	1	?	1	1	1	1	1	1	0	0	?	1	1	?

Abdomen (Fig. 1): Tergites I-VII, each with 2 folds, prodorsum with 1 *prs*, postdorsum with 1 *pds*; tergite VIII undivided with 2 setae; tergite IX undivided and reduced with 2 setae; with 6 pairs of subequal bicameral spiracles, each pair anterolaterally on tergites I-VI, VII and VII without spiracles; pleura I-VIII without setae, each sternum with 1 seta. Anal area with 2 lobes and 1 seta.

Pupal description (Figs. 16-21): Measurements (in mm): body length: 3.00-4.13, body width (metathorax): 1.22-1.50.

General: Aedeagus and exarate; body setae on tubercles, setae greatly reduced in number and size.

Colouration: Inner body pure white, except eyes, which are pale to dark.

Rostrum in ventral view (Figs. 16, 22): Apex extends past mesocoxae but not to metacoxae, mesorostrum dilated, mandibular theca projected; 1 distirostral seta (*drs*).

Head: Frons with 1 seta (*os*), similar in size as *drs*, behind eyes. Antennae basally near prosternum and apically extend to propleura, oblique to protibia.

Thorax: In dorsal view, pronotum with 1 apical pronotal seta (*aps*), 2 lateral pronotal setae (*lps* 1-2), and 1 discal pronotal seta (*dps*2); in ventral view, *aps* and *lps* 1-2 completely visible. Mesonotum without setae; metanotum with 1 seta (*mtns*). Legs (Figs. 16-21): in ventral view, metatibiae and femora covered by pterothecae, front and middle legs and metatarsomere visible; pro-, meso- and metafemora apically bearing 1 slightly out-

Table 2. Pupal chaetotaxy (of one side of the body) compared among Neotropical *Apion brevicorne* and Palearctic species of Apioninae. Question marks indicate that setae were either not mentioned, not illustrated or doubtful in descriptions and figures.

Body Part	Character	Species								
		<i>A. brevicorne</i>	<i>E. ulicis</i>	<i>E. fuscirostre</i>	<i>E. elongatissimum</i>	<i>E. laufferi</i>	<i>E. putoni</i>	<i>E. compactum</i>	<i>P. botanicum</i>	<i>S. elongatum</i>
Head	basirostral setae (<i>brs</i>)	0	0	1	1	1	2	2	0	0
	distirostral setae (<i>drs</i>)	1	0	1	1	1	1	1	1	1
	rostral lateral setae (<i>rls</i>)	0	0	0	0	0	0	0	0	0
	orbital setae (<i>os</i>)	0	1	1	1	1	0	1	1	0
	postorbital setae (<i>pos</i>)	0	0	0	0	0	1	0	0	0
	supraorbital setae (<i>sos</i>)	1	0	0	0	0	1	0	0	1
	vertical setae (<i>vs</i>)	0	0	1	1	1	1	1	0	0
Thorax	apical pronotal setae (<i>aps</i>)	1	2	1	2	1	1	1	2	2
	basal pronotal setae (<i>bps</i>)	0	0	1	1	1	1	1	1	1
	discal pronotal setae (<i>dps</i>)	1	1	1	1	1	1	2	2	1
	lateral pronotal setae (<i>lps</i>)	2	1	2	1	2	2	2	1	1
	mesonotal setae (<i>msns</i>)	0	?	1	1	1	1	1	0	0
	metanotal setae (<i>mnts</i>)	1	?	2	4	2	2	2	2	3
Legs	femoral setae (<i>fes</i>)	1-3	1-3	1-3	1-3	1-3	1-3	3	1 and 3	1
Abdomen	postdorsal setae (<i>pds</i>)	0	?	2	2	2	3	3	3-4	7
	pleural setae	0	?	1	1	1	1	1	?	0
	ventral setae	0-1	?	2	2	2	2	1	2	0

curved seta (*fes*), each seta inserted in a small rounded protuberance.

Abdomen: In ventral view, ventrites IV-IX visible (Fig. 16), in dorsal view tergites I-VII about equal in length and decreasing in width (Fig. 17), segments VII-IX clearly and gradually reduced; 5 spiracles present, positioned on pleura I-V, bicameral (Fig. 18); pseudocerci inconspicuous at segment IX, unpigmented; in ventral view, ventrites IV-VIII with 1 pair of setae; in dorsal view, tergites I-IX without setae.

Sexual dimorphism: Female with small round convexity on each side of sternum on ninth abdominal segment (Figs. 16, 22).

Biological information (Figs. 23-28): *Apion brevicorne* is associated with *C. malmei*. Adults may feed on nectar in flowers at any time, day or night. Copulation and oviposition occur on fruits at any stage of development. The oviposition is endophytic and eggs are easily found only in fruits at an early stage of development. As in most weevils, the damage caused by *A. brevicorne* to the seed is mostly by larvae that develop within the seed, where they feed on developing endocarp. Pupae were always found within seeds (without a pupal chamber) along with feces that indicate that the larvae consumed the entire endocarp, and so development is completed within the seed. The number of adults in each seed is quite variable. Seed pods that contain larvae, pupae or adults show no external evidence of presence. This suggests that for adults to disperse, they depend on the dry dehiscence of the fruit. Dead adults and pupae can be found within dry seed pods that did not dehisce.

DISCUSSION

All of the 13 species of Apioninae whose immature stages have been described are in seven of the subtribes of the Apionini (Williams, 1968; May, 1994; Sanz Benito & Gurrea Sanz, 1999; Nikulina, 2016; Wang *et al.*, 2013; Letowski *et al.*, 2015). We found that morphology of *Apion brevicorne* larvae and pupae described herein are very different from other species in these subtribes (especially the reduced chaetotaxy of the mala, thorax and abdomen) and different from other generalized endophagous Apioninae (May, 1993, 1994; Wang *et al.*, 2013; Alonso-Zarazaga & Wanat, 2014) and other Curculionoidea (Oberprieler *et al.*, 2014) (Tables 1 and 2). Establishing homologies for the reduced number of setae for the Apioninae is complicated because of the various usages of nomenclature for the Curculionoidea (Anderson, 1947, Marvaldi, 1999; Wang *et al.*, 2013). Differences in terminology and abbreviations among publications can lead to errors or inconsistencies in morphology, phylogeny and interpretation (Lira *et al.*, 2017).

Larval characteristics that vary among the Apioninae and which can be used to identify genera and species of Apionini are mainly chaetotaxy of the head and mouthparts. Variability is common interspecifically as well as intrageneric and thus may be used to diagnose species (Sanz Benito & Gurrea Sanz, 1999; Wang *et al.*, 2013; Alonso-Zarazaga & Wanat, 2014).

The indistinct frontal line and endocarina we describe for *Apion brevicorne* is the first observation of these characters in Apioninae. The frontal line may be absent in some groups of Curculionoidea (Oberprieler *et al.*, 2014) but is always present in Apioninae (Alonso-Zarazaga &

Wanat, 2014). This may be very useful in future phylogenetic analyses of Apioninae.

The hypopharyngeal bracon, a bar that connects the two hypopharyngeal margins and which supports the hypopharynx, separates larvae of the Curculionoidea from those of the Bruchidae and Chrysomelidae (Lawrence, 1991; Oberprieler et al., 2014). The hypopharyngeal bracon is present and distinct in the primitive Australo-Pacific *Neocyba* sp. (Rhadinocybitae) (May, 1993) and absent in *A. brevicorne*. Its absence in Apioninae was first noted in *P. botanicum* (Wang et al., 2013), perhaps because it was uncommon to refer to ventral characters of the epicranium when describing apionine larvae (Bennett, 1992; Emden, 1938; Gosik et al., 2010; May, 1994; Sanz Benito & Gurrea Sanz, 1999). More apionine species need to be studied to determine the importance of the hypopharyngeal bracon as a diagnostic character (Wang et al., 2013).

The number of spiracles (6 pairs) in larval *A. brevicorne* and Holarctic *Podapion* Riley, 1883 differ from that for typical Apioninae (7 pairs) as well (Emden, 1938; Scherf, 1964; Wang et al., 2013; Alonso-Zarazaga & Wanat, 2014; Letowski et al., 2015). Also, pupal *A. brevicorne* have less setae everywhere except on the femora. Pupal chaetotaxy ranges from 6 setae on the head and rostrum of *Exapion putoni* (Sanz Benito & Gurrea Sanz, 1999) to one in *Exapion ulisis* (May, 1994). Thus, pupal chaetotaxy can be used to identify pupae in other subtribes or genera of the Apionini (May, 1994; Sanz Benito & Gurrea Sanz, 1999; Wang et al., 2013).

In the female pupae, the gonotheca of the ninth abdominal sternum is divided into two parts. This pattern of sexual dimorphism seems to be common in pupae in a variety of Curculionoidea (Burke, 1968; May, 1994; Sarro et al., 2004; De Sousa et al., 2004), but has never been noted in the Apioninae (May, 1994; Sanz Benito & Gurrea Sanz, 1999; Wang et al., 2013).

Morphological and taxonomic studies of immature stages of Apioninae are still uncommon, yet such studies are fundamental for species identification, as well as for understanding their taxonomy and systematics. Apioninae biology is also poorly known in the Neotropics. Now, with our results, we were able to identify pests of unidentified genera of Neotropical Apioninae (*Apion* sp.) that are found on three species of *Copaifera*: *C. sabulicola* J. Costa & L.P. Queiroz, *Copaifera luetzelburgii* Harms and *Copaifera depilis* Dwyer (Santos et al., 2015). We confirmed that samples of these hitherto unknown species also pertain to *A. brevicorne* (Figs. 29-35). Thus, *A. brevicorne* is oligophagous in contrast with other Brazilian Apioninae in their association with several *Copaifera* spp. Pests of *Copaifera* spp. may cause reduced productivity, with important detrimental effects on plant populations (Santos et al., 2015). Studies should continue using integrative taxonomy to determine the number of species of *Copaifera* that may host *A. brevicorne*, whether each host species is associated with additional taxonomic units of the weevil, and the taxonomic position of the species as a possible new genus within the Apionini.

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