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Morphology of the larval instars of *Bagous binodulus* (Herbst, 1795) and *B. robustus* H. Brisout, 1863 with notes on their biology (Coleoptera: Curculionidae)

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Abstract. The article contains the first detailed and illustrated description of the larval instars of *Bagous binodulus* (Herbst) and *B. robustus* (H. Bris.). Their diagnostic characters are provided e. g., distribution of setae and structure of mouth parts are presented. A short note on the biology of both species is given.

Key words: entomology, morphology, *Coleoptera*, *Curculionidae*, *Bagous binodulus, B. robustus*, larva, description.

INTRODUCTION

The genus *Bagous* Germar, 1817 includes more than 200 species, of which 72 have been recorded from the Palearctic (Dieckmann 1964a, O'Brien & Askenvold 1992, Cuppen & Heijerman 1995). The mentioned weevils are in the majority tiny, rarely medium-sized beetles (2-6 mm in length), associated with plants growing in wet habitats e.g.: lakes, ponds, old river-beds, wet meadows, undergrowth of alder or floodplain forests (Burakowski et al. 1995). Most of *Bagous* species are monophagous, trophically connected with several water plants or helophytes (Cuppen & Heijerman 1995). These beetles are mainly considered as rare, stenotopic species, often endangered (Sprick 2001). As a result of the revision by Caldara & O'Brien (1994) the genus *Hydronomus* Schoenherr, 1825 was included to the genus *Bagous* Germar, 1817.

The knowledge about biology and (especially) morphology of immature stages of mentioned genus is still incomplete. The morphology of larval instars of the genus *Bagous* is described and illustrated only for 10 species: *B. alismatis* (Marsham, 1802),

B. australasiae Blackburn, *B. brevis* Gyll., *B. collignensis* Herbst, *B. frit* Herbst, *B. frivaldszkyi* Tourn., *B. lutulentus* (Gyll.), *B. nodulosus* Gyll., *B. rufimanus* Hoffmann and *B. subcarinatus* Gyll. (Scherf 1964; Leiler 1987, Mantovani et al. 1992; May 1994; Cuppen & Heijerman 1995; Staniec & Gosik 2003; Gosik 2006a, b, Gosik 2008, Gosik 2009).

Bagous binodulus (Herbst, 1795) (Fig 1) is a stenotopic and aquatic species (Koch 1992). In natural conditions it is monophagous on water soldiers (*Striatiotes aloides* L.), but in labor feeding of adults on with clover (*Trifolium repens* L.) and greater plantain (*Plantago major* L.) was observed (Dieckmann 1964b). The species is distinguishable from other *Bagous* species by the presence of two pairs of protruding calli on the back of 3rd and 5th elytral intervals and relatively large body size (4-6 mm) (Freude et al. 1983). The distribution of *B. binodulus* is strictly limited to a few localities in central and north part of Europe (Alonzo-Zarazaga 2007). Its life-cycle and a piece of information about morphology of larval instars are provided by Scherf (1964). Inclusion of *B. binodulus* to the species designated for conservation by NATURA 2000 was proposed by Sprick (2001).

B. robustus H. Brisout, 1863 (Fig. 2) is a stenotopic, hygrophilous species, which development is connected with common water plantain (*Alisma plantago-aquatica* L.) (Koch 1992). Red-brown colour of tarsi in *B. robustus* vs. black in *B. lutulentus* is the most visible difference between both species. Despite large morphological similarities to *B. lutulentus*, taxonomical status of *B. robustus* as a valid species is unquestionable (Dieckmann 1972). Distribution of *B. robustus* is poorly known, its occurrence is recorded from several localities in Europe, North Africa and Near East (Alonzo-Zarazaga 2007). The morphology of development stages and life-cycle of *B. robustus* are still unknown (Burakowski et al 1995).

MATERIAL AND METHODS

B. binodulus: 27 exx. of larvae (different stages) were collected on 16th, 25th of July, 7th and 14th of August 2008 from leafs of water soldiers growing on small water pool in Dratów near Łęczna (Central-East Poland) (UTM nets: FB48).

B. robustus: 7 exx. of larvae (different stages), were extracted from leafs and stems of common water plantain growing on old river bank of Bug river in Gródek near Hrubieszów (UTM nets: GB03). The observation of life-cycle of the species was conducted in vegetations seasons 2006 – 2008.

The material to morphological studies was preserved in a liquid of 75% alcohol. The punctured larvae were rinsed in distilled water and cleared in 10% liquid of potassium hydroxide (KOH) and finally placed in 10% glycerin to prepare microscopic slides. The drawings were made using camera lucida (MNR-1, 10^X, PZO). The terminology follows given by May (1977, 1994) and Scherf (1964).

RESULTS

Description of larva of *Bagous binodulus*

L_1 (Fig. 3), body length: 2.25 – 2.75 mm; head width: 0.37 – 0.45 mm.

L_3 (Fig. 4), body length: 6.80 – 8.40 mm; head width: 0.72 – 0.82 mm.

Body slender, elongated, rounded in cross section (in L_1 oblate dorso-ventrally); the terminal segments curved upwards: distinctly in L_1, slightly in L_3. Spiracles bicameral, placed medially, except those on VIII segment, localized dorsally. The spiracles of last pair twice as large as other (Figs 5-7). The color of all larval instars white. Cuticle minutely spiculate (Fig. 8). Prothorax smaller then meso- and metathorax. Prodorsal sclerite black, well visible in all larval instars. The thoracic segments smaller than the first segment of abdomen. Abdominal segments I-VI almost equal in length; segment VII slightly smaller than previous ones; segment VIII narrow, segment IX semicircular in dorsal view, segment X reduced to anal lobes. Segment VIII with a pair, seg. IX with two pairs of visible protuberances on posterior margin of each ones. Chaetotaxy of the body is distinctly reduced, setae, brown, short, except those on VIII and IX abdominal segments. Dorsal parts of prothorax with complex of 1 macro (prns) and 3 micro setae on each side (Fig. 13); next macro setae placed: dorsolaterally (dls), dorsopleurolaterally (dpls) and ventropleurolaterally (vpls) (Fig. 4). Mesothorax: 1 micro seta prodorsally (prs), 1 macro and 2 micro setae postdorsally (pds) and 1 macro setae dorsopleurolaterally (dpls), placed on each side of the body. Chaetotaxy of metathorax similar to those on mesothorax. Each pedal area of thoracic segments with 1 macro and 4 - 5 micro setae (ps). Each abdominal segments I-VII with 1 micro seta prodorsally, 1 macro and 2 micro setae postdorsally, 1 macro and 1 micro setae dorsopleurolaterally and one micro setae ventropleurolaterally (vpls), on each side. Segment VIII with a very long seta dorsally (ds); 2 setae of unequal length dorsolaterally and 1 micro seta ventropleurolaterally on each side. Segment IX with 4 very long setae and 2 pairs of short setae dorsally (Fig. 14). Anus placed ventrally with 4 anal lobes about almost equal size.

The shape of head suboval (in L_1 slightly oblate dorso-ventrally) (Figs 9, 10). Frontal suture distinct, Y- shaped. The single ocellus (oc) rounded, placed on each side latero-posteriorly, visibly exceed outline of the head. Chaetotaxy of the head: 3 pairs of dorsal epicranial setae 2 ($des_{1, 2, 4}$), a pair of frontal setae (2 fs_2), 3 pairs of lateral setae (2 les_{1-3}) and a pair of ventral epicranial setae 2 (ves). Des_1 located near suture coronalis, des_2 placed on suture frontalis, des_4 near ocellus; fs_2 situated close to epistoma; $les_{2, 3}$ distinctly shorter than les_1. Post epicranium with 3 pairs of short, posterior epicranial setae ($3pes_{1-3}$). Epicranial area with group of 6 pores on each side; frons with 8 pores localized along frontal suture. Antenna slender, conical, exceeding outline of the head; basal membranous area with 4 finger-like setal sensillae and one pore (Fig. 11). The color of head capsule dark brown with black knobbly (Fig. 12). Clypeus (cl) and labrum (lrm) (Fig. 15): clypeus trapezium-shaped 2.9 x as wide as long, with 4 pairs of setal sensillae (cls_{1-4}), distributed in vicinity of posterior margin; labrum about 4.0 x as wide as long; with 6 labral setae ($lrms_{1-3}$) placed mediano-laterally; $lrms_2$ twice longer than other ones; $lrms_1$ and $lrms_3$ short, almost equal of length;

the anterior margin of labrum slightly cut out in middle inside. Epipharynx (Fig. 16): anteromedian setae ($ams_{1, 2}$), 2 pairs, short, blunt, unequal of length; antero-lateral setae ($als_{1, 2}$), 3 pairs curved, finger-like, almost equal of length; median setae ($mes_{1, 2}$), 2 pairs, short, blunt; tormae rods (t) long, slightly convergent posterioly. Mandible (Fig. 17) broad, bifid, with 1 setal sensilla and a pore on the ventral area; teeth of unequal height, truncate. Maxilla (Fig. 18) consists of triangle cardo (cd), stipes (st), mala (ma) and maxillary palp (mp). Stipes and mala fused; stipes with 1 stipal ($stps_1$) and 2 palpiferal ($pfs_{1, 2}$) setae. Mala with 7 curved, unequal of length setae dorso-apically (dms_{1-7}), and 5 different in lengthy and shape setae ventro-apically (vms_{1-5}) (Fig. 19). Dms bigger than vms. Maxillary palp 2-segmented; basal segment slightly longer and distinctly wider than distal segment, length ratio of segments basal and distal - 1 : 1.3. Distal segment with 10 conical, cuticular processes apically and a pore dorsally. Basal segment with a finger-like seta. Praelabium (Fig. 20) (plb) with 3 pairs of tiny, sharp micro setae (code: 1-3) on anterior margin and a pair of long setae medially (code: 4). Anterior margin of plb slightly sinuate to inside. Labial palp (lpa) one-segmented; lpa located on visibly protuberances. Each palps with a pore and 4 conical cuticular processes apically. Premental sclerite well visible, Q-shaped. Postlabium (Fig. 21) (pslb) with 3 pairs unequal of length setae (code: 1-3), localized postero-medially and postero-laterally on each side.

Description of larva of *Bagous robustus*

L_1 (Fig. 22), body length: 1.75 – 1.87 mm; head width: 0.30 – 0.37 mm.

L_3 (Fig. 23), body length: 4.50 – 5.35 mm; head width: 0.62 – 0.67 mm.

Body slender, rounded, in cross section elongated (in L_1 moderately elongated); in L_3 the terminal segments (VIII, IX) slightly curved upwards. Spiracles bicameral, placed medially, except those on VIII segment, localized dorsally. The spiracles of last pair distinctly bigger than other ones (Figs 24-26). The color of all larval instars white. Cuticle very finely knobby (Fig. 27). Prothorax smaller then meso- and metathorax. Prodorsal sclerite light brown. The thoracic segments smaller than the first segment of abdomen. Abdominal segments I-V almost equal of length; segment VI some wider than previously ones, segments VII-IX narrowed gradually; segment X reduced to anal lobes. Segment VIII with 2 protuberances placed dorsolaterally, segment IX with 2 pairs of protuberances dorsally. Chaetotaxy of the body is extremely reduced, setae light yellow, short, except those on VIII and IX abdominal segments. Dorsal parts of prothorax with complex of 4 macro setae (prns) of unequal of length, on each side (Fig. 31). There are also: 1 macro seta dorsolaterally (dls), 1 macro and 1 micro setae dorsopleurolaterally (dpls) and 1 macro seta ventropleurolaterally (vpls) (Fig. 23). Mesothorax with: 1 micro seta prodorsally (prs), 1 macro and 2 micro setae postdorsally (pds), 1 macro and 2 micro setae ventropleurolaterally (vpls), on each side of the body. Chaetotaxy of metathorax similar to those on mesothorax. Each pedal area of thoracic segments with 2 macro and 4-5 micro setae (ps). Each abdominal segments I-VII with 1 micro seta prodorsally (prs), 1 macro and 2 micro setae postdorsally (pds), 1 macro and 1 micro setae dorsopleurolaterally (dpls) and one micro setae ventropleurolaterally

(vpls), on each side of the body. Segment VIII with a very long seta dorsally (ds) placed on protuberances; segment IX with 4 very long setae (ds) placed on protuberances and a pair of short setae dorsomedially (Fig. 32). Anus placed ventrally with 4 anal lobes about almost equal size.

The shape of head of all instars oval (Figs 28, 29). Frontal suture distinct, Y- shaped. The single ocellus (oc) placed on each side latero-posteriorly, don't exceed outline of the head. Chaetotaxy of the head: 4 pairs of dorsal epicranial setae (2 des_{1-4}), a pair of frontal setae (2 fs_2) and 2 pairs of lateral setae (2 $les_{1, 2}$). Des_1 located near suture coronalis, des_3 distinctly shorter than other ones, des_2 placed on suture frontalis, des_4 near to ocellus; fs_2 localized close to epistoma; les_2 distinctly shorter than les_1. Post epicranium with 5 pairs of short, posterior epicranial setae ($5pes_{1-5}$). Epicranial area with group of 4 pores on each side; 8 pores localized on frons. Antenna relatively broad, conical, basal membranous area with 2 very short setal sensillae and 7-8 pores (Fig. 30). Color of head capsule brown. Clypeus (cl) and labrum (lrm) (Fig. 33): clypeus trapezium-shaped 2.8 x as wide as long, with a pair of setal sensillae posterio-laterally, the anterior margin of clypeus sinuate; labrum about 4.3 x as wide as long; with 6 labral setae 2 ($lrms_{1-3}$) placed antero-laterally; $lrms_2$ thrice longer than other ones; $lrms_1$ and $lrms_3$ almost equal of length; the anterior margin of labrum straight. Epipharynx (Fig. 34): anteromedian setae ($ams_{1, 2}$), 2 pairs, short, blunt, unequal of length; antero-lateral setae (als_{1-3}), 3 pairs curved, almost equal of length; median setae ($mes_{1, 2}$), 2 pairs, short, blunt; tormae rods (t) short, kidney-shaped, slightly convergent. Mandible (Fig. 35) broad, bifid, with 2 setal sensillae on the ventral area; teeth of equal height, slightly truncate. Maxilla (Fig. 36) consists of triangle cardo (cd), stipes (st), mala (ma) and maxillary palp (mp). Stipes and mala fused; stipes with 1 stipal (stps) and 2 palpiferal setae ($pfs_{1, 2}$). Mala with 7 different of length and shape setae dorso-apically (dms_{1-7}), and 3 almost similar to each other setae ventro-apically (vms_{1-3}) (Fig. 37). Dms bigger than vms. Maxillary palp 2-segmented; basal segment slightly bigger than distal, length ratio of segments basal and distal: 1 : 1.4. Segment distal with conical, tiny cuticular processes apically. Each segment with a pore. Praelabium (Fig. 38) (plb) with 4 pairs of tiny, sharp micro setae on anterior margin and two pairs of macro setae placed medially on premental sclerite. Anterior margin of plb rounded. Labial palp (lpa) one-segmented; lpa conical, placed on small protuberances. Each palps with a pore and group of conical cuticular processes apically. Premental sclerite well visible, Q-shaped. Postlabium (Fig. 39) (pslb) with 3 pairs of unequal of length setae, localized postero-medially and postero-laterally on each side. There is also a pair of micro setae in vicinity of posterior margin of postlabium.

REMARKS ON THE ECOLOGY AND BIOLOGY

The feeding and copulations of *B. binodulus* were observed on leafs of water soldiers protruding over the water surface. The larvae inhabit only young, small plants; the adults were found also on the older exemplars of *S. aloides* L. Active specimens (adults) occurred from the first decade of May to the end of September. After that time I collected the adults by sifting of plant remains, probably a place of overwintering

for the adults. The different larval instants were collected from the first decade of July to the middle of August. At first the young larval instars hollow out along the carding of the leaves, older ones feed also on the surface of the plant. Up to nine larvae of different instars inhabited one plant specimen at the same time. Consequently, feeding of the larvae causes considerable damage to the plant.

S. aloides belongs to common plants in south-eastern Poland. However it doesn't indicate frequency of distribution of *B. binodulus*. Mentioned weevil was found only in several localities on old river banks (e. g. Bug river valley) and in old pit excavations (Poleski National Park). The species preferred biotopes with full insolations and stable water. Usually 50-70 exx. of *B. binodulus* in a sample, on pit excavation about area ca. 200m^2 were observed.

B. robustus was collected exclusively on common water plantain. The plants exemplars inhabited by larvae showed some deformation. The stems and the fresh, rolled leaves were curved in upper parts. The young larval instars feed in stem of leaf. Only one specimen could be found in the same plant. The different larval instants were collected on plants from the second half of May to the end of June. The pupae were found in the end of June. *B. robustus* prefers biotopes with full insolation and static water. It was observed on wet meadows and banks of old river banks. By using of entomological scoop, one or two specimens of *B. robustus* in one sample were usually collected.

DISCUSSION

The study of Scherf (1964) contains a description of the larva of *B. binodulus*. Unfortunately, its utility is limited due to lack of detailed description of chaetotaxy of the head, without figures. However provided by Scherf (1964) description of the life-cycle and biology of *B. binodulus* was consistent with the field observations obtained during the collection of the study material.

The diagnostic characters of the larva of *B. binodulus* are following: (1) des_3 absent; (2) fs long, placed close to epistoma; (3) antenna relatively long, conical; ocelli exceeded outline of head; (4) mandibles with 1 seta; (5) maxilla with 7 dms; (6) labial palps localized on protuberances; (7) protuberances of segment VIII with long setae; (8) abdominal segment IX with 4 long setae placed on protuberances.

Some similarities were observed between the larvae instars of both *B. binodulus* and *B. alismatis* (Gosik 2008). It was particularly visible in the case of the younger larval instars of *B. binodulus*. Some characters (those have been considered as characteristic for *B. alismatis* larvae) are found in *B. binodulus* larvae as well, namely: black colour of head and pronotal sclerite, structure of ocelli. The flatting of the head and body presents in *B. alismatis* larvae and in young instars of *B. binodulus* larvae is most probably an effect of adaptation and doesn't confirm the close kinship of the both species.

The larvae of the currently described species of the genus *Bagous* have shown very few species-related characteristics (small exception was found in the case of the *B. frivaldszkyi* larvae). On this background the *B. alismatis* larvae differed much, which

puts the inclusion of the *Hydronomus* genus in the *Bagous* genus proposed by Caldara & O'Brien (1994) in question.

The morphology of the *B. robustus* larvae shows rather any specially similarities to *B. lutulentus* larvae. This makes the distinction between the two species possible not only by the differences in habitat and plant preference, but as well as through the differences e. g., in the structure of their mouthparts. Those facts may confirm the present systematic position of the both species. The diagnostic characters of the larva of *Bagous robustus* in comparisons with larva of *B. lutulentus* are the following: (1) fs long placed close to epistoma in *B. robustus vs.* fs very short, placed near of suture frontalis in *B. lutulentus*; (2) antenna short, conical in *B. robustus vs.* antenna long, arrow-like in *B. lutulentus*; (3) clypeus with a pair of cls in *B. robustus vs.* two pairs in *B. lutulentus*; (4) epipharynx with 2 pairs of ams in *B. robustus vs*. a pair in *B. lutulentus*; (5) tormae kidney-shaped, converged in *B. robustus vs.* narrow, parallel in *B. lutulentus*; (6) mandibles with 2 setae in *B. robustus vs*. mandibles without setae in *B. lutulentus*; (7) maxilla with 7 dms in *B. robustus vs*. maxilla with 3 dms in *B. lutulentus*.

In most localities *B. robustus* occurred together with *B. alismatis* but the development of the larvae of both species on the same plant specimen was not observed. The distinction of larval instars of both species is not difficult.

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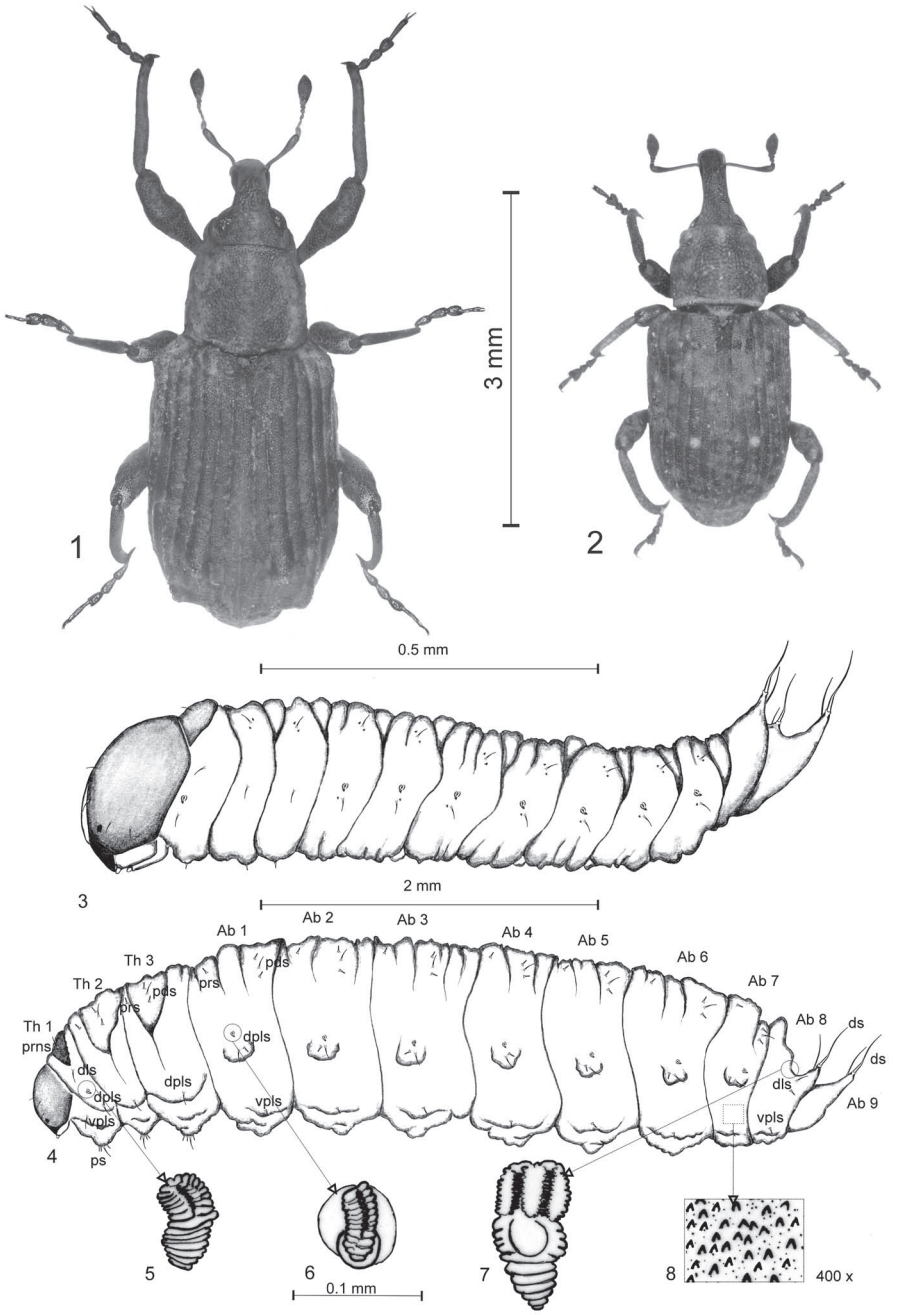
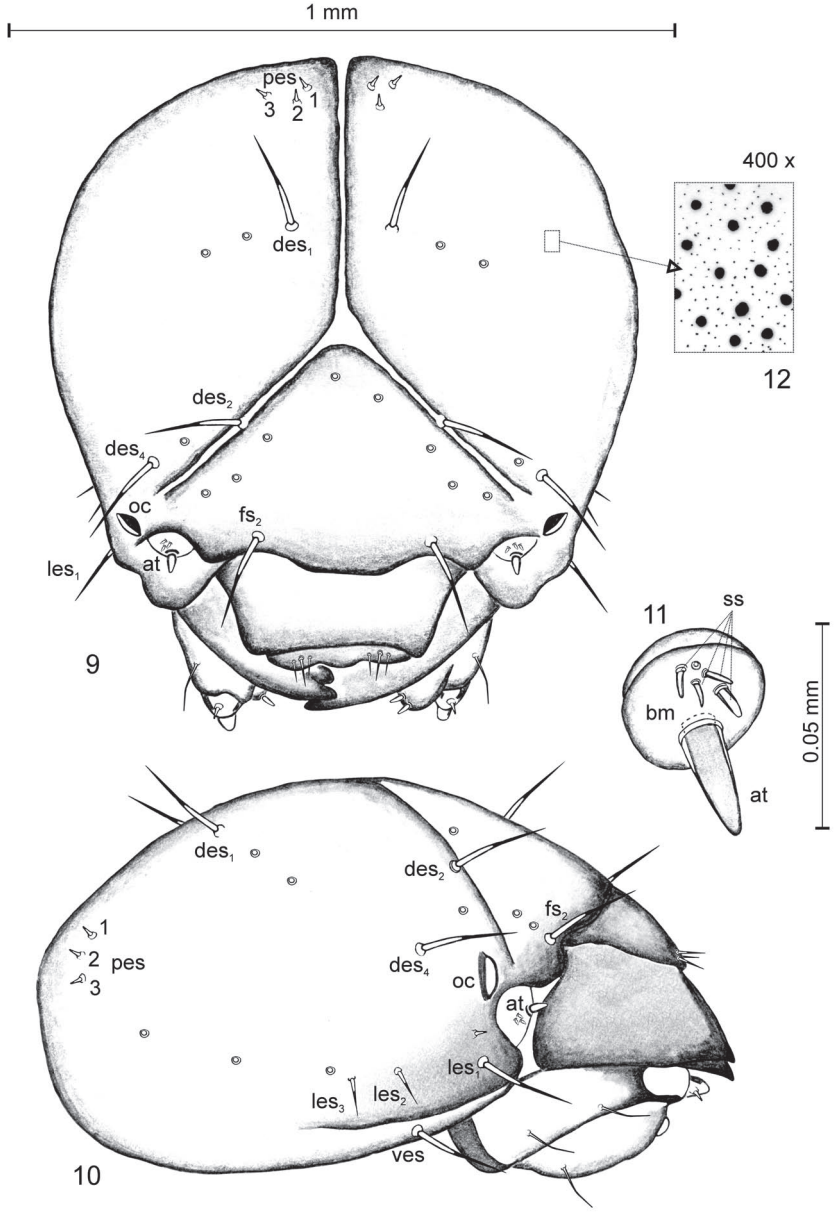
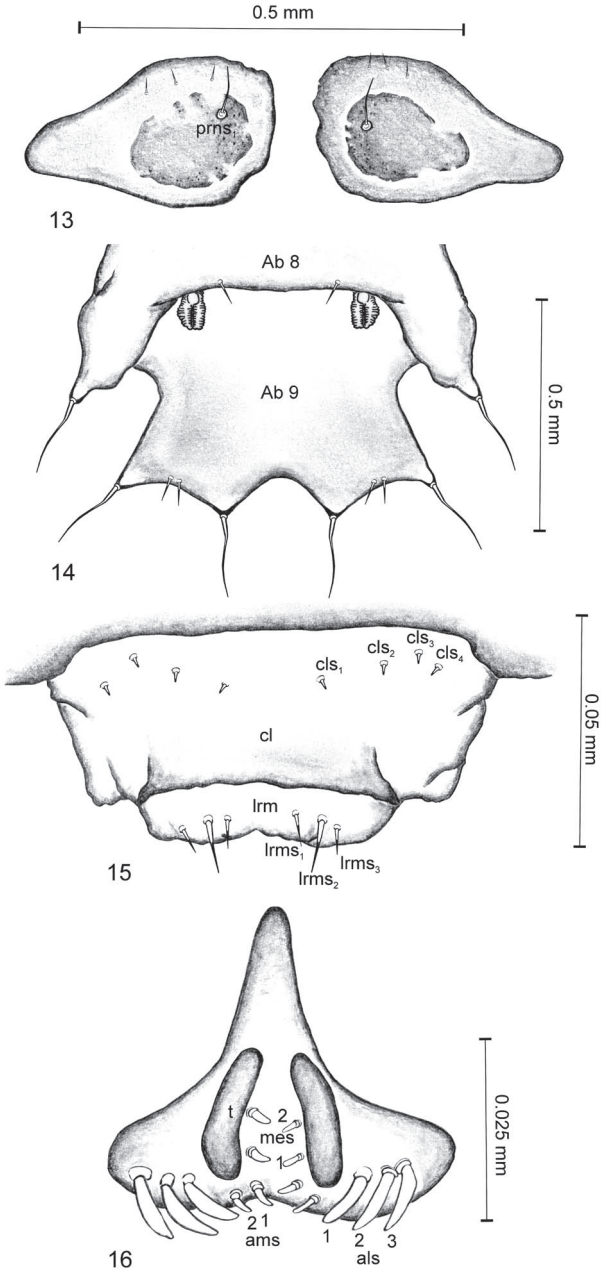


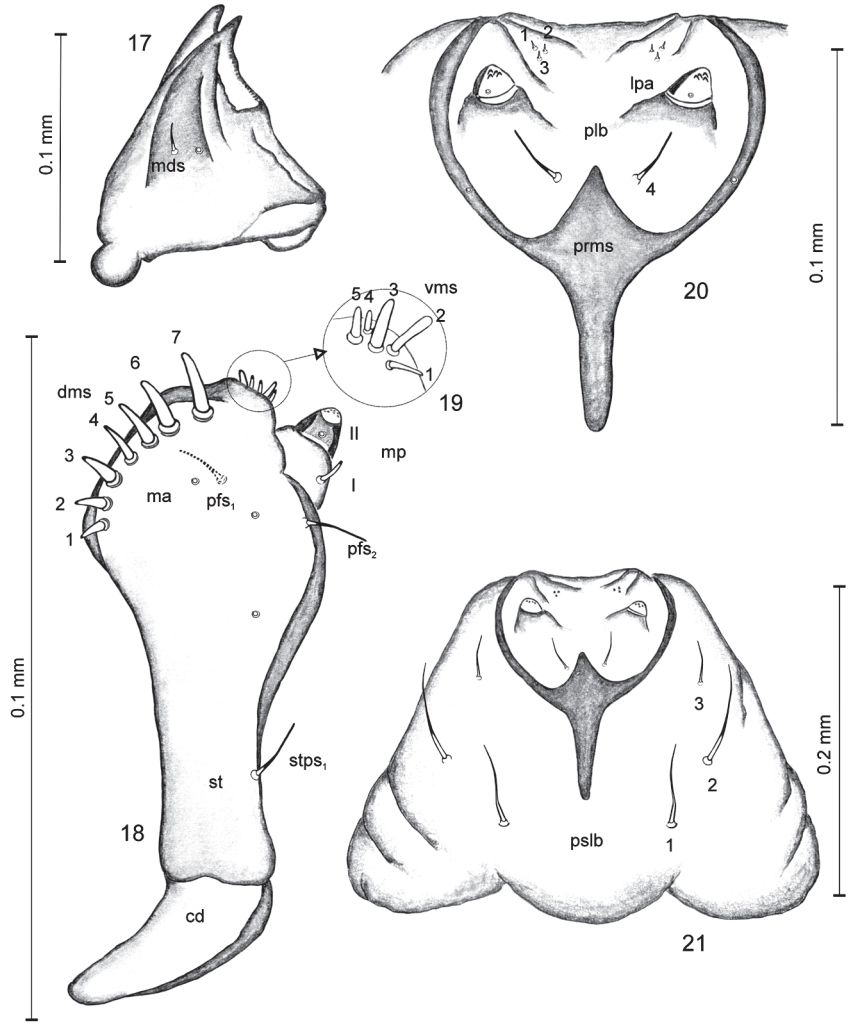
1. *Bagous binodulus* (Herbst); 2. *B. robustus* (H. Brisout), adult; 3-8. *B. binodulus* (Herbst), larva: 3 – young instars; 4 – mature larva (Th1-3, Ab1-9 –number of segments, prns – pronotal setae, dls – dorsolateral s., dpls – dorsopleurolateral s., vpls – ventropleurolateral s., prs – prodorsal s., pds – postdorsal s., ds – dorsal s., ps – pedal s.); 5 – spiracle of prothorax; 6 – spiracle of 1st abdominal seg.; 7 – spiracle of 8th abdominal seg.; 8 – structure of cuticle



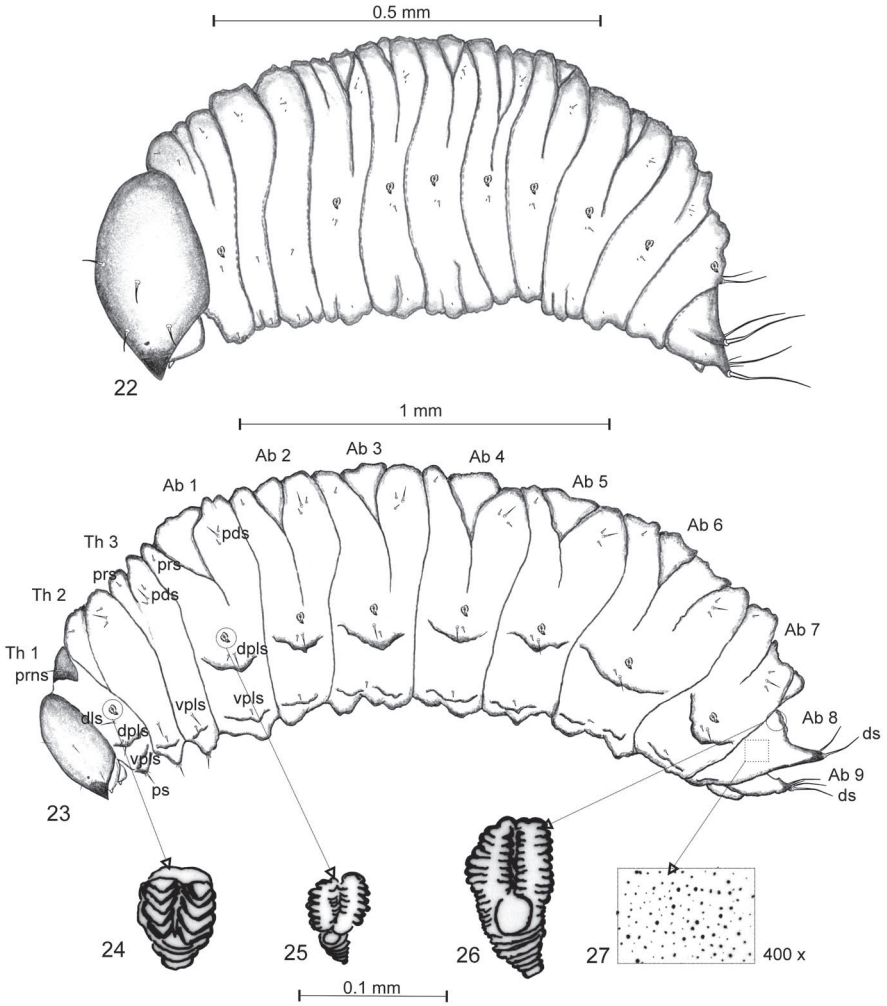
9-12. *B. binodulus* (Herbst), mature larva, head: 9 – dorsal view (des – dorsal epicranial s., fs – frontal s., les – lateral epicranial s., pes – post epicranial s., ves – ventral epicranial s., oc – ocellus, at – antenna); 10 – lateral view; 11 – antenna (ss – setae sensillae, bm – basal membranous); 12 – structure of cuticle



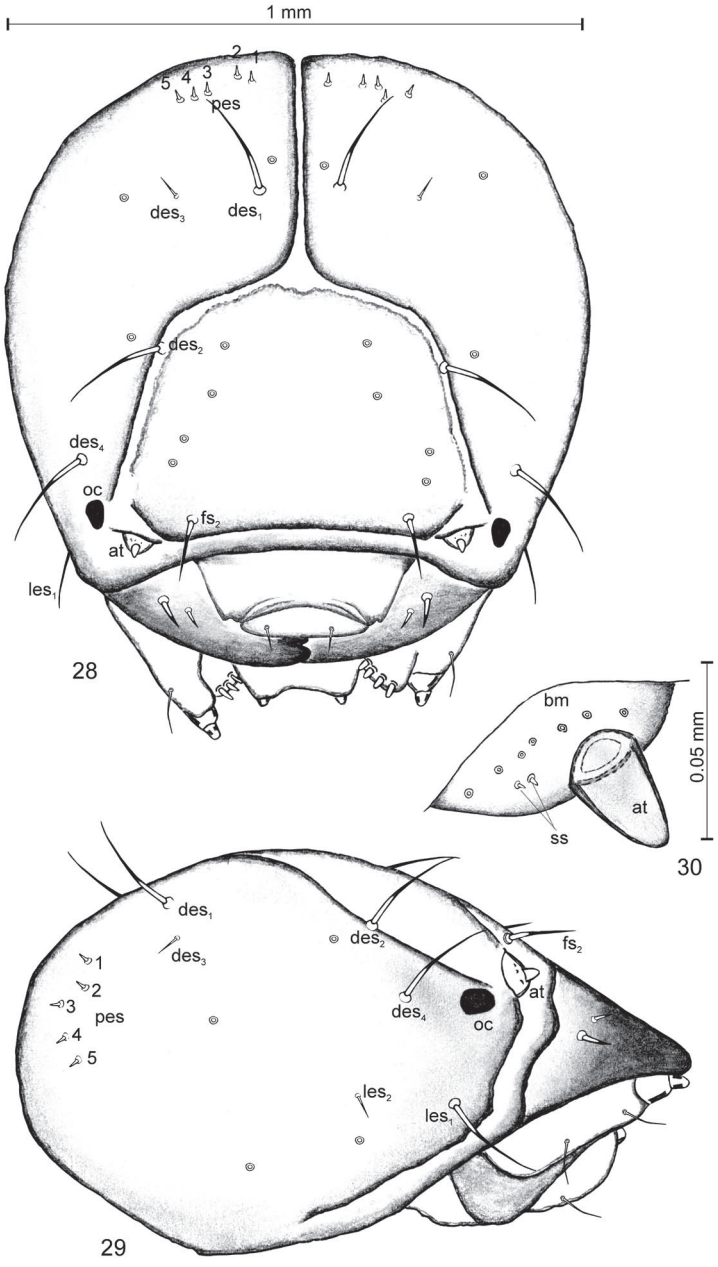
13-16. *B. binodulus* (Herbst), mature larva: 13 – chaetotaxy of pronotum; 14 – terminal segments of abdomen, dorsal view (Ab 8, 9 – number of seg.); 15 – clypeus (cl) and labrum (lrm) (cls – clypeal s., lrms – labral s.); 16 – epipharynx (ams – anteromedial s., als – anteriolateral s., mes – median s., t – torma)



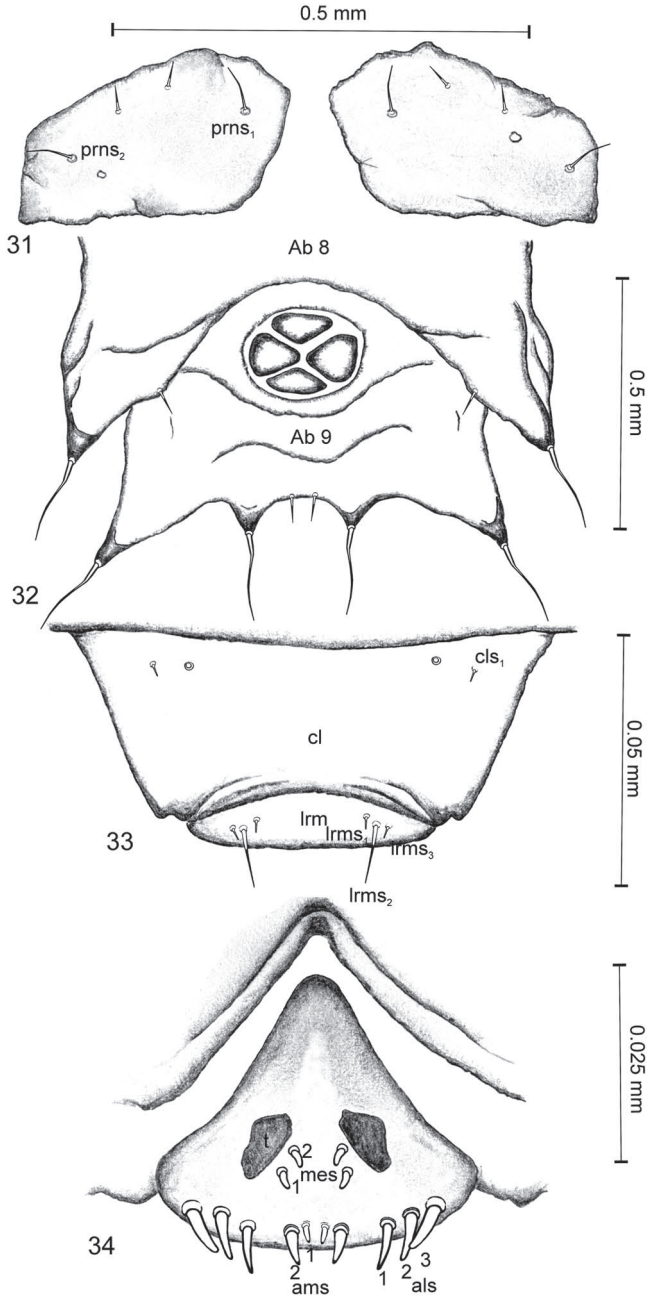
17-21. *B. binodulus* (Herbst), mature larva: 17 – left mandible (mds – mandibles dorsal s.); 18 – right maxilla (dms – dorsally malae s., vms – ventrally malae s., ma – mala, cd – cardo, st – stipes, mp – maxillary palp, pfs – palpiferal s., stps – stipal s.); 19 – shape and distribution of vms; 20 – prelabium (plb) (lpa – labial palp, prms – premental sclerite); 21 – postlabium (pslb)



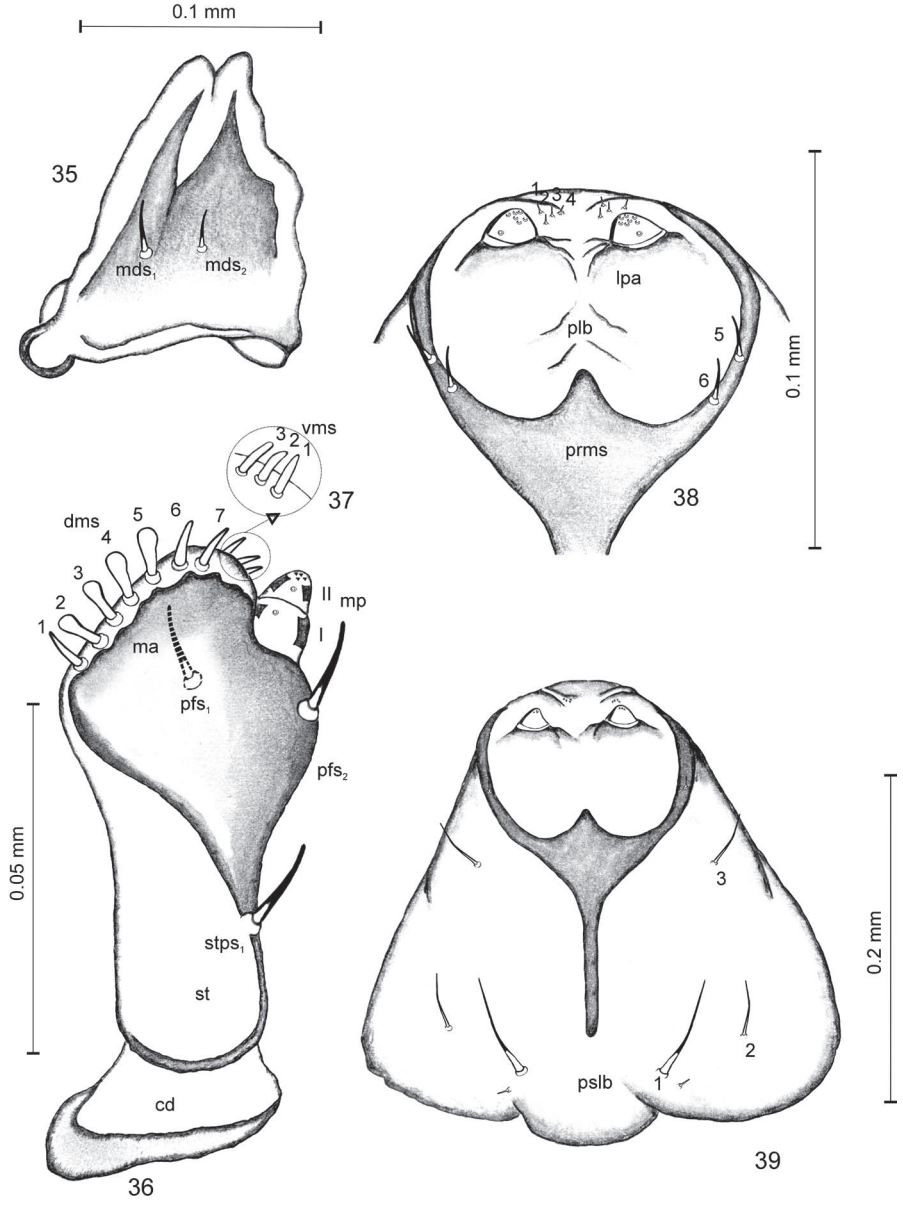
22-27. *B. robustus* (H. Brisout), larva: 22 – young instars; 23 – mature larva (Th1–3, Ab1–9 – number of segments, prns – pronotal setae, dls – dorsolateral s., dpls – dorsopleurolateral s., vpls – ventropleurolateral s., prs – prodorsal s., pds – postdorsal s., ds – dorsal s., ps – pedal s.); 24 – spiracle of prothorax; 25 – spiracle of 1st abdominal seg.; 26 – spiracle of 8th abdominal seg.; 27 – structure of cuticle



28-30. *B. robustus* (H. Brisout), mature larva, head: 28 – dorsal view (des – dorsal epicranial s., fs – frontal s., les – lateral epicranial s., pes – post epicranial s., oc – ocellus, at – antenna); 29 – lateral view; 30 – antenna (ss – setae sensillae, bm – basal membranous)



31-34. *B. robustus* (H. Brisout), mature larva: 31 – chaetotaxy of pronotum; 32 – terminal segments of abdomen, ventral view (Ab 8, 9 – number of seg.); 33 – clypeus (cl) and labrum (lrm) (cls – clypeal s., lrms – labral s.); 34 – epipharynx (ams – anteromedial s., als – anteriolateral s., mes – median s., t – torma)



35-39. *B. robustus* (H. Brisout), mature larva: 35 – left mandible (mds – mandibles dorsal s.); 36 – right maxilla (dms – dorsally malae s., vms – ventrally malae s., ma – mala, cd – cardo, st – stipes, mp – maxillary palp, pfs – palpiferal s., stps – stipal s.); 37 – shape and distribution of vms; 38 – prelabium (plb) (lpa – labial palp, prms – premental sclerite); 39 – postlabium (pslb)