Morphology of the mature larva and pupa of *Rhinusa bipustulata* (Rossi, 1792) (*Coleoptera*: *Curculionidae*) with some remarks on its biology

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The aim of this work is a detailed description of the mature larva and pupa of *Rhinusa bipustulata* (Rossi, 1792). Diagnostic features of immature stages, information about life-cycle and ecology of this species are presented. The differentiated key to larval instars of selected species is also given.

Key words**:** *Coleoptera*, *Curculionoidea, Mecini, Rhinusa bipustulata*, morphology, larva, pupa, life-cycle.

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INTRODUCTION

The current status of the curculionid genus *Rhinusa* Stephens, 1829 is a result of the classification by Caldara (2001). The weevils of the mentioned genus are black color. The medium sized body (2,5-5mm) beetles distinguishing from other *Mecinini* by the following features: the 3rd and the 6th intervals connected each other in posterior parts of each elytra; body covered by long setae (Smreczyński 1976; Freude et al. 1983). Most of them (25 species) are known from the area of Europe, several species occur in the whole Palearctics, some of them are widely distributed in the Holarctic (Alonso-Zarazaga 2009). The majority of the European *Rhinusa* species are associated with the xerothermic communities, pasture or psammophilous grass communities. Only few of them inhabit other habitats, e. g. undergrowths or meadows (Koch 1992; Burakowski et al. 1997). *Rhinusa* species are generally oligophagous weevils associated with plants from the genera: *Antirrhinum* L., *Chaenorrhinum* (DC.) Reichenb., *Kickxia* Dum., *Linaria* L., *Scrophularia* L., *Verbascum* L. Some of them are considered to be rare or extremely endangered e. g. in: Norway (Ødegaard et al. 2008), Germany (Sprick et al. 2003; Bayer & Winkelmann 2005; Schneider 2004) and Poland (Pawłowski et al. 2002).

The knowledge about morphology of immature stages of the *Rhinusa* species is fragmentary. Some records about several species have been presented by van Emden (1938) and Scherf (1964).

Rhinusa bipustulata (Rossi, 1792) is a Palearctic species, which is known in Europe from dispersed localities (apart from Scandinavia and Great Britain) (Alonso-Zarazaga 2009). The species is easily distinguishable from other *Rhinusa* species by presences of red, rounded marks on each of elytras, shape of rostrum and body densely covered by relatively long, protruding setae (Fig. 1). The plants from genus *Scophularia*

L., *Limosella aquatica* L. and *Linaria vulgaris* L. are known as host plants of this species (Koch 1992). In Poland it is recorded at several locations and considered a rare and endangered weevil (Burakowski et al. 1997; Kubisz et al. 1998, Wanat & Mokrzycki 2005). Its complete life-cycle and morphology of immature stages are only fragmentarily known (Scherf 1964; Burakowski et al. 1997).

MATERIAL AND METHODS

60 exemplars of different larval stages (including 43 specimens of mature larvae) and 40 exemplars of pupae.
The insects were collected in: Nałęczów (UTM nets: EB88), Lublin (FB 07), Wola Wereszczyńska near Urszulin (Poleski National Park) (UTM nets: FC50), on July 10th, 17th, 20th, August 01st 2009.

The specimens were collected from seeds capsules of woodland figwort (*Scrophularia nodosa* L.) growing on small water pools bank (Nałęczów), along dewatering ditch (Wola Wereszczyńska) and in gardens (Lublin). In order to correctly determine the development stages of *R. bipustulata*, some larvae were kept alive under laboratory conditions until pupation and then until metamorphosis. The preimaginal stages were preserved in liquid of 75% alcohol. The punctured larvae and pupae were rinsed in distilled water and cleared in 5% solution of potassium hydroxide (KOH) and finally stored in 5% glycerin to prepare microscopic slides. The drawings were made using microscope and camera lucida (MNR-1, 10^X, PZO). The terminology of Scherf (1964) and May (1977, 1993, 1994) was used in the morphological description of larva and pupa. The general recognizing and nomenclature of setae is similar to that presented by Skuhrovec (2004).

DESCRIPTION OF MATURE LARVA

Morphology and chaetotaxy of the body
Body length: 4.00 – 5.00 mm (mean 4.30 mm) elongated, curved, C–shaped, rounded in cross

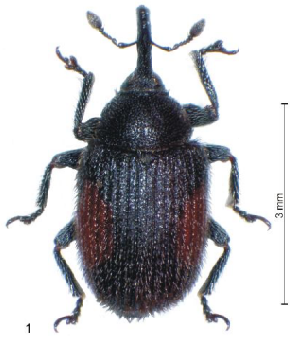
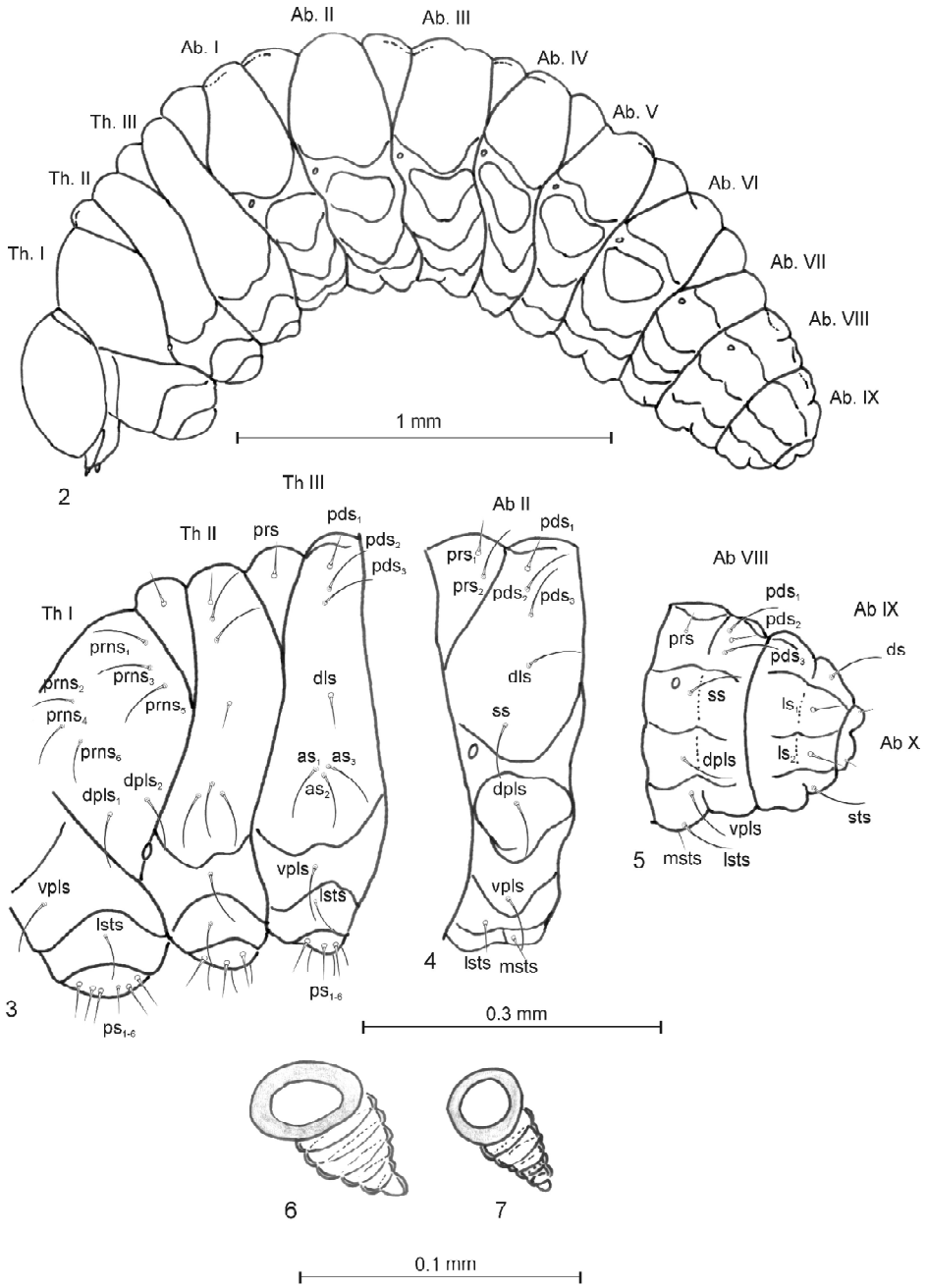


Fig. 1. *Rhinusa bipustulata* (Rossi), adult

section (Fig. 2). The greatest width (on the level of metathorax) up to 1.2 mm. All thoracic segments almost equal in length, slightly smaller than first segment of abdomen. Abdominal segments I-IV almost equal length, bigger than abd. segments V-VI; next ones decreasing gradually to the terminal parts of the body, abd. segment X reduced to four anal lobes about unequal size. Anus localized terminally.

Chaetotaxy of the body rather reduced. Setae medium long, light yellow. **Thorax.** Prothorax (Fig. 3) with 6 prodorsal setae (*prns*); 2 dorsopleural setae (*dpls*), 1 ventropleural seta (*vpls*) and 1 mediosternal seta (*msts*) on each side. Mesothorax with 1 short prodorsal seta (*prs*), 3 different in length postdorsal setae (*pds*), 1 dorslateral seta (*dls*), 3 equal length setae on alar area (*as*), 1 ventropleural seta (*vpls*) and 1 laterosternal seta (*lsts*) on each side. Each pedal area of thoracic segments well isolated, with 6 long setae (*ps*). There are also a pair of short setae between pedal lobes. Chaetotaxy of metathorax similar to that on mesothorax. **Abdomen.** Abdominal segments I-VII with 2 prodorsal seta (*prs*), 3 postdorsal setae (*pds*), 1



Figs 2-7. *Rhinusa bipustulata* (Rossi), mature larva: 2 - habitus, 3 - lateral view of thoracic segments, 4 – lateral view of second abdominal segment, 5 – lateral view of abdominal segments VIII-X (prns – pronotal setae, dpls – dorsopleural s., vpls – ventropleural s., prs – prodorsal s., pds – postdorsal s., dls – dorsolateral s., as – alar s., ss – spiracular s., lsts – laterosternal s., msts – mesosternal s., sts – sternal s., ps – pleural s., ds – dorsal s.), 6 – spiracle of thorax, 7 – spiracle of second abdominal segment

dorsolateral seta (*dls*), 1 seta on spiracular area (*ss*), 1 dorsopleural seta (*dpls*), 1 ventropleural seta (*vpls*), 1 laterosternal seta (*lsts*) and 1 short mediosternal seta (*msts*) on each side (Fig. 4). Abd. segment VIII with 1 prodorsal seta (*prs*), 3 equal length postdorsal setae (*pds*), 1 seta on spiracular area (*ss*), 1 dorsopleural seta (*dpls*), 1 ventropleural seta (*vpls*), 1 laterosternal seta (*lsts*) and 1 short mediosternal seta (*msts*) on each side. Abd. segment IX with 1 dorsal seta (*ds*), 2 equal length lateral setae (*ls*) and 1 sternal seta (*sts*) (Fig. 5). Each of anal lobes with 1 very short setae.

Spiracles (9 pairs) (Figs 6, 7) unicameral, first one placed between pro- and mesothorax, next ones placed laterally, close to anterior margins of abd. segments I-VIII. The color of the all thoracic and abdominal segments white. Cuticle minutely spiculate.

Head capsule and mouth parts

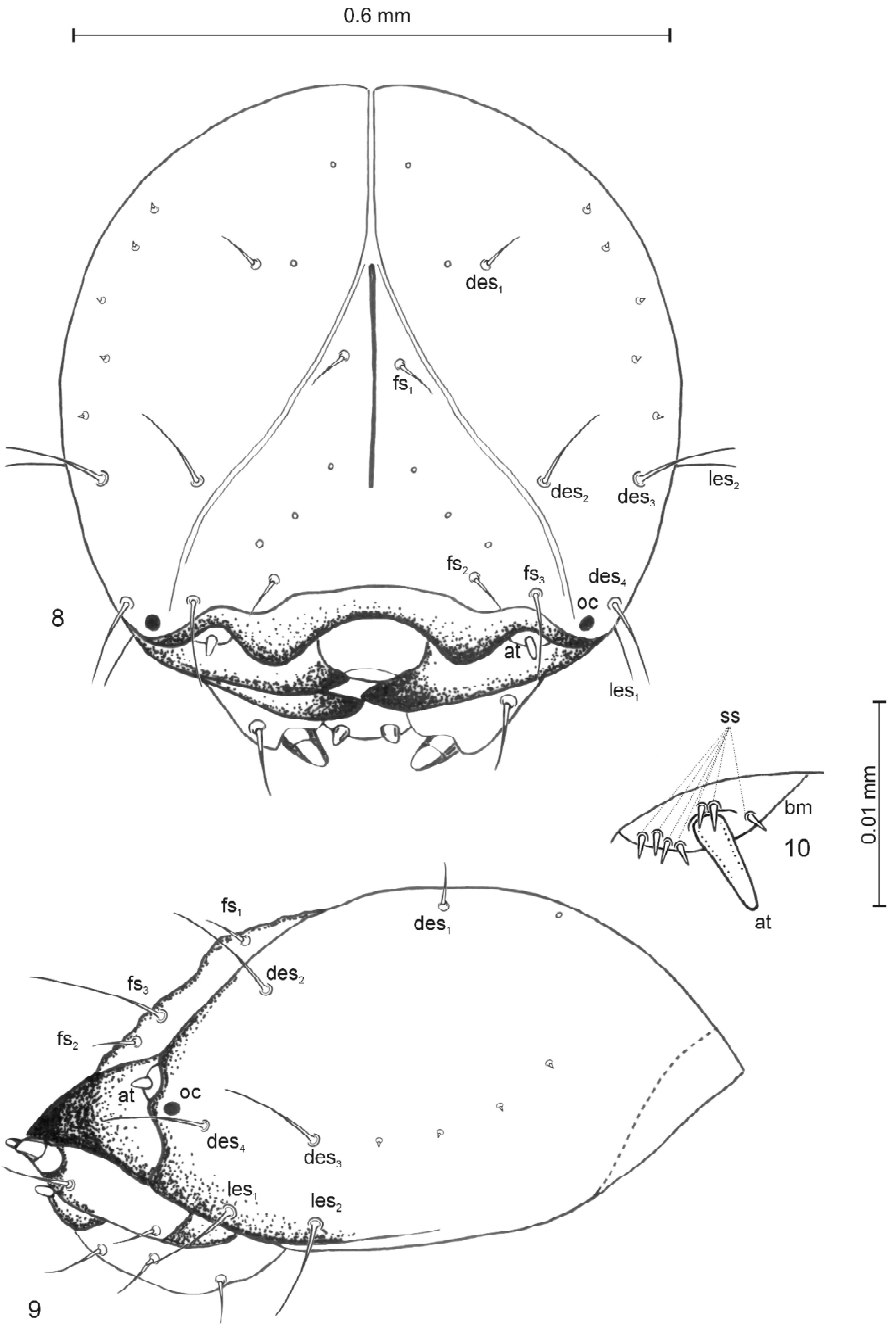
Head (Figs 8, 9) width: 0.55 - 0.65 mm (mean 0.60 mm), oval, frontal suture poorly visible, Y-shaped, extended almost to occelli, endocarinal line presents, reaches middle of the frons. Dorsal epicranial setae des_1 located almost in central part of epicranium, des_2 close to frontal suture, des_3 and des_4 placed antero-laterally; des_1 visibly shorter than other macro setae of epicranium. Frontal seta fs_1 placed antero-medially, fs_2 and fs_3 localized postero-laterally; fs_1 and fs_2 equal length, 3 times shorter than fs_3. Lateral epicranial setae $les_{1, 2}$ equal length, as long as des_2. Epicranial area with group of 2 pores: one pore in vicinity of des_1, next close to suture coronalis. There are a group of 5 setae sensillae, distributed in line, laterally, on each side of epicranium. Single ocelli (*oc*), poor visible, placed on each side antero-laterally. Antenna (Fig. 10) one-segmented, triangular, long, circular in cross-section, placed in the end of suture frontalis; basal membranous area with group of 7 very short, sharp, sensillae appendage.

Labrum (Fig. 11, left side) about 2.7 times as wide as long, with 3 pairs of long, sharp, labial setae ($lrms_{1-3}$) about different length; $lrms_1$ 2 times longer than $lrms_2$ and $lrms_3$; anterior margin of labrum double sinuate. Clypeus 3.7 times as wide as long with 3 pairs of short, unequal length setae (cls_{1-3}), localized posterior-laterally; cls_3 2 times longer than cls_2 and cls_1; anterior margin of clypeus rounded to inside. Epipharynx (Fig. 11, right side) with 3 pairs of blunt, finger-like, different length antero-lateral setae (als_{1-3}); antero-medial setae ($ams_{1, 2}$) 2 pairs, bacilliform; a pair of median seta (*mes*) very short, bacilliform, torma (*t*) easily to observed, kidney-shaped. Mandible (Fig. 12) broad, bifid, teeth of unequal height, mandibla dorsal setae ($mds_{1, 2}$) almost equal length. Maxilla (Fig. 13): stipes and mala fused, stipes with 1 stipal (*stps*) and 3 palpiferal (pfs_{1-3}) setae, different length, pfs_3 distinctly longer than other ones. Mala with 6, bacilliform, dorsal setae (dms_{1-6}), different in length; ventral mala setae (vms_{1-3}) short, almost equal length; *vms* distinctly shorter then *dms*. Maxillary palpi two-segmented; segment basal with 1 short, maxillarypalpi seta (*mpxs*); length ratio of segments basal and distal almost: 1:1; distal segment with group of 4, long conical, cuticular apical processes; each of segments with a dorsal pore. Praelabium (Fig. 14) heart-shaped, with a pair of prealabial setae (*plbs*); margin of ligula slightly sinuated. 2 pairs of ligular setae ($ligs_{1, 2}$) sharp, short; premental sclerite poorly visible. Labial palps one-segmented; each of distal segments with 5 very short, conical cuticular apical processes; each of segments with a dorsal pore. Postlabium (*pslb*) with 3 pairs, different in length macro setae ($pslbs_{1-3}$), localized medially.

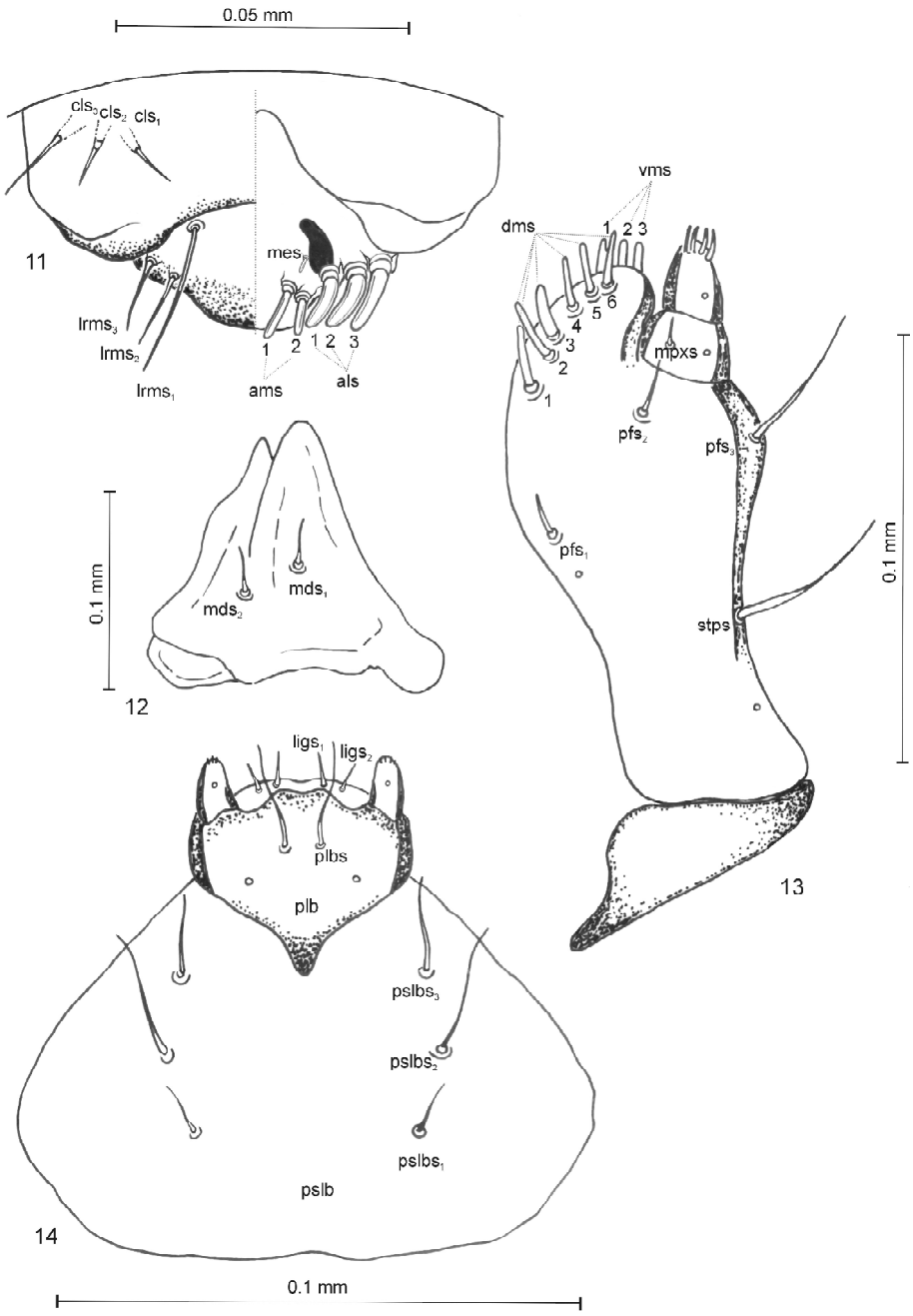
DESCRIPTION OF PUPA

Morphology. Body (Figs 15 - 17) length:
male: 2.90 - 3.40 mm, width in widest place (between end of mesofemurs): 1.50 - 1.70 mm.
female: 5.00 - 4.20 mm, width in widest place (between end of mesofemurs): 2.10 - 2.60 mm.

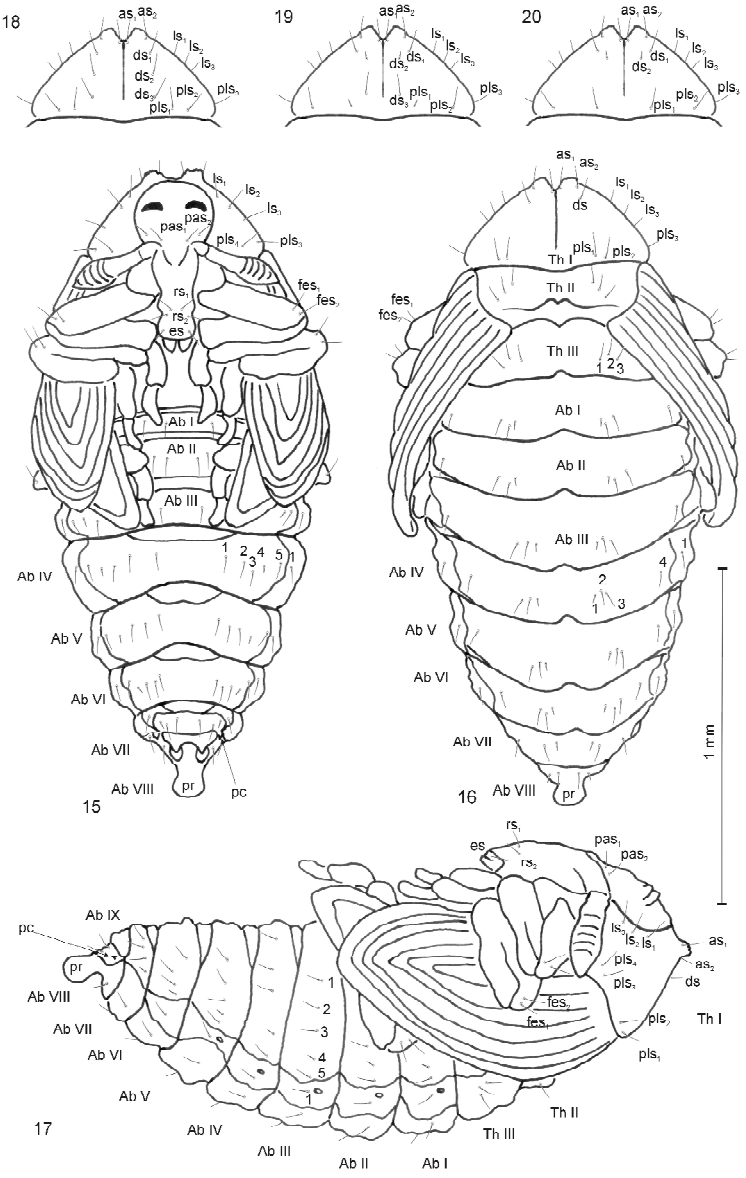
Body relatively stout, white or yellowish. The cuticle smooth. Rostrum medium long, about 3 times as long as wide, reached almost up to mesocoxae. Antennae relatively long and slender. Pronotum 1.6 times as wide as long. Mesonotum and metanotum almost of equal length. Each apex



Figs 8-10. *Rhinusa bipustulata* (Rossi), mature larva, head and antenna: 8 – dorsal view, 9 – lateral view (des - dorsal epicranial setae, fs - frontal s., les - lateral epicranial s., oc – ocellus, at - antenna), 10 – antenna (at – antenna, ss – setal sensillae, bm - basal membranous)



Figs 11-14. *Rhinusa bipustulata* (Rossi), mature larva, mount parts: 11 – epipharynx (right side), labrum and clypeus (left side) (cls – clypeal s., lrms – labral setae, ams - anteromedial s., als - anteriolateral s., mes – median s., t - tormae), 12 - right mandible (mds – mandible dorsal s.), 13 - right maxilla, dorsal view (dms - dorsally malae setae, vms - ventrally malae s., pfs – palpiferal s., stps – stipal s.), 14- praelabium and postlabium, ventral aspect (plb – praelabium, pslb – postlabium, plbs – prelabial s., pslbs – postlabial s.).



Figs 15-20. *Rhinusa bipustulata* (Rossi), pupa, habitus: 15 - ventral view, 16 – dorsal view, 17 – lateral view (pc – pseudocerci, as - apical s., ls - lateral s., ds - discal s., pls - posterolateral s., pas – postantennal s., rs – rostral s., es – epistomal s.), 18 – 20 differences in chaetotaxy of pronotum.

of femora with groups of 2 setae ($fes_{1,2}$). Abdomen includes nine segments. Segments I-IV of equal length; next segments diminish gradually to the end of body. Segment VIII almost semicircle, with rounded protuberances (pr); IX reduced, distinctly smaller than other segments. Spiracles on abdominal segments placed laterally; on abdominal segments I-V functional, on segment VI atrophic on next ones invisible.

Chaetotaxy visible, setae medium long, unequal length, on prothorax placed on small protuberances. Head includes 2 pairs of post antennal setae ($pas_{1,2}$). Rostrum with 2 pairs of rostral setae ($rs_{1,2}$), placed medially and a pair of epistomal setae (*es*). Setae on head and rostrum straight, shorter than those on prothorax (Fig. 15). Pronotum with: 2 pairs of apical setae ($as_{1,2}$) placed beside a conical protuberances; 3 pairs of lateral setae (ls_{1-3}); a pair of discal setae (ds_1) and 4 pairs of posterolateral setae (pls_{1-4}). Dorsal parts of mesothorax with 3 pairs setae placed medially. Chaetotaxy of metathorax similar to this on previously one. Dorsal parts of each abdominal segments I-VII with 3 pairs of unequal length setae placed postero-medially and next one postero-laterally. Each of abdominal segments I-VII with a seta laterally and 5 pairs of short setae ventrally, distributed in regular lines. Abdominal segment VIII with 2 pairs of micro setae ventrally and 2 pairs short, thin setae dorsally. Abdominal segment IX with 3 pairs of micro-setae ventrally. Pseudocerci very short, triangular, poorly visibly (Figs 16, 17).

Sexual dimorphism well-marked: gonotheca in female double, in male single. There are also some differences in chaetotaxy of pronotum, presented on a sparse specimens (Figs 18-20). However, in cause of 34 exemplars (85% of all examined specimens) the chaetotaxy of pronotum were as those visible on Fig. 16.

CONCLUSIONS

The features of the larva of the genus *Rhinusa* [compiled on the basis of descriptions of: *R. antirrhini*, *R. linariae* and *R. collina* (van Emden 1938; Scherf 1964) and R. *bipustulata*] are: (1) slender body; (2) greatly reduced setal numbers; (3) one –cameral spiracles; (4) thoracic spiracles placed between pro- and mesothorax; pigmented head; (5) distinct frontal suture, endocarinal line present; (6) antennae one-segmented, conical, elongated, setae sensillae present; (7) ocelli present; (8) clypeus with 3 *cls*; (9) labrum on anterior margin with 5 pairs of setae; (10) bifid mandibles; (11) maxillary palps two segmented; (12) labial palp one-segmented; (13) ligula concave; (14) praemental sclerite present; (15) terminal anus.

Diagnostic features of the mature larva of *R. bipustulata*: (1) body: 4.00-5.00 mm; (2) prothorax with 6 *prns*; (3) head (width: 0.55 - 0.65 mm) subglobose, light yellow; (4) head with: 8 *des* (des_1 2 times shorter than other ones, 6 *fs* (fs_3 3 times longer than other ones) and 2 *les*; (5) basal membranous area with 7 setal sensilles; (6) posterior margin of clypeus double sinuate, labrum with 3 pairs of *lrms*; (7) epipharynx with: 2 *mes*, 6 *als* and 4 *ams*; (8) kidney shaped tormae; (9) molar edge of mandible with conical, blunt tooth; (10) mandible with 2 setae; (11) maxilla with: 1 *stps*, 3 *pfs*; (12) mala with 6 *dms* and 3 *vms*; (13) each of maxillary palps with a seta; (14) labial palps one segmented; (15) pentagonal praelabium; (16) premental sclerite poorly developed.

Mature larvae of only *Rhinusa antirrhini*, *R. linariae*, *R. collina* and *R. bipustulata* have been already described (van Emden 1938; Scherf 1964; presented paper).

The descriptions given by Scherf (1964) are hardly illustrated and, in cause of *R. collina,* rather fragmentary. Moreover the larvae of *R. collina* and *R. linariae* are, in opinion of Scherf (1964), indiscernible. It stays in opposition to the information, concerning those larvae, as presented by van Emden (1938).

Based on descriptions of larvae of: *R. collina*, *R. linariae* (van Emden 1938), *R. antirrhini* (Scherf 1964) and *R. bipustulata*, mentioned species can be differentiated by the following key:

1(2) head dark brown or black.....*R. antirrhini*
2(1) head light brown to yellowish.

3(4) clypeus and labrum fused, tormae rods absent.....*R. linariae*
4(3) labrum free, tormae rods well developed.
5(6) labrum almost 2 times longer than clypeus, elongated tormae rods.....*R. collina*
6 (5) labrum and clypeus of almost equal length, kidney-shaped tormae rods.....*R. bipustulata*

The features of the pupa of the genus *Rhinusa* [compiled on basis of descriptions of: *R. antirrhini*, *R. linariae* and *R. collina* (Scherf 1964) and R. *bipustulata*]: (1) head with: a pair of longitudinal, horizontal protuberances, without setae; (2) rostrum with setae; (3) apical margin of pronotum with a pair of conical protuberances; (4) segment VIII with rounded cuticula process dorsally; (5) very short, triangular pseudocerci.
Diagnostic features of the pupa of *R. bipustulata*: (1) relatively stocky body (length: 2.90 - 4.20 mm); (2) head with: a pair of longitudinal, horizontal protuberances, without setae; (3) rostrum with 4 *pas* 4 *rs* and 2 *es*; (4) relatively long antennae; (5) apical margin of pronotum with a pair of conical protuberances; (6) pronotum with 4 *as*, 6 *ls*, 2 *ds* and 8 *pls*; (7) mesonotum with 6 setae; (8) matanotum with 6 setae; (9) each of abdominal segments I – VIII with 8 setae dorsally, 10 dorsally and 1 laterally (on each side); (10) segment VIII with rounded cuticula process dorsally; (11) each femora with 2 setae; (12) very short, poorly developed pseudocerci; (13) spiracles on abdominal segments I-V placed laterally.

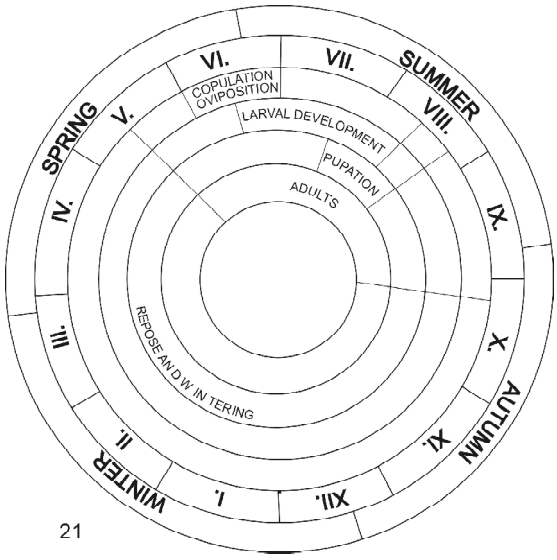


Fig. 21. Life cycle of *Rhinusa bipustulata* (Rossi), under field conditions (I.-XII. – months)

The aberrances in chaetotaxy of pronotum (presented on some specimens of *R. bipustulata*) were described also in cause of *R. collina* (Scherf 1964).

Remarks on ecology and biology

The first active specimens of *R. bipustulata* were observed in the field as early as in the middle of May, copulations and oviposition took place all June long. Also the first larval instars were found in middle of June (Fig. 21). The first mature larvae were observed as early as in first decade of July, first pupae in third decade of it. The adults of a new generation appeared from the beginning till the end of August. The information on development and life-cycle of *R. bipustulata* given by Burakowski (1997) correspond with my observations. *Scrophularia nodosa* L. is the most preferred plant species for mentioned weevil. The developmental stages were found on 50-100% of plant exemplars growing at the investigated stations; in almost 70% of capsules on a single plant. According to Burakowski (1997) a female laid many eggs to a single capsule. I found consequently: (1) up to 4 larvae of first instars developing simultaneity in a single capsule; (2) up to 2 mature larvae in a single capsule; (3) finally only a 1-2 pupa in one capsule. Immature stages infected by parasitoids (indeterminate *Hymenoptera*) were observed in several cases.

According to Koch (1992) *R. bipustulata* is a stenotopic, xerothermophilous species. I can not confirm this classification. Many of mentioned weevils were found along drainage channels, on banks of ponds, old river banks and in gardens. In my opinion it may be regarded a eurytopic species.

The numerous specimens of *R. bipustulata* were easy to observe only in June, after that

only single exemplars were found in sample. Mentioned beetles can fly very well as opposed to many other weevils species. Probably it can be the reason for only few specimens being found in the sample.

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