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Description of first instar larvae of *Cassida denticollis* SUFFRIAN, 1844, *C. prasina* ILLIGER, 1798, *C. sanguinolenta* MÜLLER, 1776, and *C. sanguinosa* SUFFRIAN, 1844 (Coleoptera: Chrysomelidae: Cassidinae)

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ABSTRACT. First instar larvae of *Cassida denticollis* SUFFRIAN, 1844, *C. prasina* ILLIGER, 1798, *C. sanguinolenta* MÜLLER, 1776, and *C. sanguinosa* SUFFRIAN, 1844 are described and illustrated. They belong to a group of closely related species, difficult to identify in the adult stage and often feeding on the same host plant of the genera *Achillea* L. or *Tanacetum* L. Results showed that morphology of first instars within a uniform species group is distinct and offers diagnostic characters.

Key words: entomology, morphology, first instar larva, Coleoptera, Chrysomelidae, Cassidinae, Cassida, Europe.

INTRODUCTION

This paper is the fourth of a series concerning descriptions of first instar larvae of the genus *Cassida* LINNAEUS, 1758. A model description of the first instar larva of the genus *Cassida* L. was proposed for *Cassida nebulosa* L. (BOROWIEC and ŚWIĘTOJAŃSKA 2002). In next papers first instar larvae were described for the following species: *Cassida stigmatica* SUFFR. and *C. rubiginosa* MÜLL. (ŚWIĘTOJAŃSKA 2004), and *C. nobilis* L. and *C. vittata* VILL. (ŚWIĘTOJAŃSKA 2005). These papers showed morphology of first instars of species belonging to two distinct subgenera: *Cassida* s. str. and *Cassidulella* STRAND, 1928, and three distinct species groups within the nominotypical subgenus.

In the present paper morphology of first instar larvae of four very closely related species is presented: *Cassida denticollis* SUFFRIAN, 1844, *C. prasina* ILLIGER, 1798, *C. sanguinolenta* MÜLLER, 1776, and *C. sanguinosa* SUFFRIAN, 1844**.** In the adult stage all these species are very similar morphologically and difficult to identify (for colour photos of all the species see BOROWIEC and ŚWIĘTOJAŃSKA 2002). They have similar spectrum of host plants, mostly Asteraceae members of the genera *Achillea* L. and *Tanacetum* L. Although some ecological differences in selection of microhabitats by particular species have been observed, in the field often on the same plant feed commonly two or three species of the group. Last instar larvae of all species of the group are also very similar but offer some diagnostic characters (BROVDII 1983, BORDY 2000). I wanted to verify if among such very closely related species first instars also have diagnostic characters or not.

MATERIAL AND METHODS

Description of first instars based on model description of first instars of Cassidini proposed by BOROWIEC and ŚWIĘTOJAŃSKA (2002). Further papers (ŚWIĘTOJAŃSKA 2004, 2005) showed that general morphology of first instar is quite constant thus in this paper I described common general morphology for all the species and distinguished diagnostic characters within particular species.

Adults of *Cassida denticollis*, *C. prasina* and *C. sanguinolenta* were collected at rural sites in Wrocław (Lower Silesia, Poland) on 5 V 2003, of *C. sanguinosa* at Kotowice near Wrocław on 1 V 2003 and placed on Petri scales. Leaves of the same *Alchemilla* sp. were offered as a food plant for adults and larvae of *Cassida denticollis*, *C. prasina* and *C. sanguinosa*. Adults and larvae of *C. sanguinolenta* were fed leaves of *Tanacetum* sp.. Hatching larvae were preserved in 75 to 80 % ethanol.

Larvae were mounted on slides with Swan's liquid (aqua distillata 20 g., gum arabic 15 g., chlorhydrate 60 g., glucose 3 g., acetic acid glacial 2 g.) and then were figured using Nikon SMZ1500 stereomicroscope, and Nikon ECLIPSE 80i microscope with phase contrast.

DIAGNOSES

The larva of *Cassida denticollis* in general body shape is similar to larvae of *C. rubiginosa* and *C. saguinolenta*. Their supra-anal processes are as long as body length; lateral processes relatively long (as long as or slightly longer than half of body width); the top of scoli 1-13 armed apically with elongate truncate setae at the apex covered with scales, setae distinctly shortened posteriorly to prominent cauliflower-shaped sensilla; the same type of "ventral organ" (usually with 8 setae in the middle of 1st, 2d and 3rd abdominal sternite). Labrum of *C. denticollis* and *C. sanguinolenta* looks similar (emargination of anterior margin with 6 short, thin setae and anterior margin on each side dorsally with 1 short, thin seta, and

ventrally with 1 short, thin and 3 long, stout setae) but differs from labrum of *C. rubiginosa* (emargination of anterior margin with 6 long, thin setae and anterior margin on each side dorsally with 1 long, thin seta, and ventrally with 1 long, thin and 3 long, stout setae). On average *C. denticollis* (length .95-1.55 mm) is slightly longer and wider than *C. sanguinolenta* (length 0.85-1.37 mm) and both of them are distinctly larger than *C. rubiginosa* (length 0.7-0.9 mm). Tops of 15th and 16th lateral processes of *C. denticollis* are bulb like without any setae, of *C. rubiginosa* unisetose whereas of C. *sanguinolenta* with elongate truncate seta covered with scales.

In *Cassida prasina* ratio of lateral processes length to the body width is similar as in *C. sanguinosa*, *C. nobilis* and *C. vittata* (lateral processes as long as or slightly shorter than half of body width). All of those species possess lateral scoli 1-14 armed apically with elongate truncate or clubate setae which are distinctly shortened posteriorly to prominent cauliflower-shaped sensilla. In body length *C. prasina* is the most similar to *C. vittata* (0.76-1.40 mm, 0.8-1.4 mm respectively) but differs in apical setae of thoracic lateral processes (elongate truncate, clubate respectively) and tops of 15th and 16th lateral processes (stout cone like sensilla, top with one simple seta respectively). They also have different types of labrum: in *C. prasina* emargination of anterior margin of labrum has 6 long, thin setae and anterior margin on each side dorsally 1 short, thin seta, and ventrally 1 short, thin and 3 long, stout setae whereas in *C. vittata* emargination of anterior margin has 6 long, stout setae and anterior margin on each side dorsally 1 short, thin seta, and ventrally 1 long, thin and 2 long, stout setae. *C. nobilis* (length 0.7-1.2 mm) is smaller than *C. prasina*, with tops of 15th and 16th lateral processes with one simple seta like in *C. vittata* and its labrum differs from labrum of *C. prasina* (emargination of anterior margin with 6 long, stout setae and anterior margin on each side dorsally with 1 long, thin seta, and ventrally with 1 long, thin and 2 long, stout setae). *C. sanguinosa* (0.82-.95 mm) is smaller than *C. prasina*, with thoracic lateral processes armed apically with clubate setae (in *C. prasina* elongate truncate) those setae are distinctly longer than in *C. prasina*, the tops of 15th and 16th lateral processes are with one simple seta (in *C. prasina* stout cone like sensilla) and its labrum is different from that of *C. prasina* (emargination of anterior margin with 6 long, stout setae and anterior margin on each side dorsally with 1 long, thin seta, and ventrally with 1 long, thin and 3 long, stout setae).

GENERAL DESCRIPTION OF FIRST INSTAR OF *C. DENTICOLLIS*, *C. PRASINA*, *C. SANGUINOLENTA*, AND *C. SANGUINOSA*

Body flattened dorso-ventrally, oval, moderately narrowed posteriorly, widest across meso- and metanotum (figs 33-40).

Body with 16 pairs of lateral scoli and a single pair of long supra-anal processes. Scoli shortened posteriorly from 1st to 13th. Scoli 14th distinctly longer than 13th except *C. sanguinosa* where scoli 14th as long as 13th or only slightly

longer. Scoli 15th and 16th the longest but 16th slightly longer than 15th. First and second lateral scoli placed very close and directed anteriorly (figs 1-4). First lateral scoli with one long and one shorter lateral branch, other scoli simple, without lateral branches but with a few cauliflower-shaped sensilla. Lateral scoli from first to 14th and lateral branches of first scoli armed apically with elongate sensilla widened at the top (figs 1-4, 8, 9, 13, 14, 18, 19, 23, 24). Sensilla at the top of 1st to 13th lateral scoli gradually shortened posteriorly. Supra-anal processes sinuate, bent dorsally, apically flask-shaped without apical setae (figs 5, 10, 15, 20).

Spiracles 9, elevated, annular but biforous at the top (figs 25-32). Close to pronotal spiracle one minute seta is placed, close to each abdominal spiracle two very short setae and one small cauliflower-shaped sensillum are placed.

Dorsal and ventral sides of the body distinctly granulate (figs 33-40). Dorsal side without long pointed setae (only a pair of very minute setae at border of tergites and minute setae placed close to spiracles) but with numerous cauliflower sensilla, whose size slightly decreases posteriorly. Ventral side of pro-, meso-, metanotum, and first three abdominal segments with long pointed setae (except a pair of very minute setae at border of each tergite). Ventral side of remaining abdominal segments with a pair of very minute setae at border of tergite and cauliflower-shaped sensilla, which are distinctly larger than sensilla on dorsal side of abdomen but more or less as large as or slightly larger than sensilla on dorsal side of pro-, meso- and metanotum.

Pronotum dorsally on each side with one campaniform sensillum anteriorly, one very short seta antero-laterally and 10 cauliflower-shaped sensilla (figs 33, 35, 37, 39). Dorsal side of meso- and metanotum close to anterior border of tergite with a pair of very minute setae, antero-medially with a pair of cauliflower sensilla, and on each side posteriorly with five cauliflower sensilla arranged in row. Each antero-lateral side with single cauliflower sensillum and a very short seta. On each side of mesonotum close to posterior border laterally one minute seta. All abdominal segments dorsally, close to anterior border, with a pair of minute setae. Two minute setae placed close to each spiracle. First abdominal segment on each side with three (rarely with two) cauliflower-shaped sensilla anteriorly and a pair of cauliflower-shape sensilla postero-laterally. Remaining abdominal segments on each side, with two pairs of cauliflower sensilla: first placed antero-laterally and the second postero-medially. Each side of tergite with one cauliflower sensillum placed laterally very close to spiracle.

Ventral side of pro-, meso- and metanotum with two pairs of long pointed setae in the middle, and two pairs of minutae setae at anterior border (figs 34, 36, 38, 40). Anterior margin of all abdominal segments with a pair of very minute setae. On ventral side of first three abdominal segments a group of strong setae forming “ventral organ” (figs 41-44). First two abdominal segments medially usually with 8 long pointed setae. Third abdominal segment medially the most changeable with 8, 7 or 6 long pointed setae or with 2, 4 or 6 long pointed setae and 2 elongate cauliflower-shaped sensilla. Moreover on each side of first three

abdominal sterna two sensilla postero-laterally and one sensillum antero-laterally. Segments from 4th to 6th on each side with two pairs of cauliflower sensilla: first pair placed antero-medially, the second postero-laterally. One additional sensillum is placed antero-laterally. Ventral side of abdominal segment 7 on each side with one sensillum placed antero-medially, another one placed antero-laterally and a pair of sensilla placed postero-laterally. Ventral side of abdominal segment 8 with sensilla arranged like on sternite 7, but without one sensillum placed postero-laterally.

Ocelli black, five on each side of head, arranged in a constant pattern: four in one row, three of them placed very close, and one at a distance, fifth placed in the gap between three and one ocellus.

Frontal side of head with four small, vertical, pointed setae (V 1-4) and five frontal rows of setae: row Fa with three setae, Fb with four setae, Fc with three setae, Fd with single seta, Fe with two setae. In area between seta Fc1 and Fc2 (usually closer to Fc1) one campaniform sensillum (figs 45, 52, 59, 66). Temporal side of head with three blunt setae (T 1-3) and single campaniform sensillum (figs 46, 53, 60, 67).

Antennae 2- segmented, set in membraneous ring.

Labrum wider than long, with distinctly emarginate anterior margin. Mid part of ventral surface (epipharyngeal area) with a pair of small setae, four campaniform sensilla, and two irregular groups of a few (usually eight) small sensilla (figs 48, 55, 62, 69). Central and lateral parts of ventral side of labrum armed with numerous small spines. Emargination on ventral surface with six setae, and anterior margin on each side ventrally with four long setae: three stout and one slightly thinner (figs 47, 54, 61, 68). Dorsally at margin two setae. Across dorsal side of labrum run four setae, and central part of labrum with two pairs of campaniform sensilla.

Mandibles heavily sclerotized, palmate, with six triangular, apical teeth, five in row and sixth slightly retracted (figs 49, 50, 56, 57, 63, 64, 70, 71). Dorsal side of mandibles at base with two setae and two campaniform sensilla. Dorsal margin of second to fourth mandibular tooth finely crenulate.

Maxillae and labium connate (figs 51, 58, 65, 72). Palpiger distinct, at basal part heavily sclerotised with two setae and three campaniform sensilla and numerous spines dorso-laterally which look like the spines covering ligula. Maxillary palp two-segmented: first segment with two setae and one campaniform sensillum, second segment with a group of 12 sensilla at apex, and below the apex with campaniform sensillum, digitiform sensillum and seta. Galea fused with lacinia. Mala bear three long pointed at the apex setae, three short pointed setae, one long blunt at the apex seta and one short sensillum (short blunt seta or peg like sensillum). Labial palp one-segmented with a group of 9 sensilla at the apex and one campaniform sensillum below the apex. Ligula divided, covered with spines, and with six large campaniform sensilla at base. Prementum with two long and two short setae and four campaniform sensilla. Postmentum with two long and four shorter setae.

Legs stout, with constant chaetotaxy (figs 73-76). Tibiotarsus apically with heavily sclerotized, short and curved, single and simple claw armed basally with a pointed seta. Claw surrounded by a complex of 6 setae. Tibiotarsus above claw with two campaniform sensilla and one small seta, and in dorso-medial part with two long setae. Femur with 11 long setae and one short seta close to the base dorsally. Basally on internal side a group of five campaniform sensilla and one short pointed seta, and at base ventrally one campaniform sensillum. Also basally but on external side two campaniform sensilla. Coxa with setae arranged in three groups: first with three short setae and one long prominent seta (pointed at the top or widened apically), second with three short setae, and third with two short setae. Dorsally, close to first group of setae occurs additional one short seta. At base of each coxa two minutae setae. At base of first pair one additional minutae seta.

DISCUSSION

Description of first instars of European *Cassida* showed that all possess distinct diagnostic characters. For previously described five species these characters were summarized in Table 1 in ŚWIĘTOJAŃSKA (2005). The level of difference in the species of distinct groups depends on type of defense mechanism of the beetle. Species representing the same defense mechanism differ mostly in combination of the following characters (Table 1): shape of frontal setae on head (cauliflower or pointed), shape of apical setae of lateral scoli 1-14 (elongate trunctate, elongate clubate or cone-shaped), structure of apex of lateral scoli 15 and 16 (shape, setose or not setose), number and arrangement of setae of "ventral organ", shape of setae of labrum (long or short), and shape of femoral setae (cauliflower or pointed).

Some of noted above characters are constant and some variable. The most variable character is the number and arrangement of setae in "ventral organ". This phenomenon was observed also in previously described species (see ŚWIĘTOJAŃSKA 2005). I did not observe correlation between body size of adult and body size of first instar larva. The largest species – *Cassida sanguinosa* has first instar larvae almost the same size as distinctly smaller *C. prasina* and *C. sanguinolenta*, and distinctly smaller larvae than slightly smaller in adult stage *C. denticollis* (Table 1).

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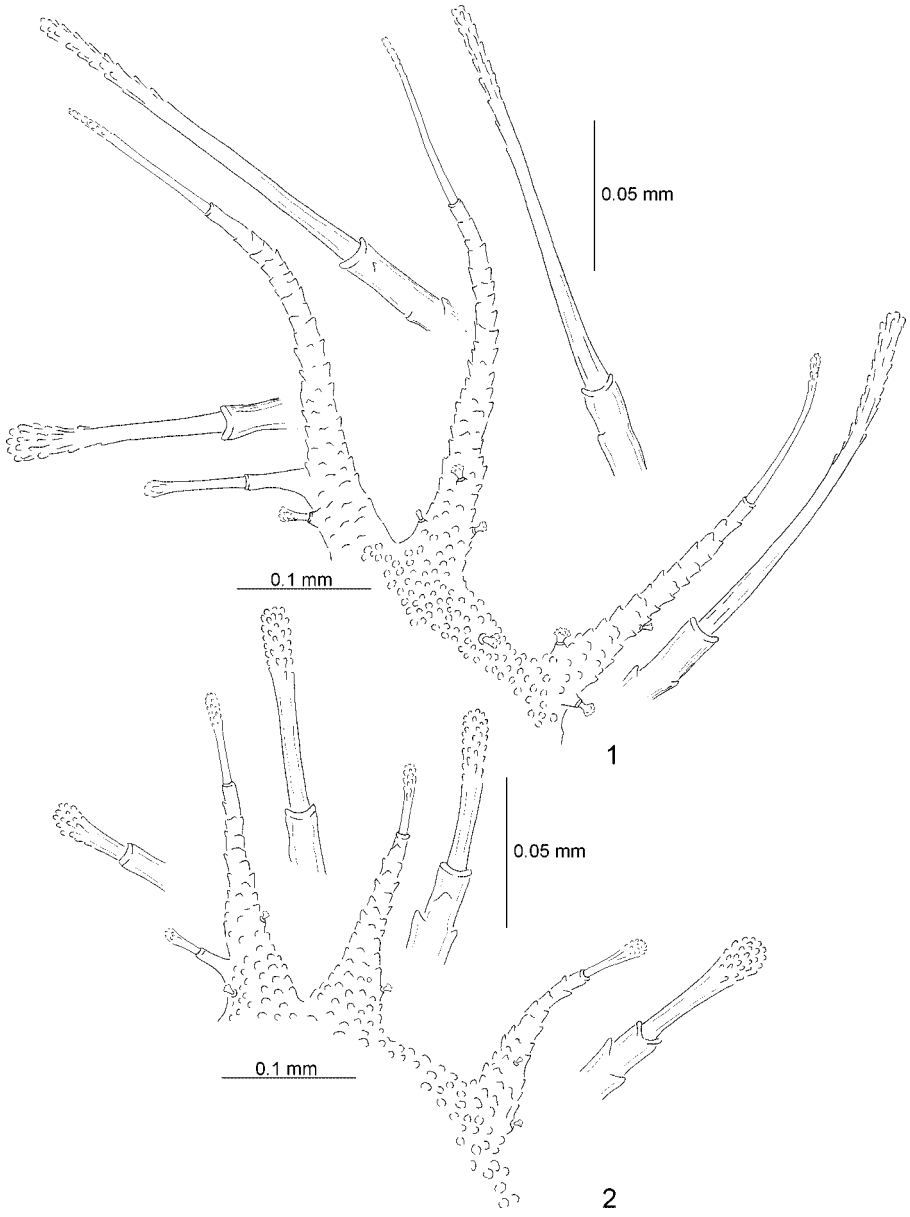
Table 1. Comparative characters of first instar larvae of four species of European *Cassida prasina* group.

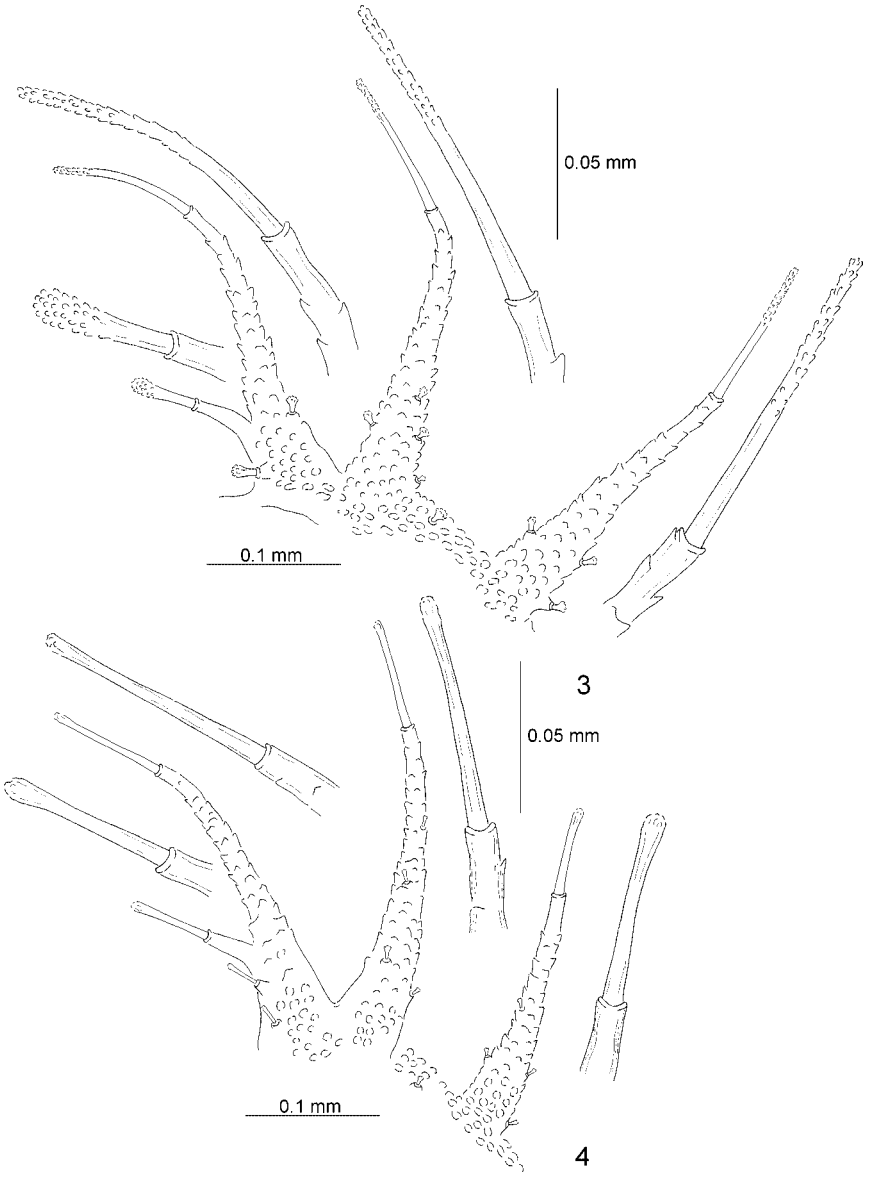
Character\Species	***C. denticollis***	***C. prasina***	***C. sanguinolenta***	***C. sanguinosa***
length of body [mm]	0.95-1.55 (n=20)	0.76-1.40 (n=20)	0.85-1.37 (n=20)	0.82-.95 (n=5)
width of body [mm]	0.50-0.70 (n=20)	0.47-0.60 (n=20)	0.47-0.67 (n=20)	0.45-0.53 (n=5)
length of supra-anal prosesses	as long as body length	as long as body length	as long as body length	as long as body length
lateral scoli 1-14 armed apically with:	**elongate truncate setae** at the apex covered with scales, distinctly shortened posteriorly to prominent cauliflower-shaped sensilla	**elongate truncate setae** at the apex covered with scales, distinctly shortened posteriorly to prominent cauliflower-shaped sensilla	**elongate truncate setae** at the apex covered with scales, slightly shortened posteriorly to 13th scoli, 14th scoli slightly longer than of 13th	**elongate clubate setae**, distinctly shortened posteriorly to short prominent cauliflower-shaped sensilla
apex of 15th and 16th lateral scoli	**bulb like without any seta**	with stout prominent **cone-shaped sensilla**	**elongate truncate setae** at the apex covered with scales, longer than setae of 13th scoli	**unisetose** sometimes some of this scoli bisetose
"ventral organ" number of setae in the middle of: 1st / 2nd / 3rd abdominal segments	the most often: **8 / 8 / 8** or **8 / 8 / 6 +2** elongate cauliflower-shaped sensilla, sometimes: **8 / 8 / 7** or **8 / 7 / 8**	the most often: **8 / 8 / 6** or **8 / 8 / 4 + 2** elongate cauliflower-shaped sensilla, sometimes: **8 / 8 / 8** or **10 / 8 /6** or **8 / 8 / 2 + 2** elongate cauliflower-shaped sensilla	the most often: **8 / 8 / 8** sometimes: **8 / 6 / 6**	**8 / 8 / 7** or **8 / 8 / 6** or **8 / 8 / 8**

Table 1. Continuation

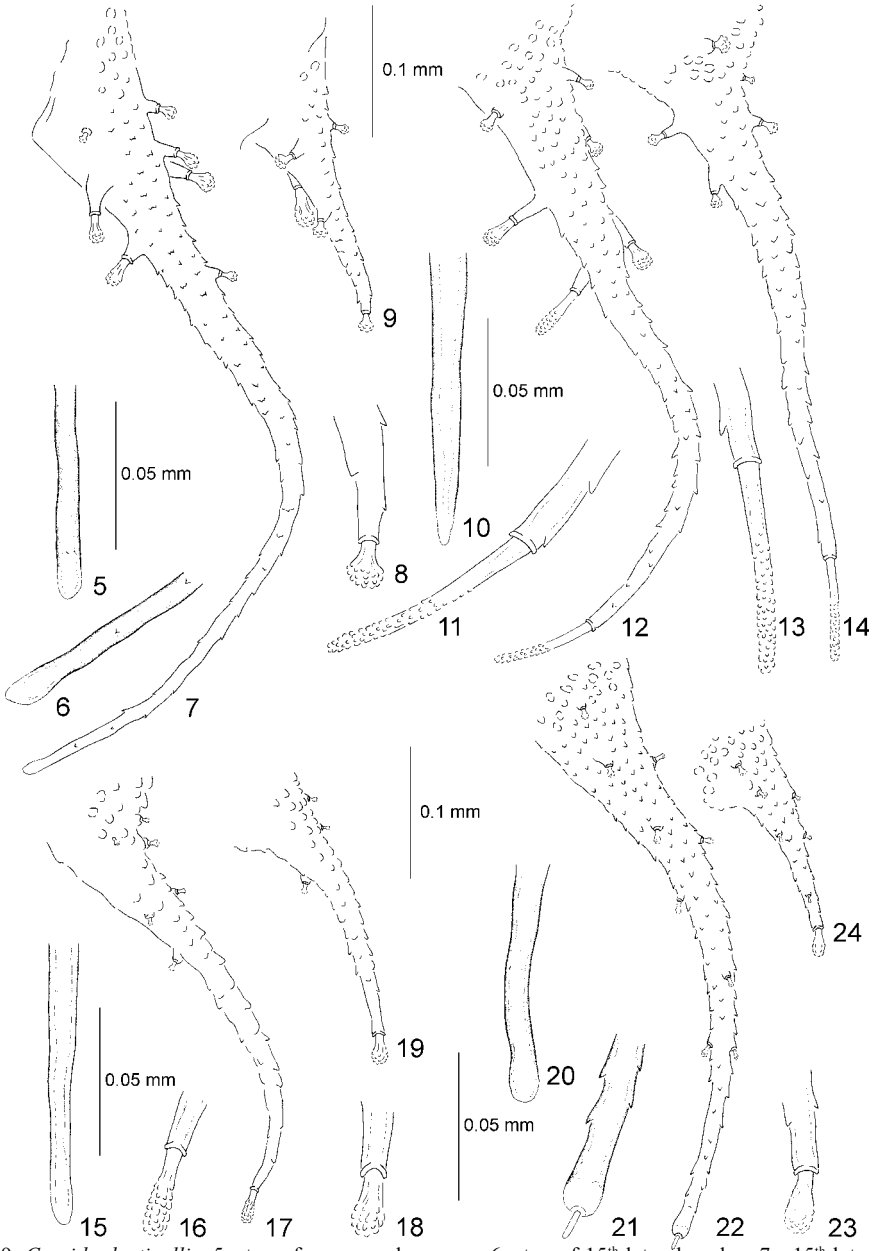
head with five frontal rows (Fa-Fe) of	elongate **cauliflower-shaped sensilla**	elongate **cauliflower-shaped sensilla**	elongate **cauliflower-shaped sensilla**	pointed **setae**
emargination of anterior margin of labrum with setae:	6 **short** thin	6 **long** thin	6 **short** thin	6 **long** stout
anterior margin of labrum on each side dorsally/ventrally with setae:	1 short thin / 1 short thin + 3 long stout	1 short thin / 1 short thin + 3 long stout	1 short thin / 1 short thin + 3 long stout	1 long thin / 1 long thin + 3 long stout
femur of all species with one short seta at base dorsally and with:	**7** long pointed **setae** + **4** elongate **cauliflower-shaped sensilla**	**7** long pointed **setae** + **4** elongate **cauliflower-shaped sensilla**	**7** long pointed **setae** + **4** elongate **cauliflower-shaped sensilla**	**11** long pointed **setae**
close to each pronotal spiracle placed:	1 very minute seta	1 very minute seta	1 very minute seta	1 very minute seta
close to each abdominal spiracle placed:	2 very minute setae + 1 cauliflower-shaped sensillum	2 very minute setae + 1 cauliflower-shaped sensillum	2 very minute setae + 1 cauliflower-shaped sensillum	2 very minute setae + 1 cauliflower-shaped sensillum



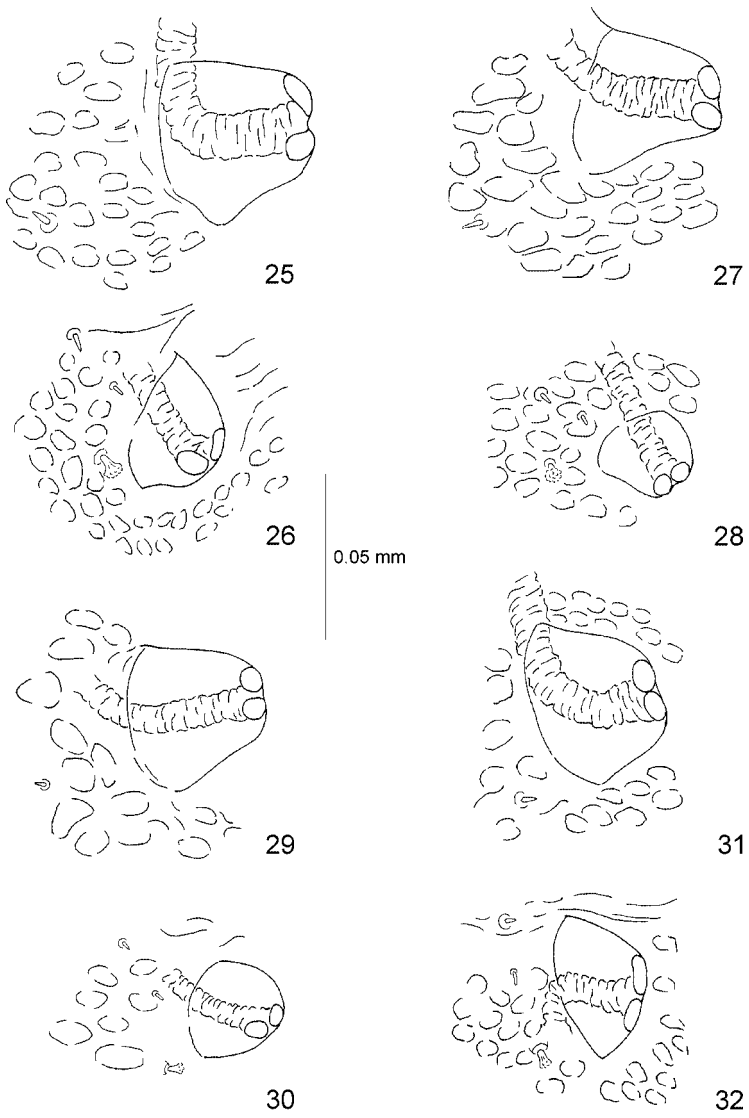
1, 2. First three lateral scoli: 1 - *Cassida denticollis*; 2 - *Cassida prasina*



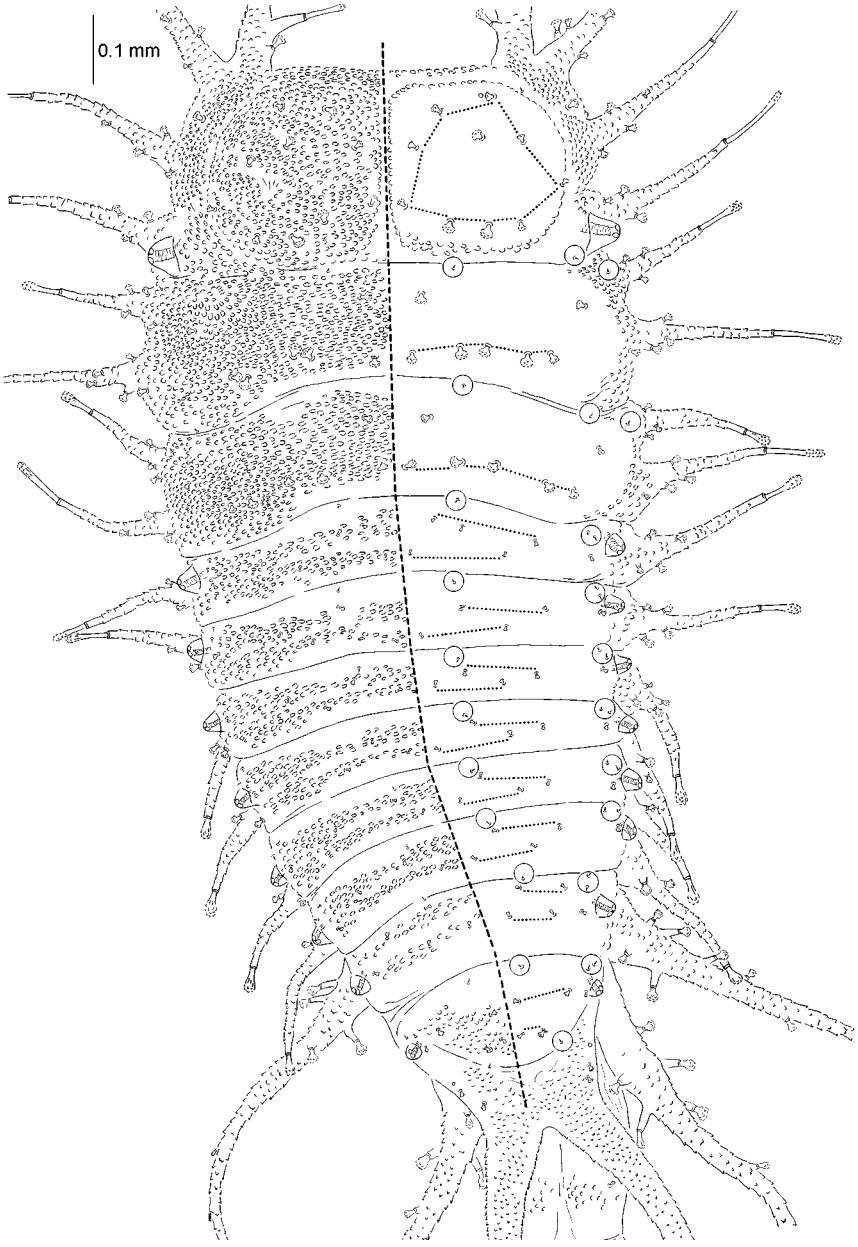
3, 4. First three lateral scoli: 3 - *Cassida sanguinolenta*; 4 - *Cassida sanguinosa*



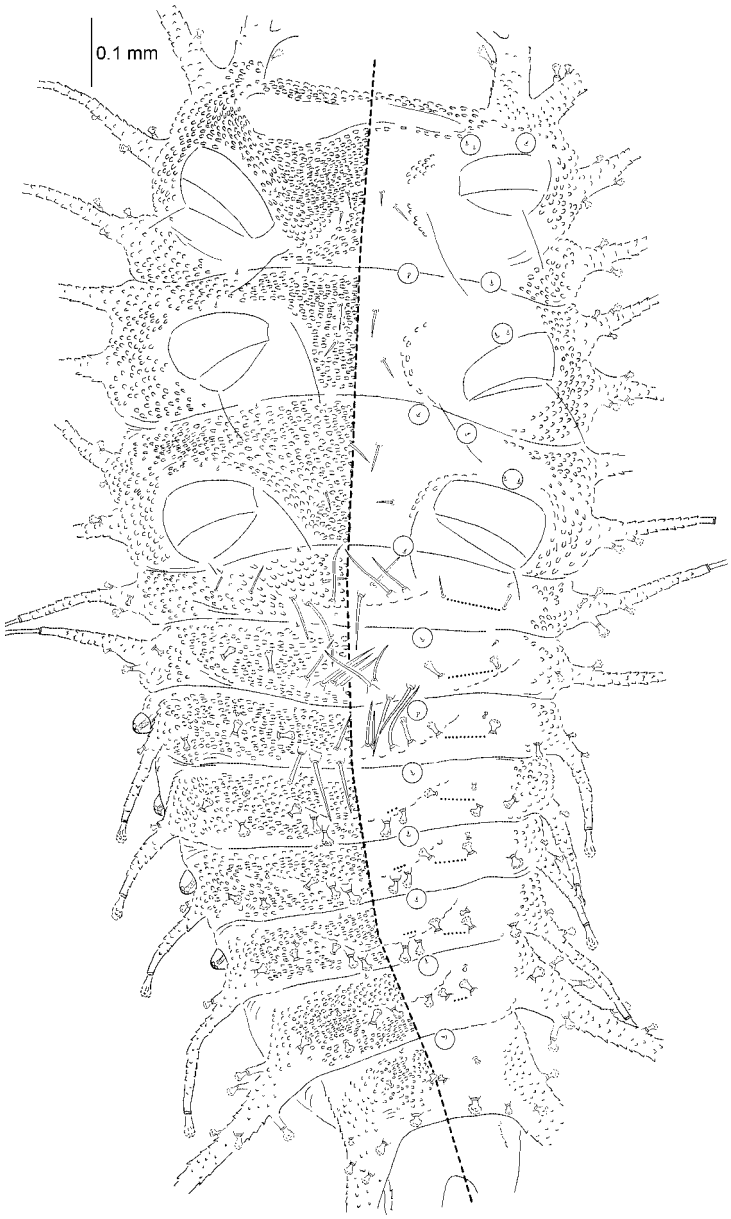
5-9. *Cassida denticollis*: 5 - top of supra-anal process; 6 - top of 15th lateral scolus; 7 - 15th lateral scolus; 8 - top of 14th lateral scolus; 9 - 14th lateral scolus. 10-14. *Cassida sanguinolenta*: 10 - top of supra-anal process; 11 - top of 15th lateral scolus; 12 - 15th lateral scolus; 13 - top of 14th lateral scolus; 14 - 14th lateral scolus. 15-19. *Cassida prasina*: 15 - top of supra-anal process; 16 - top of 15th lateral scolus; 17 - 15th lateral scolus; 18 - top of 14th lateral scolus; 19 - 14th lateral scolus. 20-24. *Cassida sanguinosa*: 20 - top of supra-anal process; 21 - top of 15th lateral scolus; 22 - 15th lateral scolus; 23 - top of 14th lateral scolus; 24 - 14th lateral scolus



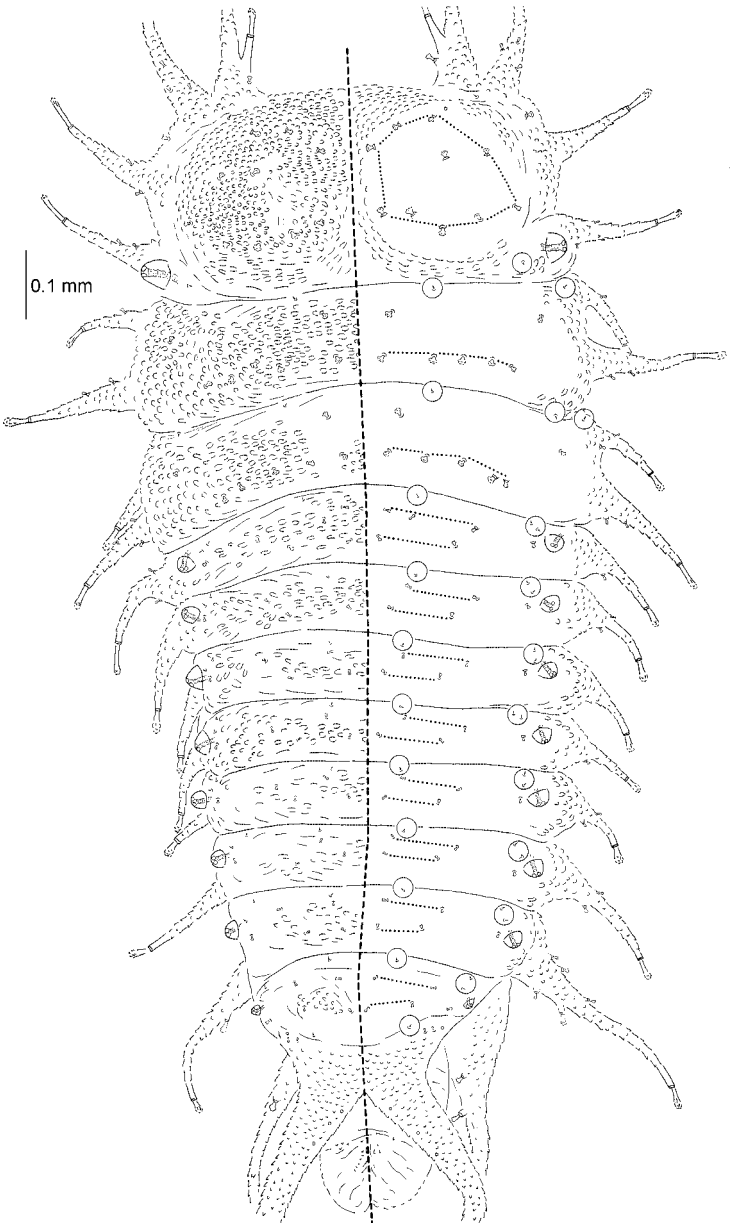
25, 26. *Cassida denticollis*: 25 - spiracle of pronotum; 26 - spiracle of first abdominal segment. 27, 28. *Cassida sanguinolenta*: 27 - spiracle of pronotum; 28 - spiracle of first abdominal segment. 29, 30. *Cassida prasina*: 29 - spiracle of pronotum; 30 - spiracle of first abdominal segment. 31, 32. *Cassida sanguinosa*: 31 - spiracle of pronotum; 32 - spiracle of first abdominal segment



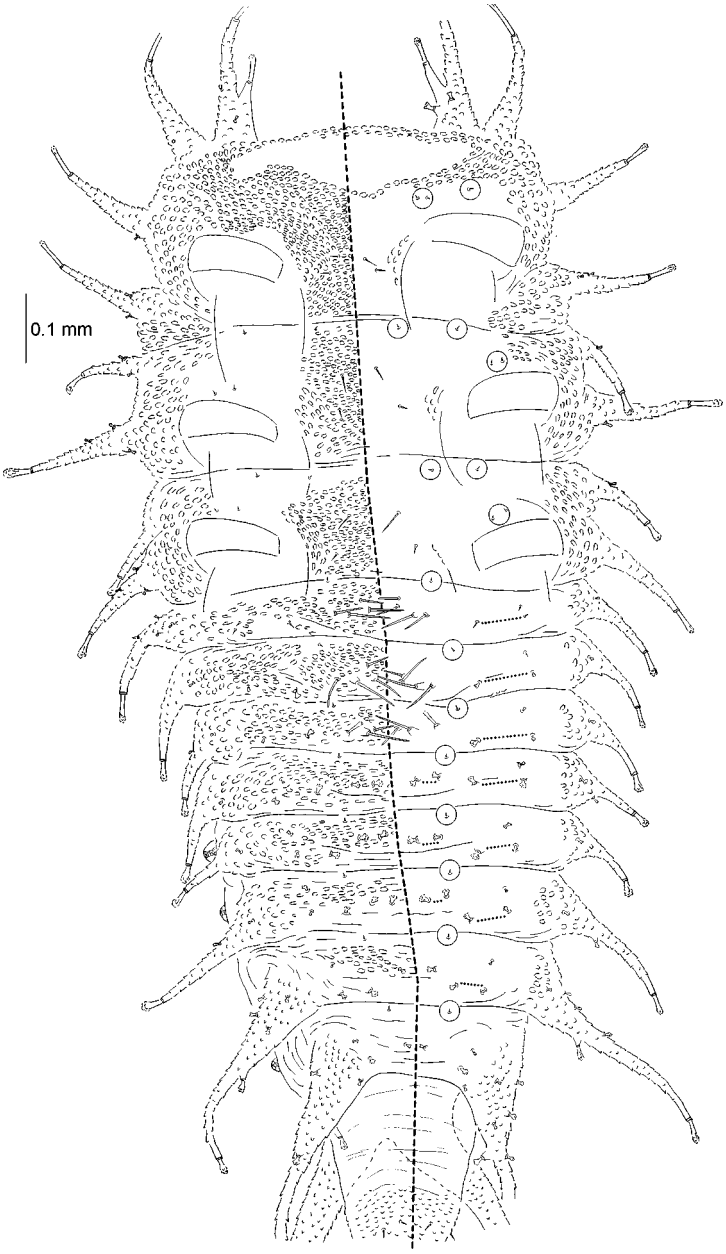
33. *Cassida denticollis*, chaetotaxy of dorsal side of body



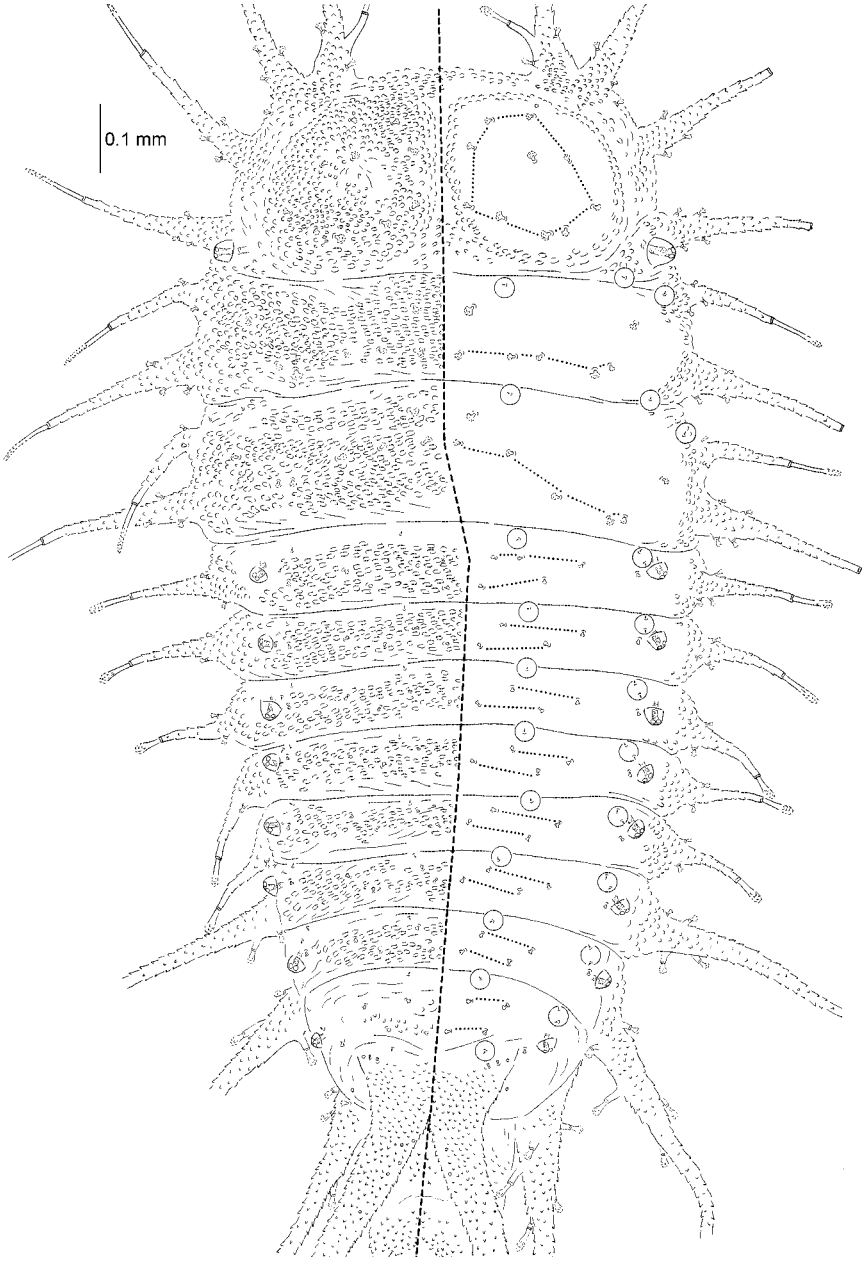
34. *Cassida denticollis*, chaetotaxy of ventral side of body



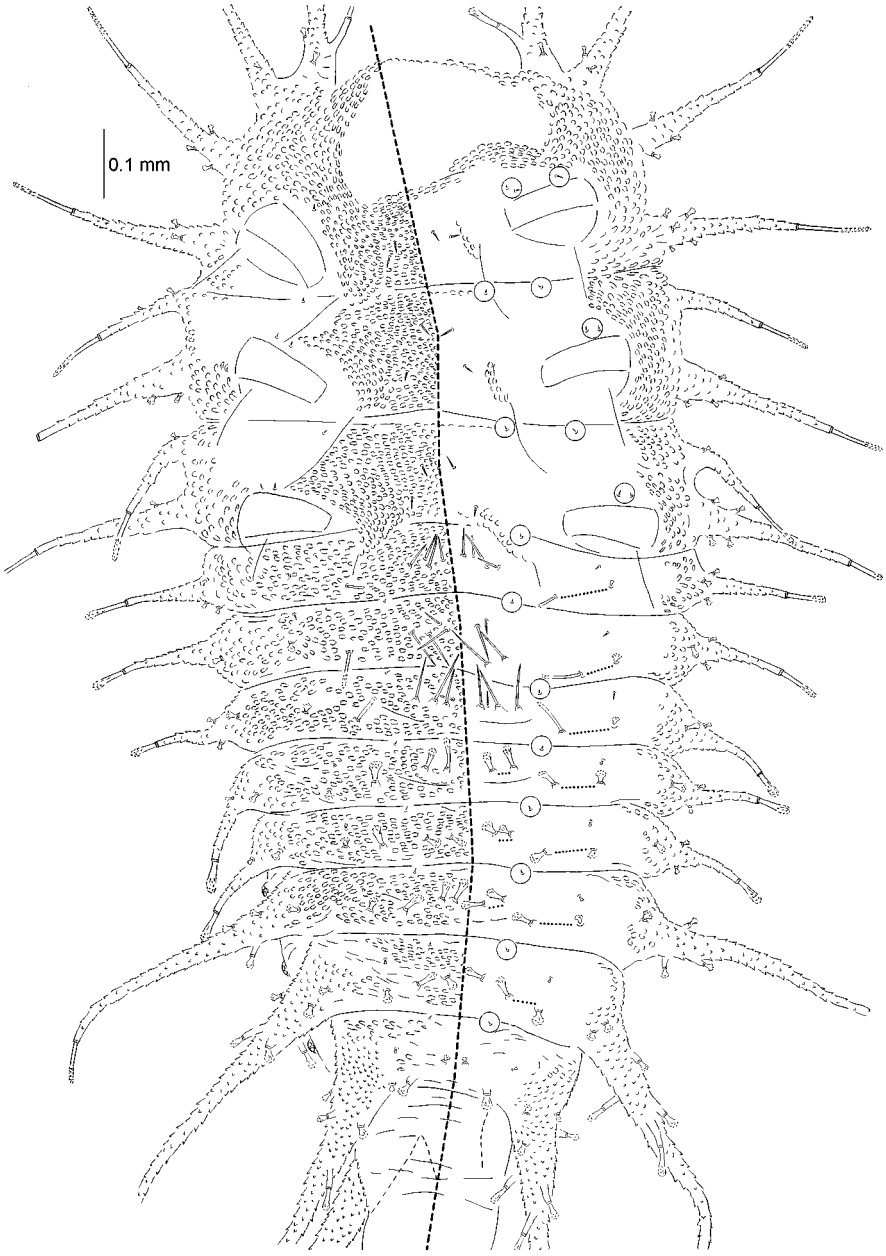
35. *Cassida prasina*, chaetotaxy of dorsal side of body



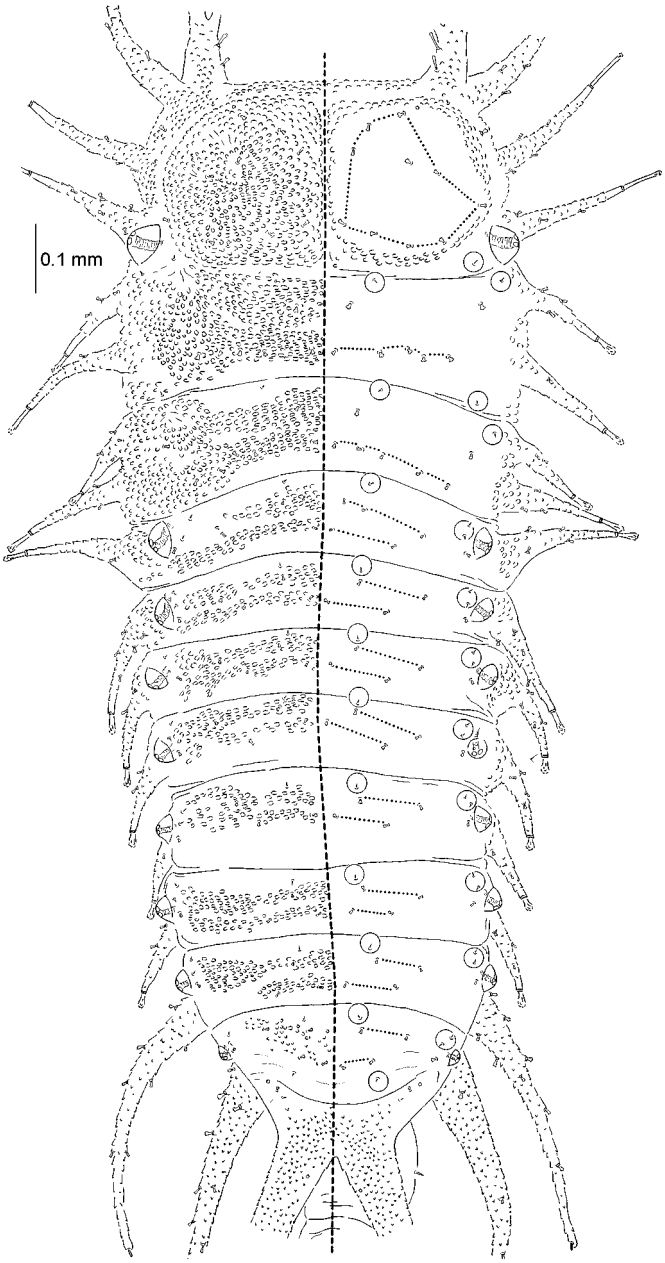
36. *Cassida prasina*, chaetotaxy of ventral side of body



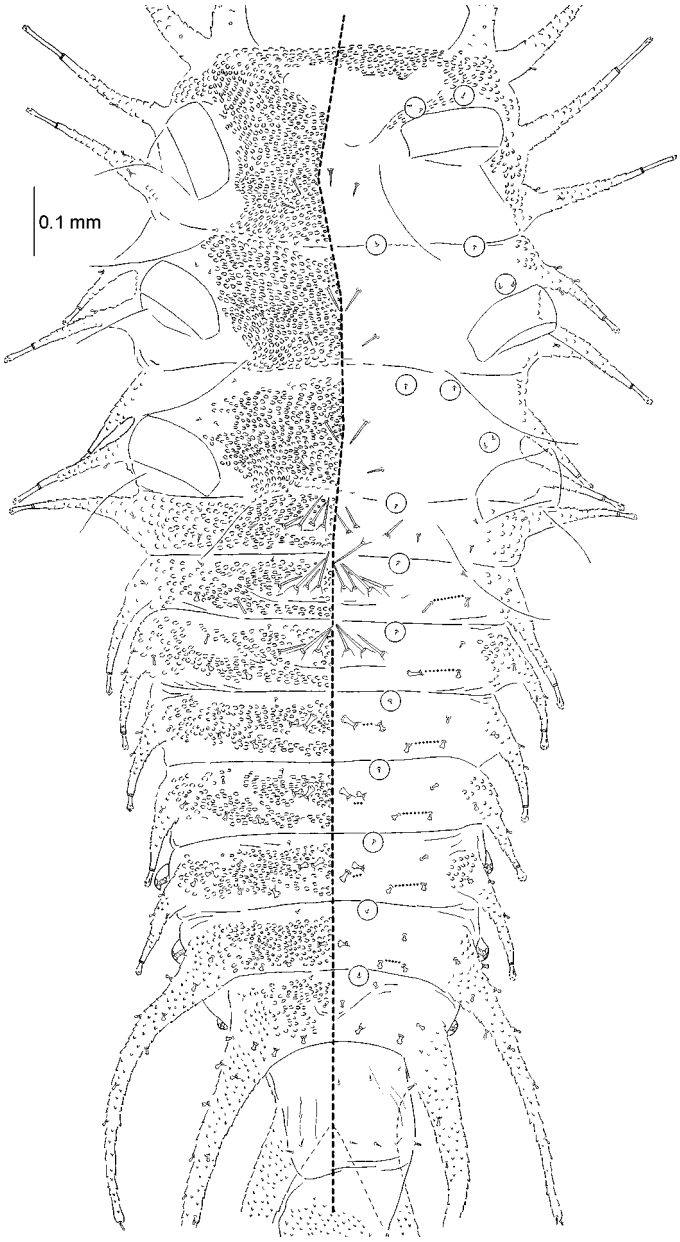
37. *Cassida sanguinolenta*, chaetotaxy of dorsal side of body



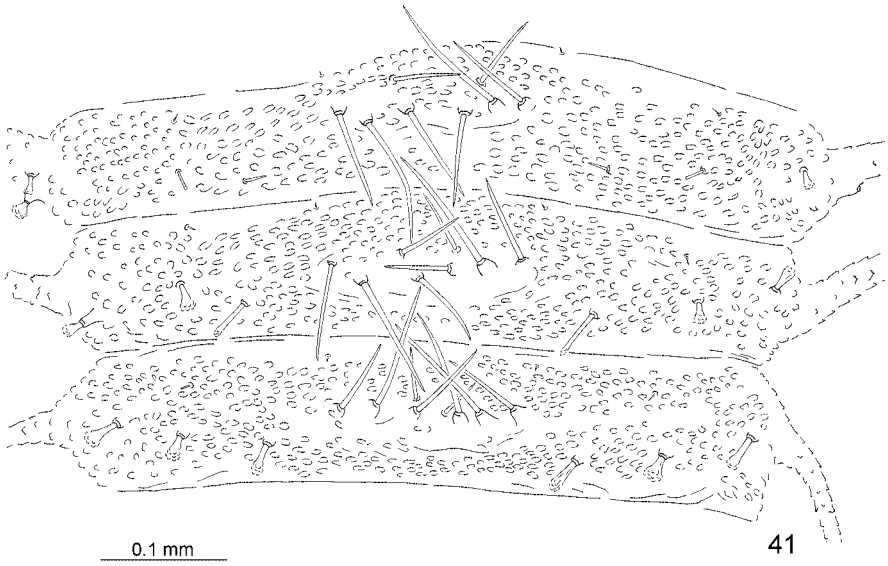
38. *Cassida sanguinolenta*, chaetotaxy of ventral side of body

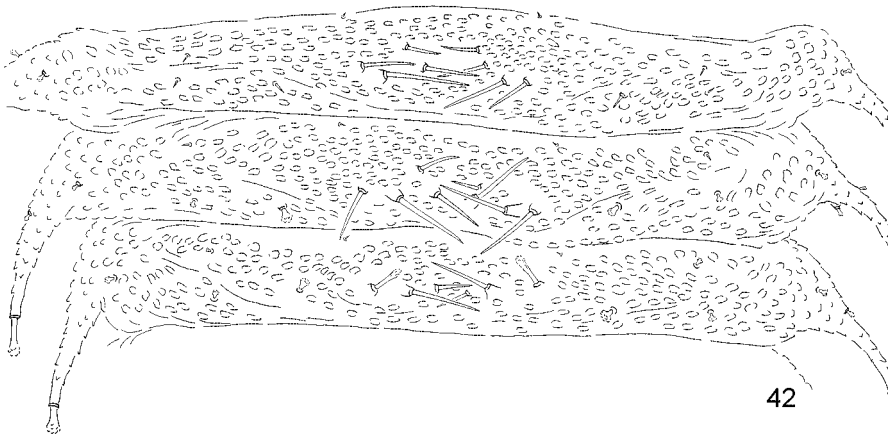


39. *Cassida sanguinosa*, chaetotaxy of dorsal side of body

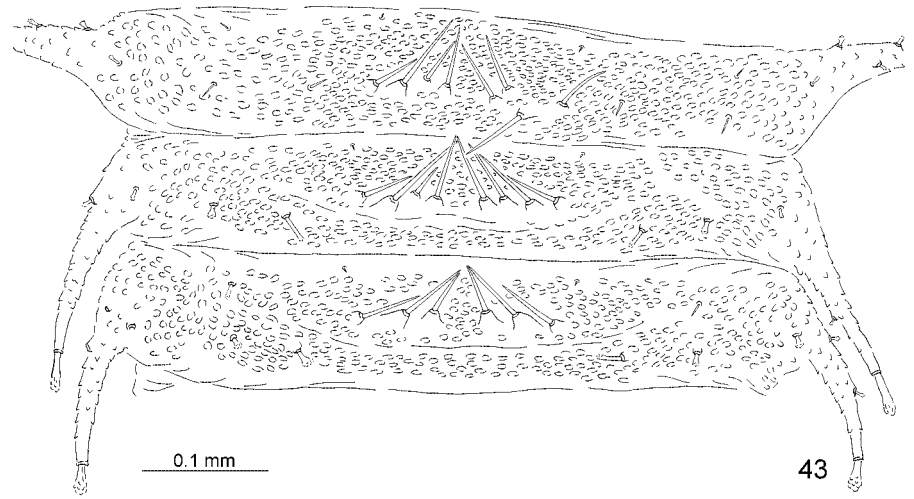


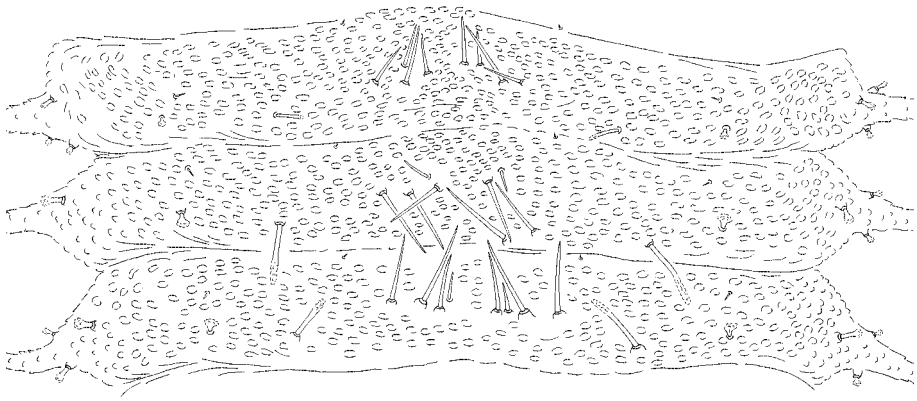
40. *Cassida sanguinosa*, chaetotaxy of ventral side of body



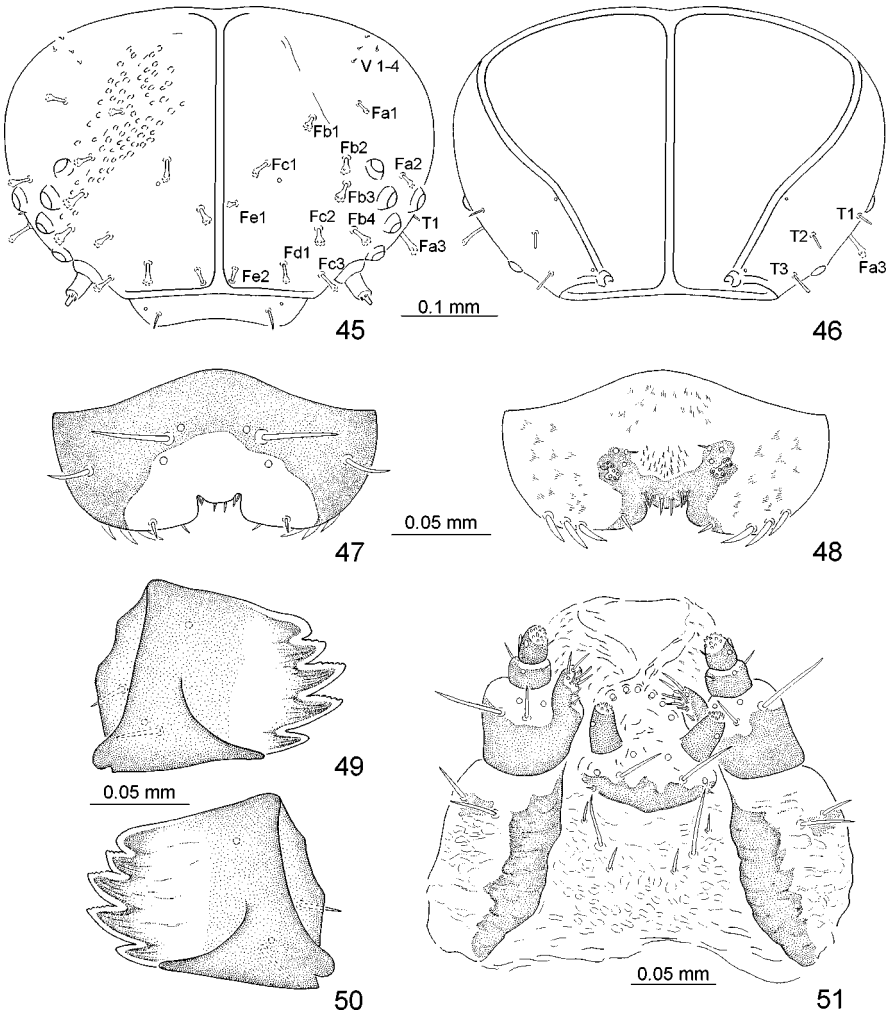


41, 42. Chaetotaxy of ventral side of first three abdominal segments: 41 - *Cassida denticollis*; 42 - *Cassida prasina*

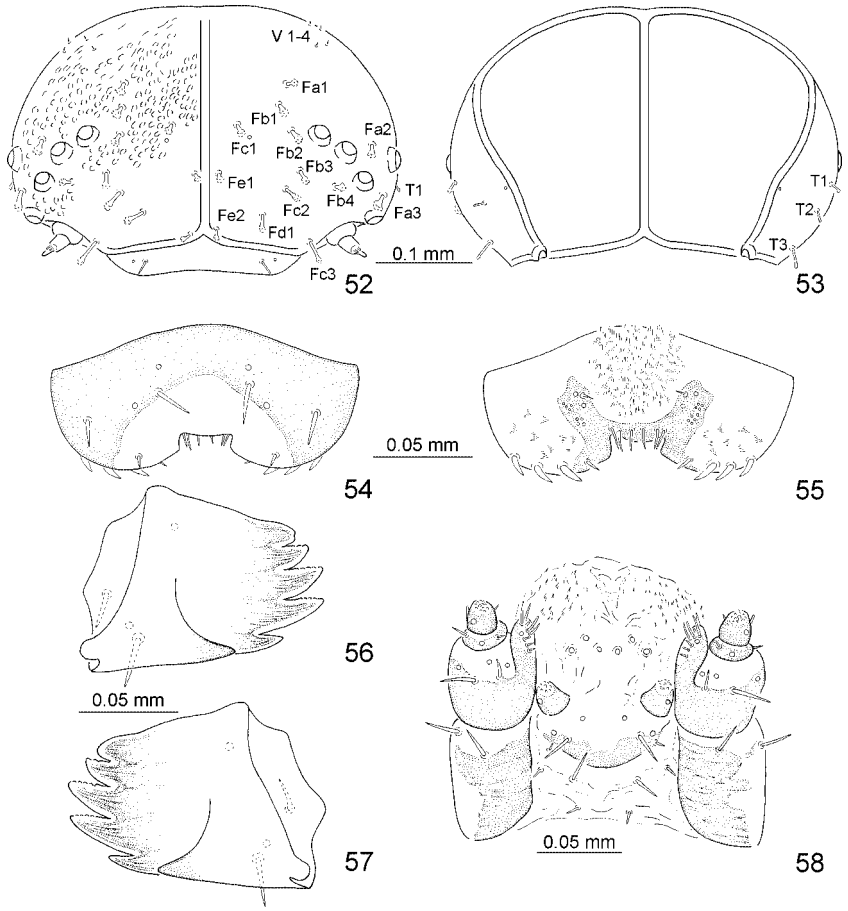




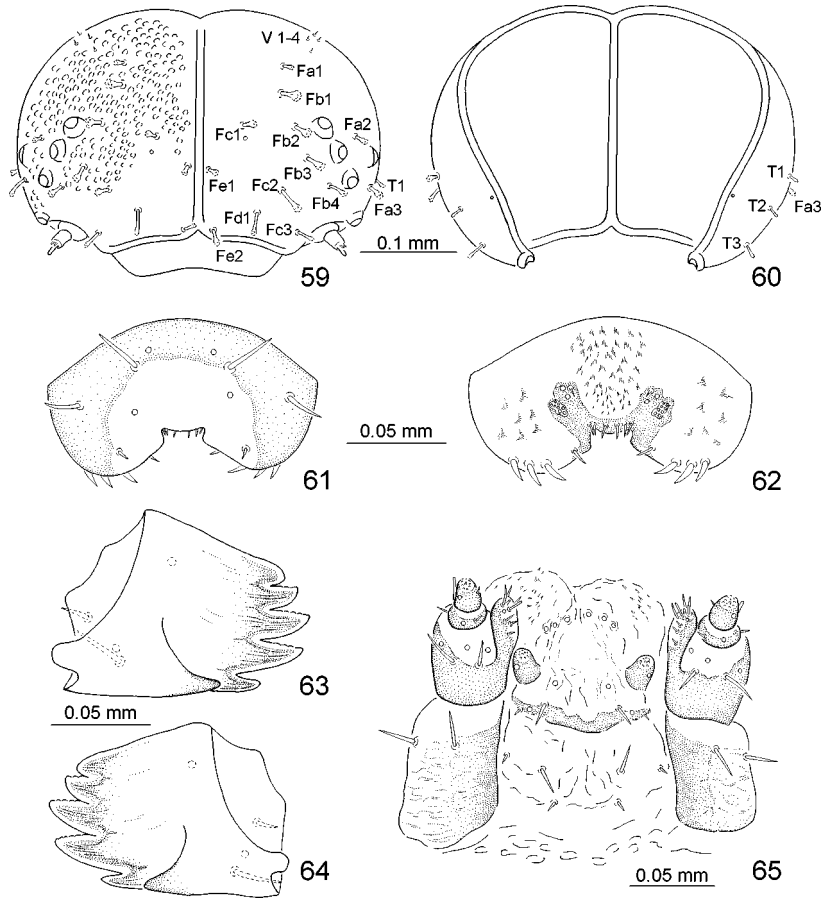
43, 44. Chaetotaxy of ventral side of first three abdominal segments: 43 - *Cassida sanguinosa*; 44 - *Cassida sanguinolenta*



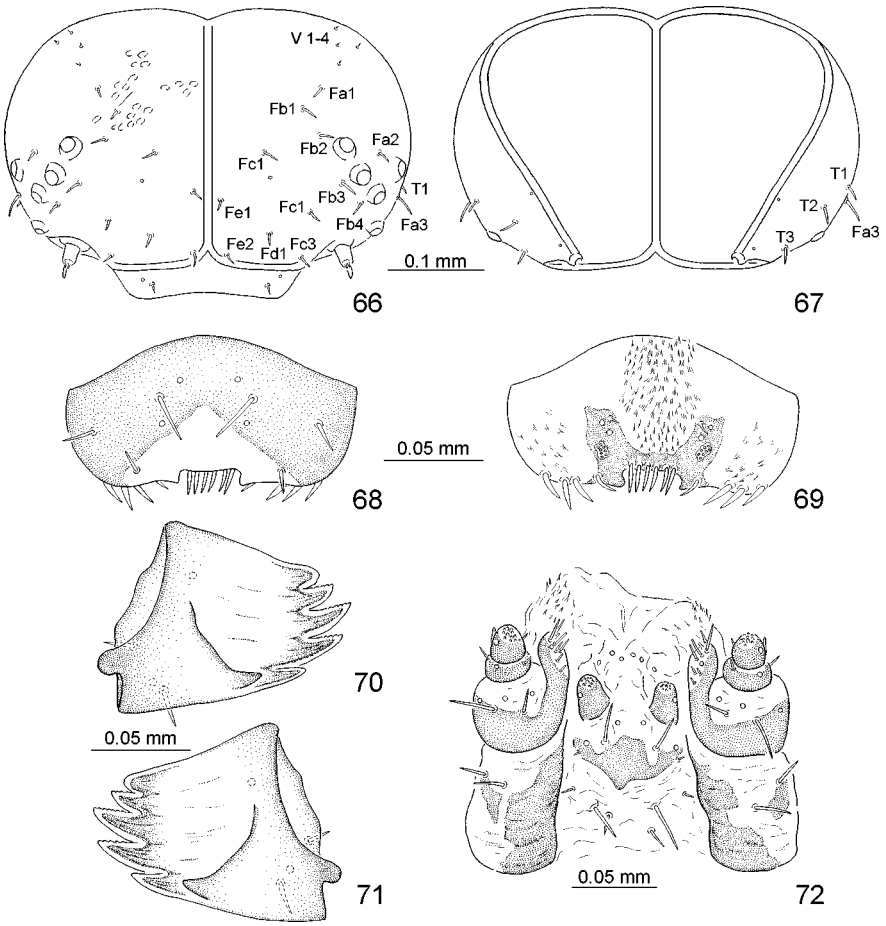
45-51. *Cassida denticollis*: 45 - frontal side of head; 46 - temporal side of head; 47 - dorsal side of labrum; 48 - ventral side of labrum; 49, 50 - mandibles; 51 - maxillae and labium



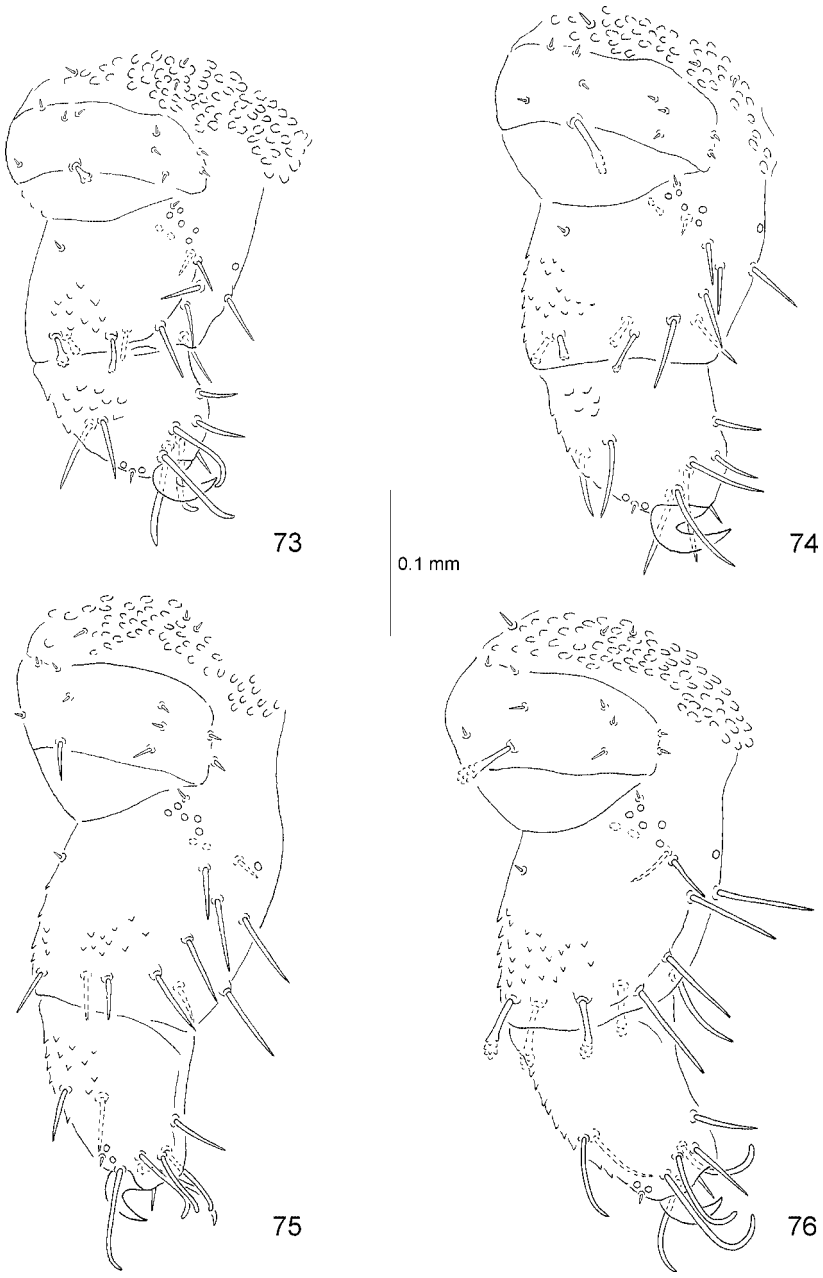
52-58. *Cassida prasina*: 52 - frontal side of head; 53 - temporal side of head; 54 - dorsal side of labrum; 55 - ventral side of labrum; 56, 57 - mandibles; 58 - maxillae and labium



59-65. *Cassida sanguinolenta*: 59 - frontal side of head; 60 - temporal side of head; 61 - dorsal side of labrum; 62 - ventral side of labrum; 63, 64 - mandibles; 65 - maxillae and labium



66-72. *Cassida sanguinosa*: 66 - frontal side of head; 67 - temporal side of head; 68 - dorsal side of labrum; 69 - ventral side of labrum; 70, 71 - mandibles; 72 - maxillae and labium



73-76. Leg of first pair: 73 - *Cassida prasina*; 74 - *Cassida sanguinolenta*; 75 - *Cassida sanguinosa*; 76 - *Cassida denticollis*