Genus	Vol. 16 (1): 49-68	Wrocław, 31 III 2005

Comparative description of first instar larvae of *Cassida nobilis* LINNAEUS, 1758 and *Cassida vittata* VILLERS, 1789 (Coleoptera: Chrysomelidae: Cassidinae)

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ABSTRACT. First instar larvae of *Cassida nobilis* and *Cassida vittata* from Palaearctic Region are described and illustrated. Comparative diagnosis of *Cassida nobilis*, *Cassida vittata*, *Cassida nebulosa*, *Cassida stigmatica* and *Cassida rubiginosa* is given. Results suggested that morphology of first instars within a single genus is correlated with defensive behaviour and that first instars of closely related species have diagnostic characters.

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

INTRODUCTION

Till now first instar larvae of nine Cassidinae species were described in detail, seven from the tribe Cassidini (BOROWIEC and ŚWIĘTOJAŃSKA 2002, GHATE et al. 2004, ŚWIĘTOJAŃSKA 2004), one from Basiprionotini (ŚWIĘTOJAŃSKA and GHATE 2003), and another one from Aspidimorphini (RANADE et al. 2004). From European species first instars of three species belonging to the genus *Cassida* were described: *Cassida nebulosa* LINNAEUS, 1758 (BOROWIEC and ŚWIĘTOJAŃSKA 2002), *Cassida stigmatica* SUFFRIAN, 1844 and *Cassida rubiginosa* MÜLLER, 1776 (ŚWIĘTOJAŃSKA 2004). All papers suggested that first instars possess important characters of both diagnostic and phylogenetic value but all hitherto described larvae represent distinct and mostly not closely related species.

In this paper I wanted to verify if in very closely related species first instars have diagnostic characters or not. The present paper contains description of first

instar larvae of another two species of European *Cassida*: *Cassida nobilis* LINNAEUS, 1758 and *Cassida vittata* VILLERS, 1789. Adults of both are very similar externally and were classified in the subgenus *Cassidulella* STRAND, 1928. Both are associated biologically with Chenopodiaceae plants. The larvae also seem to have the same type of defensive behaviour, similar to that described for the larvae of *C. rubiginosa*. In a previous paper (ŚWIĘTOJAŃSKA 2004) I suggested that defence behaviour in larvae could be manifested by the different structure of sensory chaetae. In this paper I would like to verify this hypothesis by comparising of first instars of *C. nobilis* and *C. vittata* with *C. nebulosa*, *C. rubiginosa* and *C. stigmatica*, species of three different defensive systems.

MATERIAL AND METHODS

Description of first instars of *Cassida nobilis* and *C. vittata* based on model description of first instars of Cassidini proposed by BOROWIEC and ŚWIĘTOJAŃSKA (2002). Further papers (GHATE et al. 2004, ŚWIĘTOJAŃSKA 2004) showed that general morphology of the first instar is quite constant thus in this paper I described the common general morphology for both *C. nobilis* and *C. vittata* and distinguished diagnostic characters.

Adults of *Cassida nobilis* and *C. vittata* were collected at rural sites in Wrocław (Lower Silesia, Poland) in June 2004 and placed on Petri scale. Leaves of the same *Chenopodium* sp. were offered as a food plant for adults and larvae of both species. Hatching larvae were preserved in 75 to 80% ethanol. Nine specimens of *Cassida nobilis* and six of *Cassida vittata* were studied in detail.

Larvae were mounted on slides with Swan's liquid (aqua distillata 20 g., arabic gum 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.

DIAGNOSIS

On average *Cassida vittata* is slightly longer and wider than *C. nobilis*. *Cassida vittata* (n = 6) length: 0.8-1.4 mm, width: 0.5-0.6 mm while *C. nobilis* (n = 9) length: 07-1.2 mm, width: 0.3-0.6 mm.

Living specimens of *Cassida nobilis* are uniformly green whereas specimens of *C. vittata* are green with black head, legs, lateral scoli, supra-anal processes, spiracles, and pronotal patches which cover practically the whole surface of dorsal part of pronotum. Prepared specimens of *Cassida nobilis* are uniformly transparent yellow, while in *C. vittata* dark parts of the body are preserved thus the larva is contrasting transparent yellow with dark brown appendages (figs 1, 2).

Supra-anal processes of *Cassida vittata* are as long as half body length, of *C. nobilis* 3/5 body length (figs 1, 2). In *Cassida vittata* basal half of supra-anal

processes is covered with numerous spikes, in *Cassida nobilis* basal 2/3 are covered with spikes.

Dorsal part of pronotum of both species usually possesses 10 cauliflower-shaped sensilla but in *Cassida nobilis* sometimes some of them are doubled (figs 25, 26).

In *Cassida nobilis* setae which form frontal rows of head at the top are slightly widened apically, in *C. vittata* frontal setae of head are pointed to slightly widened apically.

Chaetotaxy of the femur of both species is the same: 11 long and 1 short setae. Long setae of *Cassida vittata* are pointed whereas in *C. nobilis* often 2-4 of them are slightly widened apically.

GENERAL DESCRIPTION OF FIRST INSTAR OF BOTH SPECIES

Body flattened dorso-ventrally, oval, moderately narrowed posteriorly, widest across meso- and metanotum (figs 1, 2). Living specimens are green.

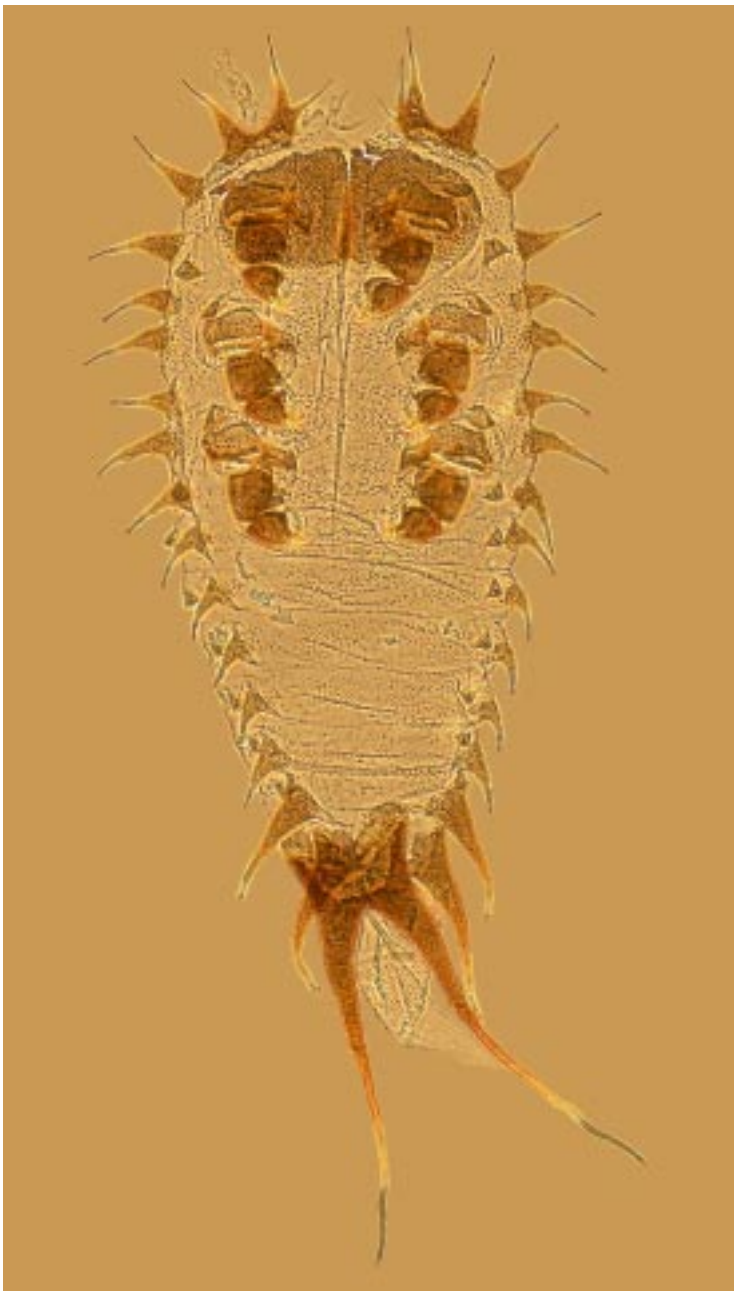
Body with 16 pairs of lateral scoli and a single pair of long supra-anal processes. Scoli from first to 14th short. Abdominal scoli very slightly shortened posteriorly. Scoli 15th and 16th the longest but 16th slightly longer than 15th (figs 1, 2). First and second lateral scoli placed very close and directed anteriorly. First lateral scoli with one long and one shorter lateral branch, other scoli simple, without lateral branches but with a few cauliflower-shaped sensilla (figs 6, 10, 11, 12, 18, 19). Lateral scoli from first to 14th and lateral branches of first scoli armed apically with elongate sensilla widened at the top (figs 3-12, 14, 18, 19, 21). Sensilla at the top of 1st to 14th lateral scoli gradually shortened posteriorly (figs 3-5, 7-9, 14, 21). Lateral scoli 15th and 16th armed apically with single seta (figs 15, 22). Supra-anal processes sinuate, bent dorsally, apically flask-shaped without apical setae (figs 13, 20).

Spiracles 9, elevated, annular but biforous at the top. Close to pronotal spiracle one minute seta is placed (figs 16, 23), close to each abdominal spiracle one very short seta and one small cauliflower-shape sensillum are placed (Fig. 17, 24).

Dorsal and ventral side of the body distinctly granulate (figs 25-28). 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 (except a pair of very minute setae at the border of each tergite) with long pointed setae. Ventral side of the remaining abdominal segments with a pair of very minute setae at border of tergite and cauliflower-shaped sensilla, which are slightly larger than sensilla on dorsal side of abdomen but more or less as large as sensilla on dorsal side of pro-, meso- and metanotum.

Pronotum dorsally on each side with one (sometimes two) campaniform sensillum anteriorly, one very short seta antero-laterally and with 10 cauliflower-

1. *Cassida nobilis*, first instar, ventral side without head; photo from slide



2. *Cassida vittata*, first instar, ventral side without head; photo from slide

shaped sensilla (figs 25, 26). Dorsal side of meso- and metanotum close to anterior border of tergite with a pair of very minute setae, antero-medially with 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 and with very short seta placed close to each spiracle. First abdominal segment on each side with three cauliflower-shaped sensilla anteriorly and a pair of cauliflower-shaped 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 27, 28). 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 29, 30). First two abdominal segments medially with 8 long pointed setae. Third abdominal segment medially with four setae and four prominent cauliflower sensilla. Moreover on each side of first three abdominal sterna two cauliflower sensilla postero-laterally and one cauliflower sensillum antero-laterally. On first two abdominal segments sensilla placed antero-laterally often shaped like blunt seta. Segments from 4th to 6th on each side with two pairs of cauliflower sensilla: first pair placed antero-medially, 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 the 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. Temporal side of head with three blunt setae (T 1-3) and single campaniform sensillum (figs 31, 32, 38, 39).

Antennae 2- segmented, set in membraneous ring.

Labrum wider than long, with distinctly emarginate anterior margin (figs 33, 34, 40, 41). Mid part of ventral surface (epipharyngeal area) with a pair of small setae, four campaniform sensilla, and two irregular groups of a few small sensilla. 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 three long setae: two stout and one slightly thinner.

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 35, 36, 42, 43). 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 37, 44). Palpiger distinct, at basal part heavily sclerotised with three setae and two campaniform sensilla ventrally and numerous short and long 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 sensilla at apex, and below the apex with campaniform sensillum, digitiform sensillum and seta. Galea fused with lacinia. Mala bear eigth setae and campaniform sensillum. Labial palp one-segmented with a group of 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, mentum and postmentum form one membraneous structure with four campaniform sensilla, four long and four short setae.

Legs stout, with constant chaetotaxy (figs 46, 48). 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 of a shape from straight or slightly curved at the top to almost hockey-stick-shaped. 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 with one long prominent seta 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. Close to the long prominent seta there is one very small sensillum or a group of sensilla as in figs 45, 47. At base of each coxa one short seta close to first group of setae on coxa.

DISCUSSION

Description of first instars of five species of European *Cassida* showed that all possess distinct diagnostic characters summarized in Table 1. The level of difference depends on the type of defense mechanism of the beetle. The most distinct defense behaviour was observed in *Cassida stigmatica*. Larvae of this species produce defense shields only from larval skins without faeces, and when the beetle is not disturbed the shield is placed linearly behind the abdomen and does not form an umbrella. In other species the defense shield is more or less supplemented by faeces, the most compact faecal shield occurs in *C. rubiginosa*, the least compact in *C. nobilis*. In all species, except *C. stigmatica*, the shield in

Table 1. Comparative characters of first instar larvae of five species of European *Cassida*.

Character\Species	***C. nebulosa***	***C. stigmatica***	***C. rubiginosa***	***C. nobilis***	***C. vittata***
length of body [mm]	1.25-1.35	0.7-1.1	0.7-0.9	0.7-1.2	0.8-1.4
length of supra-anal processes	2/3 length of body	1/2 length of body	as long as body length	3/5 of body length	1/2 of body length
lateral scoli 1-14 armed apically with:	**elongate** at the apex clubate setae which shortened posteriorly	**stout** prominent cone-shaped sensilla	**elongate** truncate setae at the apex covered with scales, distinctly shortened posteriorly to prominent cauliflower-shaped sensilla	**elongate** truncate seta at the apex covered with scales distinctly shortened posteriorly to prominent cauliflower-shaped sensilla	**elongate** clubate setae distinctly shortened posteriorly to short prominent sensilla
apex of 15th and 16th lateral scoli	**bisetose** (one seta placed apically and the second slightly below the apex) **seldom unisetose**	with stout prominent **cone-shaped sensilla** (like at the top of remaining lateral scoli)	**unisetose**	**unisetose**	**unisetose**
"ventral organ" number of setae in the middle of: 1st / 2nd / 3nd abdominal segments	most often: **10 / 8 / 4 + 4** elongate cauliflower-shaped sensilla; sometimes: **10 / 8 / 6 + 2** elongate cauliflower sensilla or **10 / 8 / 8**	most often: **8 / 8 / 0 + 6** cauliflower-shaped sensilla as large as remaining cauli-flower-sensilla of the segment; sometimes some of seta doubled or lacking: **7 / 7 / 0** **8 / 9 / 0** **9 / 7 / 0** **9 / 8 / 0** **9 / 10 / 0** **10 / 8 / 0**	**8 / 8 / 8**	**8 / 8 / 4 + 4** elongate cauliflower-shaped sensilla	**8 / 8 / 4 + 4** elongate cauliflower-shaped sensilla

Table 1. Continuation

head with five frontal rows (Fa-Fe) of	**setae** pointed to slightly widened apically	**cauliflower-shaped sensilla**	**setae** slightly widened apically	**setae** slightly widened apically	**setae** pointed to slightly widened apically
emargination of anterior margin of labrum with setae:	6 **long** stout	6 **short** thin	6 **long** thin	6 **long** stout	6 **long** stout
anterior margin of labrum on each side dorsally/ventrally with setae:	1 **short** thin / 2 long stout	1 **short** thin / 1 short thin + 3 long stout	1 **long** thin / 1 long thin + 3 long stout	1 **long** thin / 1 long thin + 2 long stout	1 **short** thin / 1 long thin + 2 long stout
femur of all species with one short seta at base dorsally and with:	11 long pointed **setae**	7 long pointed **setae** + 4 elongate **cauliflower-shaped sensilla**	11 long pointed **setae** often 1-4 of them blunt at the apex	11 long pointed **setae** often 2-4 of them slightly widened apically	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	1 very minute seta
close to each abdominal spiracle placed:	1 very minute seta + 1 slightly longer than the first one seta + 1 cauliflower-shaped sensillum	2 very minute setae + 1 cauliflower-shaped sensillum	1 very minute seta + 1 slightly longer than the first one seta + 1 cauliflower-shaped sensillum	1 seta slightly longer than minute seta + 1 cauliflower-shaped sensillum	1 seta slightly longer than minute seta + 1 cauliflower-shaped sensillum
faecal shield	**exuvia + faeces**	only **exuvia**	**exuviae + faeces**; shield thick, triangular in shape, feaces strongly covered excuvia	**exuviae + faeces**; feaces very easily drop out; often shield only with exuvia	**exuviae + faeces**; feaces loosely connected with exuvia easily drop out

undisturbed specimens forms an umbrella which covers 1/2-3/4 body length. Larvae of *C. stigmatica* are characterized by the largest number of cauliflower sensilla e.g. all setae on head are of cauliflower type while in other species setae on head are pointed or at most slightly widened apically. Apices of all lateral scoli in *C. stigmatica* are stout and strongly cone-shaped while in the remaining species in the anterior half of body scoli are elongate with only apex clubate or shortly coniform. It suggests that in species with more active defense behaviour sensoral setae are less cauliflower-shaped with smaller sensory area at apex of setae. This hypothesis needs verification by the study of ultrastructure of sensory setae and sensory glands in electron microscope.

I observed also differences in the number and arrangement of setae in the "ventral organ". The most constant arrangement was observed in *C. rubiginosa*, *C. nobilis*, and *C. vittata*, the least constant in *C. stigmatica* (see Table 1). I have no hypothesis to explain this phenomenon. The function of the "ventral organ" in first instar is unknown, in last instar I observed (unpublished data) that the larva uses this part of ventrites to attach the abdomen to the substrate (leaf surface or twig).

After the study of the first instars of five species of *Cassida* I have concluded that the best diagnostic characters in this instar are: 1. Shape of setae on head, 2. Shape of apices of lateral scoli, 3. Number and arrangement of setae in "ventral organ", and 4. Number and shape of the setae of labrum.

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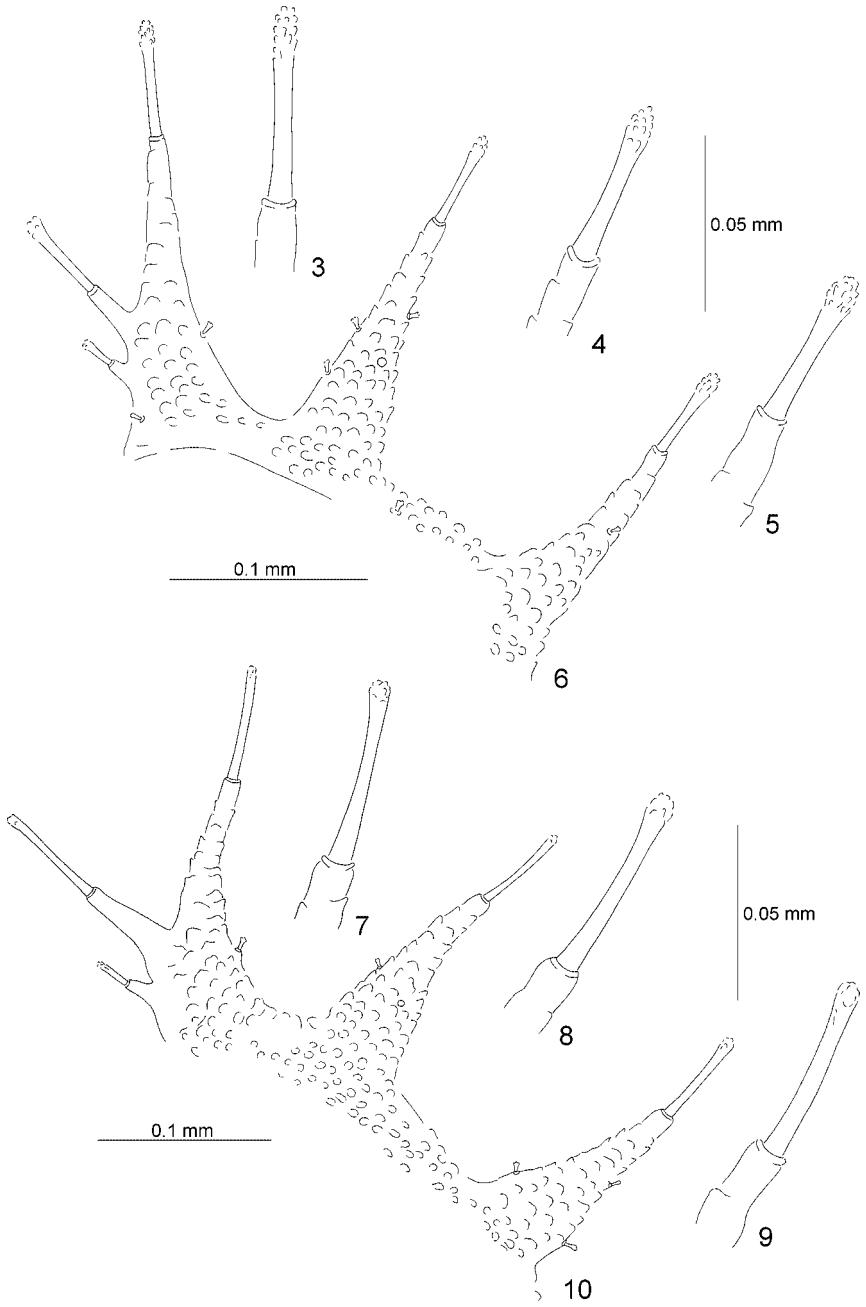
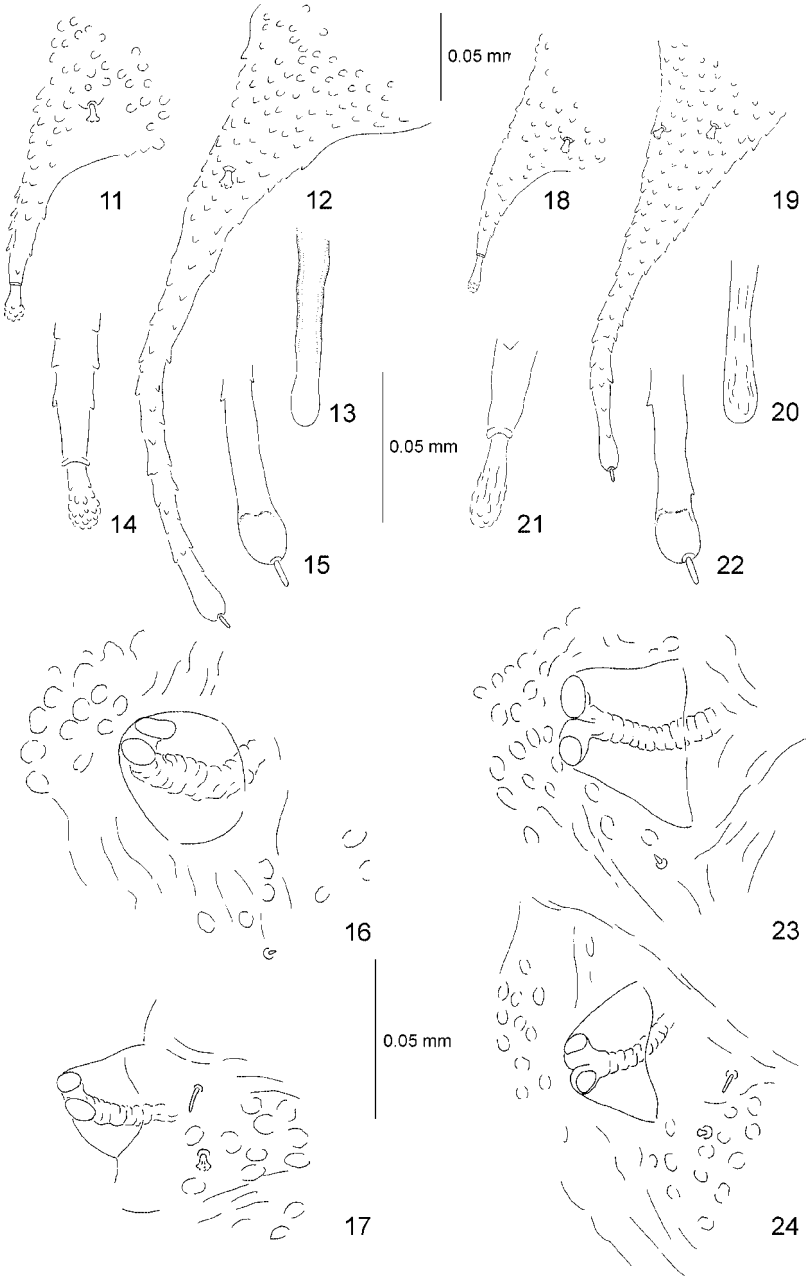
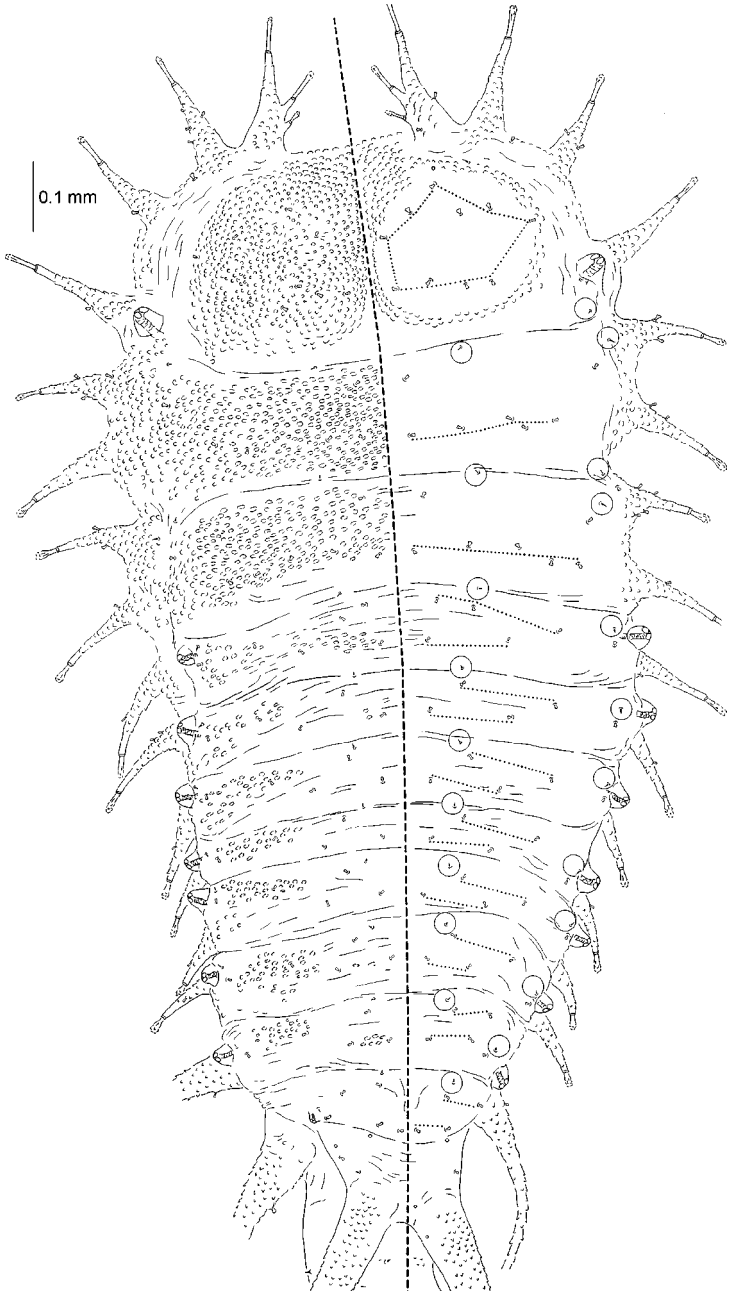


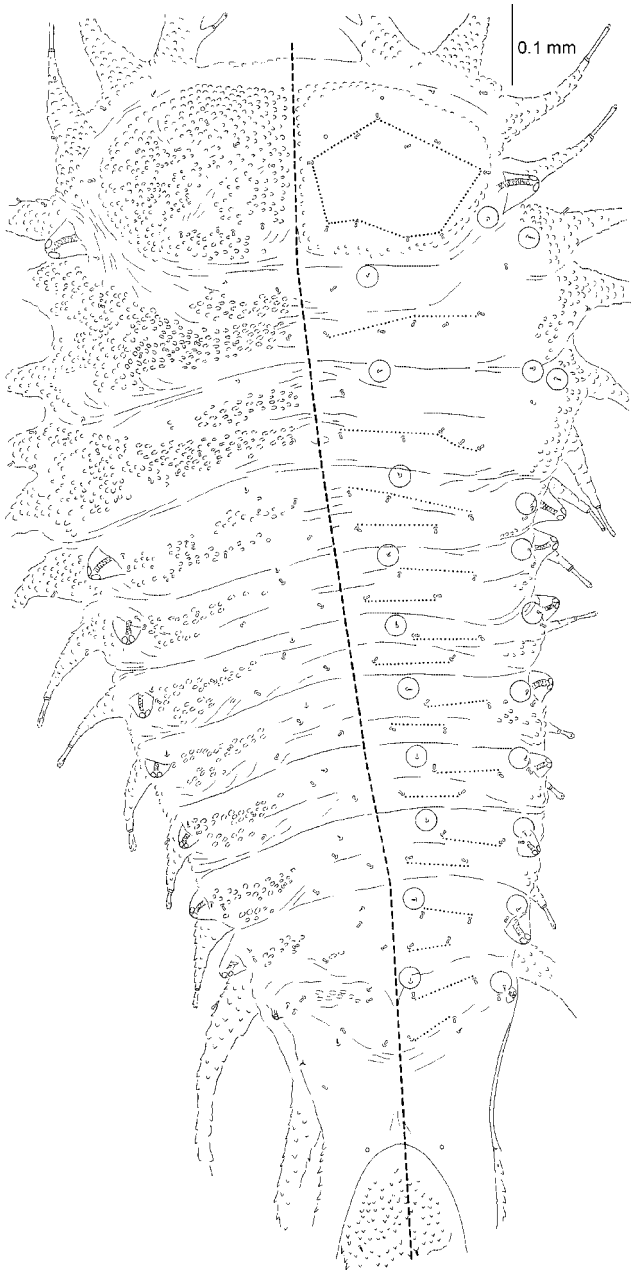
3-6. *Cassida nobilis*. (3) Top of first lateral scolus; (4) top of second lateral scolus; (5) top of third lateral scolus; (6) first three lateral scoli. 7-10. *Cassida vittata*. (7) Top of first lateral scolus; (8) top of second lateral scolus; (9) top of third lateral scolus; (10) first three lateral scoli



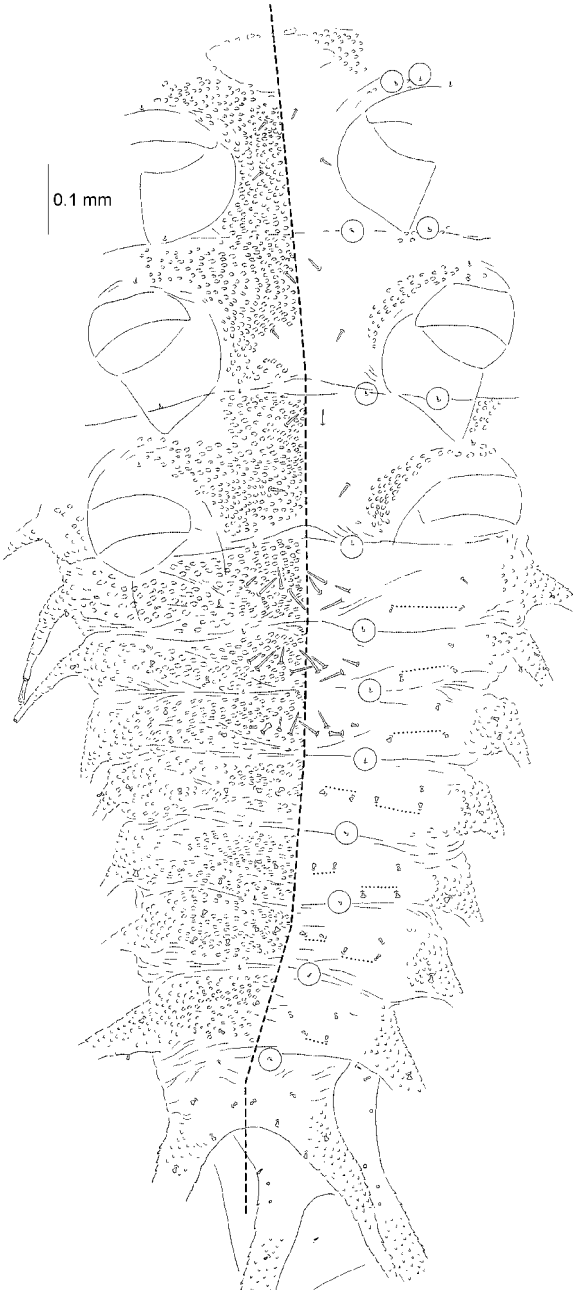
11-17. *Cassida nobilis*. (11) 14th lateral scolus; (12) 15th lateral scolus; (13) top of supra-anal process; (14) top of 14th lateral scolus; (15) top of 15th lateral scolus; (16) spiracle of pronotum; (17) spiracle of first abdominal segment. 18-24. *Cassida vittata* (18) 14th lateral scolus; (19) 15th lateral scolus; (20) top of supra-anal process; (21) top of 14th lateral scolus; (22) top of 15th lateral scolus; (23) spiracle of pronotum; (24) spiracle of first abdominal segment



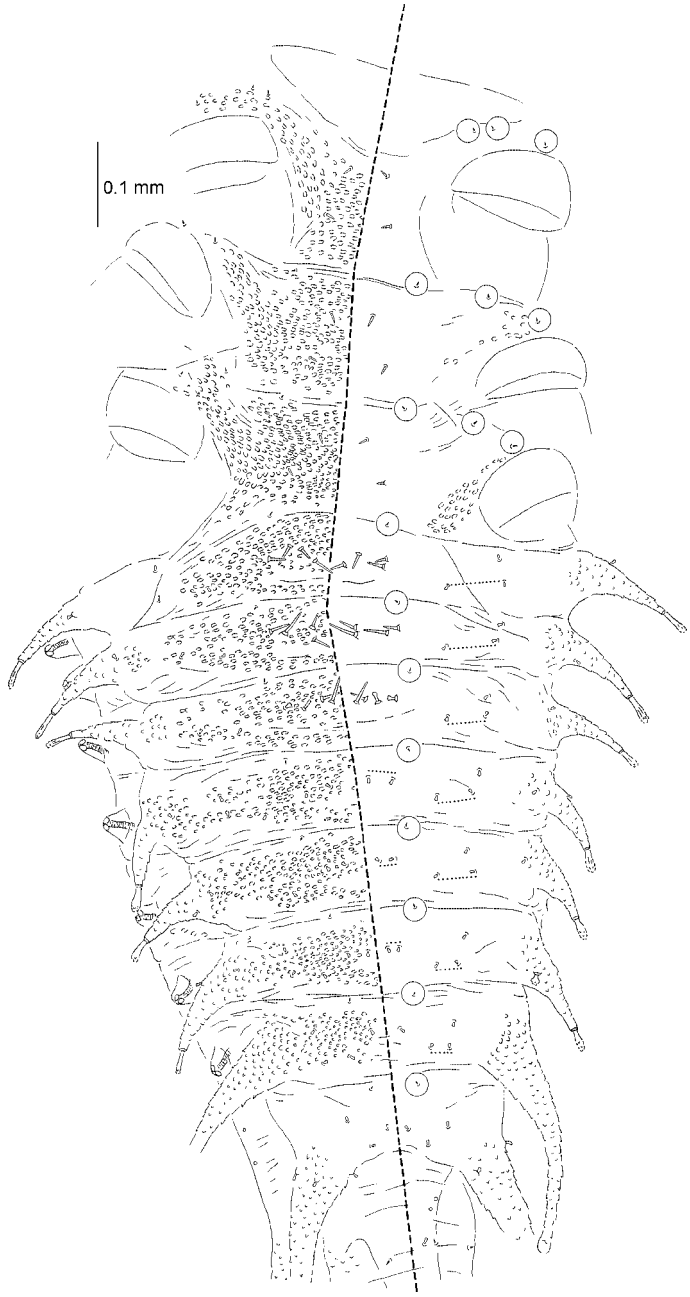
25. *Cassida nobilis*, chaetotaxy of dorsal side of body



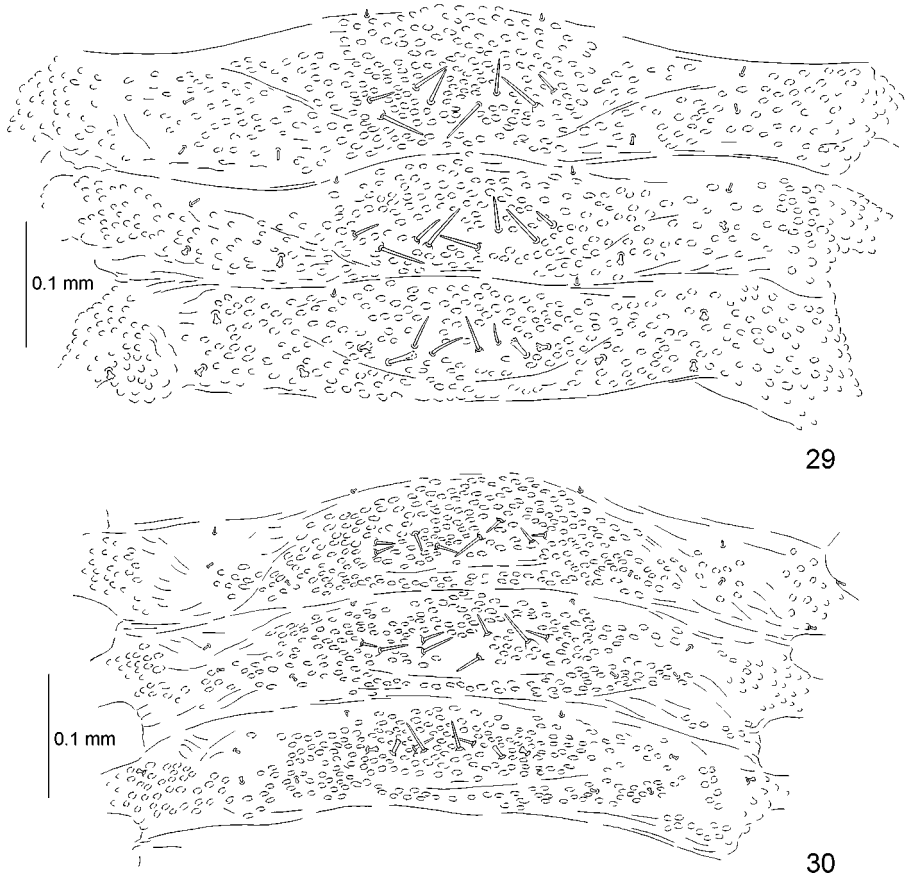
26. *Cassida vittata*, chaetotaxy of dorsal side of body



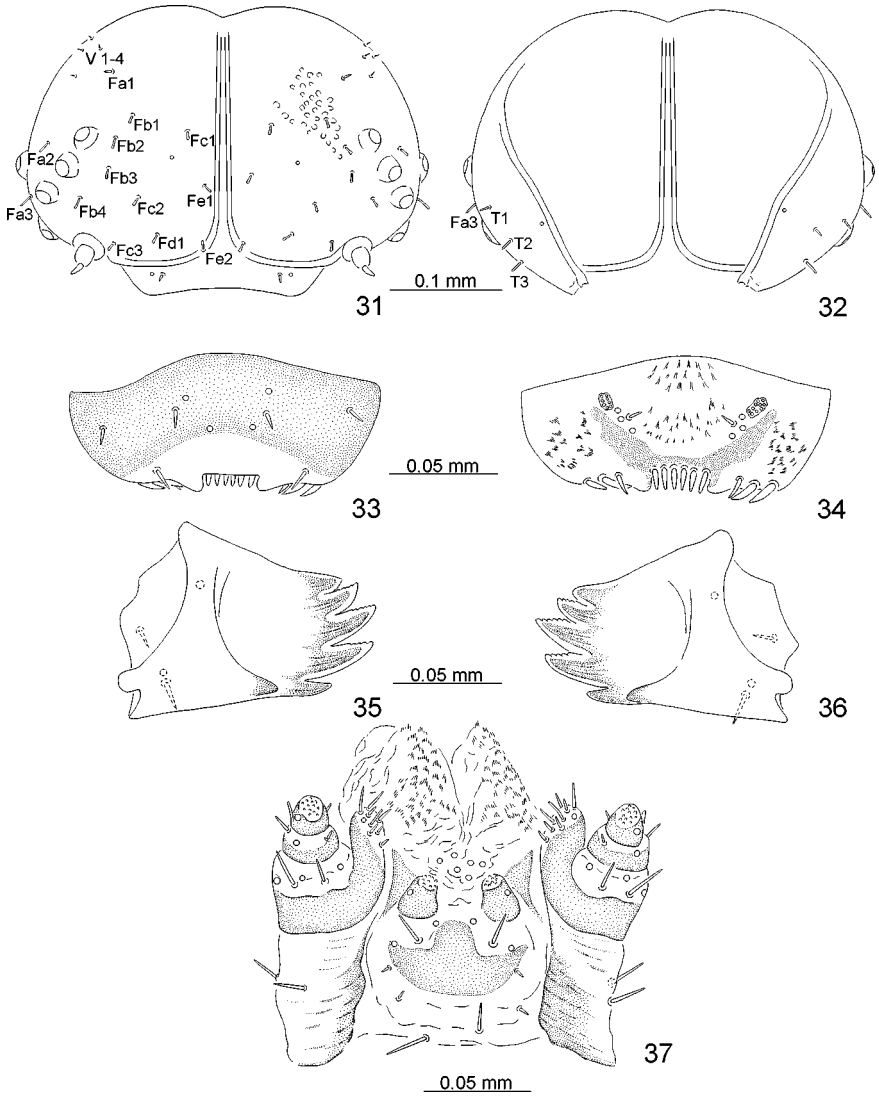
27. *Cassida nobilis*, chaetotaxy of ventral side of body



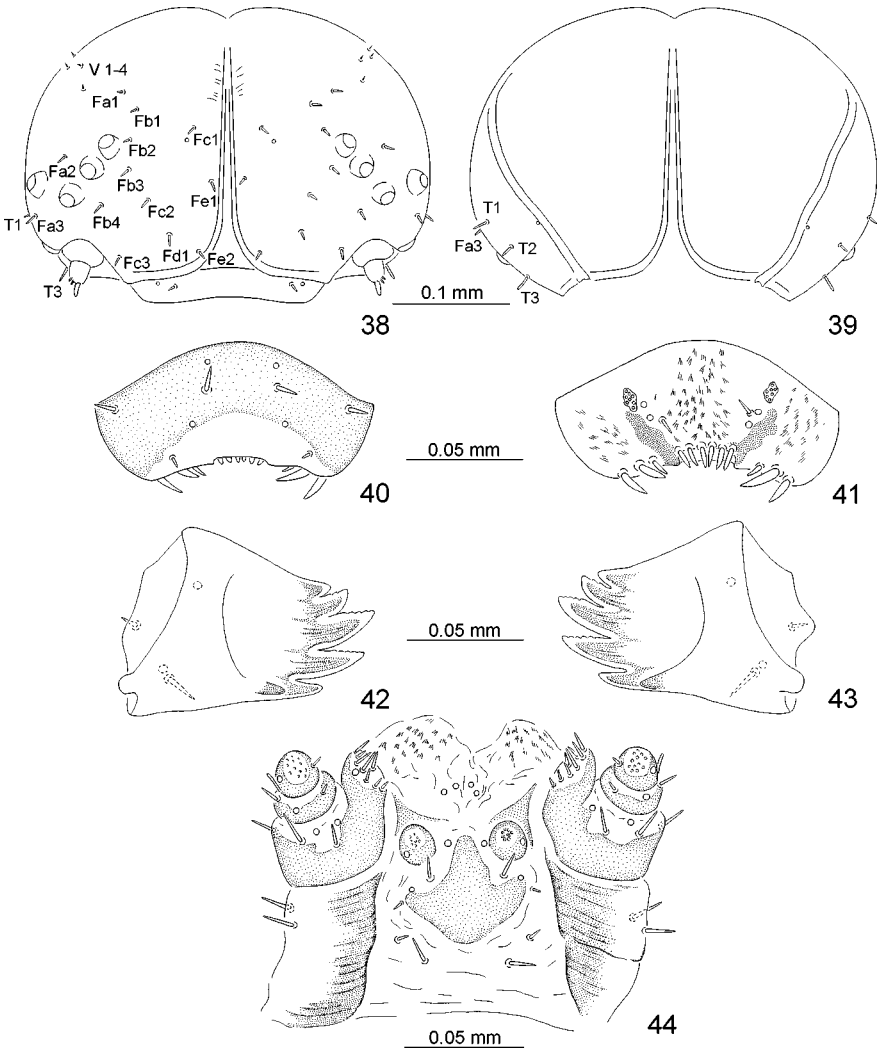
28. *Cassida vittata*, chaetotaxy of ventral side of body



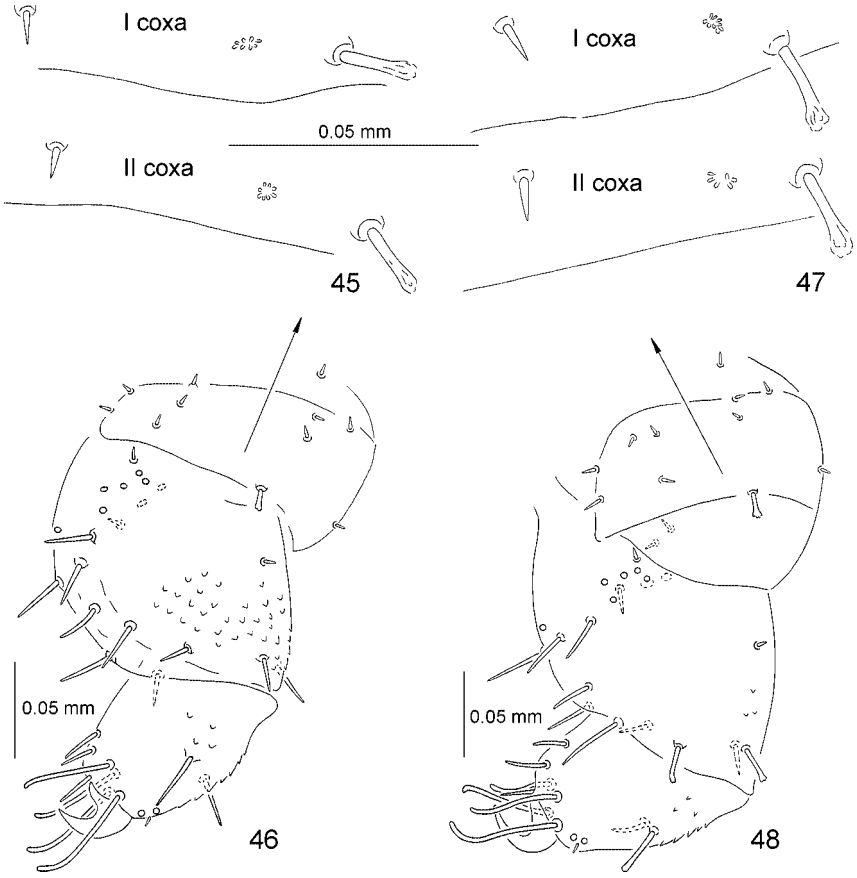
29, 30. Chaetotaxy of ventral side of first three abdominal segments. (29) *Cassida nobilis*; (30) *Cassida vittata*



31-37. *Cassida nobilis*. (31) Frontal side of head; (32) temporal side of head; (33) dorsal side of labrum; (34) ventral side of labrum; (35, 36) mandibles; (37) maxillae and labium



38-44. *Cassida vittata*. (38) Frontal side of head; (39) temporal side of head; (40) dorsal side of labrum; (41) ventral side of labrum; (42, 43) mandibles; (44) maxillae and labium



45, 46. *Cassida nobilis*. (45) Sensillum or a group of sensilla on first and second coxa, (46) external side of leg. 47, 48. *Cassida vittata* (47) sensillum or a group of sensilla on first and second coxa, (48) external side of leg