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Redescription of immatures and bionomy of the Palearctic species *Dicladispa testacea* (Linnaeus, 1767) (Coleoptera: Chrysomelidae: Cassidinae: Hispini), a leaf-mining hispine beetle

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Abstract

Dicladispa testacea (Linnaeus, 1767) is a member of the tribe Hispini Gyllenhal, 1813 associated with plants of the family Cistaceae Juss. and is widely distributed in the Mediterranean Basin. Immature stages are described in detail, including line drawings, chaetotaxy, sculpture of integument, and SEM photos of morphological details. This is the first detailed description of immatures in the tribe Hispini, and this can be regarded as a model description for studies of other species in the tribe. Diagnostic characters for this species in comparison with other described larvae and pupae of the genus *Di-cladispa* and the sympatric *Hispia atra* Linnaeus, 1767 are discussed. Some remarks on the biology of *Di-cladispa testacea*, such as host plants, feeding patterns of adults, structure of larval and pupal mines, are also given.

Key words: morphology, immature, larva, pupa, Coleoptera, Chrysomelidae, Cassidinae, *Di-cladispa testacea*, *Cistus* sp., Mediterranean region

Introduction

The leaf beetle genus *Di-cladispa* Gestro, 1897 is a member of the speciose tribe Hispini Gyllenhal, 1813 (Chrysomelidae: Cassidinae) and comprises 125 species occurring mostly in tropics and subtropics of the Old World with a few species in the warm parts of the Palearctic Region (Staines 2012). Six of them occur in the western Palearctic, especially in the Mediterranean subregion and the Middle East (Borowiec & Sekerka 2010). The most widely distributed species is *Di-cladispa testacea* (Linnaeus, 1767) recorded from Europe: Albania, Bulgaria, Croatia, France, Greece, Italy, Portugal, Spain, Switzerland, European part of Turkey, Serbia and Montenegro; from North Africa: Algeria, Morocco, Tunisia; and from Asia: Syria and Turkey (Borowiec & Sekerka 2010).

Di-cladispa testacea is associated with plants of the family Cistaceae Juss., especially the genus *Cistus* Linnaeus. Like other members in the tribe Hispini larvae, are leaf-miners and the whole life cycle is completed on the leaves of plants belonging to *Cistus*. The following species of *Cistus* were reported as food plants: *Cistus albidus* L. (Lesne 1906; Gestro 1919; Magistretti & Ruffo 1959), *C. albidus crispus* Del. (Grandi 1935), *C. florentinus* Lam. (Grandi 1935), *C. hirsutus* Lam. (Grandi 1935), *C. incanus* Rch. (Grandi 1935), *C. ladanifer* L. (García-Ocejo *et al.* 1992), *C. monspeliensis* L. (Lesne 1906; Gestro 1919; Buhr 1930; Magistretti & Ruffo 1959; Hering 1967), *C. salvifolius* L. (Laporte & Laporte 1852; Lesne 1906; Gestro 1919; Frost 1924; Needham *et al.* 1928; Maulik 1937; Magistretti & Ruffo 1959; Hering 1967), and *C. villosus* L. (Amsel & Hering 1931; Magistretti & Ruffo 1959; Hering 1967). Biondi *et al.* (1995) noted that it is associated also with other Cistaceae genera such as *Halimium* (Dunal) Spach and *Helianthemum* Mill., but without giving references, they also noted that adults were found in leaf litter under trees of *Pistacia lentiscus* L. (Anacardiaceae).

Although *Di-cladispa testacea* has a relatively large distribution area, and the host plants of this species are common in the Mediterranean subregion and were reported by various authors, different immatures stages of

D. testacea were described only a few times: under the name *Hispa testacea* by Perris 1855: 260–270, pl. V figs 79–92 (biology, larva, pupa); Lesne 1906: 68–70 (biology, larva); Grandi 1935: 19 (larva); Cox 1996: 172, fig. 300 (pupa); under the name *Dicladispa testacea* by Uhmann 1965: 118–121 (pupa); Steinhausen 1994: 306, fig. 355 (key to the larvae), 2002: 22, fig. 170 (pupa); Bordy 2000: 63–67 (biology, larva, pupa). All these descriptions are not detailed, i.e. without information on chaetotaxy, body sculpture, morphological details, and do not describe good diagnostic and potential phylogenetic characters. Some biological data were noted by Perris (1855), Lesne (1906), Needham *et al.* (1928), and Bordy (2000).

In this publication, we describe larvae and pupae of *Dicladispa testacea* and present some aspects of its biology. This first detailed description of immatures of a representative of the tribe Hispini is suggested to be a model description for other species in the tribe and completes the study of immatures of hispoid cassidines presented in previous works on representatives of the tribes Callispini (Lee *et al.* 2012) and Oncocephalini (Świętojańska *et al.* 2006, Świętojańska & Kovac 2007, Lee *et al.* 2009).

Material and methods

Immatures of *Dicladispa testacea* were collected at first on the host plants *Cistus monspeliensis* L. and *C. albidus* L. on Majorca Island from 07–20th May 2009, leg. Jolanta Świętojańska and Lech Borowiec. Both mature larvae and pupae were collected in the localities Eremita San Salvador and Eremita Betle'em; in Ses Salines and Cala Mondrago only pupae were collected. In all of these areas there were plenty of adults. Another sample of immatures were collected from *Cistus incanus* agg from 24th August to 05th September 2009 in Greece, Macedonia Province, Halkidiki, Holomontas Mts., n. Arnea, 703 m, 40.28N/23.34E, leg. Jolanta Świętojańska and Lech Borowiec. On faded blossoms of host plants numerous adult beetles were observed, most of them during copulation. Leaves possessed numerous traces of feeding, eggs and larval mines, and several specimens of first and second instar larvae were collected.

Collected larvae and pupae were preserved in 75 to 80% ethanol.

Seven first instar larvae, one larva older than second instar but younger than mature larva, and eight specimens each of the second instar, mature larva and pupa of *Dicladispa testacea* were measured and examined. For detailed examination larvae were mounted on slides. For preparing slides, larvae which were initially killed and preserved in 75 to 80 % ethanol were removed and boiled in 10% NaOH solution, cleared in distilled water and then mounted on slides with Swan's liquid (distilled water 20 g, gum arabic 15 g, chlorhydrate 60 g, glucose 3 g, glacial acetic acid 2 g) and glycerine for light microscopy. Heads of the larvae were separated from the rest of the body and then the mouthparts were dissected.

Slides and measurements of larvae were made using a Nikon SMZ 1500 stereomicroscope. A Nikon ECLIPSE 80i light microscope with phase contrast was used for specimen examination and drawing figures.

The photos of mature larvae were made using a Nikon digital camera D 5100 and Nikon SMZ 1500 stereomicroscope and Helicon Focus software.

Larvae for SEM examination were transferred from 75% to 100% ethanol and dried using HMDS (Hexamethyldisilazane). After fixing on stubs with carbon tabs they were sputter-coated with gold and examined with a LEO 435 VP scanning microscope at magnifications up to 20 000 x.

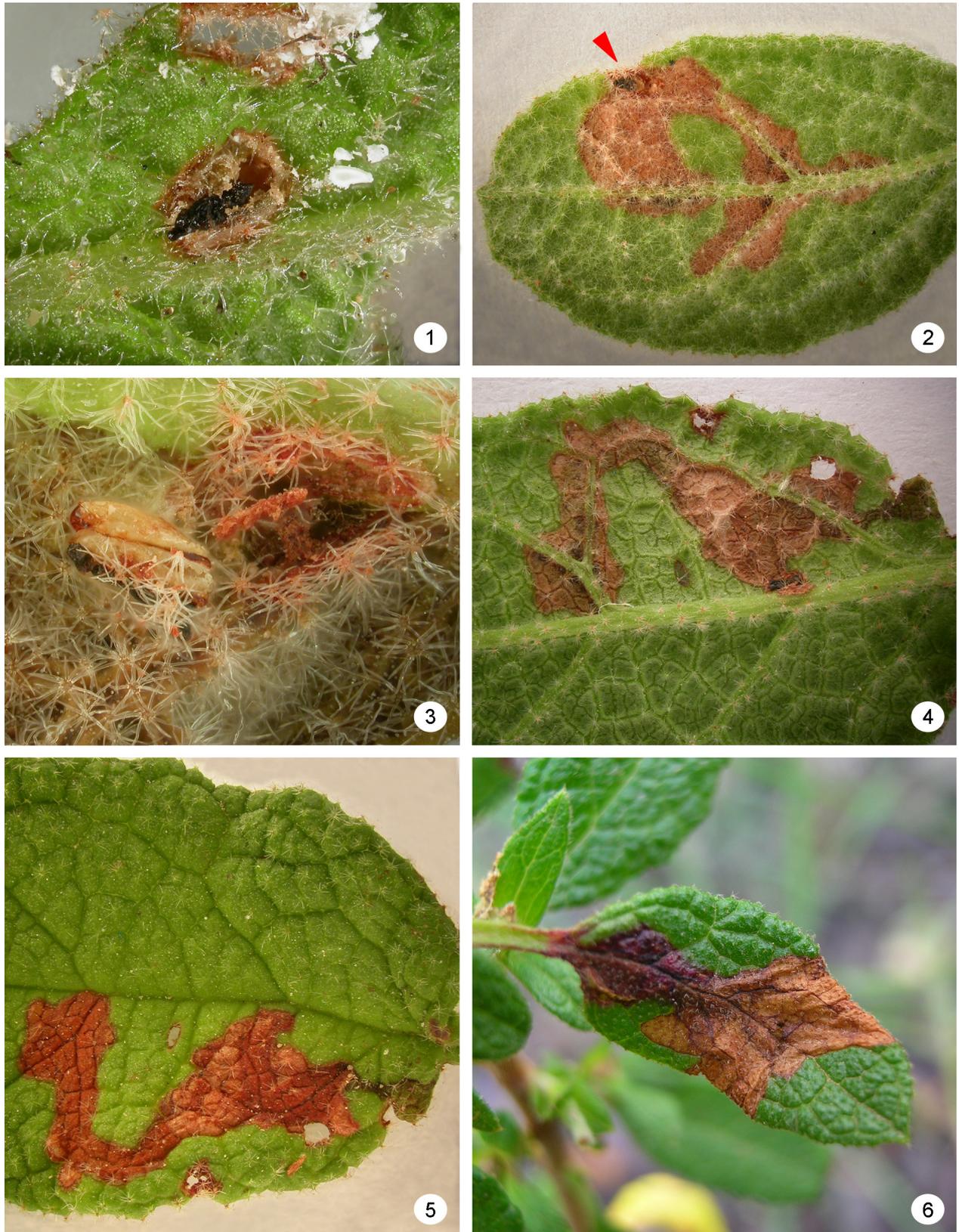
All studied materials have been deposited at Department of Biodiversity and Evolutionary Taxonomy, University of Wrocław, Poland.

Description

Dicladispa testacea (Linnaeus 1767)

Egg (Figs. 1–3).

Elongate-oval, yellowish-white (Figs. 3), deposited singly or quite often two eggs within the hole produced by female on the underside of host plant leaf (Figs 1–3). The only one egg was measured. Length of measured egg: 0.97 mm, width 0.42 mm.



FIGURES 1–6. *Dicladispa testacea*. **1, 3.** eggs; **2, 4–6.** mines of first instar larva, in Fig. 2 red arrow indicate place where egg was deposited.



FIGURES 7–12. *Dicladispa testacea*. 7–11. eggs; 12. second instar larva.

Larva (Figs 12, 44–60, 63–116).

General body structure is the same in all examined larvae, the difference between each instar are pointed in the description given below.

Measurements are presented in Table 1. Length was measured without head, from anterior border of pronotum to the tip of body; width of body was measured across pronotum (at base) and abdominal segment IV (in the middle).

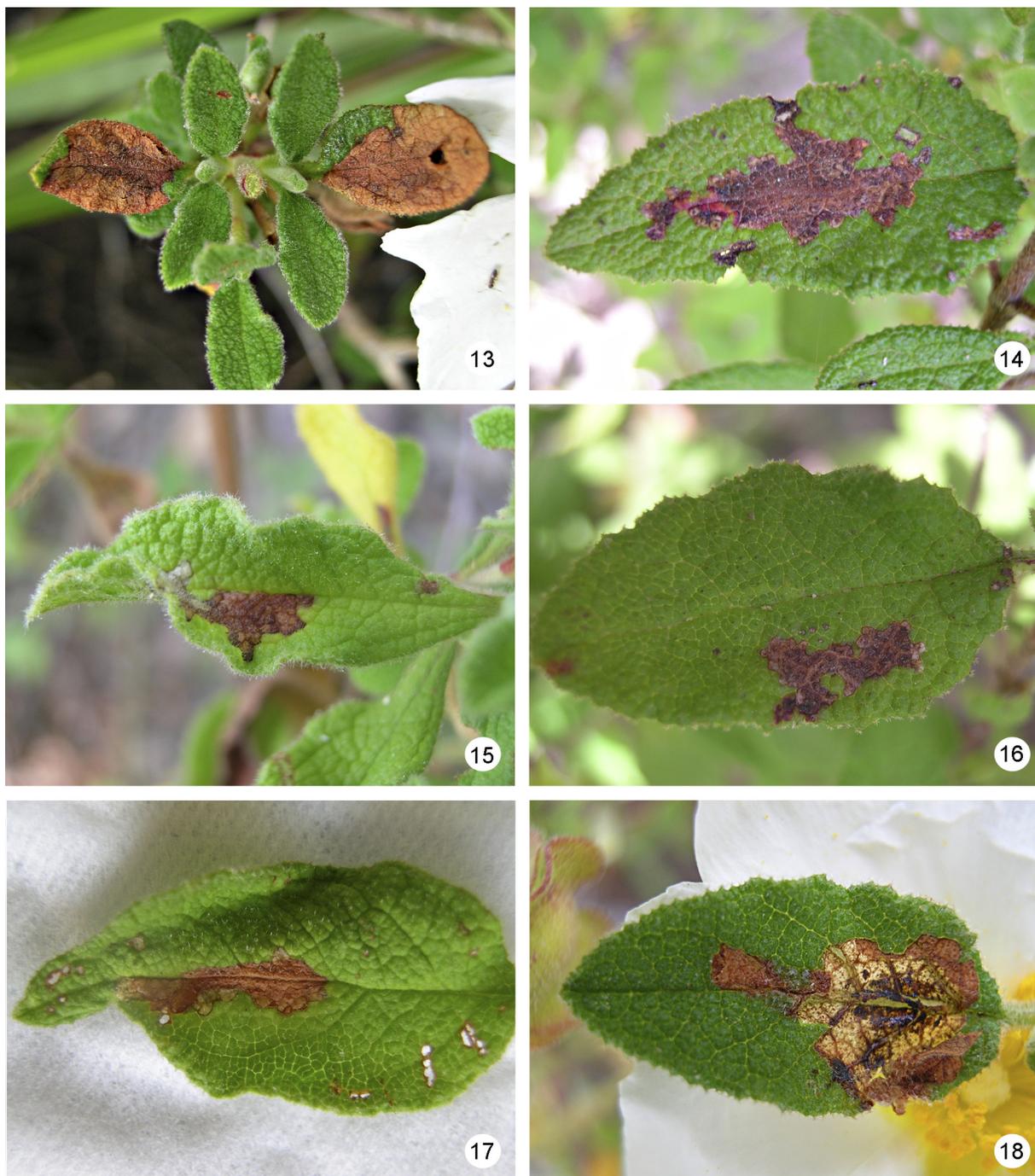
Body distinctly flattened dorso-ventrally (Fig. 65), larva of instar 1, 2 and 3 (or 4) elongate (Figs 12, 44, 45, 63, 64), in instar 1 and 2 the pronotum slightly wider than abdominal segments (Figs 63, 64, 72), in instar 3 (or 4) body parallelsided (pronotum as wide as abdominal segments) whereas mature larvae are elongate-oval and widest across abdominal segment IV–V (Figs 46, 47, 59, 60, 84). Body of alive as well as alcohol-preserved larvae yellowish-white with two irregular brown patches on pronotum, brown posterior half of abdominal segment VIII, brown triangular patch on prosternum, dark brown spiracles, brown head and legs (Figs 59, 60).

TABLE 1. Measurements of examined immature instars of *Dicladispa testacea*.

<i>Dicladispa testacea</i>	length of body [mm]	width of prothorax at base [mm]	width of abdominal segment IV [mm]	width of head [mm]
first instar larva				
1.	1.00	0.59	0.53	0.36
2.	1.12	0.58	0.51	0.40
3.	0.92	0.55	0.46	0.40
4.	1.32	0.58	0.54	0.40
5.	0.92	0.60	0.52	0.41
6.	1.03	0.63	0.56	0.41
7.	1.23	0.65	0.56	0.41
second instar				
1.	1.00	0.69	0.63	0.46
2.	1.03	0.66	0.58	0.46
3.	1.20	0.68	0.56	0.46
4.	1.28	0.68	0.61	0.46
5.	1.92	0.70	0.60	0.46
6.	1.64	0.68	0.56	0.46
7.	1.55	0.71	0.65	0.48
8.	1.60	0.70	0.50	0.48
third or fourth (?) instar				
1.	2.73	0.93	0.93	0.60
mature larva				
1.	3.00	1.17	1.60	0.65
2.	2.70	1.20	1.65	0.65
3.	4.25	1.25	2.15	0.67
4.	4.10	1.25	2.25	0.70
5.	3.70	1.25	2.30	0.72
6.	3.95	1.25	2.27	0.68
7.	4.00	1.25	2.25	0.72
8.	4.00	1.25	2.10	0.68
pupa				
1.	4.50	1.60	2.15	-
2.	4.60	1.60	2.00	-
3.	4.45	1.55	2.15	-
4.	4.00	1.35	1.85	-
5.	4.30	1.40	1.85	-
6.	4.00	1.40	2.00	-
7.	4.25	1.45	2.10	-
8.	4.00	1.30	1.65	-

Body with 8 pairs of lateral scoli placed only on abdominal segments (Figs 44–47, 59, 60, 63, 64, 68, 69, 97, 98). Lateral scoli short, approximately the same length, without lateral branches but with 2 setae at base: one placed anteriorly, second—ventrally. All lateral scoli apically armed with seta (Fig. 99).

Granulation of body distinct in all examined instars (Figs 44, 45, 63–65, 68, 69, 72–78, 80–102). Minute setae at anterior border of each tergite and sternite (except for sternite of VIII segment); tergites and sternites covered with short pointed setae (Figs 44–47, 75, 81–83). Tergites of meso-, metathorax, abdominal segments I–VII and sternites I–VII of abdomen with transverse grooves (Figs 44–47, 59, 60, 63, 64, 72, 74–76, 82, 84, 86, 89, 90). Sternites of meso- and metathorax with two structures shaped similarly to the transverse grooves of other sternites and tergites but distinctly shorter (Figs 45, 47, 64, 73, 85, 87, 88). Posterior border of each transverse groove with distinct asperites (Figs 68, 74, 75, 77, 88). Asperites also present at the anterior border of pronotum (Figs 78, 79).



FIGURES 13–18. *Dicladispa testacea*, larval mines. **18.** opened larval mine.

Pronotum on each side with 12 setae arranged in constant pattern as in Figs 44, 46. Meso-, metanotum with 6 minute setae at anterior border (a pair in the middle and a pair on each lateral side); a row of 10 setae running across segment; and a group of 5 setae on each side of tergite laterally. One seta—two times longer than other setae of tergites and sternites—placed on slightly visible protuberance on each side of meso- and metanotum. Abdominal tergites I–VII with 4 minute setae at anterior border (sometimes in mature larvae abdominal tergite I with 6 minute setae at anterior border); two rows of setae running across segment, both with 4 setae; 2 setae and campaniform sensillum placed close to each spiracle. Abdominal tergite VIII with 2 minute setae at anterior border medially; three rows of setae running across segment: anterior with 4 setae, next with 8 setae; and posterior with 2 setae (placed between spiracles). Posterior border of abdominal segment VIII in instar 1 and 2 with 8 pointed setae, in mature larvae each seta placed at the top of short process (Figs 44–47, 77, 91–94).



FIGURES 19–24. *Dicladispa testacea*, mines of pupae.



FIGURES 25–32. *Dicladispa testacea*, leaves of host plant with feeding patterns of adults.



FIGURES 33–38. *Dicladispa testacea*. 33, 34. exuviae of pupae; 35. freshly emerged adult; 36–38. adults.



39



41



40



42



43

FIGURES 39–43. *Dicladispa testacea*, adults. 40–43. mating adults.

Prosternum in posterior half with two pairs of pointed setae medially and 2 setae on each lateral side at base of leg (Figs 45, 47). Meso- and metasternum with 4 minute setae at anterior border; two pairs of pointed setae medially; and one seta on each lateral side at base of leg. Abdominal sternites I–VII with a pair of minute setae at anterior border medially; with row of 6 setae running across segment medially; and 3 setae on each side of sternite laterally. Abdominal sternite VIII with rows of 4 setae running across segment in anterior part and one seta in posterior part at base of each spiracle. Four setae along anterior base of anus. Segments which build anus not elevated (Figs 45, 47, 60, 64, 77, 84, 93).

Nine pairs of distinct spiracles: one on thorax and 8 on abdomen (Figs 63, 70, 71, 95, 96). In instar 1 and 2 diameter of spiracles approximately the same size, including spiracles of VIII abdominal segment, only spiracles of thorax slightly more elevated than the others. In mature larvae as in younger instars, spiracles of thorax slightly more elevated than those on abdomen, diameter of spiracles of abdominal segments I–VII approximately the same size, but spiracles of abdominal segment VIII distinct, elevated and conical (Figs 91–94).

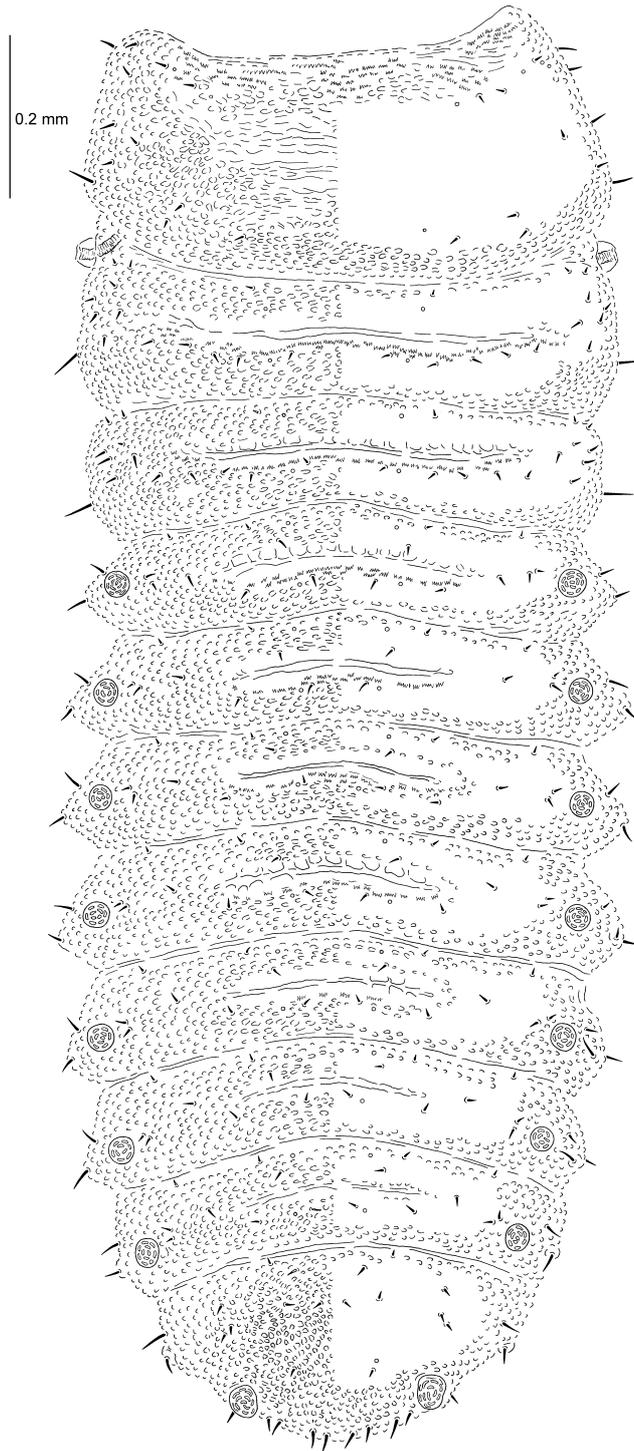


FIGURE 44. *Dicladispa testacea*, first instar larva, dorsal aspect.

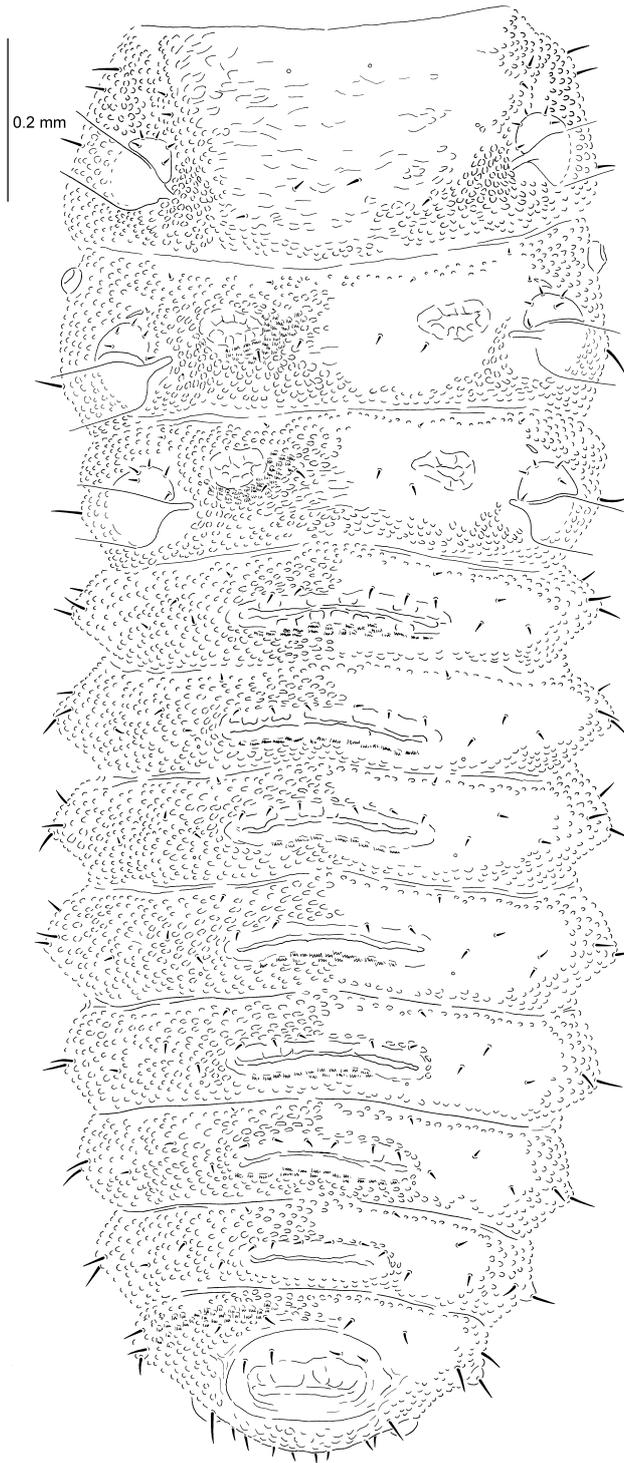


FIGURE 45. *Dicladispa testacea*, first instar larva, ventral aspect.

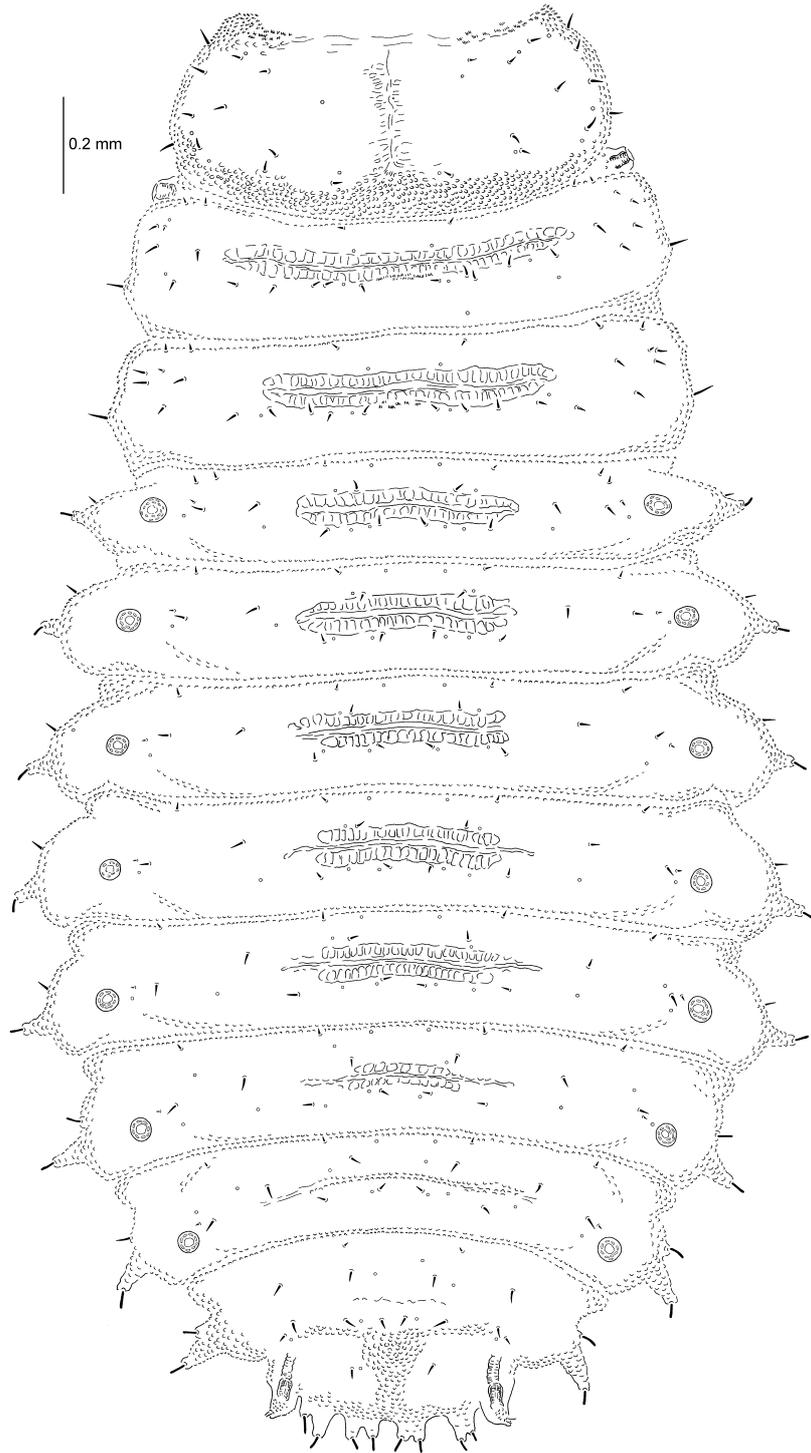


FIGURE 46. *Dicladispa testacea*, fifth instar larva, dorsal aspect.

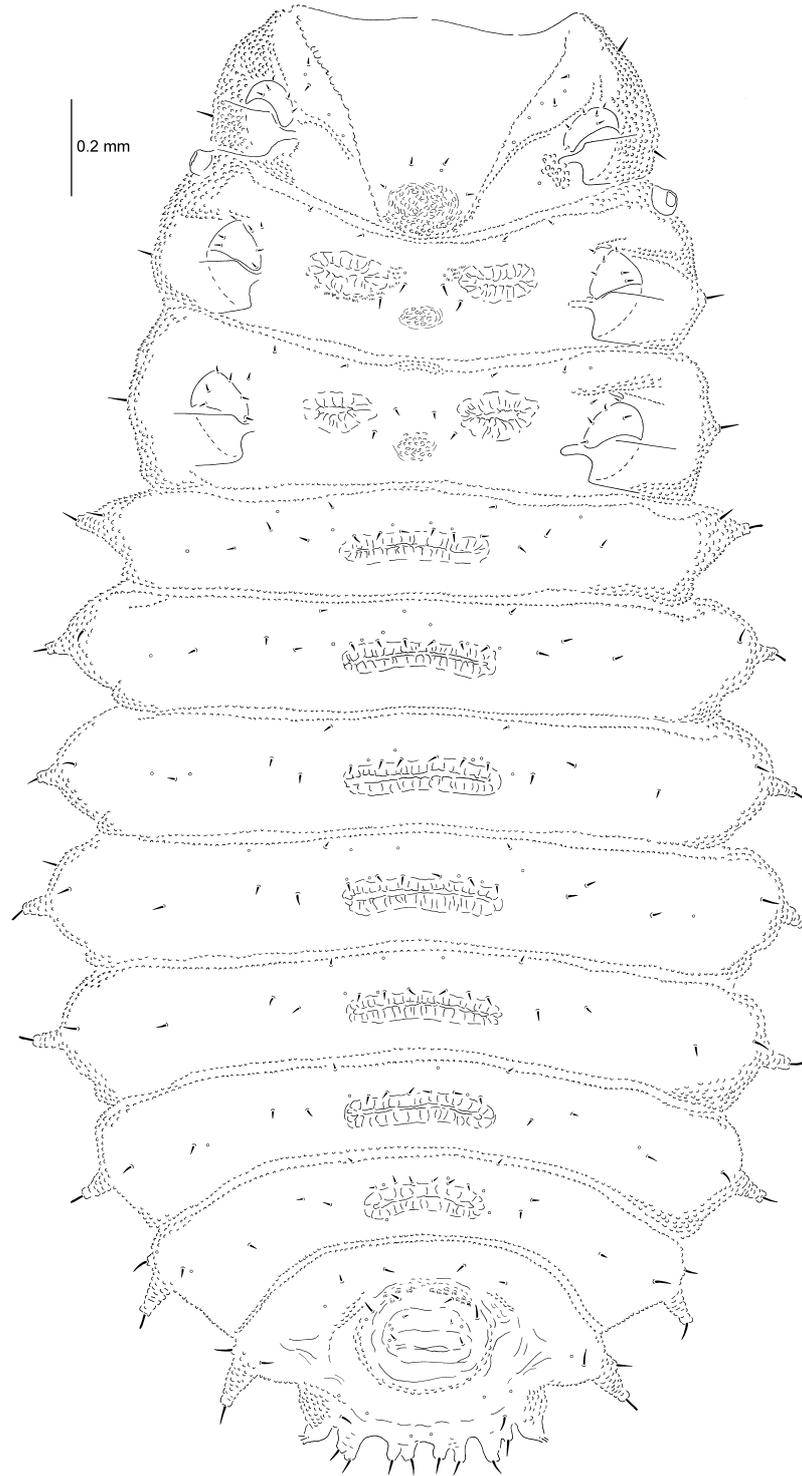
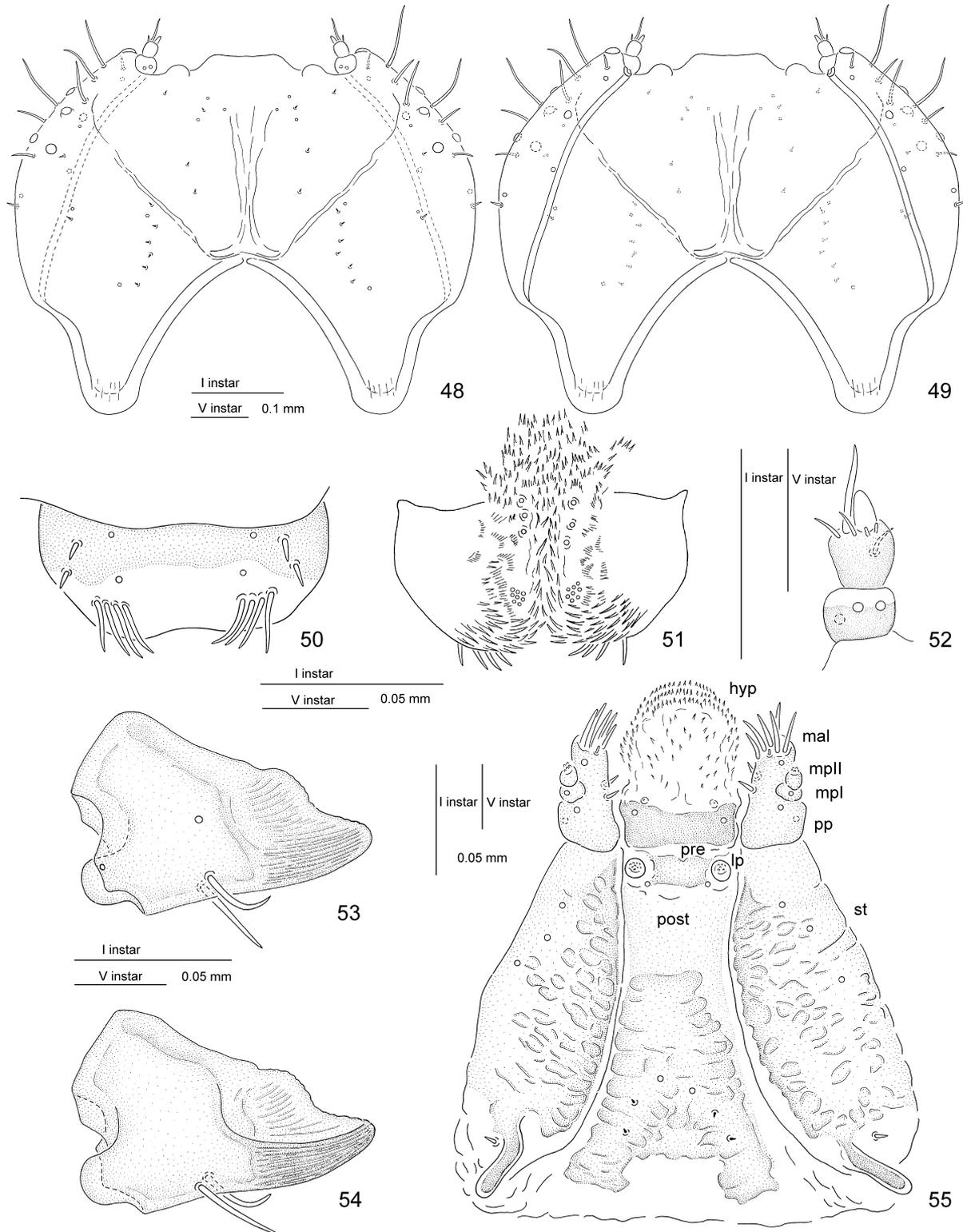
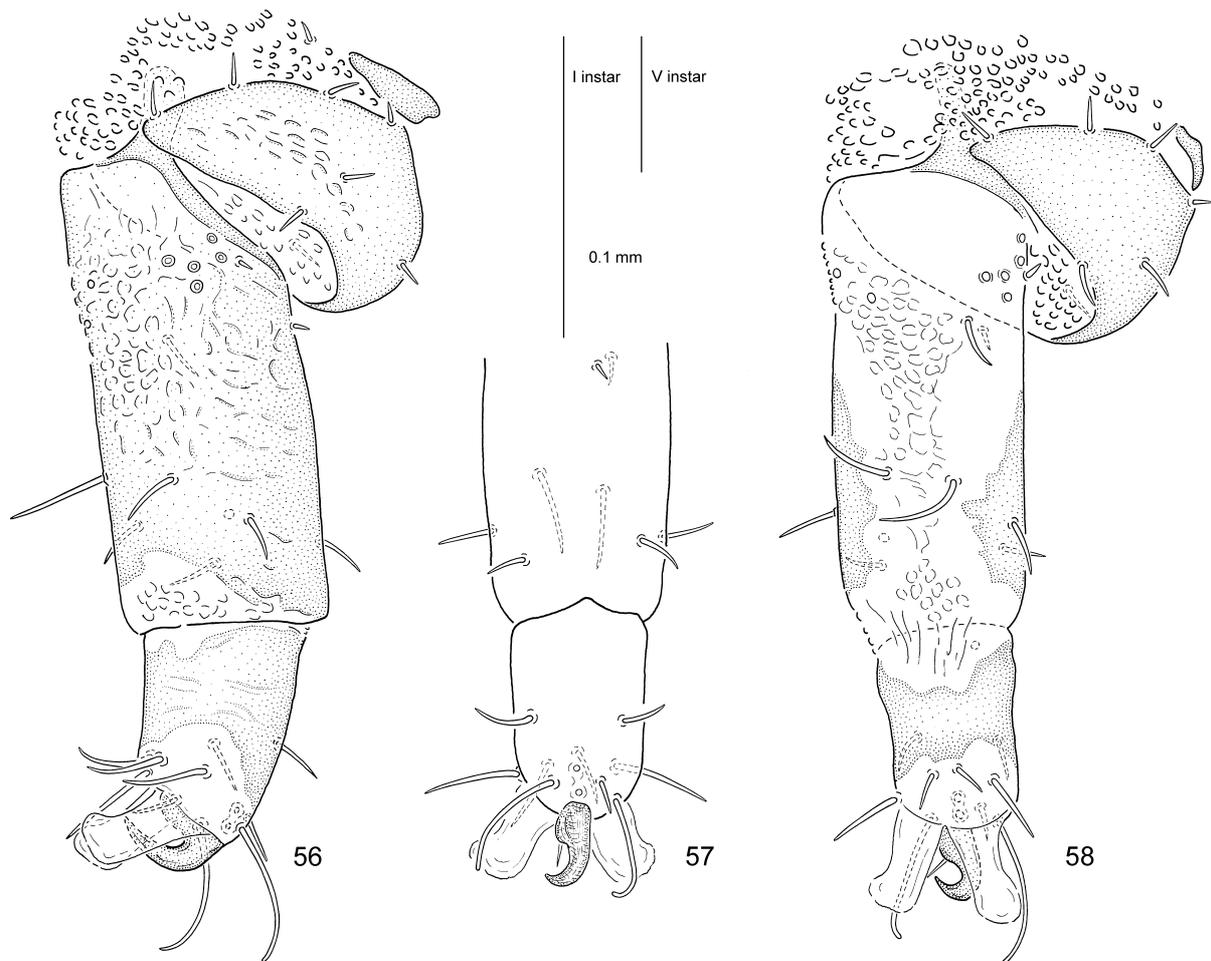


FIGURE 47. *Dicladispa testacea*, fifth instar larva, ventral aspect.



FIGURES 48–55. *Dicladispa testacea*, larva, scale show differences in size between first and fifth instar larvae. **48.** frontal side of head; **49.** temporal side of head; **50.** dorsal side of labrum; **51.** ventral side of labrum; **52.** antenna; **53, 54.** mandibles; **55.** maxillae and labium, ventrally. Abbreviations: **hyp**—hypopharynx; **lp**—labial palp; **mal**—mala; **mpI**—first segment of maxillary palp; **mpII**—second segment of maxillary palp; **post**—postmentum; **pp**—palpifer; **pre**—prementum; **st**—stipes.



FIGURES 56–58. *Dicladispa testacea*, leg of larva, scale show differences in size between first and fifth instar larvae. **56.** internal side of leg; **57.** top of femur and tibiotarsus dorsally; **58.** femur and tibiotarsus ventrally.

Head well sclerotized, prognathous, partially retracted into pronotum (Figs 48, 49, 59, 60, 63–65, 104, 106, 108). Epicranial stem absent; median endocarina wide, extending between frontal arms; frontal arms V-shaped, fronto-clypeal suture absent, clypeo-labral suture well developed (Fig. 109). Clypeus wider than long, slightly marked, without setae and campaniform sensilla.

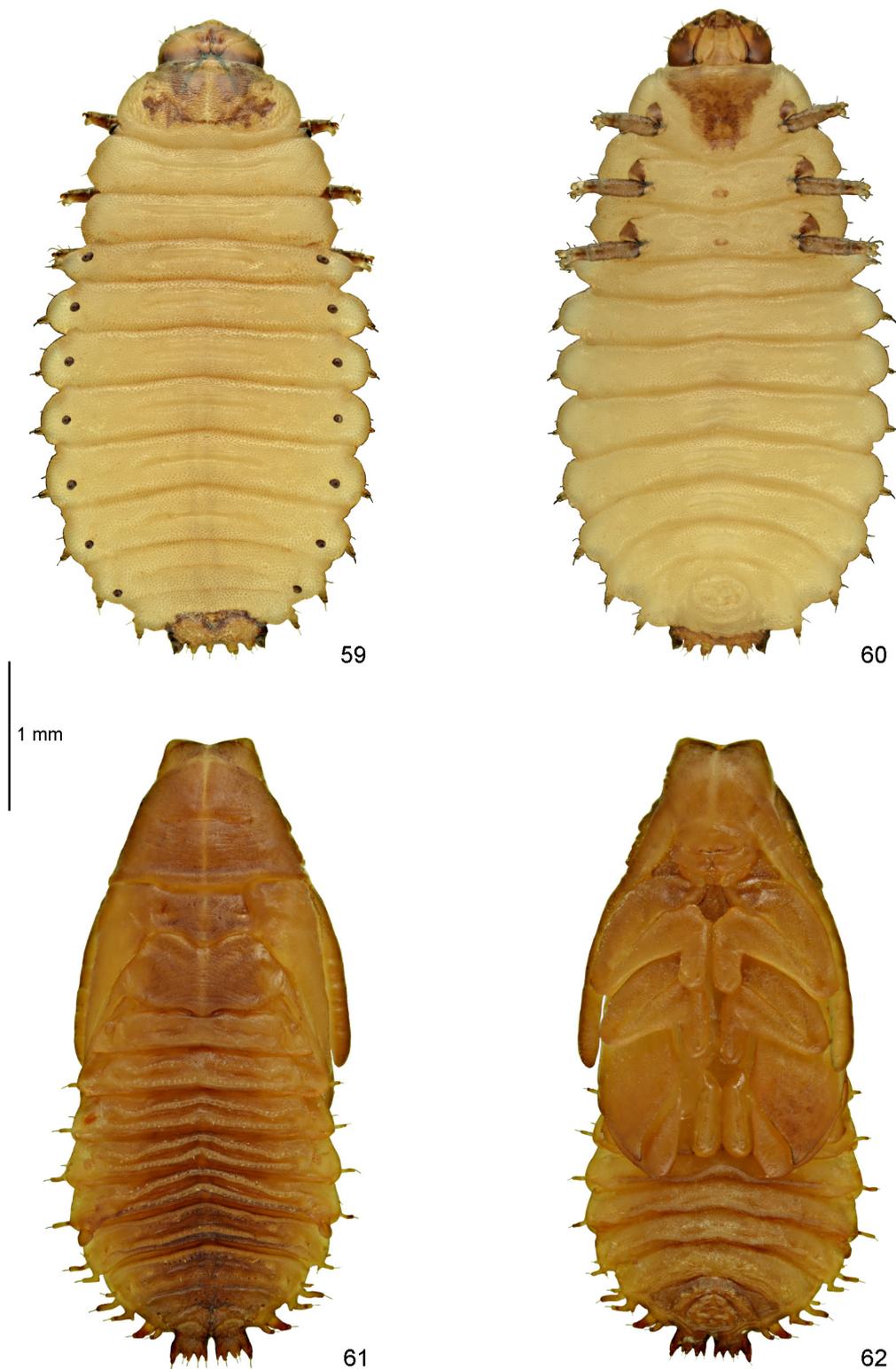
Six stemmata on each side of head, the smallest placed ventrally, hardly visible (Figs 48, 49, 107).

Head on each side with 4 small vertical pointed setae (V 1–4) and four frontal rows of pointed setae: row Fa with 3 setae, Fb with 5 setae, Fc with three setae, Fd with single seta and Fe absent. Temporal side of head with 4 setae (T 1–4) and three campaniform sensilla. Several campaniform sensilla present: one close to setae on vertex, one close to seta Fa1, one or two close to setae Fb1 and Fb2, one between setae Fb3 and Fb4, three close to seta Fd1, three setae on temporal side (Figs 48, 49, 104–108).

Antennae 2-segmented, set in membranous ring (Figs 52, 116). Both segments stout, approximately as wide as long. First segment with 3 campaniform sensilla, second segment with a group of sensilla at the apex: one prominent (sensory appendix), one long seta, 3 shorter setae and 2 small peg-like sensilla.

Labrum approximately two times longer than wide, anterior margin shallowly emarginate (Figs 50, 51, 66, 104, 109–111), on dorsal surface: two setae on each lateral side; two pairs of campaniform sensilla medially; and four stout setae on each side close to anterior margin. Mid part of ventral surface (epipharyngeal area) with three pairs of campaniform sensilla at base; two irregular groups of a few small sensilla in anterior part; and numerous spines medially and on each lateral side.

Mandibles heavily sclerotized, with two apical teeth: one distinct, conical, the second blunt at the top and slightly moved back (Figs 53, 54). Two setae placed very close to each other and two campaniform sensilla (Figs 106, 116).

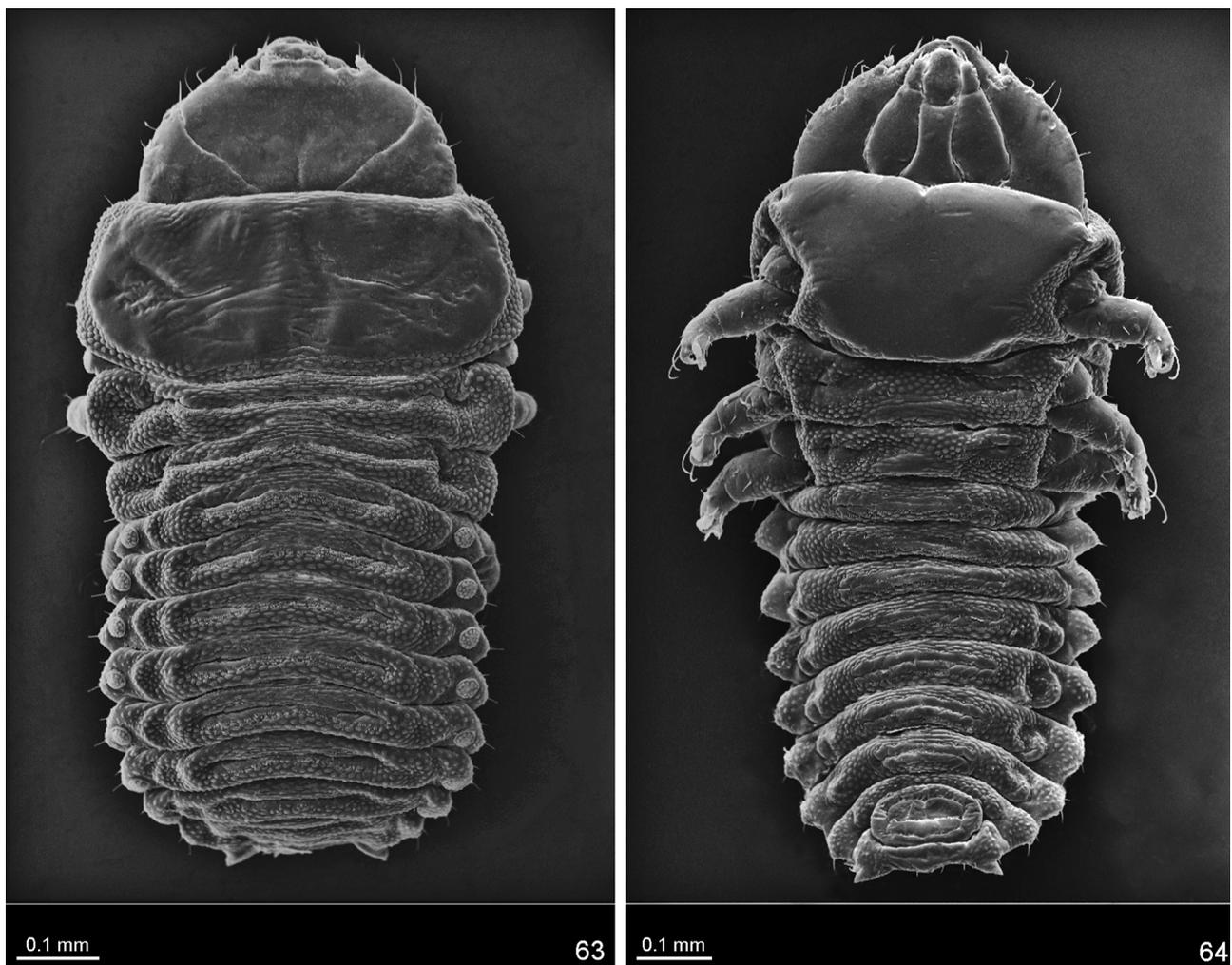


FIGURES 59–62. *Dicladispa testacea*. **59.** mature larva dorsally; **60.** mature larva ventrally; **61.** pupa dorsally; **62.** pupa ventrally.

Maxillae and labium connate (Figs 55, 108, 112). Each stipes (st) with seta at base and 3 campaniform sensilla in anterior part. Palpifer (pp) with 2 campaniform sensilla. Mala (mal) not distinctly bordered from palpifer (Figs 67, 114), bears: six long stout setae, one short blunt seta (or peg like sensillum?) and one campaniform sensillum at the top; and two setae below apex. Maxillary palp two-segmented: first segment with one campaniform sensillum;

second segment with a group of small peg-like sensilla at the apex. Labial palp (lp) one-segmented with a group of small peg-like sensilla at the apex (Figs 55, 112, 113). Hypopharynx (hyp) covered with numerous spines, at base with: two or three pairs of sensilla (campaniform sensilla or sensilla celoconica—difficult to diagnose without histological examination) (Figs 55, 113, 115). Prementum (pre) with two campaniform sensilla, each placed at base of labial palp. Postmentum (post) with four setae and two campaniform sensilla.

Legs stout, consist of three segments: coxa, femur and tibiotsarsus. Tibiotsarsus armed apically with claw (Figs 56–58, 100–103). Coxa with four setae placed along base on internal side, two setae placed dorsally and two setae on external side. One seta at base externally. Femur with 8 pointed setae: dorsally two setae and one campaniform sensillum placed in apical half and one seta in basal half; ventrally two setae placed in apical half and one seta in basal half; also in apical half one seta externally and one internally. A group of five campaniform sensilla and one short pointed seta on internal side of femur basally; two campaniform sensilla at base ventrally. Tibiotsarsus at apex with heavily sclerotised, curved, single and simple claw armed with a pointed seta basally. Claw and pointed seta surrounded by a complex of six setae. Two campaniform sensilla and seta above claw. Tibiotsarsus also with two setae dorsally. At base of claw distinct pulvilli.

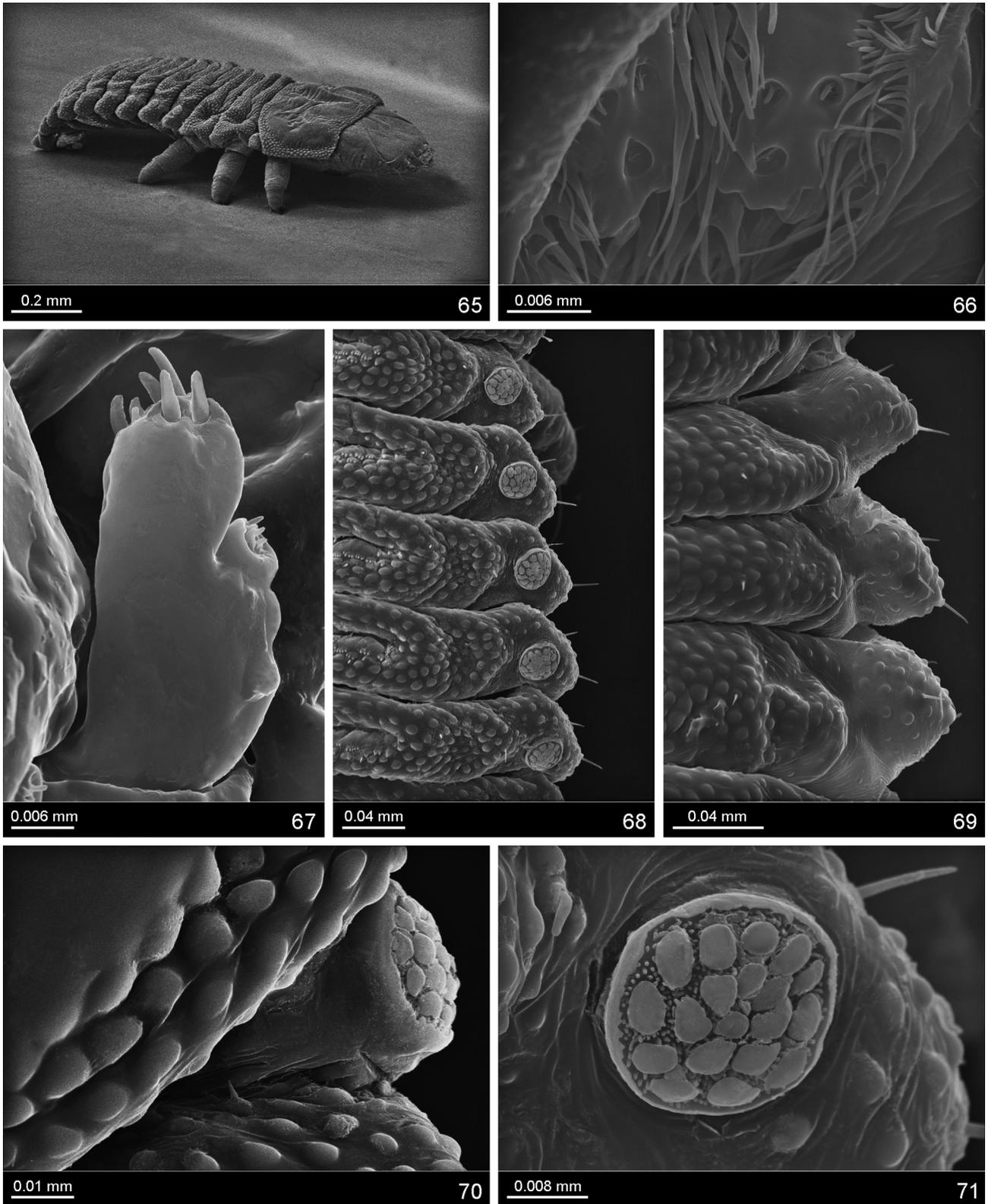


FIGURES 63, 64. *Dicladispa testacea*, first instar larva. **63.** dorsal aspect; **64.** ventral aspect.

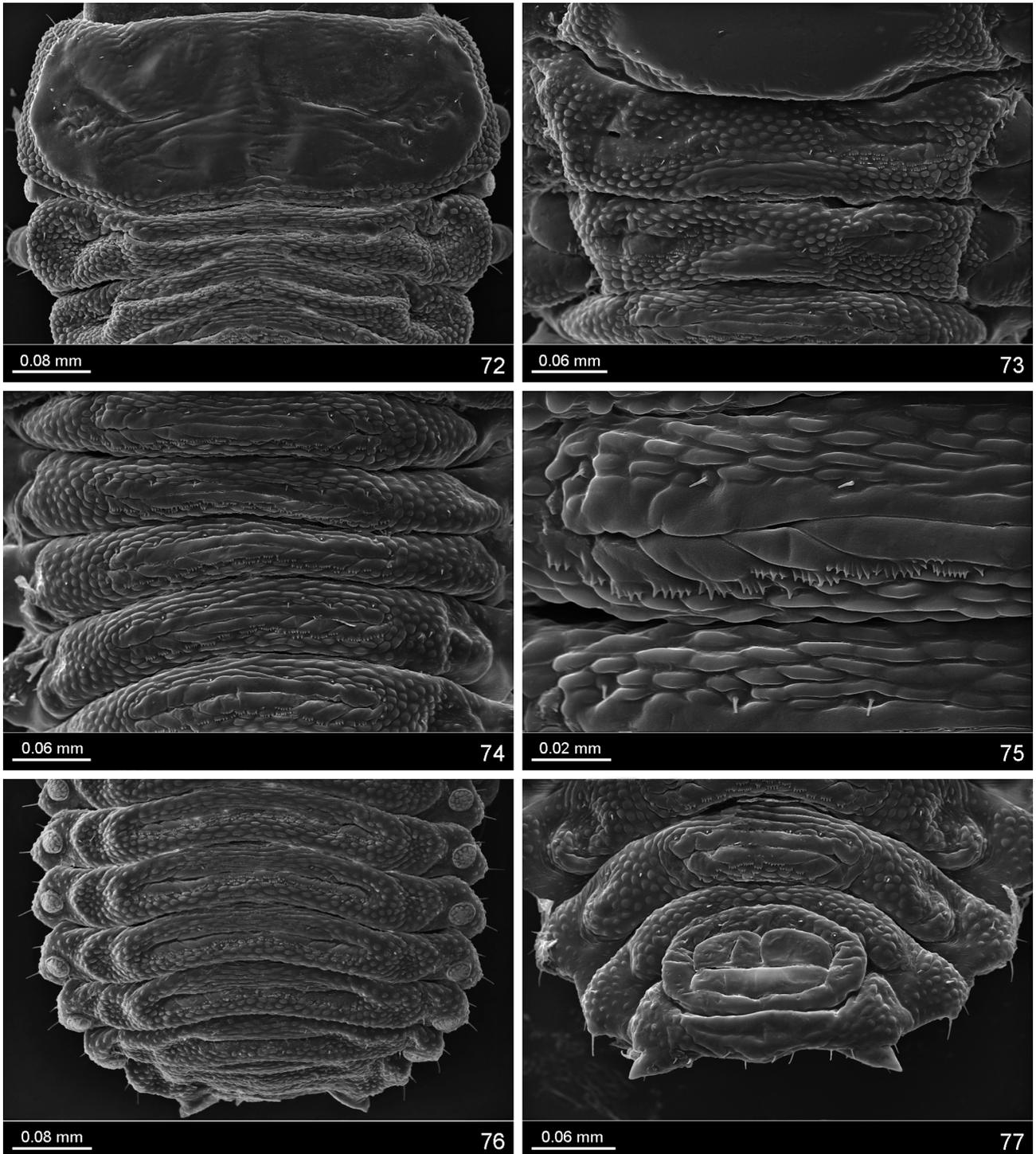
Pupa (Figs 61, 62, 117–136).

Measurements are presented in Table 1. Length was measured without head, from anterior border of pronotum to the tip of body; width of body was measured across pronotum (at base) and abdominal segment IV (in the middle).

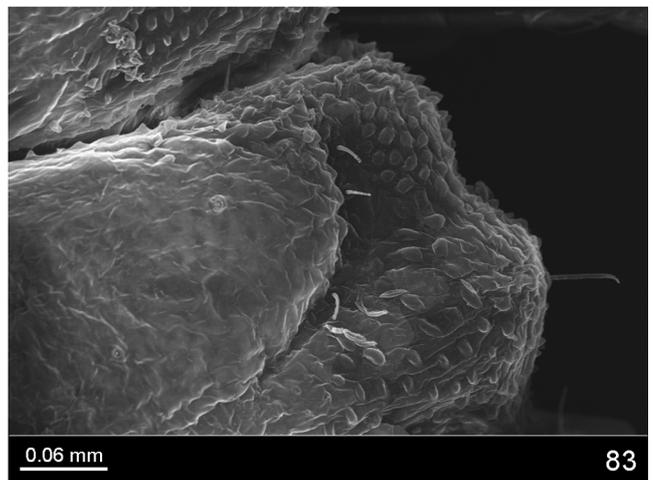
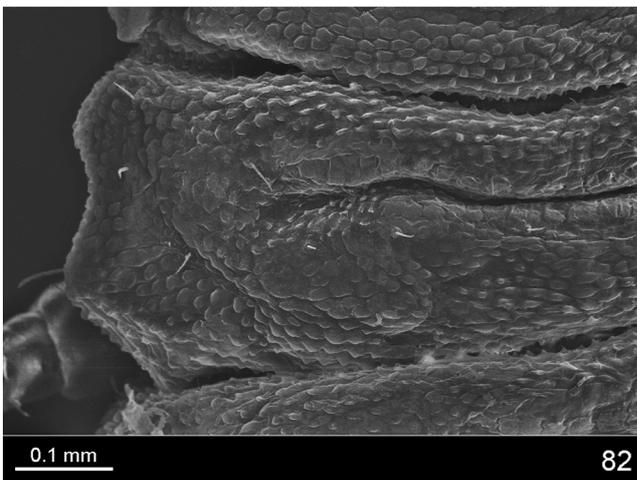
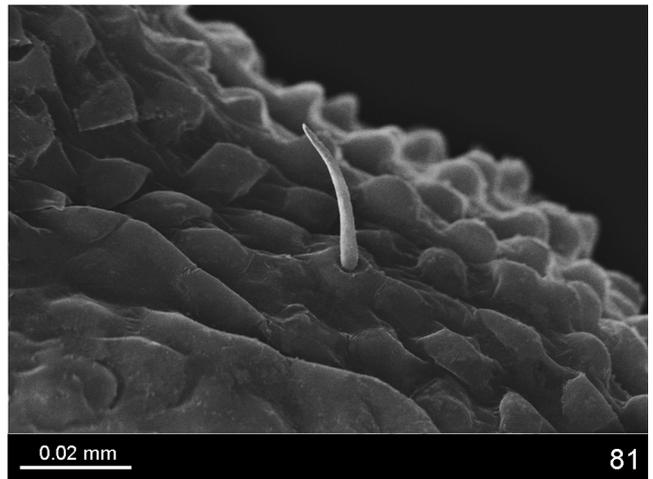
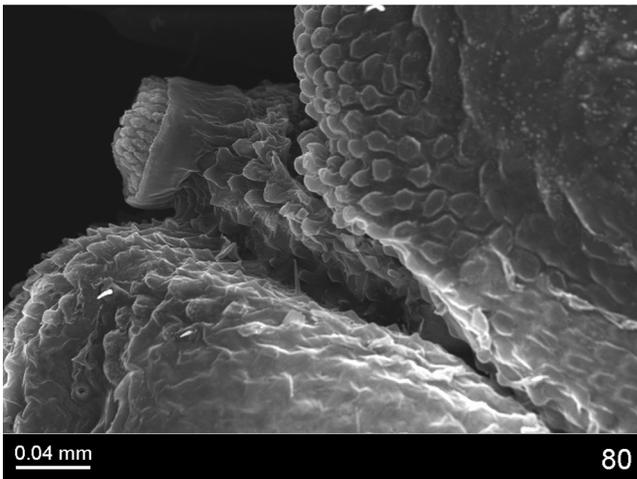
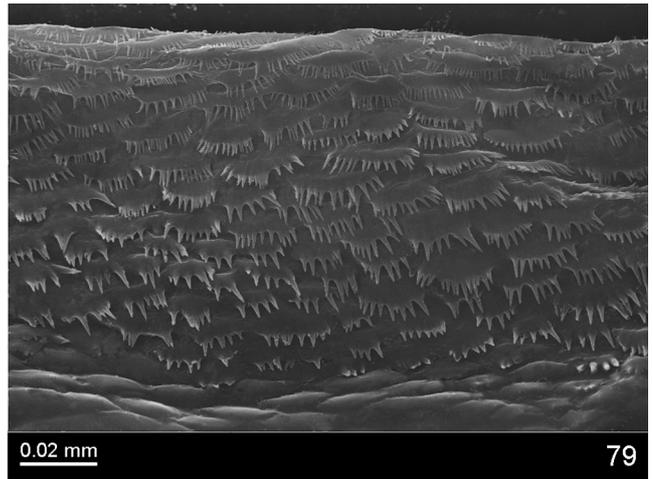
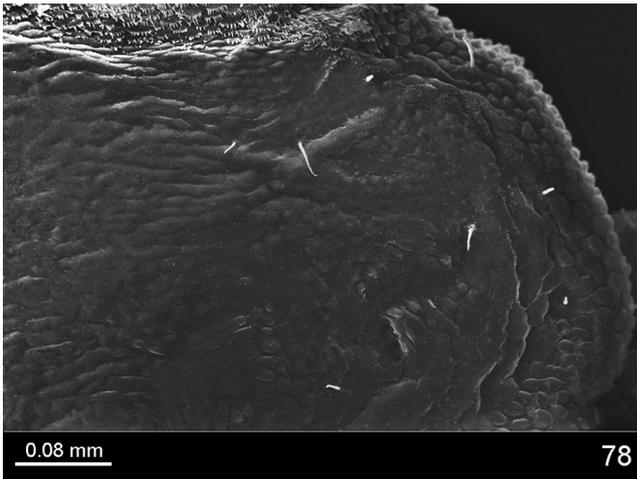
Body flattened dorso-ventrally, elongate-oval, the widest across abdominal segment IV and V (Figs 61, 62). Colour of alive as well as alcohol preserved pupa yellowish-brown.



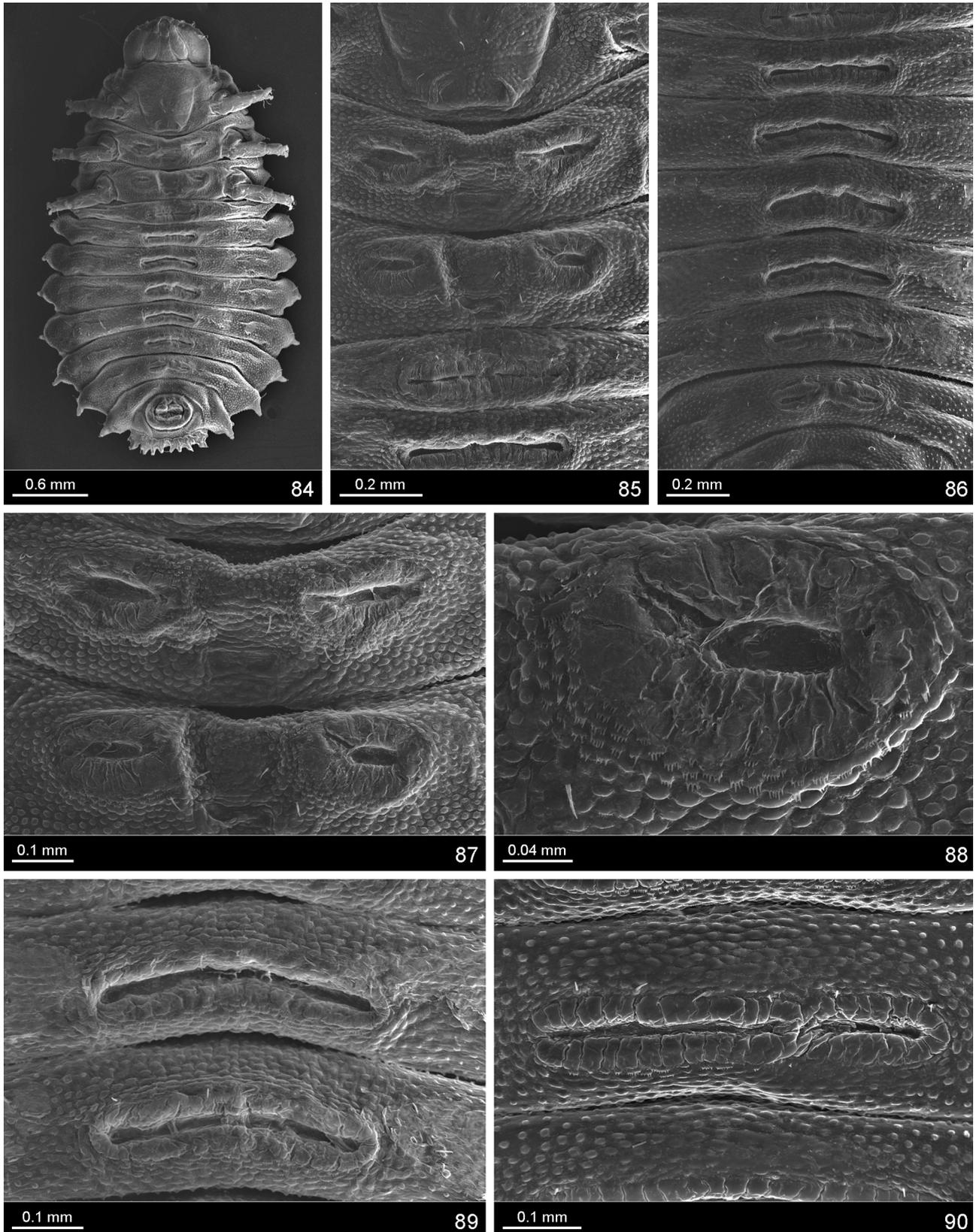
FIGURES 65–71. *Dicladispa testacea*, first instar larva. **65.** body laterally; **66.** ventral side of labrum; **67.** palpi and mala, maxillary palp artificially collapsed; **68.** spiracles of abdominal segments I–V; **69.** lateral scoli of abdomen, ventral aspect; **70.** spiracle of thorax; **71.** spiracle of second abdominal segment.



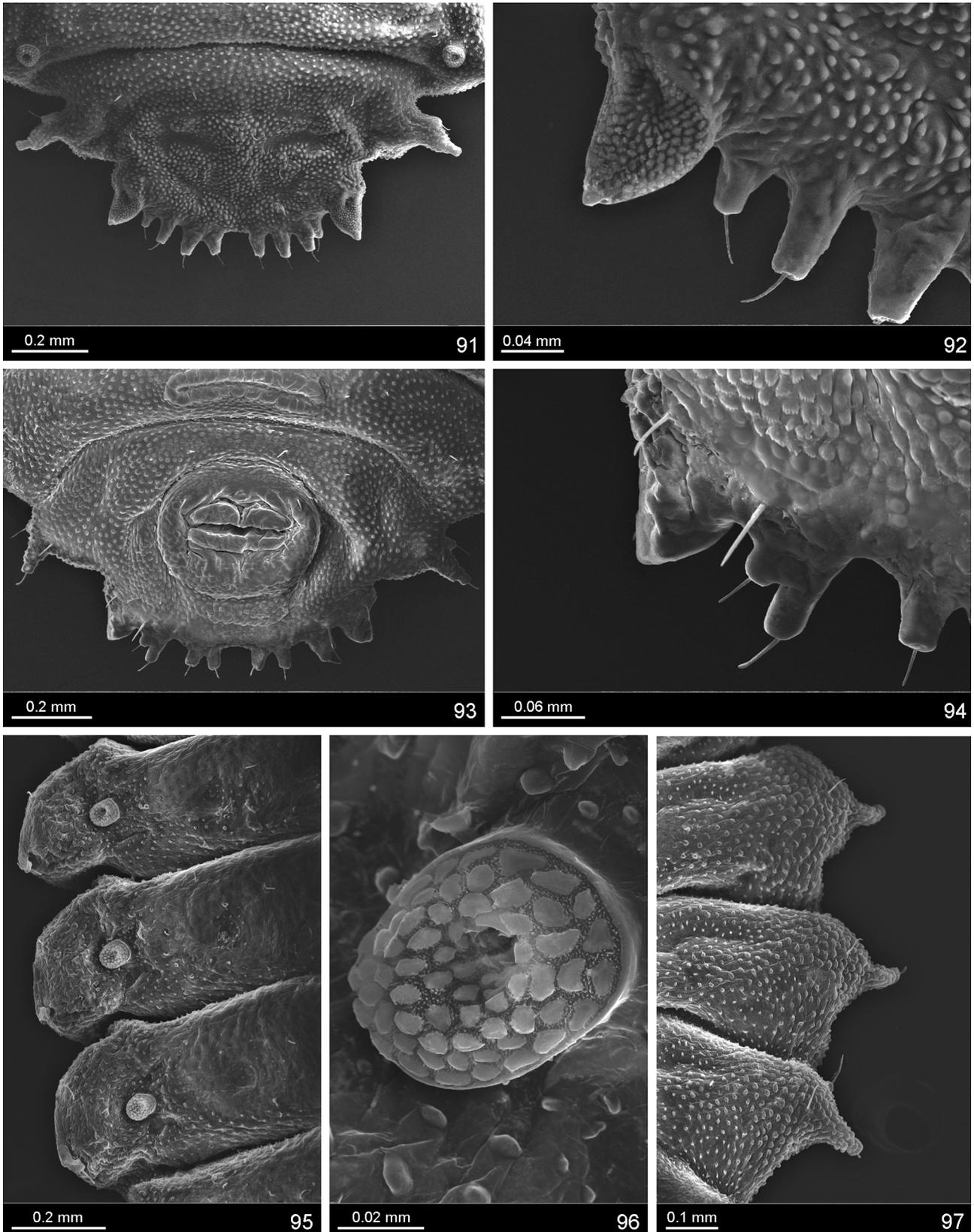
FIGURES 72–77. *Dicladispa testacea*, first instar larva. **72.** pro-, meso- and metanotum; **73.** meso- and metasternum; **74.** abdominal sternites; **75.** abdominal sternite I; **76.** abdominal tergites; **77.** top of body ventrally.



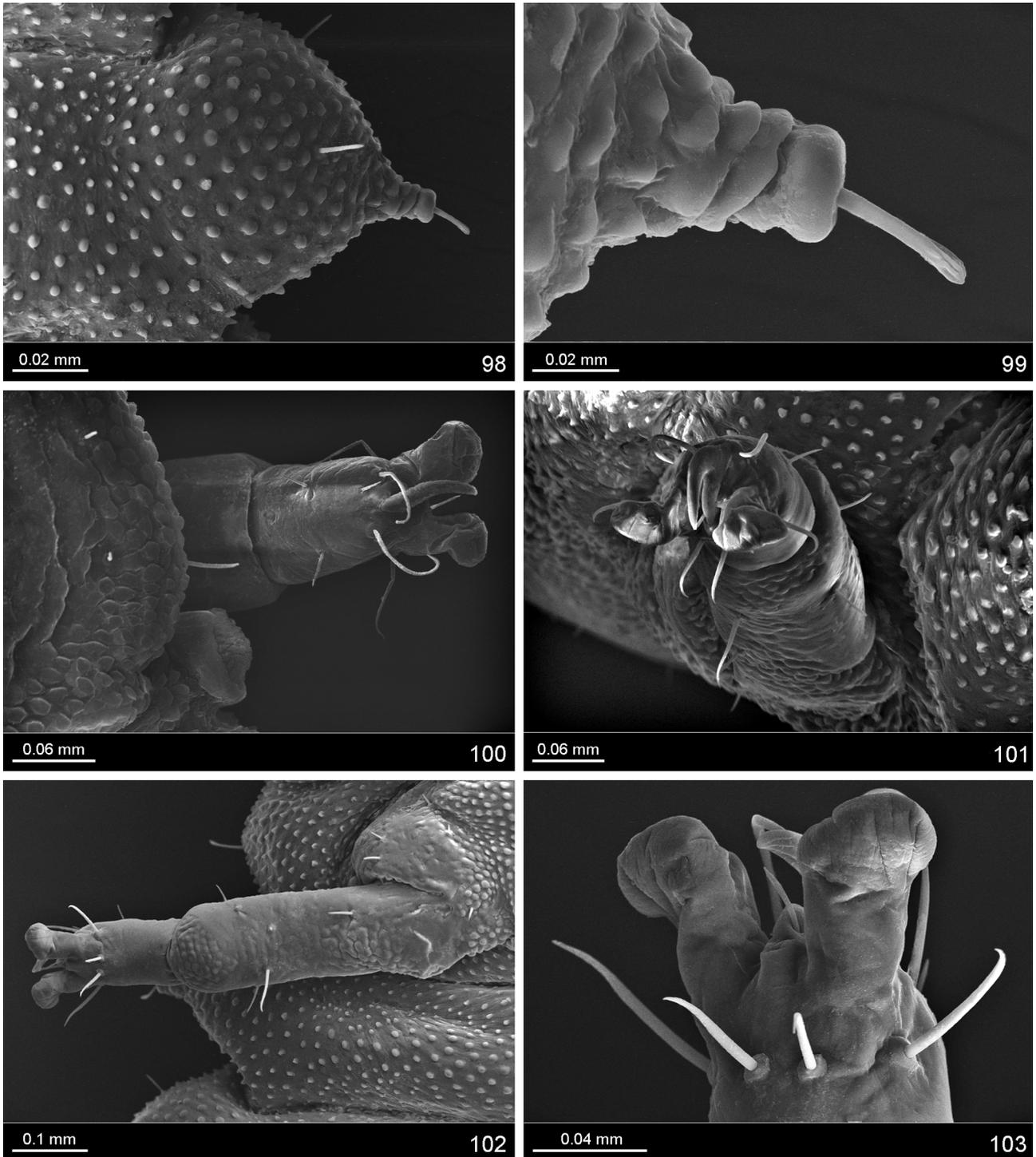
FIGURES 78–83. *Dicladispa testacea*, fifth instar larva. **78.** pronotum; **79.** anterior margin of pronotum; **80.** spiracle of thorax; **81.** seta of pronotum; **82.** mesonotum; **83.** metanotum.



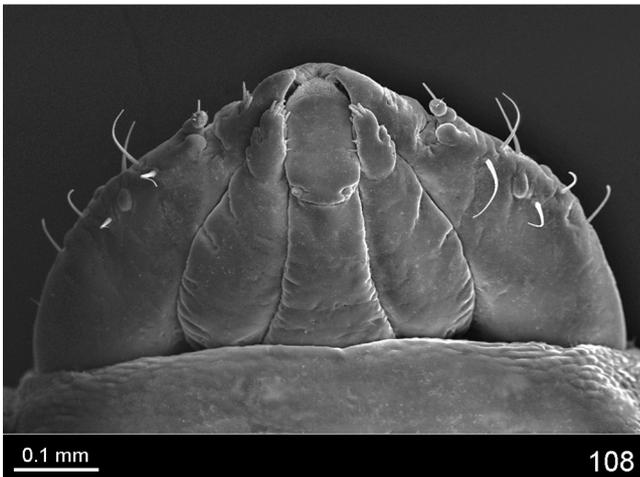
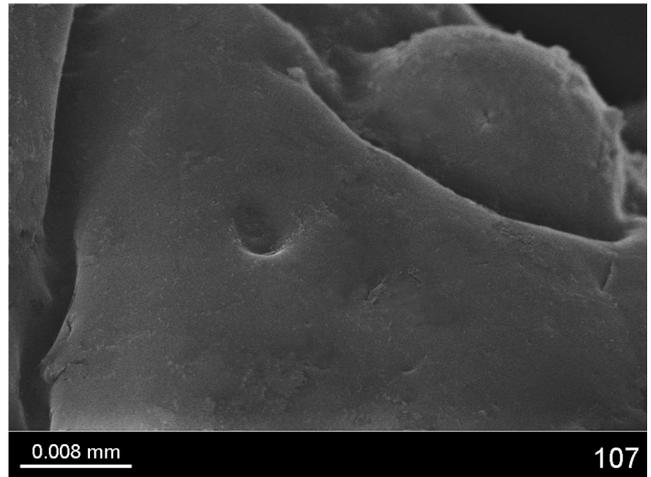
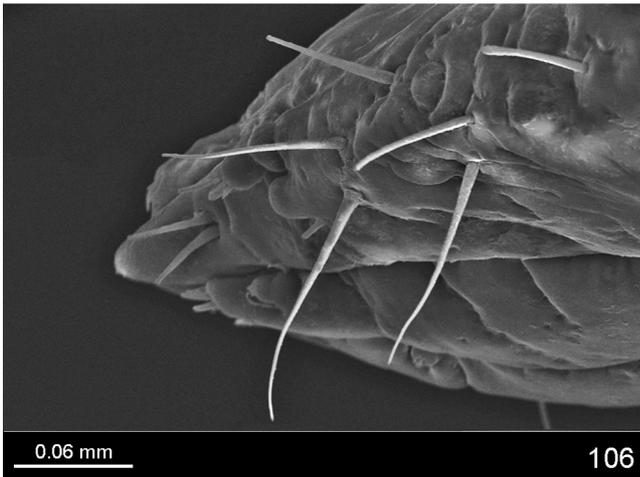
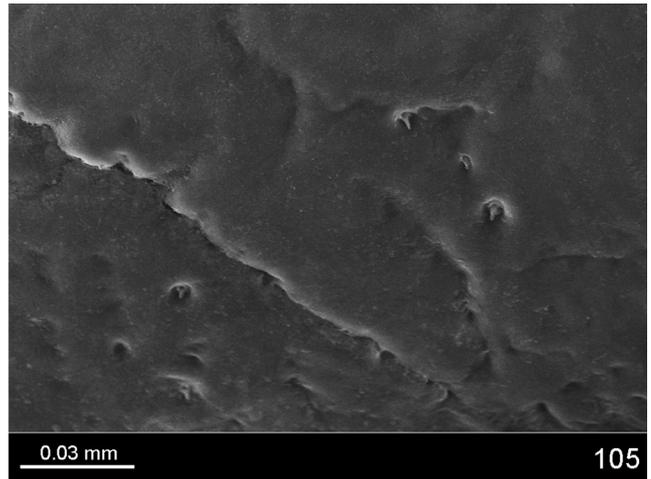
FIGURES 84–90. *Dicladispa testacea*, fifth instar larva. **84.** body visible ventrally; **85.** thoracic sternites and first two abdominal sternites medially; **86.** abdominal sternites; **87.** meso- and metasternum medially; **88.** metasternum; **89.** abdominal sternite V and VI, medially; **90.** abdominal sternite II, medially;



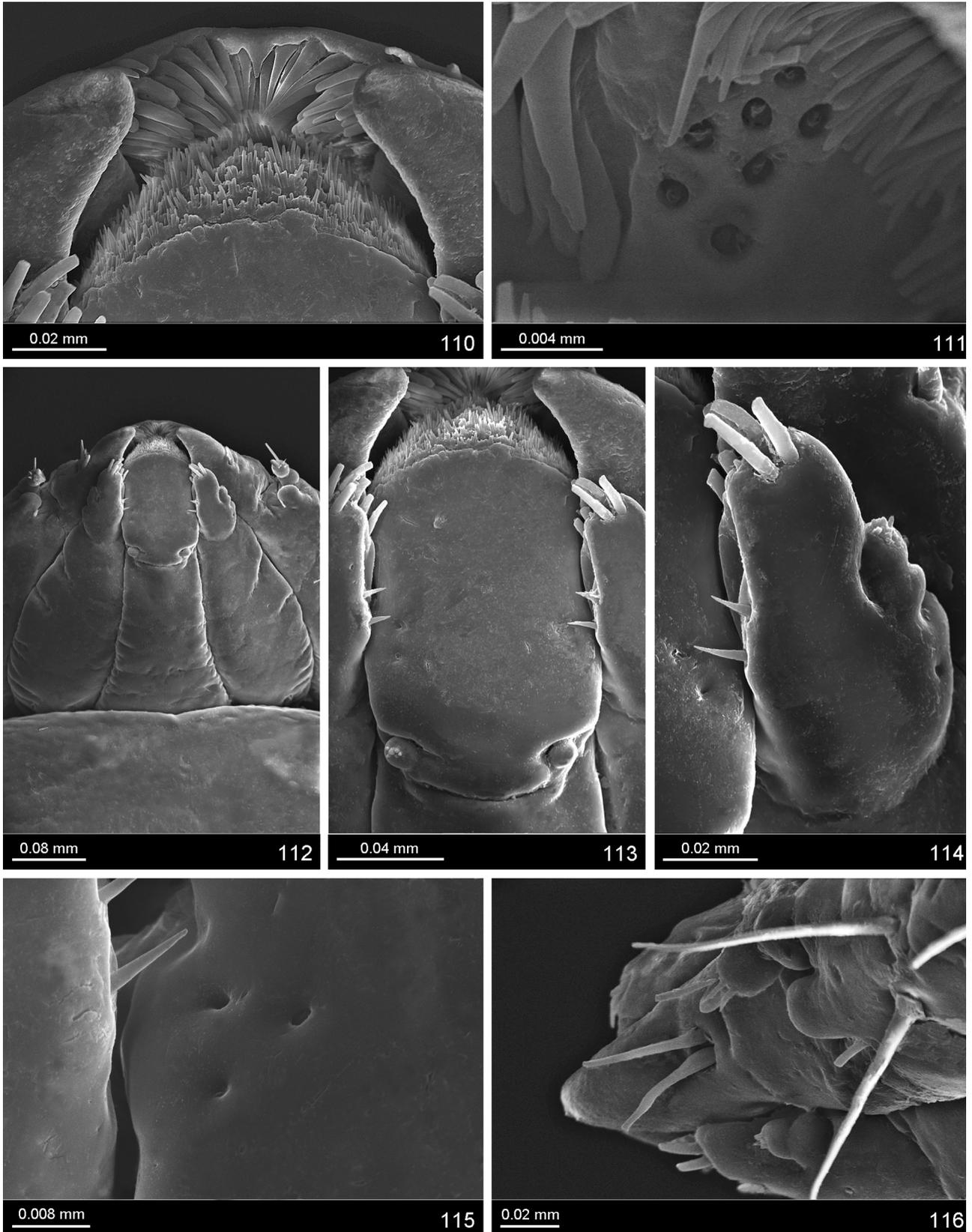
FIGURES 91–97. *Dicladispa testacea*, fifth instar larva. **91.** top of body dorsally; **92.** spiracle at the top of body dorsally; **93.** top of body, ventrally; **94.** spiracle at the top of body, ventrally; **95.** abdominal tergites II–IV, laterally; **96.** spiracle of III abdominal tergite; **97.** lateral scoli of abdominal segments V–VII, laterally.



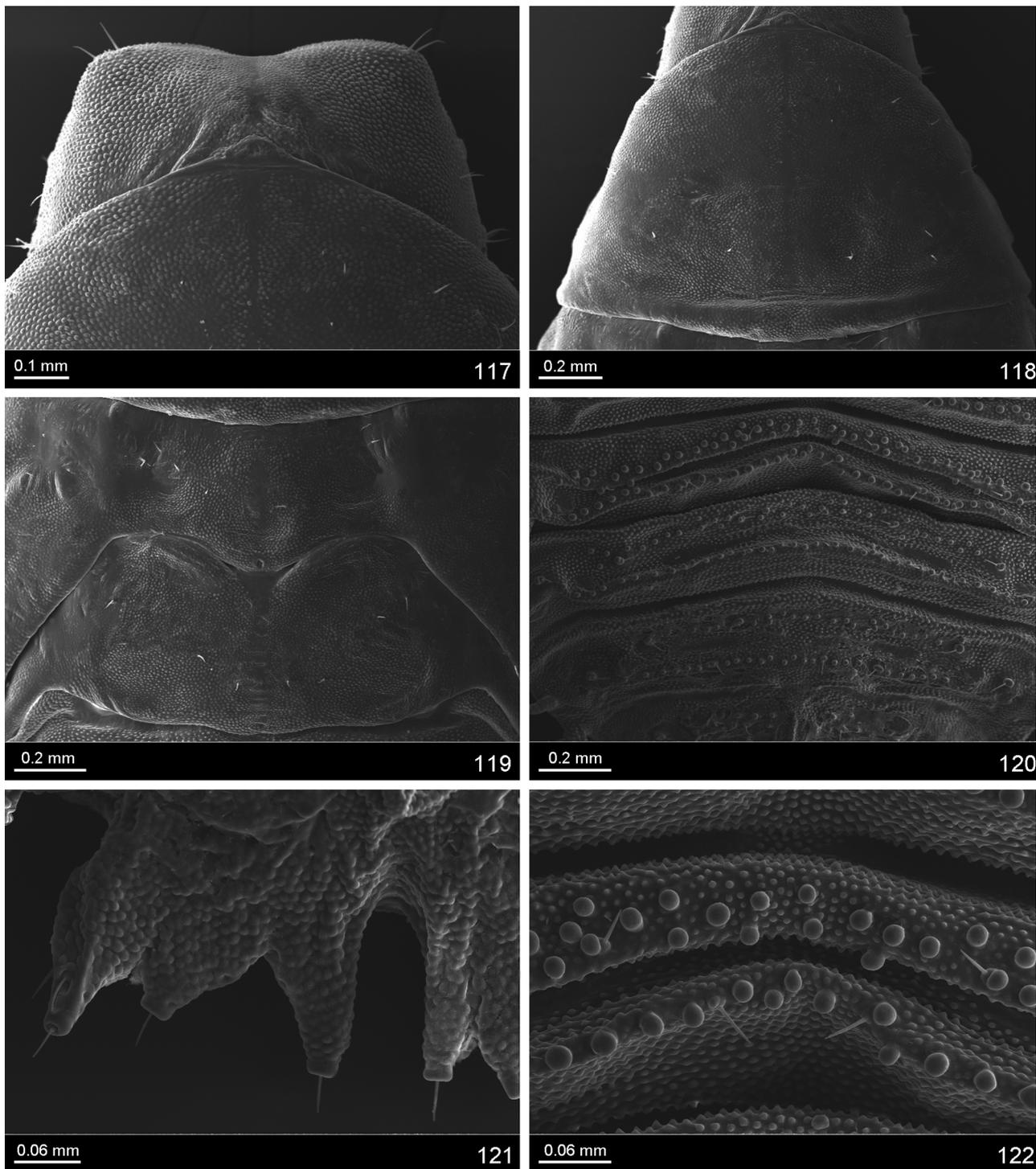
FIGURES 98–103. *Dicladispa testacea*, fifth instar larva. **98.** lateral sclerite of I abdominal segment, ventrally; **99.** seta at the top of lateral sclerite of I abdominal segment; **100.** leg, dorsal aspect; **101.** tibiotarsus and pulvilli; **102.** leg, ventral aspect; **103.** pulvilli at the top of tibiotarsus.



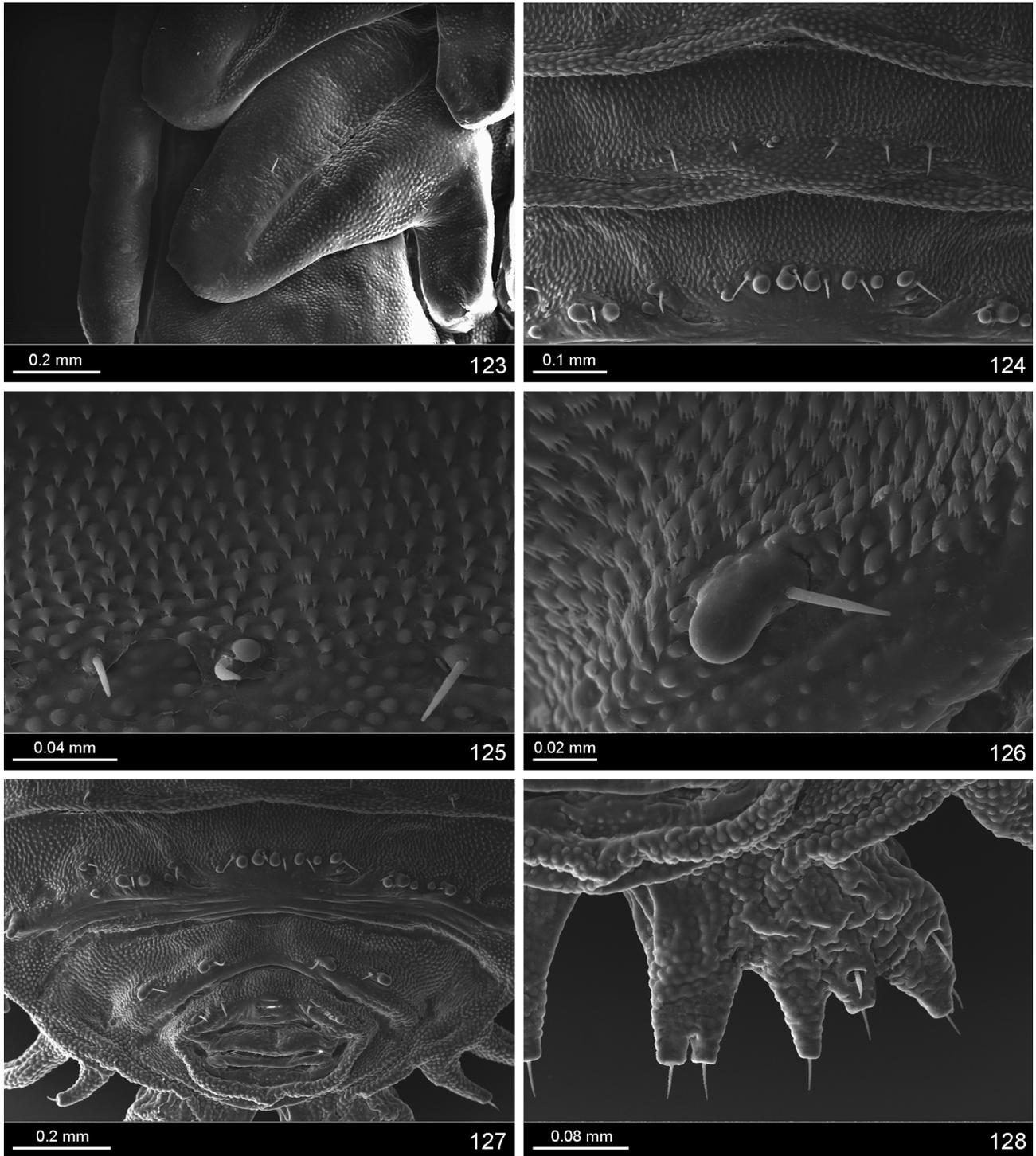
FIGURES 104–109. *Dicladispa testacea*, fifth instar larva. **104.** head; **105.** minute setae at the top of head; **106.** head laterally; **107.** stemma and campaniform sensilla; **108.** maxillae and labium; **109.** labrum dorsally.



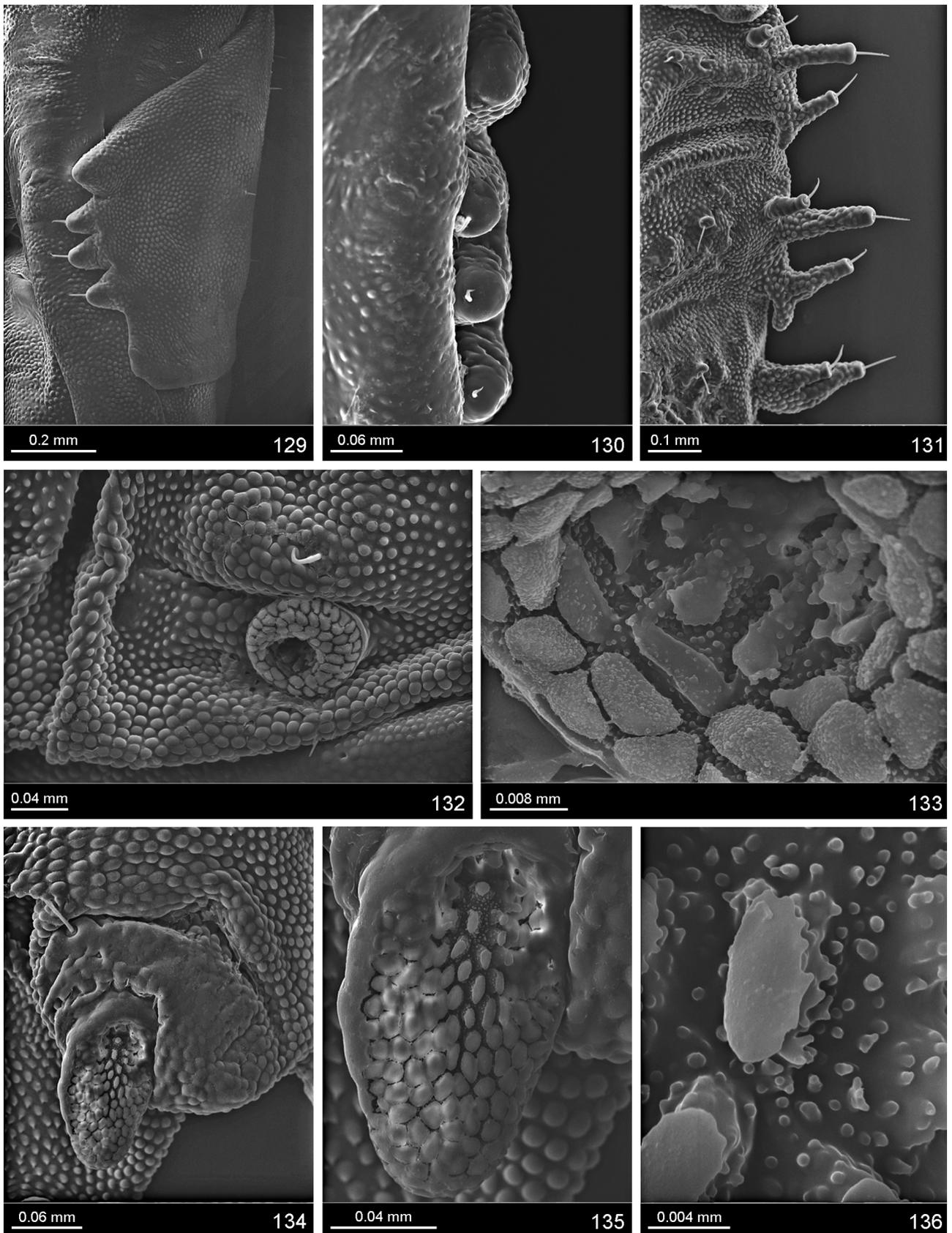
FIGURES 110–116. *Dicladispa testacea*, fifth instar larva. **110.** labrum ventrally; **111.** sensilla of ventral part of labrum; **112.** maxillae and labium; **113.** prementum and hypopharynx; **114.** palpifer, mala and two-segmented maxillary palp; **115.** sensilla of prementum; **116.** mandibular with two distinct setae.



FIGURES 117–122. *Dicladispa testacea*, pupa. **117.** top of head; **118.** pronotum; **119.** meso- and metanotum medially; **120.** setae and sculpture of abdominal tergites V–VII; **121.** top of body dorsally; **122.** abdominal tergite V.



FIGURES 123–128. *Dicladispa testacea*, pupa. **123.** leg; **124.** abdominal sternite VI, VII; **125.** setae of abdominal sternite VI; **126.** seta of abdominal sternite VIII; **127.** top of body ventrally; **128.** processes of the top of body ventrally.



FIGURES 129–136. *Dicladispa testacea*, pupa. **129.** lateral margin of pronotum; **130.** processes with setae on lateral margin of pronotum; **131.** lateral scoli of abdominal segments VI–VIII; **132.** spiracle of first abdominal tergite; **133.** inside spiracle of I abdominal tergite; **134–135.** spiracle of V abdominal tergite; **136.** inside spiracle of V abdominal tergite.

Head visible in dorsal view (Figs 61, 117).

Prothorax trapezoidal in shape, lateral edge of pronotum with four processes: three of them armed apically with single seta (Figs 118, 129, 130).

Meso- and metathorax without lateral scoli. Elytral portion distinctly visible (Figs 61, 62, 119).

First abdominal segment without lateral scoli (Figs 61, 62). Each lateral side of segments II–V with single and simple scolus and one pointed seta which on segments IV and V is placed on low tubercle. Each lateral side of segments VI–VIII with 2 scoli and pointed seta on prominent tubercle (Fig. 131), more distinct than on segments IV and V (in shape similar to lateral scoli). Each scolus apically armed with one seta. Posterior lateral scolus of segments VI and VII with lateral branch directed posteriorly without any seta. Segment VIII additionally with two broad, dorso-ventrally flattened processes placed at posterior border (Figs 61, 62, 121, 128). Each flat process apically with usually 5 tubercles (rarely 4 or 3). Each tubercle apically armed with single seta.

Abdominal segments I–V with a pair of spiracles (Figs 132–136). Spiracles of first four segments of similar diameter, distinctly marked and elevated but spiracles of the segment V are the most prominent, elongated into cylindrical appendage (respiratory horns) with elongate-oval outer spiracular opening.

Whole body including head, antennae, legs and elytral portion distinctly granulate (Figs 117–123, 128–132, 134), abdominal sternites also with distinct asperites (Figs 124–127). Head and thoracic tergites with sparse pointed setae (Figs 117, 119). First abdominal tergite with one row of tubercles placed posteriorly. Tergites of abdominal segments II–VIII with two rows of distinct tubercles, some of them with pointed seta apically (Figs 120, 122). Abdominal sternites with row of setae placed on tubercles running along posterior border (Figs 124, 125). Sternites VII and VIII with row of setae and with additional tubercles without setae (Figs 124, 126, 127). Tubercles without setae, look like tubercles on abdominal tergites.

Habitat and biological notes.

The biology of *Dicladispa testacea* is still poorly known. Beetles or characteristic feeding patterns and mines were observed in southern Spain, Balears, Greece and western Turkey coast in all localities with host plants from sea level up to 1018 m a.s.l. (the highest locality was in Termessos, Antalya Prov., Turkey). Beetles prefer sunny localities on the outskirts of the pine forests and on Mediterranean undergrowth, but they were also observed in the more shady areas within the luminous pine forests. So far, the full developmental cycle of this species has not been described and we still have some doubts if this species is mono- or bivoltine. Our observations concerning the developmental cycle coincide with the Bordy's (2000) observations. On Majorca Island we collected from 07–20th May 2009 only mature larvae, pupae and adults (but not in copula). There were not any larval instars younger than mature larvae. In Greece from 24th August to 05th September 2009 we collected only eggs and specimens of first and second instar larvae (Fig. 12) but no older larvae nor pupae. Moreover in Greece on faded blossoms of host plants numerous adult beetles were observed, most of them during copulation (Figs 39–43). According to Bordy (2000) in the southern part of France, imagines of *D. testacea* appear in May to June. Two or three weeks later imagines start to copulate and the eggs are deposited in June to July and few days later appear first larvae. Imagines in France were observed from the beginning of August to September. Our observations on August–September in Greece (adults in copula, eggs and first and second instar larvae without mature larvae and pupae) indicate that *D. testacea* is a bivoltine species.

During field observation we found that the female of *Dicladispa testacea* bites a small hole on the underside of the leaf, and then lays an egg in the hole, and covers it with faeces (Figs 1, 2, 7–11). Often two eggs were found within one hole (Fig. 3). Very often in a mine a dead larva or remnants of a *D. testacea* larva along with pupae of a parasitoid wasp were found, which confirms that parasitism of immature *D. testacea* by parasitoid wasps can be of importance.

Larvae are solitary, each develops in a separate mine (Figs 2, 4–6, 12–18). The mature larva leaves its mine and builds a new one placed on the midrib of the leaf, in which develops the pupa (Figs 19–24) and then turning into the adult (Figs 35–43). The larval mine is broad and irregular in shape. The central part of the larval mine is distinctly darker than other parts due to larval excrement (Fig. 18). The pupal mine is an elongated channel, slightly wider than the pupa with a distinct opening and often with two short lateral channels which run at a right angle from the main channel. The short lateral channels are distinctly narrower than the main channel. Often at the opening of the pupal mine were found a skin of last instar larva (Fig. 21). The pupal mine in contrast to the larval

mine is narrow, and the pupal body fills the entire interior of its mine. The pupa in mine is orientated so that the posterior part of its body is located at the inlet opening. Imago emerges from the pupal cuticle through the crack running along the dorsal side of the head and pronotum (Figs 33, 34).

The freshly emerged adult has black eyes and antennae, yellowish-brown pronotum and legs, and white elytra with black spines (Fig. 35), and its hind wings distinctly protrude from behind elytra. After 12 to 24 hours from emergence the body of adults becomes darker and harder and then they started to feed (Figs 28, 36–38). Imagines produce on the leaves characteristic feeding patterns: closely located small circular holes tightly grouped in rows (Figs 25–32).

Discussion

The tribe Hispini is the second most speciose within the hispoid Cassidinae. The last world catalogue (Staines 2012) lists 625 species divided into 24 genera. Various immature stages were described only for 35 species: one species has only the description of the egg (in general eggs of 4 species were described—Suzuki & Hara 1976), 15 species with larval descriptions (Fukuda & Kurosa 1959; Gressitt 1963; Maulik 1932; Medvedev 1968; Paulian 1949; Uhmman 1956, 1957b, 1962; Yano 1965), 14 descriptions of pupae (Cox 1996; Uhmman 1953, 1955, 1956, 1957a, 1958), and only for 5 species both larva and pupa were described, including *Dicladispa testacea* (Chen *et al.* 1986; Cox 1996; Grandi 1935; Lesne 1906; Maulik 1932; Medvedev 1968; Perris 1855; Steinhausen 1994, 2002; Uhmman 1954, 1955, 1956, 1965). Immature stages in *Dicladispa* were described for the following species: *Dicladispa armigera* (Olivier, 1808)—larva and pupa (Chen *et al.* 1986), *D. kapauku* Gressitt, 1957 (under name *Dicladispa linnei* sensu Gressitt, 1957 not Weise, 1905)—larva (Gressitt 1963); *D. malvernia* Péringuey, 1908—pupa (Uhmman 1957a); *D. ornata* (Uhmman, 1939)—pupa (Uhmman 1958); *D. testacea* (Linnaeus, 1767)—larva and pupa (Bordy 2000; Grandi 1935; Lesne 1906; Perris 1855; Steinhausen 1994, 2002; Uhmman 1965); *D. varii* Uhmman, 1957a—pupa (Uhmman 1958); and *D. vicinalis* (Péringuey, 1898)—pupa (Uhmman 1957a).

In comparing the description of the larva of *Dicladispa testacea* with two described congeners, *D. kapauku* and *D. armigera*, all three species have the thoracic segments without lateral scoli, the character of larvae of specialized Hispini. Descriptions and figures of *D. kapauku* in Gressitt (1963) and *D. armigera* in Chen *et al.* (1986) are superficial but one character easily distinguished the larva of *D. testacea* from both congeners. In New Guinean and Chinese members the body of the last instar is narrow, parallelsided while in Palearctic member it is elongate-oval, widest across fourth and fifth abdominal segments. The difference depends probably on the structure of leaves mined by larvae because *D. kapauku* and *D. armigera* produce mines in very narrow leaves of grasses while *D. testacea* mines broad leaves of *Cistus*. The cutting edge of mandibles in *D. testacea* has no additional teeth while *D. armigera* possesses three additional teeth. The pupa of *D. testacea* differs from the only well described pupa of *D. armigera* in the stouter, oval body (parallelsided in *D. armigera*), the very short spiracles of fifth abdominal segment not extending to the half of length of the sixth segment (in *D. armigera* the spiracles are elongate and extend to the posterior margin of the sixth segment), and the lateral the processes of the pronotum run downwards, not visible from above (in *D. armigera* the processes run lateral and are well visible from above).

Among hispine species sympatric with *Dicladispa testacea*, immatures were described for the commonest species *Hispa atra* Linnaeus, 1767 (Grandi 1935, Uhmman 1954, Medvedev 1968, Steinhausen 1994, 2002, Bordy 2000). The larva of *H. atra* is easily distinguishable from *D. testacea* in its narrow, parallelsided body (this species is mining the narrow leaves of grasses), the lateral scoli of abdomen, the very short with rounded apex and the apex of last abdominal segment is rounded without serration.

Acknowledgements

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References

- Amsel, H.G. & Hering, M. (1931) Beitrag zur Kenntnis der Minenfauna Palästinas. *Deutsche Entomologische Zeitschrift*, 1931, 113–152.
- Biondi, M., Regalin, R., Daccordi, M. & Poggi, R. (1995) I Crisomelidi (esclusi Alticini) delle Isole Circumsarde. *Annali del Museo Civico di Storia Naturale "G. Doria"*, XC. Genova, Erga Edizioni, pp. 629–651.
- Bordy, B. (2000) Coléoptères Chrysomelidae. Volume 3. Hispinae et Cassidinae. *Faune de France*, 85, 1–250, pls. i–xxvi.
- Borowiec, L. & Sekerka, L. (2010) Cassidinae. In: Löbl, I. & Smetana A. (Ed.), *Catalogue of Palaearctic Coleoptera. Vol. 6. Chrysomeloidea*. Apollo Books, pp. 64–65, 368–390.
- Buhr, H. (1930) Einige Blattminen und Gallen von der Insel Lesina (Hvar) in Dalmatien. *Sitzungsberichte Abhandlungen der Naturforschenden Gesellschaft zu Rostock*, (3) 2, 125–148.
- Chen, S.H., Yu, P.Y., Sun, C.H., T'an, C.H. & Zia, Y. (1986) *Fauna Sinica (Insecta: Coleoptera: Hispidae)*. Science Press, Beijing, 653 pp. [in Chinese]
- Cox, M.L. (1996) The pupae of Chrysomeloidea. In: Jolivet, P.H.A. & Cox, M.L. *Chrysomelidae Biology. Vol. 1. The Classification, Phylogeny and Genetics*. Academic Publishing, Amsterdam, pp. 119–265.
- Frost, S.W. (1924) The leaf mining habit in the Coleoptera. Part I. *Annals of the Entomological Society of America*, 17, 457–467.
- Fukuda, A., Kurosa, K. & Hayashi, N. (1959) Coleoptera. In: Kawada A., Esaki T., Ishii T., Shiraki T. & Yuasa, H. (Ed.), *Illustrated insect larvae of Japan*. Hokuryukan Co. Ltd., Tokyo, pp. 392–545 [in Japanese]
- García-Ocejo, A., Gurrea, P. & Petitpierre, E. (1992) Chrysomelidae (Coleoptera) de La Sierra de Gredos (Sistema Central): Datos faunísticos, ecológicos, y fenológicos. *Miscellanea Zoológica*, 16, 81–92.
- Gestro, R. (1897) Materiali per lo studio delle Hispidae. *Annali del Museo Civico di Storia Naturale di Genova*, Series 2, 18 (38), 37–138.
- Gestro, R. (1919) Materiali per lo studio delle Hispidae LII. *Annali del Museo Civico Naturale di Genova*, Series 3, 8 (48), 338–349.
- Grandi, G. (1935) Morfologia ed etologia comparata di insetti a regime specializzato. VI. La morfologia delle larve minatrici degli Hispini dei gen. *Hispa* L. e *Hispella* Chap. (Coleoptera - Chrysomelidae). *Memorie della R. Accademia delle scienze dell'Istituto di Bologna, Classe di scienze fisiche*, 2 (9), 19–26.
- Gressitt, J.L. (1963). Hispine beetles (Chrysomelidae) from New Guinea. *Pacific Insects*, 5, 591–714.
- Gyllenhal, L. (1813) *Insecta Suecica*. Tomo I, pars III. F.J. Leverentz, Scaris, 730 pp.
- Hering, E.M. (1967) Blattminen der Insel Hvar (Col., Dipt., Hym., Lep.). *Deutsche Entomologische Zeitschrift*, Series 2, 14, 1–80.
- Laporte, J.L. & Laporte, E. (1852) Faune Entomologique, ou Histoire naturelle des Insectes qui se trouvent dans le département de la Gironde. *Actes Société Linneenne de Bordeaux*, Series 2, 8, 162–187.
- Lee, C.-F., Świętojańska, J. & Staines C.L. (2009) *Prionispa houjayi* (Coleoptera: Chrysomelidae: Cassidinae: Oncocephalini), a newly recorded genus and new species from Taiwan, with a description of its immature stages and notes on its bionomy. *Zoological Studies*, 51, 832–861.
- Lee, C.-F., Świętojańska, J. & Staines C. L. (2012) A Review of the Genus *Callispa* Baly, 1858 in Taiwan (Coleoptera: Chrysomelidae: Cassidinae: Callispini), with descriptions of two new species and their immature stages, and notes on their bionomy. *Zoological Studies*, 51, 832–861.
- Lesne, P. (1906) Notes biologiques sur l'*Hispa testacea* L. [Col.]. *Bulletin de la Société Entomologique de France*, 1904, 68–70.
- Linnaeus, C. (1767) *Systema naturae*. Volume 1, pars 2. Holmiae. pp. 533–1327.
- Magistretti, M. & Rufo, S. (1959) Primo contributo alla conoscenza della fauna delle oasi xerothermiche prealpine (Coleotteri Carabidi, Scarabeidi, Crisomelidi). *Memorie del Museo Civico di Storia Naturale-Verona*, 7, 99–125.
- Maulik, S. (1932) On the structure of larvae of hispine beetles-II. *Proceedings of the Zoological Society of London*, 293–322.
- Maulik, S. (1937) Distributional correlation between Hispine beetles and their host plants. *Proceedings of the Zoological Society of London*, Ser. A, 129–159.
- Medvedev, L.N. (1968) On larvae of Hispinae (Coleoptera, Chrysomelidae) of the fauna of the USSR. *Zoologicheskyy Zhurnal*, 47, 79–84. [in Russian]
- Needham, J.G., Frost, S.W. & Tothill, B.H. (1928) *Leaf-mining insects*. Williams and Wilkins Company, Baltimore, 351 pp.
- Paulian, R. (1949) Recherches sur les insectes d'importance biologique de Madagascar I. *Mémoires de l'Institut Scientifique de Madagascar*, 3 (A), 348–391.
- Péringuey, L. (1898) Catalogue of the South African Hispinae (Coleoptera), with descriptions of new species. *Annals of the South African Museum*, 1, 113–130.
- Péringuey, L. (1908) Seventh contribution to the South African coleopterous fauna. *Annals of the South African Museum*, Cape Town, 5, 271–346.
- Perris, E. (1855) Histoire des métamorphoses de divers insectes (*Liodes castanea*, *Cryptohypnus riparius*, *Ebaeus albifrons*, *Lagria lata*, etc.). *Memoires de la Société Scientifique de Leige*, 10, 233–280.
- Staines, C.L. (2012) Tribe Hispini. Catalog of the hispines of the World (Coleoptera: Chrysomelidae: Cassidinae). Available from: http://entomology.si.edu/Collections_Coleoptera-Hispines.html (Accessed 29 May 2014)

- Steinhausen, W.R (1994) Chrysomelidae. In: Klausnitzer, B. (Ed.), *Die Larven der Käfer Mitteleuropas, 2. Band, Myxophaga, Polyphaga, Teil 1*. Goecke & Evers Verlag, Krefeld, pp. 231–314.
- Steinhausen, W.R. (2002) Die Puppen mitteleuropäischer Blättkäfer Bestimmungstabelle 2. Teil (Coleoptera: Chrysomelidae). *Mitteilungen Muenchener Entomologischen Gesellschaft*, 92, 5–36.
- Suzuki, K. & Hara, A. (1976) Comparative study of the egg size in relation to the egg number in the family Chrysomelidae (Insecta: Coleoptera). *Journal of the College of Liberal Arts*, 9, 39–81.
- Świętojańska, J. & Kovac, D. (2007) Description of immatures and the bionomy of the Oriental leaf beetle *Chaeridiona thailandica* Kimoto, 1998 (Coleoptera: Chrysomelidae: Cassidinae: Oncocephalini), a leaf-mining hispine beetle. *Zootaxa*, 1637, 21–36.
- Świętojańska, J., Chorzępa, M. & Ghate, H. (2006) Description of last instar larva and pupa of *Chaeridiona picea* Baly, 1869 and *Oncocephala quadrilobata* (Guérin, 1844) (Coleoptera: Chrysomelidae: Cassidinae: Oncocephalini) from India. *Zootaxa*, 1341, 49–68.
- Uhmann, E. (1939) Hispinen des Deutschen Entomologischen Instituts, Berlin-Dahlem. III. Teil. (Coleoptera: Chrysomelidae). 82. Beitrag zur Kenntnis der Hispinen (Coleoptera: Chrysomelidae). *Arbeiten über morphologische und taxonomische Entomologie aus Berlin-Dahlem*, 6, 151–156.
- Uhmann, E. (1953) Eine Puppenexuvie von *Dactylispa pubicollis* Chap. 138. Beitrag zur Kenntnis der Hispinae (Coleopt. Chrysomel.). *Mitteilungen aus dem Zoologischen Museum in Berlin*, 29, 134–136.
- Uhmann, E. (1954) Die Puppe von *Hispa atra* L. 155. Beitrag zur Kenntnis der Hispinae (Coleoptera, Chrysomelidae). *Deutsche Entomologische Zeitschrift N.F.*, 1, 38–41.
<http://dx.doi.org/10.1002/mmnd.19540010105>
- Uhmann, E. (1955) Die Puppenhäute zweier *Dactylispa*-Arten aus Java. 157. Beitrag zur Kenntnis der Hispinae (Col., Chrysomelidae). *Idea*, 10, 53–56.
- Uhmann, E. (1956) Hispinae aus Indonesia. 170. Beitrag zur Kenntnis der Hispinae (Coleoptera, Chrysomelidae). *Beaufortia, Series of Miscellaneous Publications*, 5 (50), 61–72.
- Uhmann, E. (1957a) Hispinae aus Südafrika. 172. Beitrag zur Kenntnis der Hispinae (Col. Chrysomelidae). *Annals of the Transvaal Museum*, 23 (1), 87–102.
- Uhmann, E. (1957b) Hispinae aus dem Britischen Museum. IX. Teil. 184. Beitrag zur Kenntnis der Hispinae (Coleopt. Chrysomelidae). *The Annals and Magazine of Natural History*, Series 12, 10, 364–368.
- Uhmann, E. (1958) Hispinae aus Südafrika. III. Teil. 183. Beitrag zur Kenntnis der Hispinae (Coleopt.: Chrysomelidae). *Journal of the Entomological Society of South Africa*, 21 (1), 214–226.
- Uhmann, E. (1962) *Dactylispa capicola* (Péringuey) und Verwandte. (203. Beitrag zur Kenntnis der Hispinae (Coleoptera, Chrysomelidae)). *Annals of the South African Museum*, 46 (8), 223–230.
- Uhmann, E. (1965) Die Puppe von *Dicladispa testacea* (Linné) (Coleoptera: Chrysomelidae). 219. Beitrag zur Kenntnis der Hispinae. *Nachrichtenblatt der Bayerischen Entomologen*, 14, 118–121.
- Yano, T. (1965) Larval stages of leaf-miners found in Shikoku (Coleopterous leaf-miners of Japan, VII). *Transactions of the Shikoku Entomological Society*, Marsuyama, 8 (4), 115–132.