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Morphology of preimaginal stages of *Lauxania* and *Calliopum* (Diptera: Lauxaniidae)

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Abstract

Scanning electron microscopy (SEM) analysis of all preimaginal stages of the following lauxaniid species is presented for the first time: *Lauxania cylindricornis* (Fabricius), *L. minor* Martinek, *Calliopum aeneum* (Fallén), *C. elisae* (Meigen), *C. splendidum* Papp and *C. simillimum* (Collin). Detailed images of the pseudocephalon, antenna, maxillary palpus, facial mask, cirri, mouth hooks, body segments and anterior and posterior spiracles of all larval instars are provided along with illustrations of the cephaloskeleton for all three instars. Eggs bear longitudinal ridges and a perforated tubercle on the posterior end. Larvae have several rows of cirri, a pair of abdominal ambulatory lobes and the distal part of Malpighian tubules is filled with white matter. Identification of species or genera is problematic especially in the second instar. Puparia are barrel-shaped, while the calcareous deposit over the puparium typical for other lauxaniids is missing. The morphology of the immature stages supports the notion that *Lauxania* may be not monophyletic.

Key words: Diptera, Lauxaniidae, *Lauxania cylindricornis*, *L. minor*, *Calliopum aeneum*, *C. elisae*, *C. simillimum*, *C. splendidum*, egg, larva, puparium, morphology

Introduction

Lauxaniidae is a common and species rich family of acalyptrate flies. Adults often occur on vegetation in shaded habitats and near streams (Papp & Shatalkin, 1998). Only a negligible proportion of the described species have known larvae and larval biology, although larvae may represent a valuable source of phylogenetic data (Meier 1995, 1996, Meier & Lim 2009, Pape 2001, Szpila & Pape 2007, 2008). Larvae of lauxaniids are saprophagous, usually occurring in decaying vegetation such as fallen leaves or bird nests (Miller & Foote 1976). Immature morphology and biology of some lauxaniid species were briefly studied by Meijere (1909), Hennig (1952) and Sasakawa & Ikeuchi (1982, 1983). More detailed records of immature biology were given by Miller (1977a, 1977b, 1977c) and Miller & Foote (1975). Miller & Foote (1976) studied morphology of eggs, all three larval instars and puparia for eight common North American species. Gaimari & Silva (2010) summarized recent knowledge of lauxaniid larval morphology and biology.

The genus *Lauxania* Latreille is commonly considered to be related to the genus *Calliopum* Strand. Both these genera share metallic black coloration, yellowish wings and a transverse depression on the lower part of face, though in *Calliopum* the last character is less pronounced. Pérusse & Wheeler (2000) suggest that based on the structure of the genitalia, *Lauxania* and *Calliopum* are not monophyletic in relation one to another.

The genus *Lauxania* is represented by 11 species in Palearctic region (Shatalkin 2000, Merz 2001). Species of *Lauxania* share an elongated third antennal segment, a white arista and usually black knob of the halter (Shatalkin 2000). The genus is morphologically heterogeneous and currently is divided into three subgenera. Papp (1978) erected the subgenus *Callixania* Papp for *L. minor* Martinek 1974, which differs from the nominate subgenus by having a protruding facial angle, the short scape, inclinate anterior fronto-orbital setae, and by the male genitalia. Later, Papp & Shatalkin (1998) proposed that *Callixania* should be considered as a genus, which currently has two species (Shatalkin 2000).

Lauxania s. str. is the most species rich subgenus of *Lauxania*, with most of the included species described only in the last three decades (Shatalkin 2000, Merz 2001).

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Shatalkin (2000) described a separate subgenus *Czernushka* Shatalkin for *L. albomaculata* Strobl 1909. This species is unique among other *Lauxania* species by having a bare arista.

The genus *Calliopum* is represented by 15 species in Europe (Shatalkin 2000, Carles-Torlá 2001). The arista is brown and the third antennal segment is shorter in comparison with *Lauxania*.

In the present paper the preimaginal stages are described for two species of *Lauxania* representing two subgenera: *L.* (*Callixania*) *minor* and *L.* (*Lauxania*) *cylindricornis* (Fabricius 1794); and four species of the genus *Calliopum*, namely *C. aeneum* (Fallén 1820), *C. elisae* (Meigen 1826), *C. simillimum* (Collin 1933) and *C. splendidum* Papp 1978.

Material and methods

Adult flies were obtained by sweeping vegetation at several localities in western and northern Slovakia. The keys of Merz (2001) and Shatalkin (2000) and its English translation (Schacht et al. 2004) were used for determination. Both males and females were kept in plastic jars filled with moss (*Hypnum* sp.) to maintain suitable humidity. A mixture of honey and yeasts was used as an adult food source (Miller 1977b, 1977c). Eggs were laid singly by females on the moss. Eggs were transferred in glass dishes and kept at a temperature of 10±2°C. Rotting leaves of wild cherry (*Cerasus avium*) were added to glass dishes as a larval food source immediately after the first larvae appeared. Larvae were reared in glass dishes until pupariation. In winter 2009 we also collected larvae from nature to acquire puparia. Living larvae were washed in a stream of liquid water from a vaporizer except for the tiny first instars. Larvae were put in water and slowly heated until the temperature reached 45°C, consequently retaining an exposed pseudocephalon and a pair of extendable ambulatory lobes. Eggs, individual larvae and puparia were stored in 80% ethanol. Preparation of larvae for SEM involved dehydratation through 80, 90, and 99.5% ethanol series and soaking in hexamethyldisilazane (HMDS) (Brown 1993). We removed reduntant HMDS so that all larvae remained immersed and remaining HMDS slowly evaporated by atmospheric pressure and room temperature. For eggs, the ethanol series was replaced by soaking directly in 99.5% ethanol and then in HMDS. Larvae and eggs were coated with gold or a gold-palladium mixture. SEM pictures were taken with a Quanta 3D 200i. Cephaloskeletons were re-drawn from photographs taken by Nikon Coolpix 990 digital camera mounted on a Nikon Eclipse E600 microscope under differential interference contrast. Cephaloskeletons were extracted mechanically; for the first instar we used the whole anterior half of body. Soaking in 4% KOH for at least one hour removed soft tissues. The line drawings were scanned and further processed in Adobe Photoshop, version 6.0.1 CE.

Photographs of puparia were taken by Micrometrics camera mounted on Zeiss binocular microscope Stemi 2000-C, in the program Micrometrics SE Premium—318-318004BF to acquire morphometric data. The dead larvae were measured before the dehydration process.

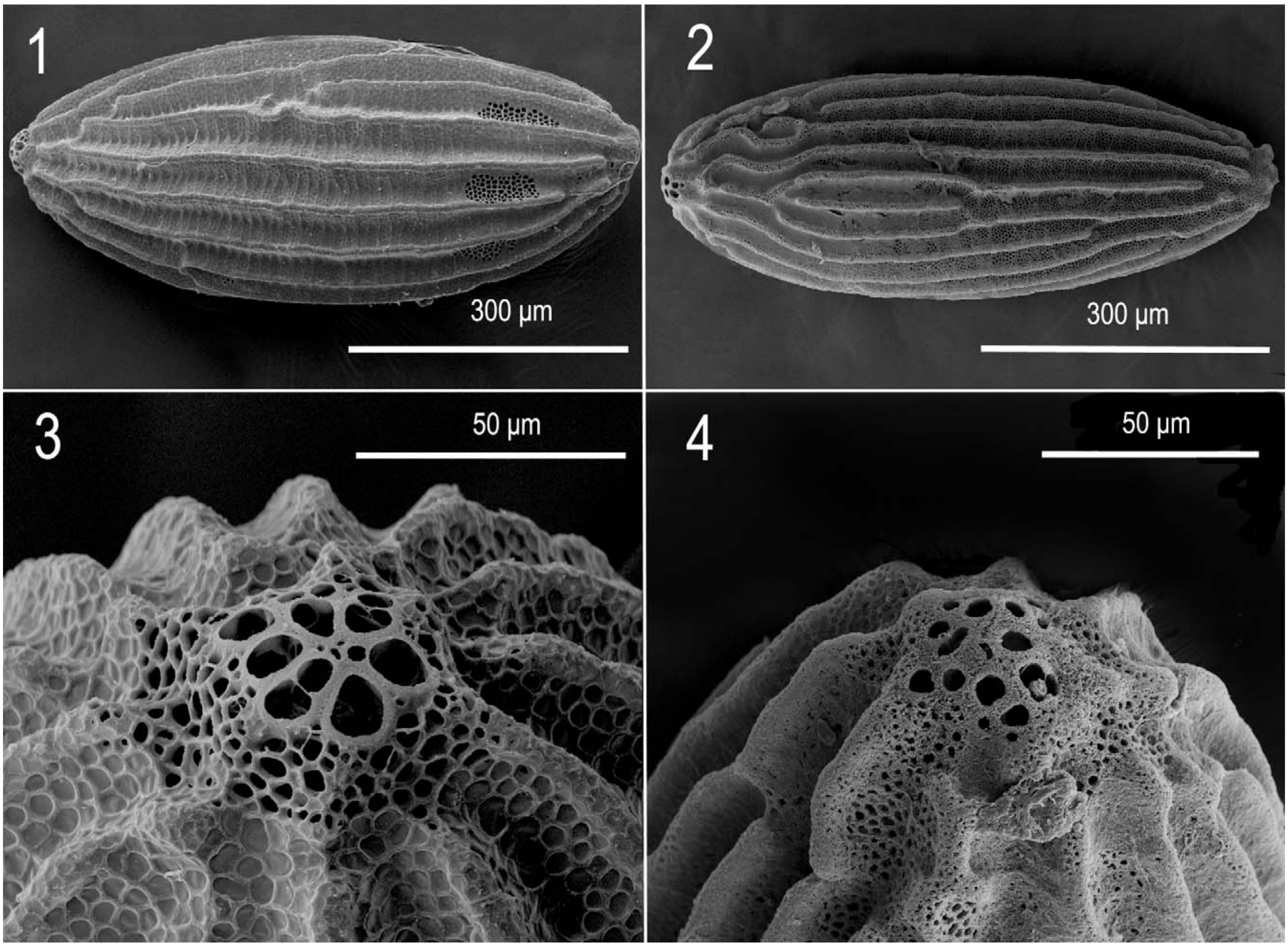
Terminology follows Courtney et al. (2000) and Miller & Foote (1976).

Results

From every instar and species we prepared up to 30 specimens for SEM and 3 to 10 cephaloskeletons. Puparia were obtained in sufficient number from laboratory rearing only for the species of *Lauxania*, and *Calliopum splendidum*. Puparia for *C. simillimum* were obtained both from laboratory rearing and from larvae collected in nature. We reared only one puparium of *C. aeneum* from a larva collected in nature. Larvae of *C. elisae* during the time of study did not pupariate. Several specimens of *C. splendidum* emerged from reared puparia.

In the laboratory conditions, first instar larvae appeared several days after egg laying. The first instar was short, lasting only for a few days. Third-instar larvae appeared after two–three weeks. After the third-instar larvae reached full length, they stop ingesting food and become less active. Some quiescent larvae stay alive for three months, but only a few pupated. Pupariation takes places typically between leaves. The developmental rate greatly varies according to the temperature, but it seems that larvae are more likely to complete development in cold conditions. Larvae kept at room temperature did not pupariate at all.

Eggs, larvae and puparium show great similarity in all species, so features held in common will be described first.



FIGURES 1–4. Eggs of *Lauxania cylindricornis* and *L. minor.* 1 – *L. cylindricornis*, oriented with posterior end to left. 2. *L. minor*, oriented with posterior end to left. 3. *L. cylindricornis*, detail of posterior tubercle. 4. *L. minor,* detail of posterior tubercle.

Characteristics of preimaginal stages of genera *Lauxania* and *Calliopum*

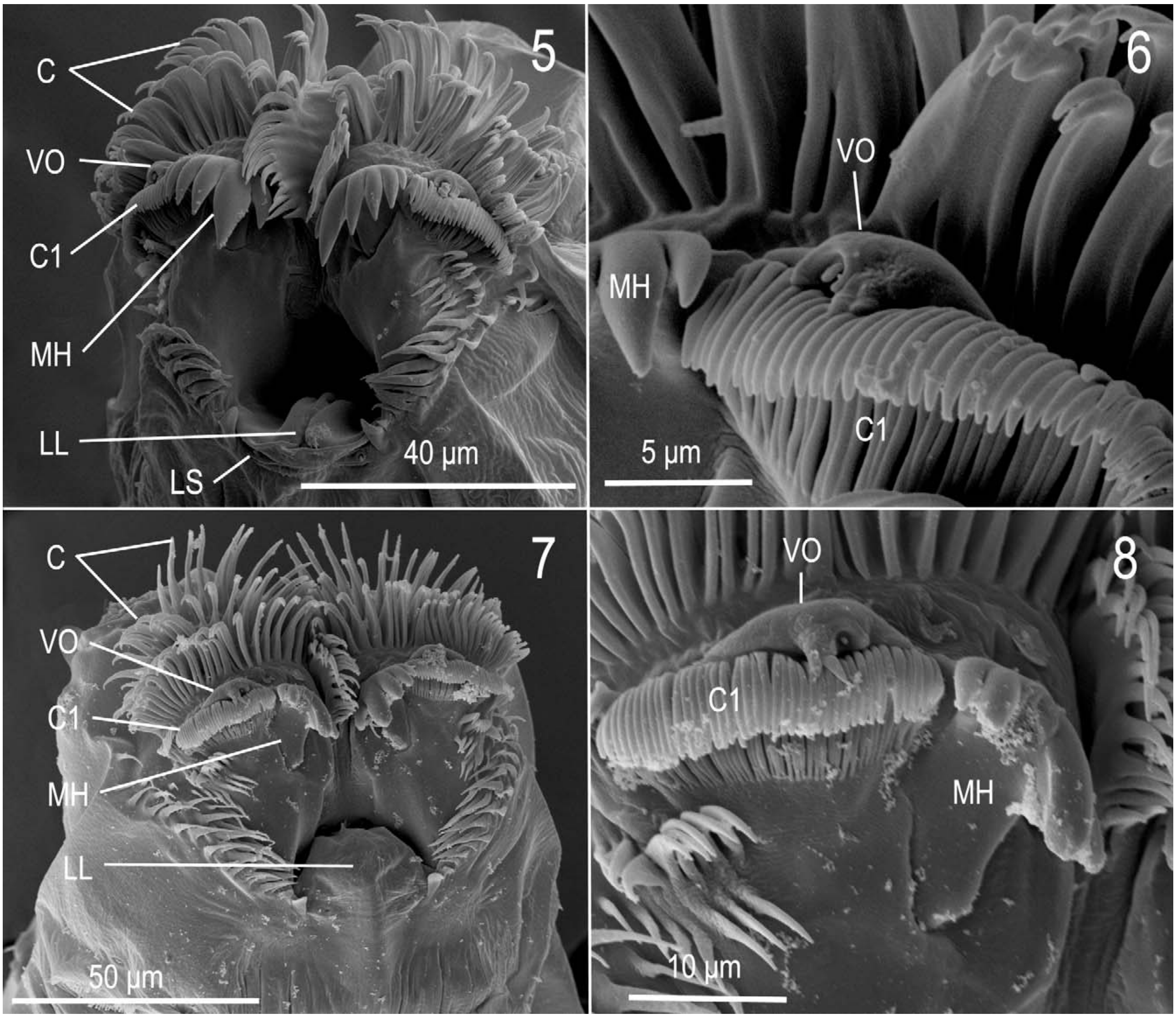
Egg. (Figs 1–4, 35–46) White, oval to carinate, covered by longitudinal ridges. Posterior tip of egg shielded by ball-shaped tubercle with circular openings. Micropylar pole surrounded by asymmetric collar.

Larva. Body cylindrical and moderately dorsoventrally flattened, tapering both on anterior and posterior ends, integument translucent. Distal part of Malpighian tubules thin and filled with white substance. Body segments differentiated into pseudocephalon, three thoracic segments, seven abdominal segments and the anal division.

Pseudocephalon bilobed, bearing antenna, maxillary palpus, cirri and ventral organ. Antenna simple, dome shaped, longer than wide (Fig. 10). Maxillary palpus consists of a cluster of several sensilla; sensilla arrangement similar across instars and species (four sensilla coeloconica, four sensilla basiconica and two accessory sensilla, Figs 14, 24, 64). Cirri form 5–7 transverse rows, at base extended considerably and laterally flattened. Medially, rows of cirri somewhat irregular. Labial lobe equipped with two basal sensilla. Internal surface of labial lobe with fibrous outgrowths (e.g. Fig. 23).

Thoracic segments without fully developed crevices and welts. Dorsally and ventrally with comb spines on anterior border (Figs 16, 19, 26, 29). First thoracic segment has anteroventrally several rows of special comb spines aligned in diagonal direction (e.g. Figs 49, 51). Dorsally, there are always three pairs of tubercles on each segment; in the second and the third thoracic segments they are arranged in one transverse line; in the first thoracic segment the second pair is moved posteriad (e.g. Figs 26, 96).

The integument of abdominal segments and anal division wrinkled intricately, creating numerous crevices and welts. The basic plan of crevices, welts and projections held in common in all instars.



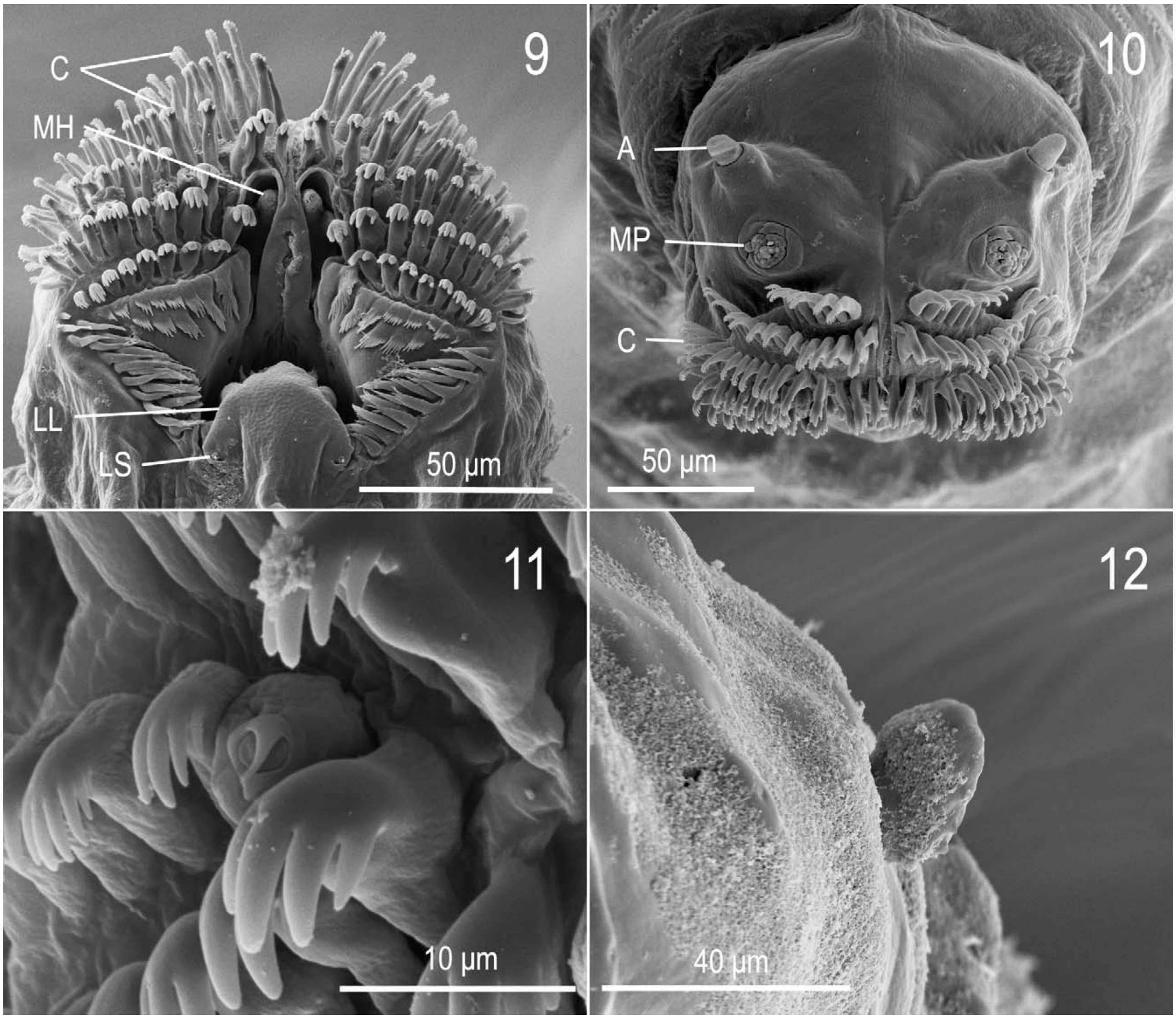
FIGURES 5–8. First instar of *L. cylindricornis* and *L. minor.* 5. *L. minor*, pseudocephalon, ventral view. 6. *L. minor*, detail of ventral organ. 7. *L. cylindricornis*, pseudocephalon, ventral view. 8. *L. cylindricornis*, detail of ventral organ and mouth hook. C, cirri; C1, first row of cirri; LL, labial lobe; LS, labial sensilla; MH, mouth hook; VO, ventral organ.

Abdominal segments dorsally with anterior spinous welt. Middle welt with four tubercules, posteriorly delimited by strong arched crevice, followed by a narrow area and a posterior creeping welt (Fig. 57). The latter two parts may not be well delimited. Lateral parts of abdominal segments with three to four paired tubercles; the upper-most probably corresponds to the lateral processes of the anal division (Figs 21, 104). An additional small lateral welt without spines present (e.g. Fig. 104). Ventrally, abdominal segments with anteriorly spinous and posteriorly smooth creeping welt (Figs 22, 32, 103). The posterior welt weakened in the middle. There are seven abdominal and one anal spinous creeping welt both on dorsal and ventral side.

The anal division bears 4 pairs of processes: two directed laterally, one dorsally and one tiny pair behind ambulatory lobes directed ventrally. Pair of extendable ambulatory lobes surrounds the anus (Figs 31, 33). Posterior spiracles similar across all instars, placed on closely adjacent cylindrical prominences. Spiracular plate of posterior spiracle with three spiracular slits placed on ovoid elevations and with four peristigmatic tufts (first instar with two slits and three tufts), (Figs 34, 60).

The integument is equipped by sensilla of various types. They are located mostly on body extremities, typically on creeping welts, lateral processes of abdominal segments and processes of anal division.

Cephaloskeleton consists of mouth-hooks, dental sclerites, labrum, epistomal sclerite, intermediate sclerite, parastomal bars, rami, ligulate sclerites, subhypostomal sclerites and basal sclerite, which consists of vertical plate, ventral and dorsal cornua; dorsal cornua connected via dorsal bridge (Figs 107–115).



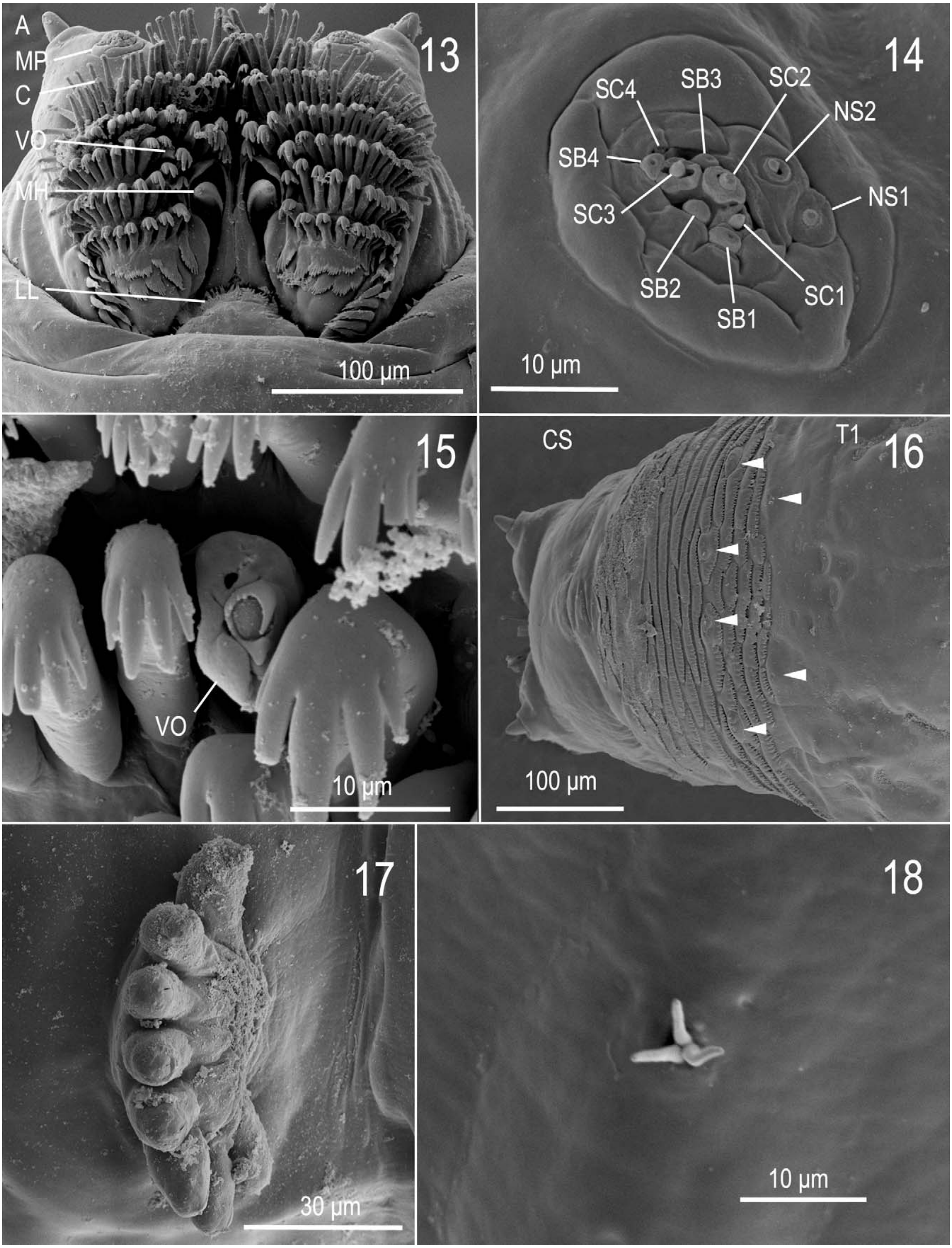
FIGURES 9–12. *L. cylindricornis*, second instar. 9. Pseudocephalon, ventral view. 10. Pseudocephalon, anterodorsal view. 11. Ventral organ, detail. 12. Anterior spiracle. A, antenna; C, cirri; LL, labial lobe; LS, labial sensilla; MH, mouth hook; MP, maxillary palpus.

All instars can be easily distinguished by the absolute length of the cephaloskeleton and the form of spiracles: the first instar has no visible anterior spiracles (thought they are present, note ecdysial scar in the anterior spiracle of second instar, Fig. 68, all instars are thus amphipneustic), the second instar with anterior spiracles simple and the third instar with anterior spiracles fan-shaped (Fig. 17).

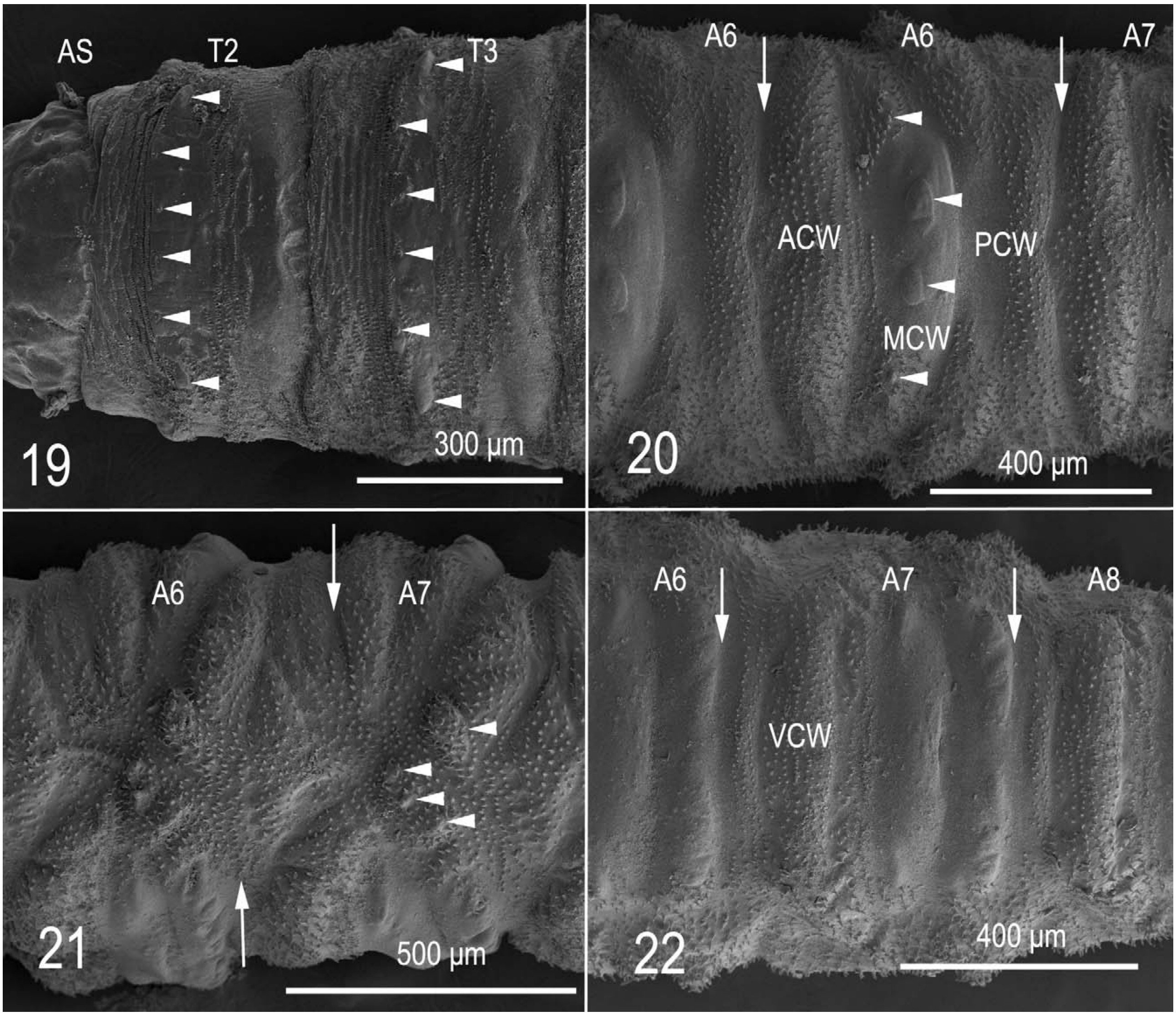
First larval instar. Cirri aligned in five transverse rows. First row sclerotised, very compact and loosely attaching to mouth-hooks. Remaining cirri soft, slender and apically pointed. Paired vertical row of cirri-resembling outgrowths arises between mouth-hooks. Slender outgrowths located opposite to sclerotic row of cirri (e.g. Figs 5, 7). Lobe-like ventral organ lying on first row of cirri (Figs 6, 8).

Thoracic segments covered anteroventrally by comb spines (Figs 55, 56). Abdominal segments and anal division smooth except for creeping welts (Figs 57–59).

Cephaloskeleton (Figs 107, 110, 113). Mouth-hooks parallel, divided into distal dentate and proximal stalk parts; dentate part equipped with 4 to 6 teeth; stalked part sclerotised, ventrally with dental sclerites, basally touching to labrum. Labrum rounded on anterior edge, slender. Epistomal sclerite broad, convex, laterally with two openings, posteriorly projected into sharp corners. Ligulate sclerites simple. Subhypostomal sclerites strongly converging, extending from tip of intermediate sclerite. Intermediate sclerite slender and extending ventrally from strong parastomal bars. Dorsal cornu slender, distal part hyaline. Dorsal edge of ventral cornu hyaline. Bottom of pharynx covered with fine dendroid structure.



FIGURES 13–18. *Lauxania minor*, third instar, arrowheads indicate the tubercles. 13. Pseudocephalon, ventral view. 14. Maxillary palpus. 15. Ventral organ. 16. Pseudocephalon and first thoracic segment, dorsal view. 17. Anterior spiracle. 18. Keilin's organ. A, antenna; C, cirri; CS, pseudocephalon; LL, labial lobe; MH, mouth hook; MP, maxillary palpus; NS1, NS2, first and second additional sensilla; SB1–SB4, first to fourth sensilla basiconica; SC1–SC4, first to fourth sensilla coeloconica; VO—ventral organ.



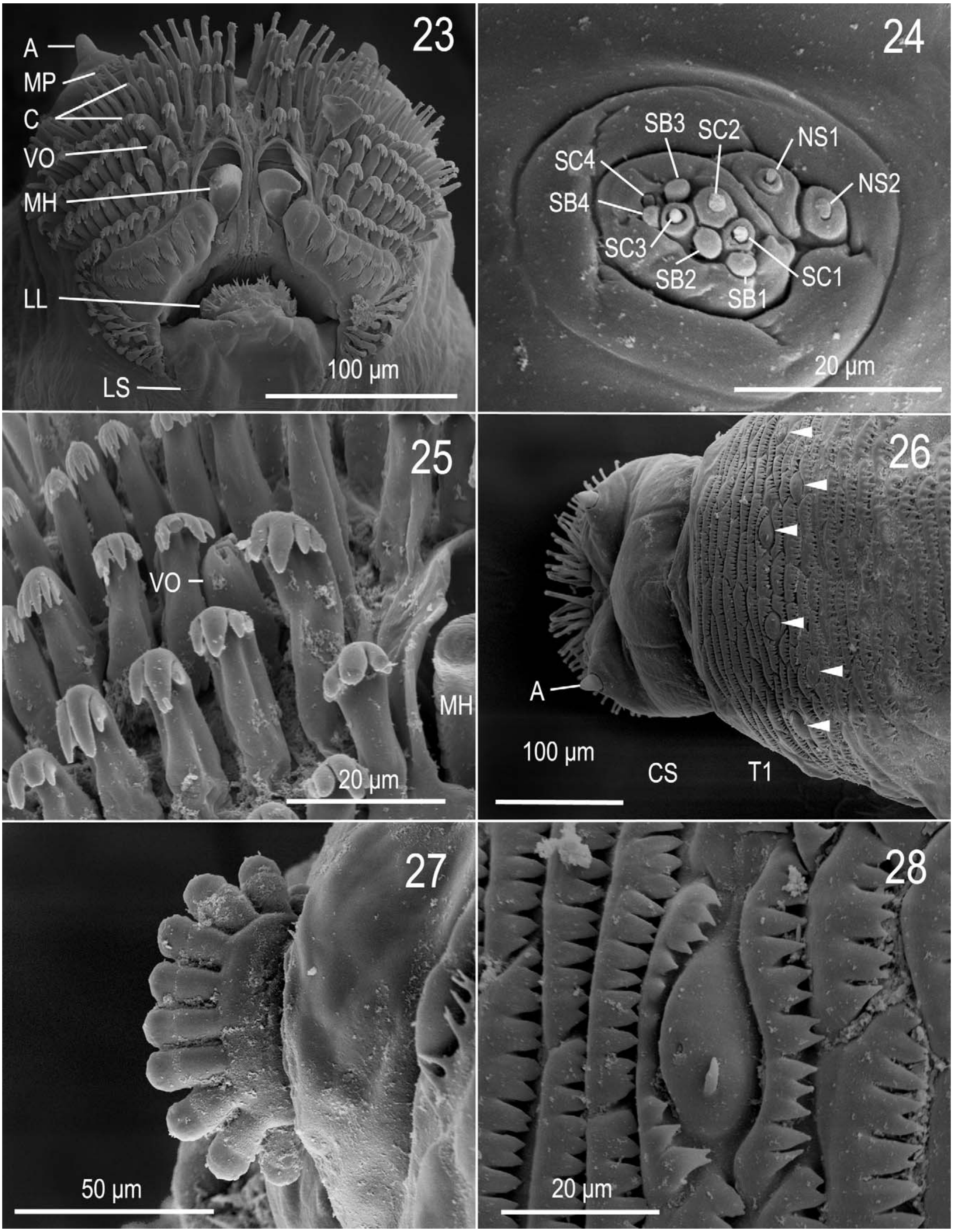
FIGURES 19–22. *Lauxania minor*, third instar, arrows indicate the borders between segments, arrowheads indicate the tubercles. 19. Second and third thoracic segment, dorsal view. 20. Abdominal segments, dorsal view. 21. Abdominal segments, lateral view. 22. Abdominal segments, ventral view. A6–A8, sixth to eighth abdominal segments; ACW, anterior creeping welt; AS, anterior spiracle; PCW, posterior creeping welt; MCW, medial creeping welt; VCW, ventral creeping welt.

Second larval instar. Cirri organized in six rows; apical part of cirri hand-shaped, with sclerotised digitiform projections (Figs 9, 10). Soft, fimbriated outgrowths surround the mouth (Fig. 9). Ventral organ cylindrical, visible in third row of cirri (Fig. 11).

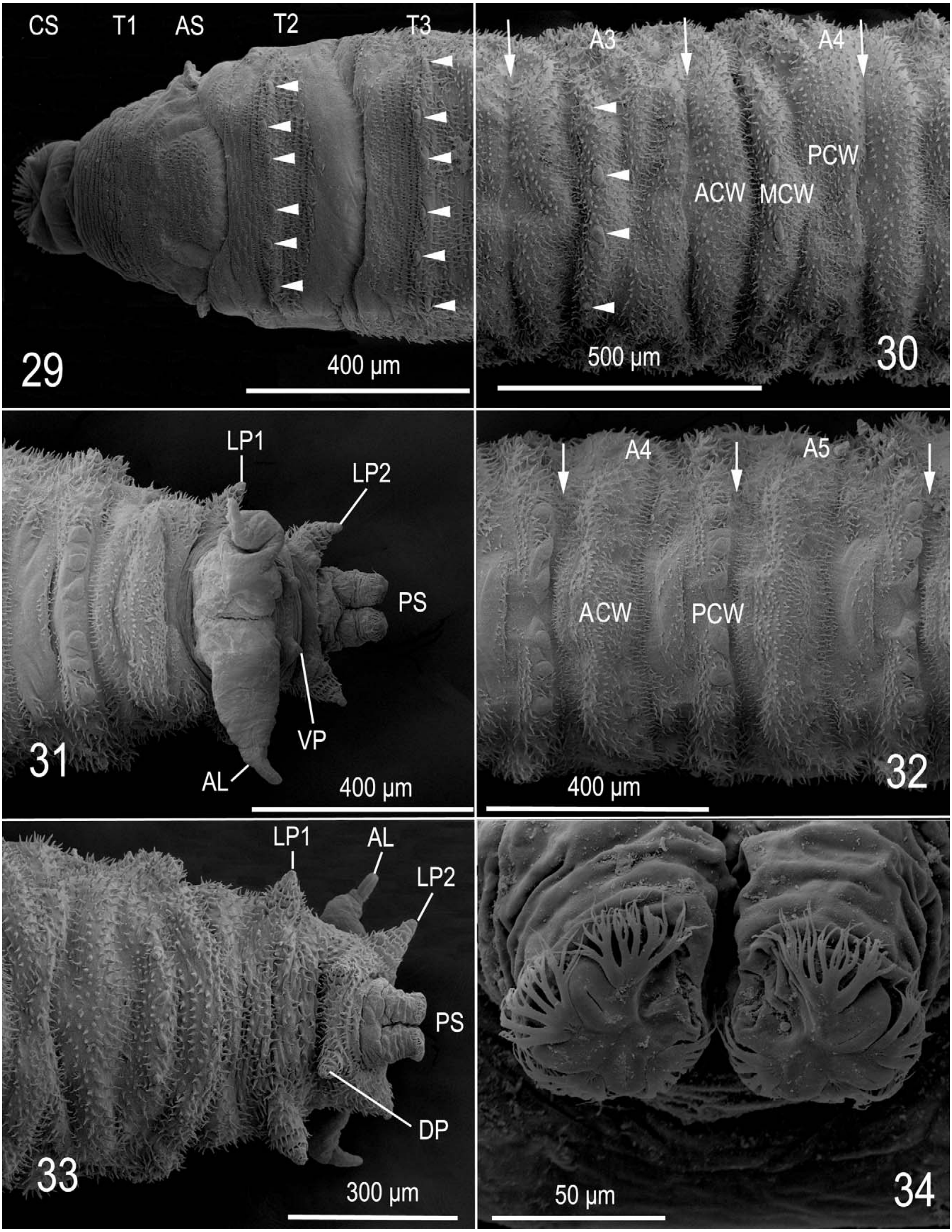
Anterior spiracles simple, ovate, with ecdysial scar at base and tiny holes at periphery (Figs 12, 68). Thoracic segments armed with strong comb-like spines anteriorly, otherwise smooth. Integument vestiture resembling that of third instar, but less developed (Figs 69–73).

Cephaloskeleton (Figs 108, 111, 114) strongly sclerotised comparing to the first instar. Mouth-hooks converging, with two unequal amber-coloured teeth and dorsal apodeme near base; basal part of mouth-hook articulating with intermediate sclerite; two small openings visible from lateral view.. Dental sclerites present. Epistomal sclerite rather narrow, arched and convex, with paired lateral openings. Subhypostomal sclerites rod-like, converging and ascending anteriorly. Ligulate sclerites short, simple. Rami slightly bent from lateral view. Vertical plates narrower compared to the third instar. Dorsal bridge trapezoidal to semilunar. Ventral cornu with long and hyaline apodeme, posterodorsal edge of ventral cornua sclerotised. Seven pharyngeal ridges present.

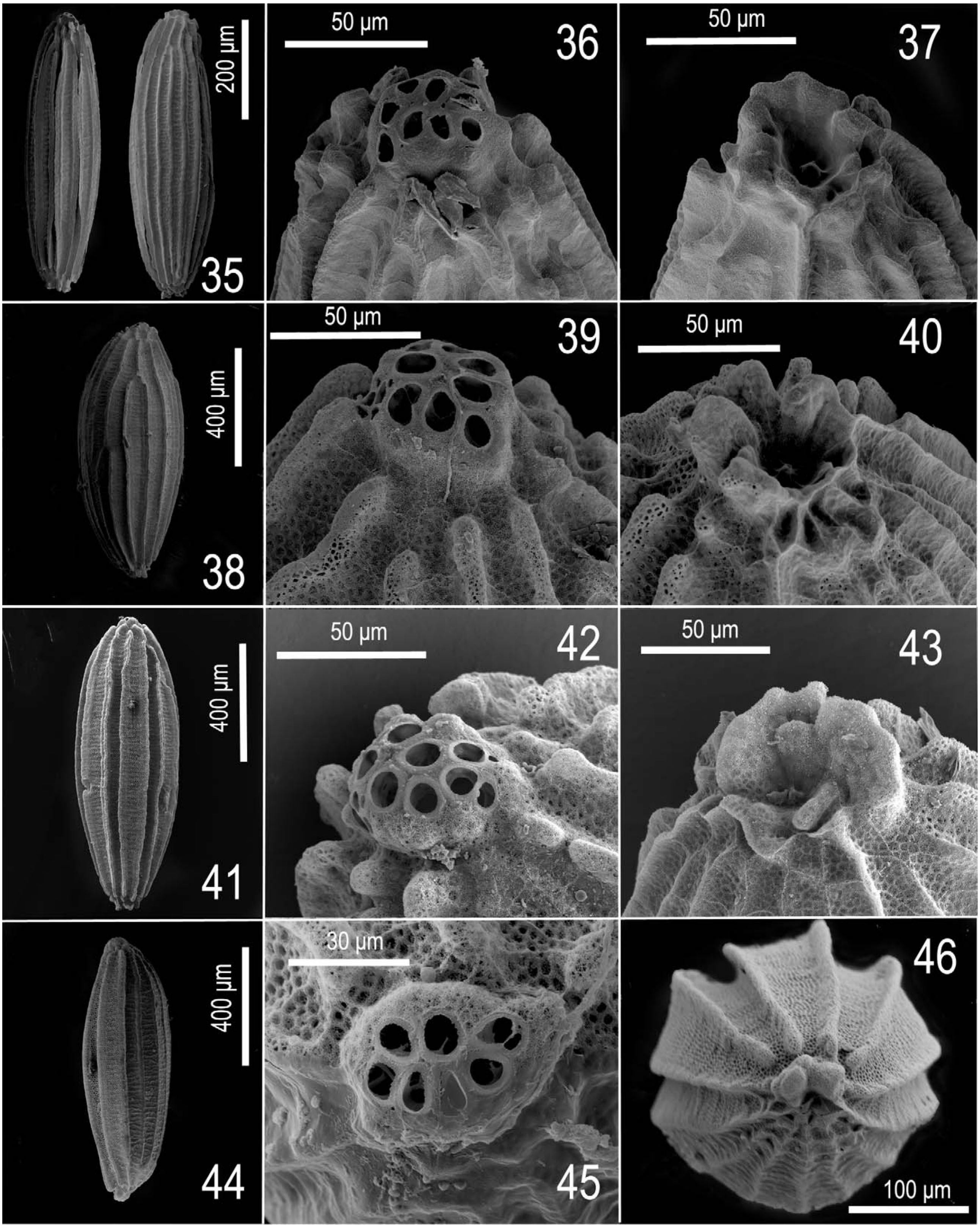
Third larval instar. Facial mask organized by the same manner, as in second instar, but cirri in seven rows (Figs 15, 25).



FIGURES 23–28. *Lauxania cylindricornis*, third instar, arrowheads indicate the tubercles. 23. Pseudocephalon, ventral view. 24. Maxillary palpus. 25. Ventral organ. 26. Pseudocephalon and first thoracic segment, dorsal view. 27. Anterior spiracle, anterior view. 28. Medial tubercle on first thoracic segment, dorsal view. A, antenna; C, cirri; CS, pseudocephalon; LL, labial lobe; LS, labial sensilla; MH, mouth hook; MP, maxillary palpus; NS1, NS2, first and second additional sensilla; SB1–SB4, first to fourth sensilla basiconica; SC1–SC4, first to fourth sensilla coeloconica; VO, ventral organ.

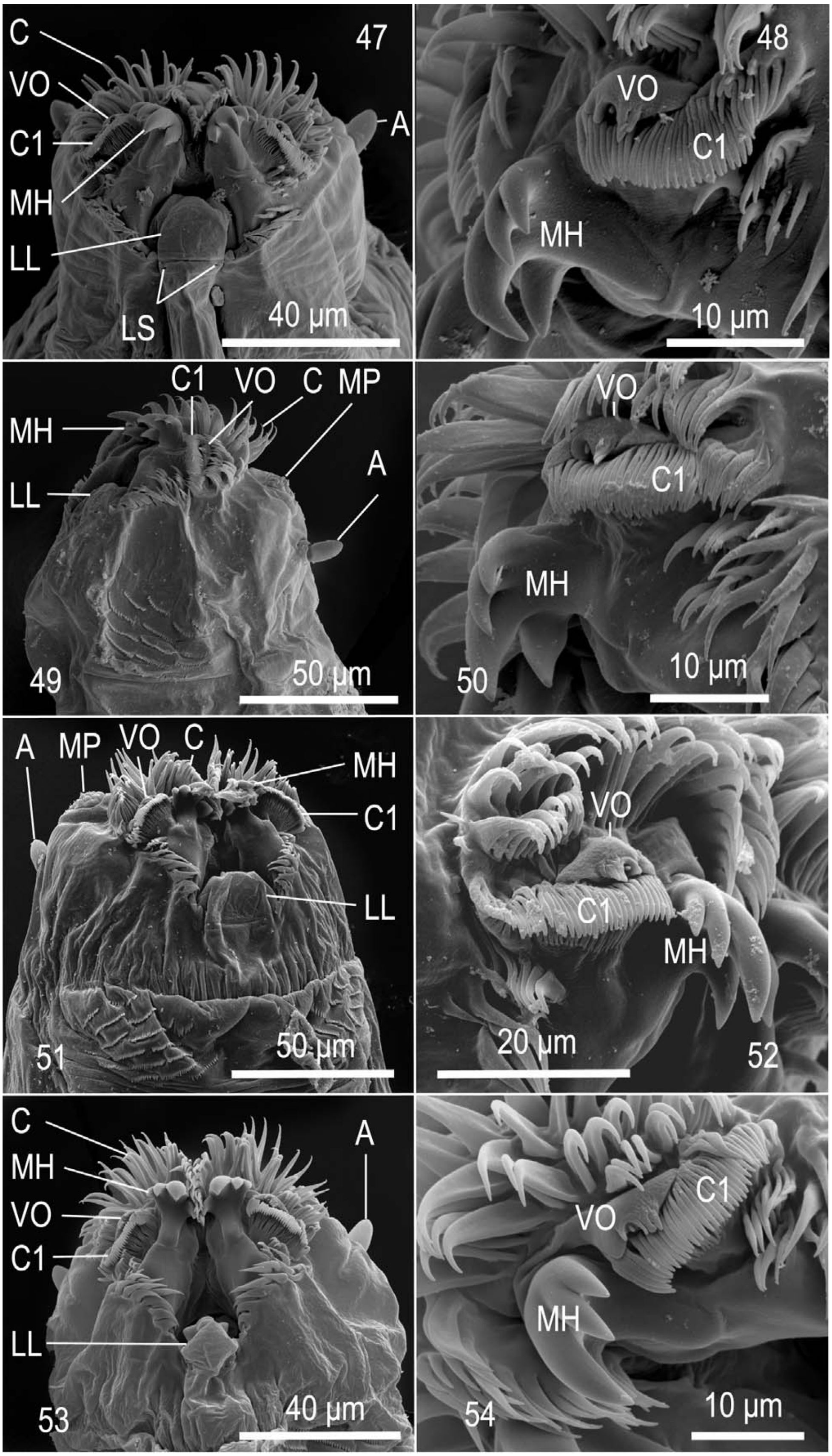


FIGURES 29–34. *Lauxania cylindricornis*, third instar, arrows indicate borders between segments, arrowheads indicate the tubercles. 29. Pseudocephalon and thoracic segments, dorsal view. 30. Abdominal segments, dorsal view. 31. Anal division, ventral view. 32. Abdominal segments, ventral view. 33. Anal division, dorsal view. 34. Posterior spiracles, posterior view. A3–A5, third to fifth abdominal segments; ACW, anterior creeping welt; AL, ambulatory lobe; AS, anterior spiracle; DP, dorsal process; LP1, LP2, first and second lateral process; PCW, posterior creeping welt; MCW, medial creeping welt; PS, posterior spiracles; T1–T3, first to third thoracic segments; VP, ventral process.

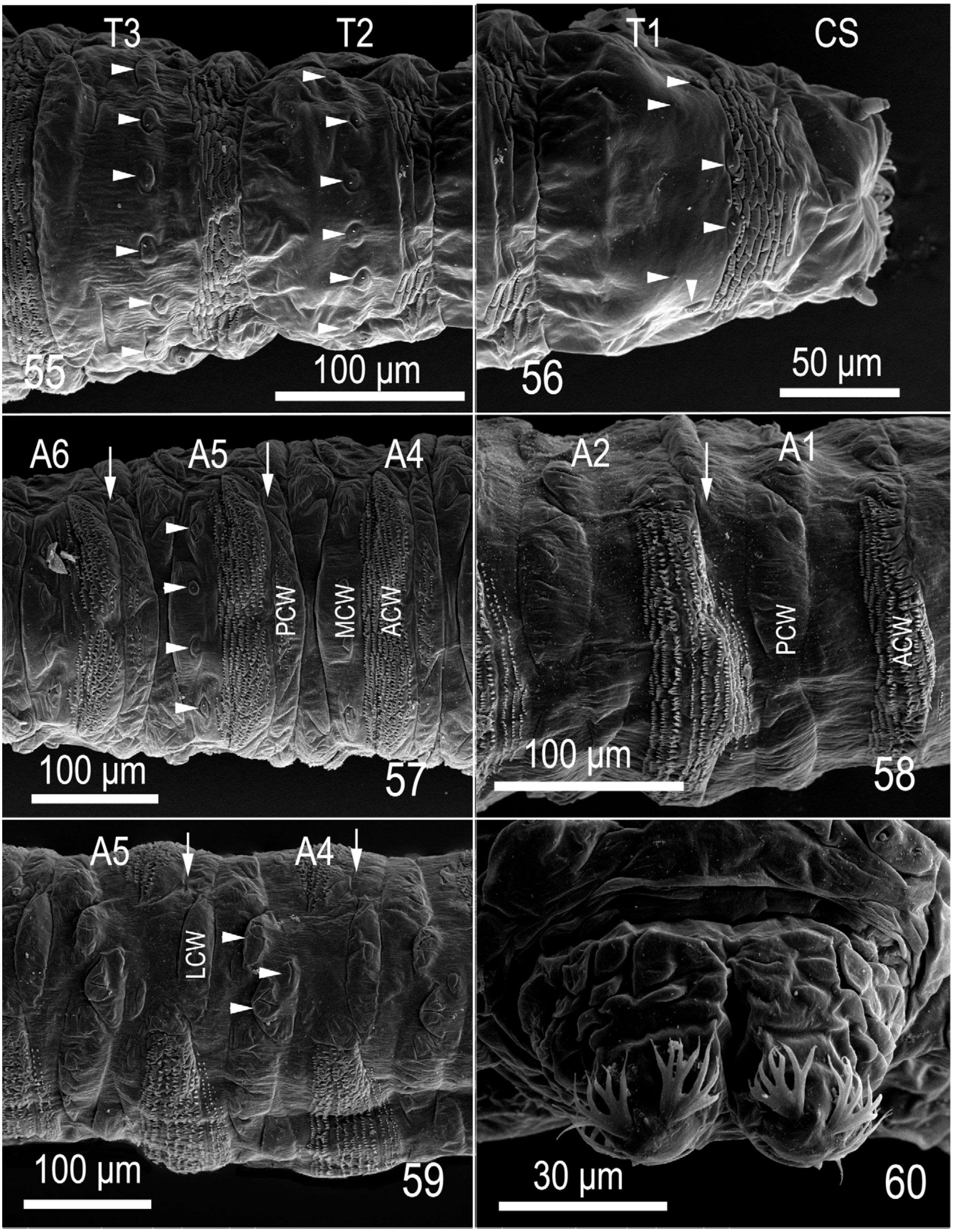


FIGURES 35–46. Eggs of *Calliopum* species, columns from left to right: whole egg oriented with posterior end up; posterior tubercle; mycropyle. 35–37. *C. aeneum*. 35. Left, dorsal view; right, ventral view. 38–40. *C. elisae*. 38. Dorsal view. 41–43. *C. splendidium*. 41. Dorsal view. 44–46. *C. simillimum*. 44. Lateral view.

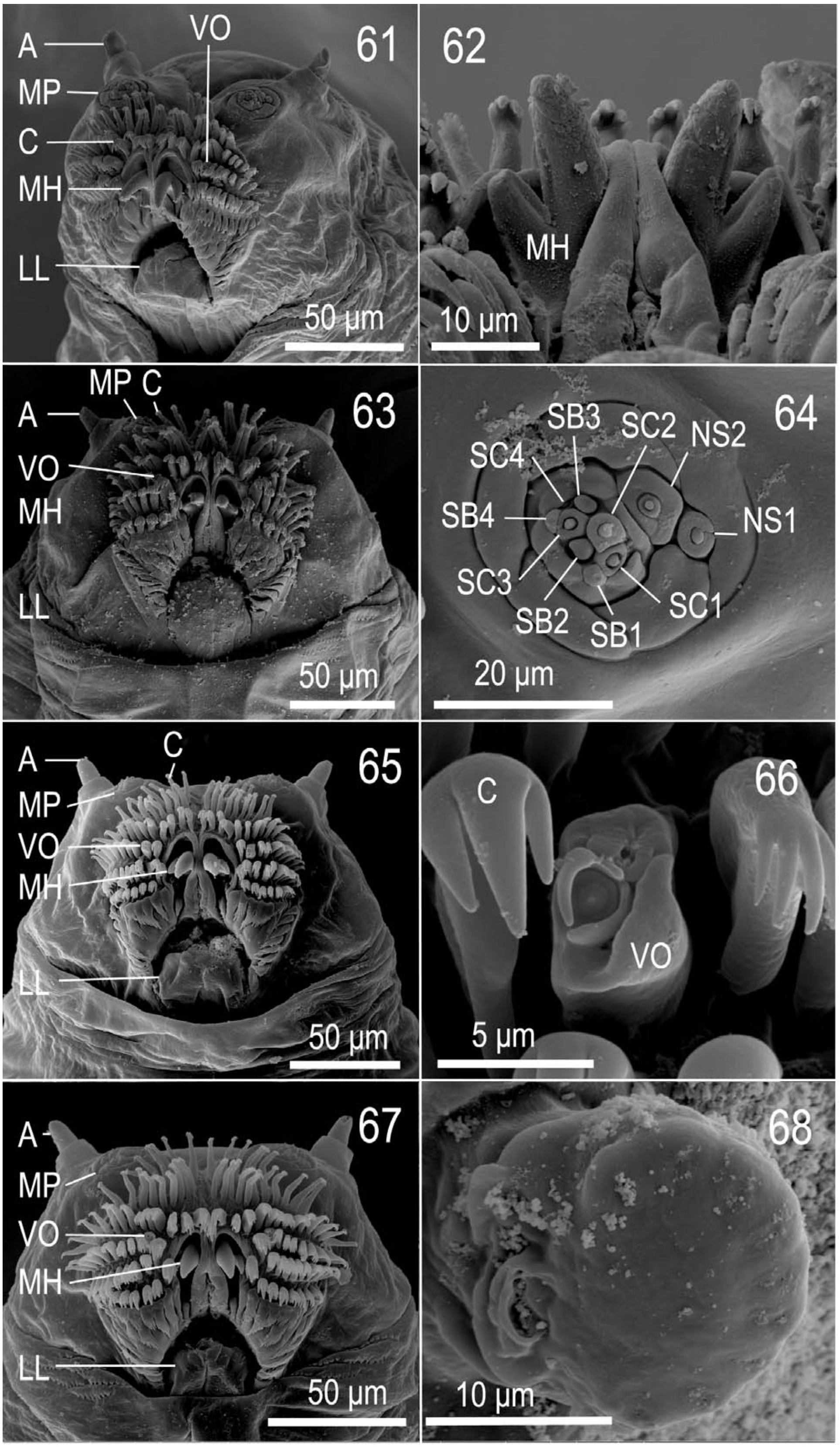
Anterior spiracles fan-shaped, directed laterally with 7–10 papillae (Figs 17, 27). Thoracic segments covered by comb-like spines, with posterior smooth area. Three pairs of oval tubercles occur on every thoracic segment dorsally (Figs 16, 19, 26, 28, 29).



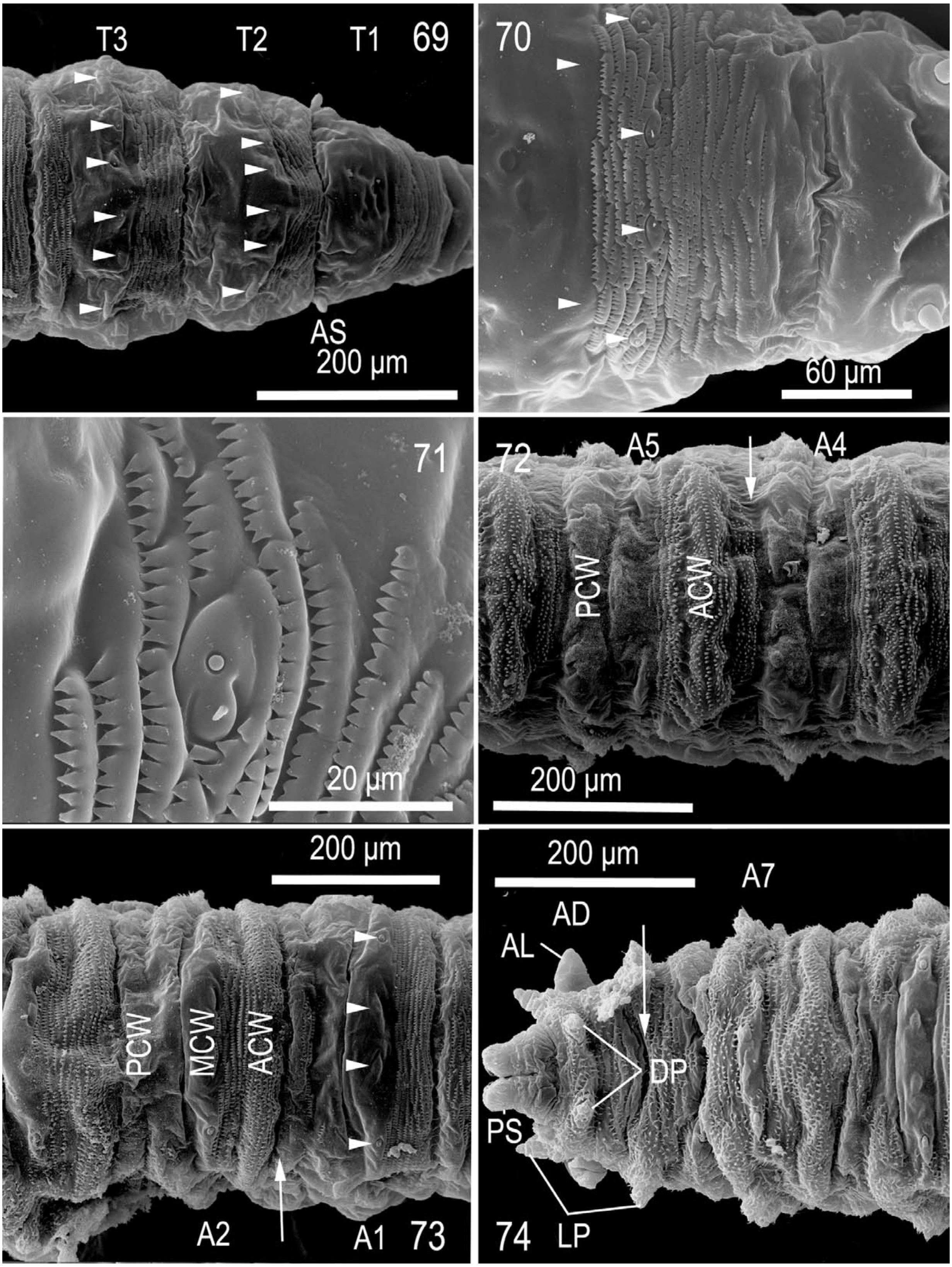
FIGURES 47–54. *Calliopum* species, first instar, with left column, pseudocephalon, ventral view (except 49, lateral view); right column, details of mouth hook, first row of cirri and ventral organ. 47–48. *C. aeneum*. 49–50. *C. elisae.* 51–52. *C. splendidum.* 53–54. *C. simillimum.* A, antenna; C, cirri; C1, first row of cirri; LL, labial lobe; LS, labial sensilla; MH, mouth hook; MP, maxillary palpus; VO, ventral organ.



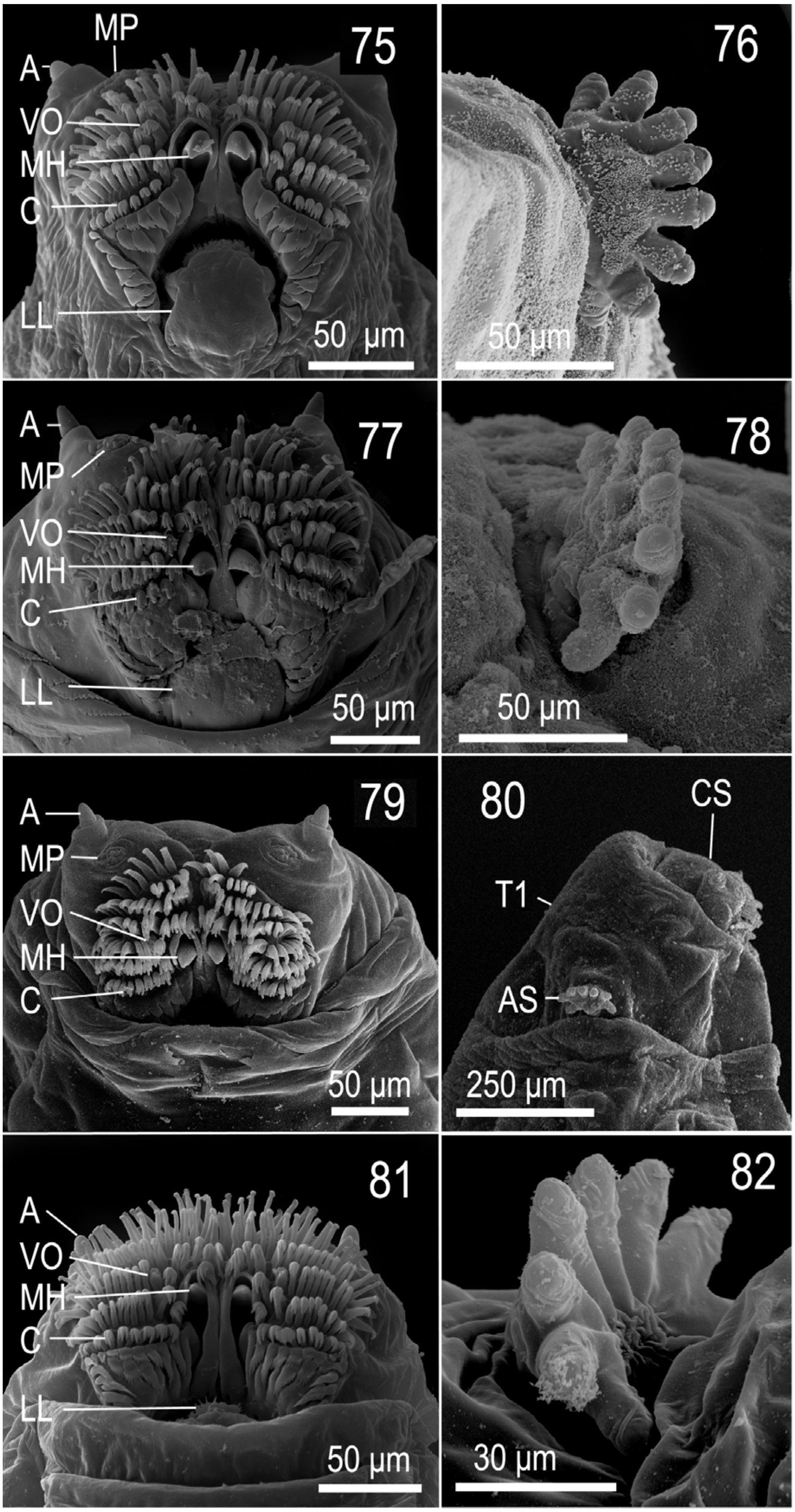
FIGURES 55–60. *Calliopum splendidum*, first instar, arrowheads indicate the tubercles. 55. Second and third thoracic segment, dorsal view. 56. Pseudocephalon and first thoracic segment, dorsal view. 57. Abdomnal segments, dorsal view. 58. Abdominal segments, ventral view. 59. Abdominal segments, lateral view. 60. Posterior spiracles, posterodorsal view.



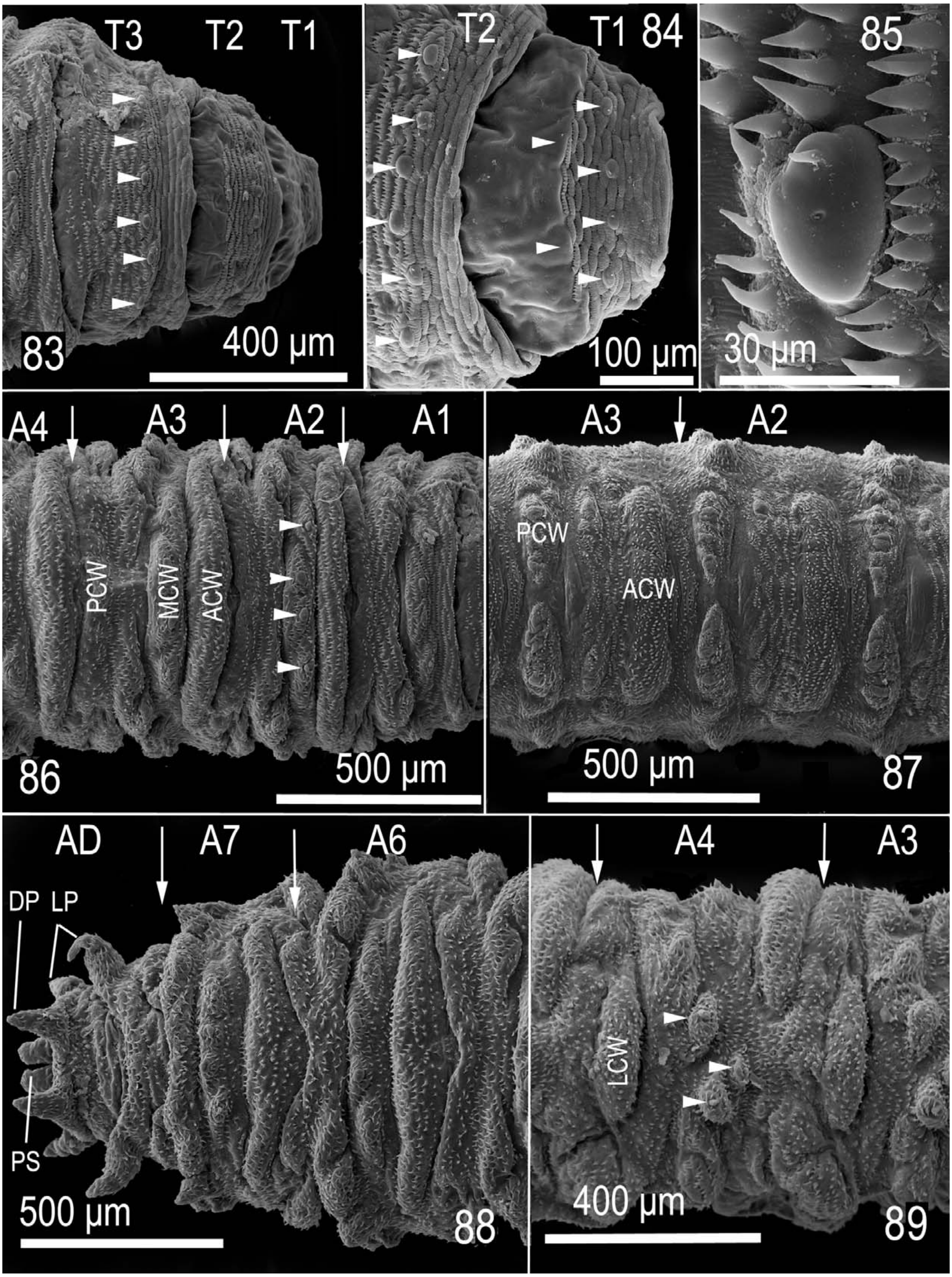
FIGURES 61–68. *Calliopum* species, second instar. 61–62. *C. aeneum*. 61. Pseudocephalon, ventral view. 62. Detail of mouth hooks, posterior view. 63–64. *C. elisae*. 63. Pseudocephalon, ventral view. 64. Maxillary palpus. 65–66. *C. splendidum*. 65. Pseudocephalon, ventral view. 66. Detail of ventral organ. 67–68. *C. simillimum*. 67. Pseudocephalon, ventral view. 68. Anterior spiracle, posterior view. A, antenna; C, cirri; LL, labial lobe; MH, mouth hook; MP, maxillary palpus; NS1–NS2, first and second additional sensilla. SB1–SB4, first to fourth sensilla basiconica; SC1–SC4, first to fouth sensilla coeloconica; VO, ventral organ.



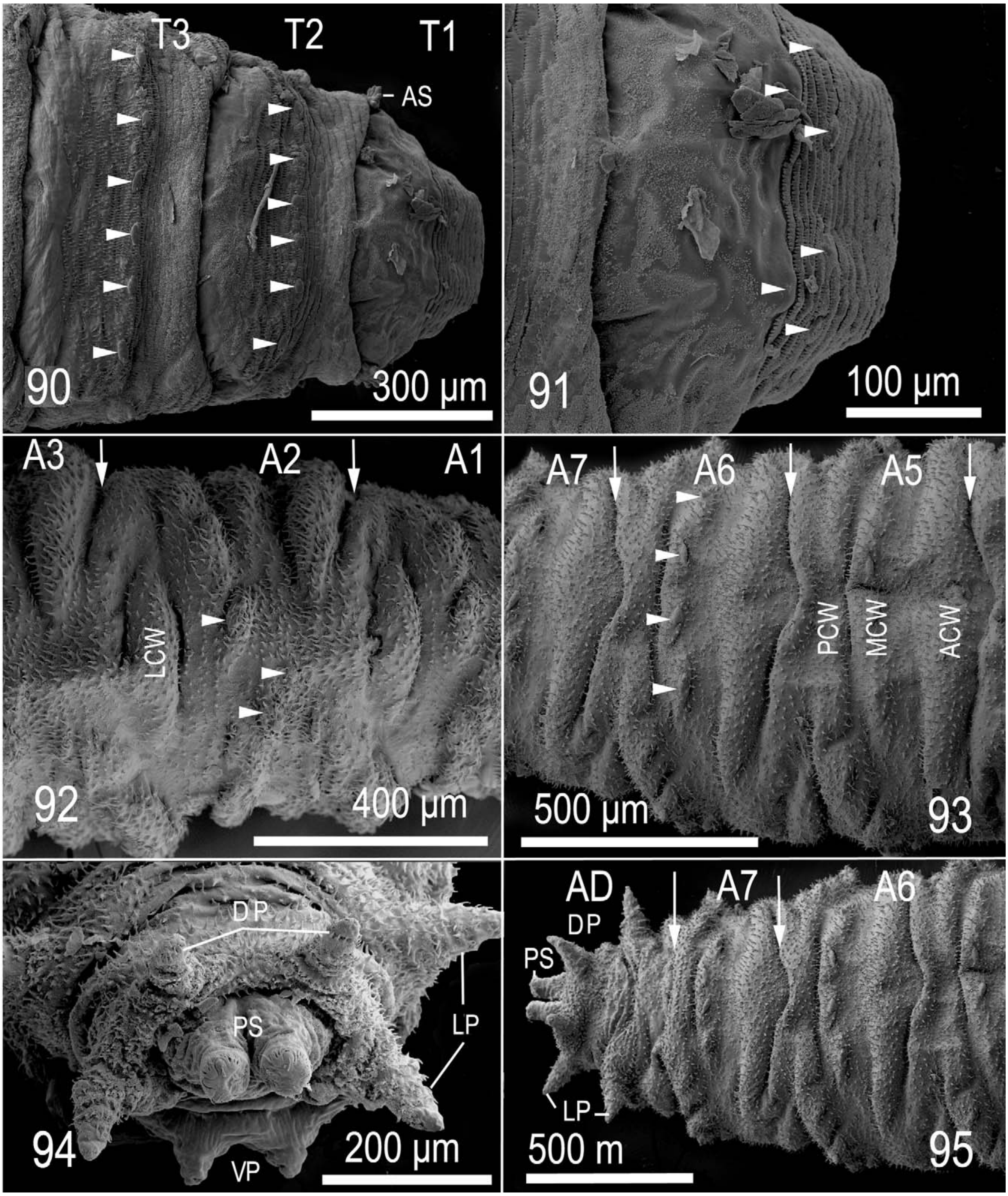
FIGURES 69–74. *Calliopum splendidum*, second instar, arrowheads indicate the dorsal tubercles, head is always oriented to the right. 69. Thoracic segments, dorsal view. 70. First thoracic segment, dorsal view. 71. Left sensilla of middle pair from the first thoracic segment. 72. Abdominal segments, ventral view. 73. Abdominal segments, dorsal view. 74. Anal division and last abdominal segments, dorsal view. A1–A7, first to seventh abdominal segments; ACW, anterior creeping welt; AD, anal division; AL, ambulatory lobes; AS, anterior spiracle; DP, dorsal process; LP, lateral process; MCW, medial creeping welt; PCW, posterior creeping welt; PS, posterior spiracles; T1–T3, first to third thoracic segments.



FIGURES 75–82. *Calliopum* species, third instar. 75–76. *C. aeneum*. 75. Pseudocephalon, ventral view. 76. Anterior spiracle, anterior view. 77–78. *C. elisae*. 77. Pseudocephalon, ventral view. 78. Anterior spiracle, lateral view. 79–80. *C. splendidum*. 79. Pseudocephalon, ventral view. 80. Lateral view of the pseudocephalon and first thoracic segments. 81–82. *C. simillimum*. 81. Pseudocephalon, ventral view. 82. Anterior spiracle, posterodorsal view. A, antenna; AS, anterior spiracle; C, cirri; CS, pseudocephalon; LL, labial lobe; MH, mouth hook; MP, maxillary palpus; T1, first thoracic segments; VO, ventral organ.

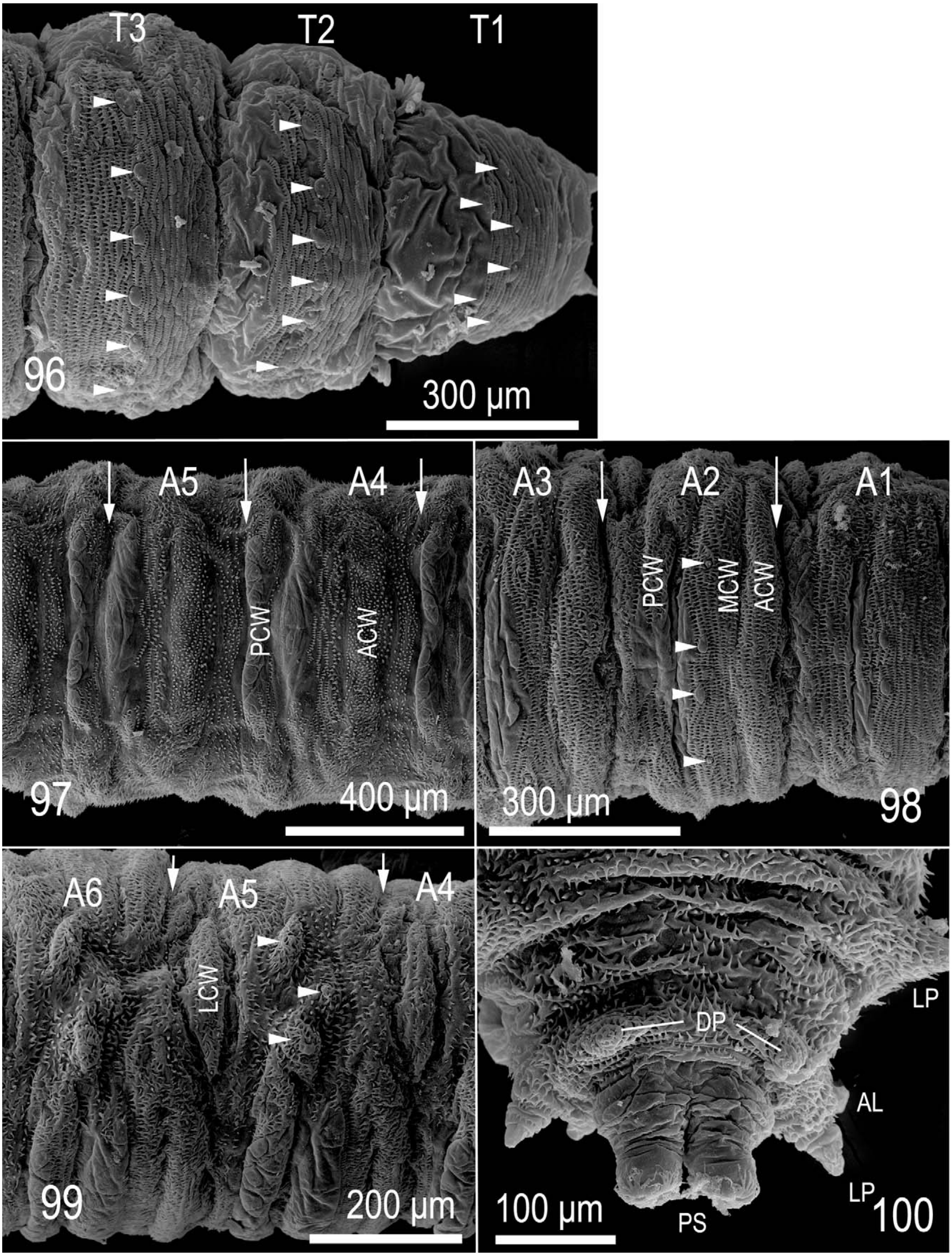


FIGURES 83–89. *Calliopum aeneum*, third instar, arrowheads indicate the tubercles. 83. Thoracic segments, dorsal view. 84. First and second thoracic segments, dorsal view. 85. Right dorsal tubercule (medial pair) of first thoracic segment. 86. Abdominal segments, dorsal view. 87. Abdominal segments, ventral view. 88. Anal division and last abdominal segments, dorsal view. 89. Abdominal segments, lateral view. A1–A7, first to seventh abdominal segments; ACW, anterior creeping welt; AD, anal division; DP, dorsal process; LCW, lateral creeping welt; LP, lateral process; MCW, medial creeping welt; PCW, posterior creeping welt; PS, posterior spiracles, T1–T3, first to third thoracic segments.

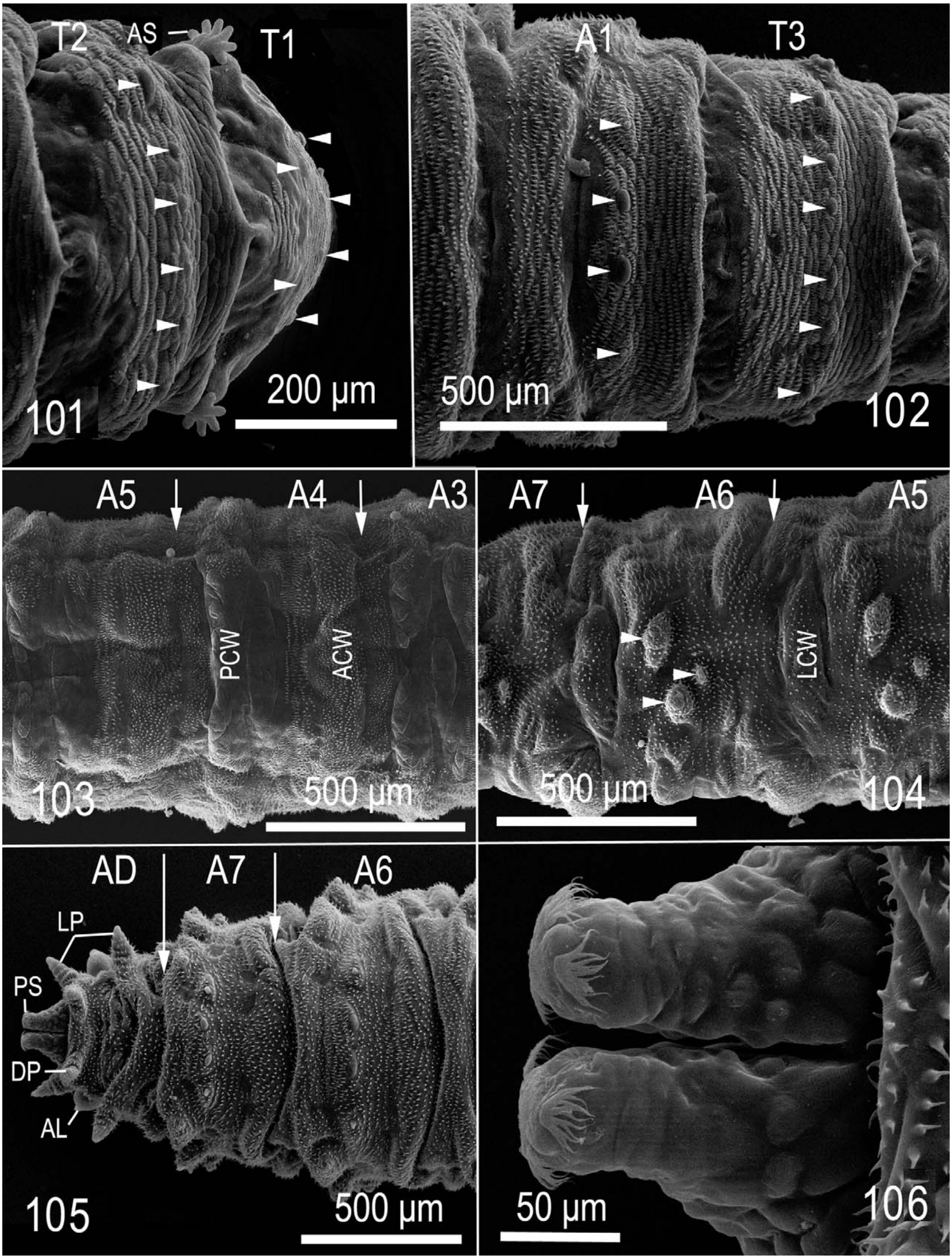


FIGURES 90–95. *Calliopum elisae*, third instar, arrowheads indicate the tubercles. 90. Thoracic segments, dorsal view. 91. First thoracic segment, dorsal view. 92–93. Abdominal segments, lateral view. 94. Anal division, postero-dorsal view. 95. Anal division and last abdominal segments, dorsal view. A1–A7, first to seventh abdominal segments; ACW, anterior creeping welt; AD, anal division; AS, anterior spiracle; DP, dorsal process; LCW, lateral creeping welt; LP, lateral process; MCW, medial creeping welt; PCW, posterior creeping welt; PS, posterior spiracles; T1–T3, first to third thoracic segments; VP, ventral process.

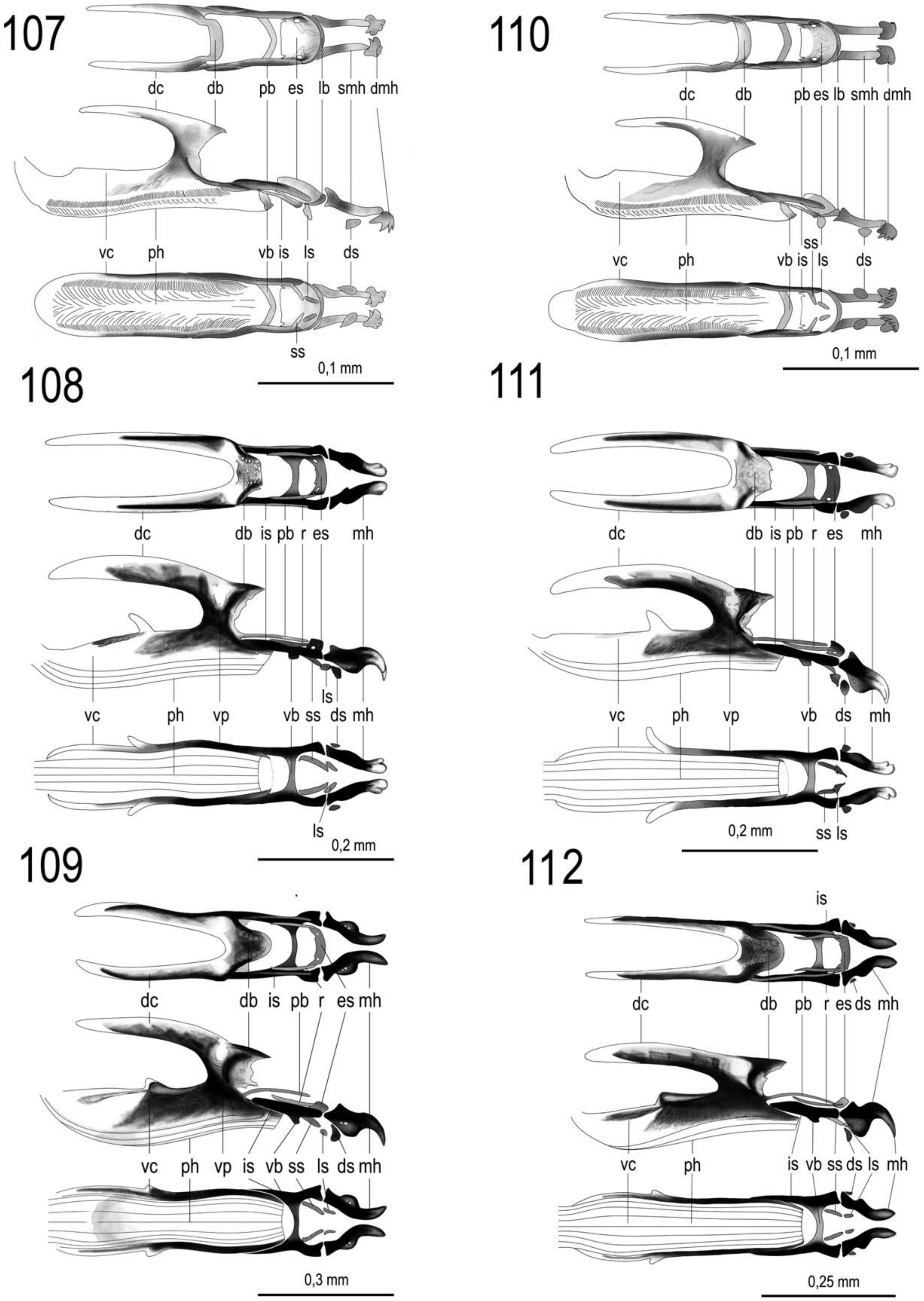
Integument of abdominal segments and anal division mostly villous, with smooth area behind ventral creeping welt (Figs 20–22, 30, 32).



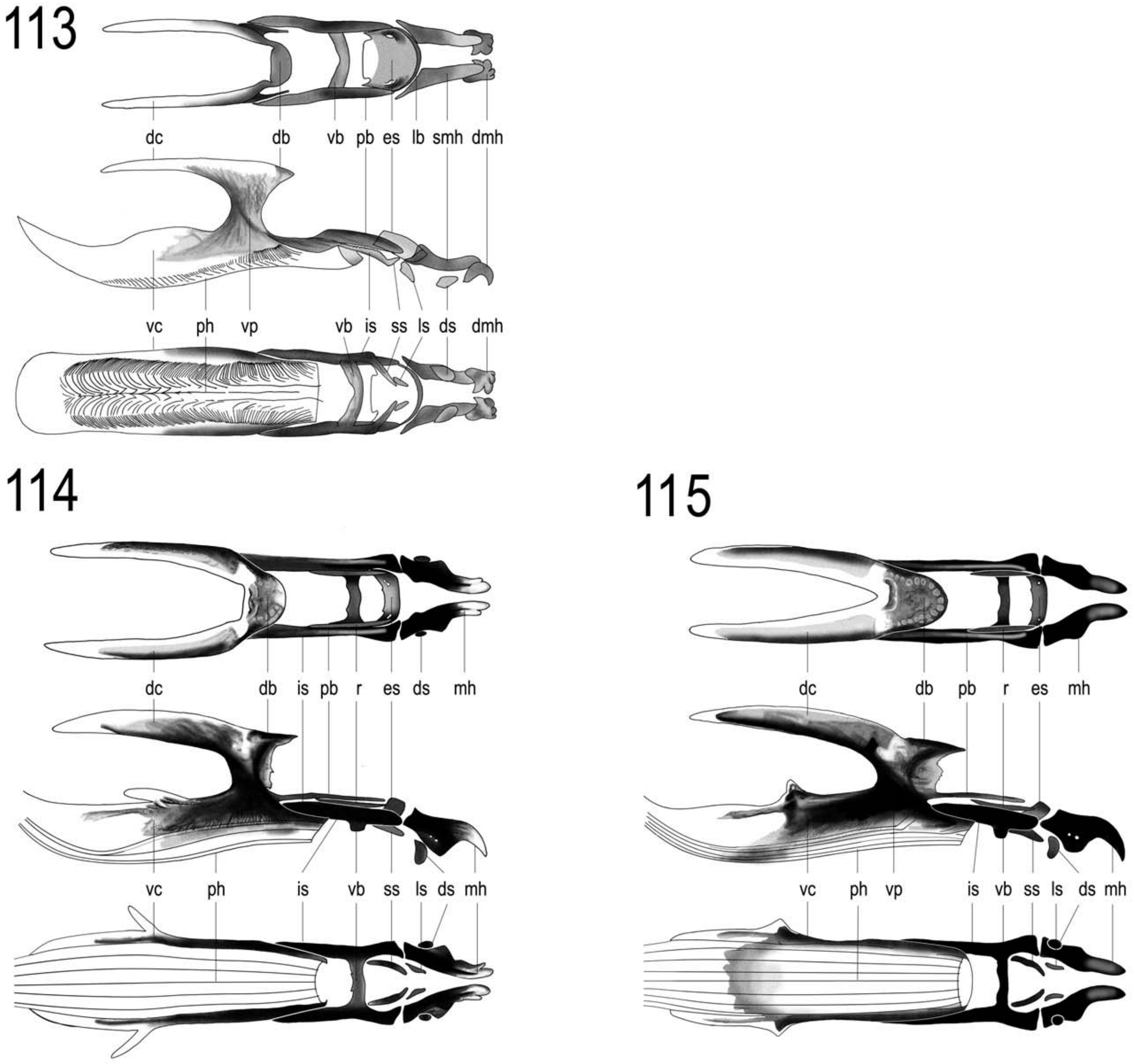
FIGURES 96–100. *Calliopum simillimum*, third instar, arrowheads indicate the tubercules. 96. Thoracic segments, dorsal view. 97. Abdominal segments, ventral view. 98. Abdominal segments, dorsal view. 99. Abdominal segments, lateral view. 100. Anal division, dorsal view. A1–A6, first to sixth abdominal segments; ACW, anterior creeping welt; AL, ambulatory lobe; DP, dorsal pricess; LCW, lateral creeping welt; LP, lateral process; MCW, medial creeping welt; PCW, posterior creeping welt; PS, posterior spiracles; T1–T3, first to third thoracic segments.



FIGURES 101–106. *Calliopum splendidum*, third instar, arrowheads indicate the tubercles. 101. First and second thoracic segment, dorsal view. 102. Third thoracic and first abdominal segments, dorsal view. 103. Abdominal segments, ventral view. 104. Abdominal segments, lateral view. 105. Anal division and last abdominal segments, dorsal view. 106. Posterior spiracles, dorsal view. A1–A7, first to seventh abdominal segments; ACW, anterior creeping welt; AL, ambulatory lobe; AS, anterior spiracle; DP, dorsal process; LCW, lateral creeping welt; LP, lateral process; PCW, posterior creeping welt; PS, posterior spiracles; T1–T3, first to third thoracic segments.



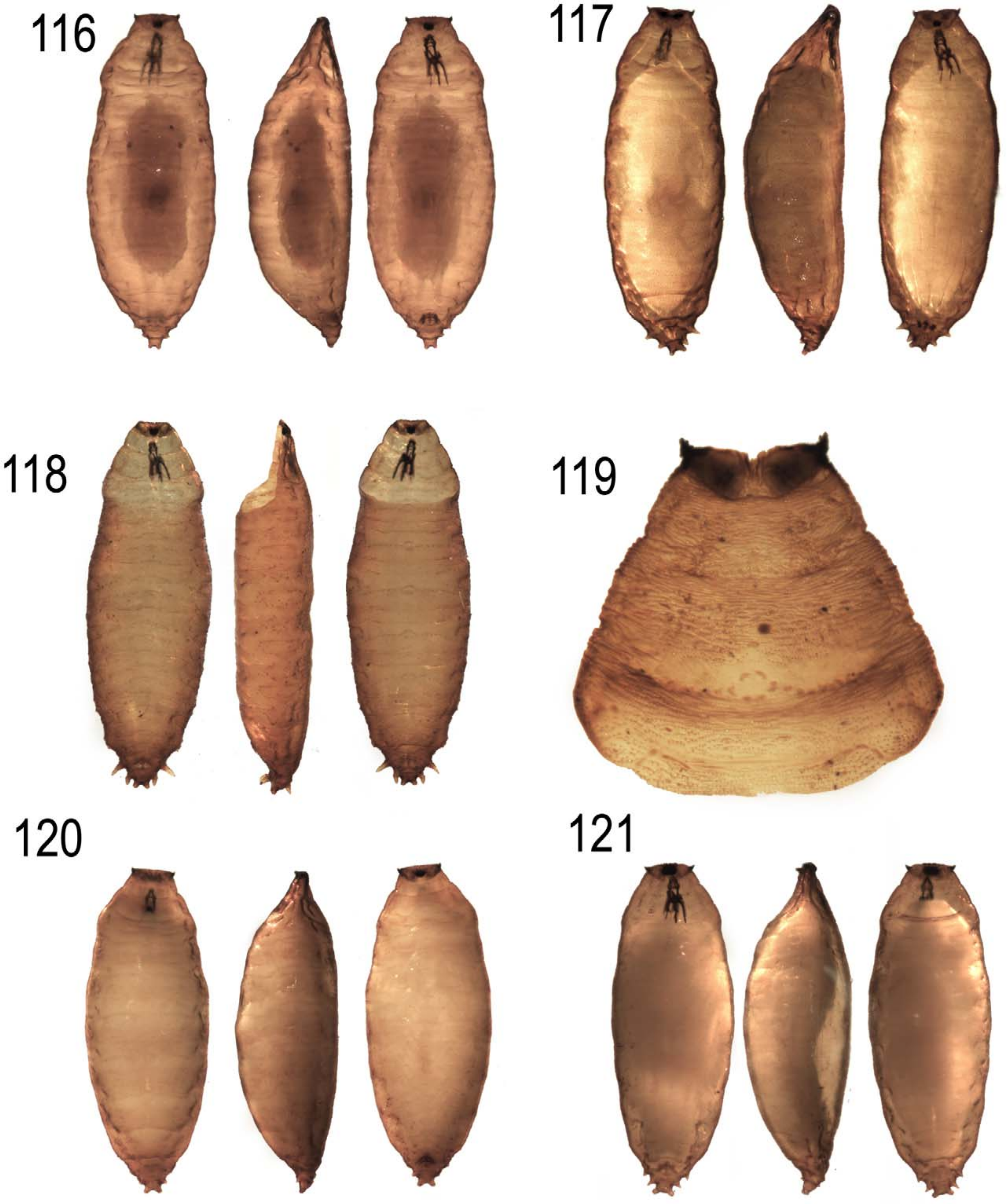
FIGURES 107–112. Cephaloskeletons of *L. cylindricornis* (left column) an *L. minor* (right colum). From the top for each figure: dorsal, lateral and ventral view. 107. *L. cylindricornis*, first instar.110. *L. minor*, first instar. 108. *L. cylindricornis* second instar. 111. *L. minor*, second instar. 109. *L. cylindricornis*, third instar 112. *L. minor*, third instar. db, dorsal bridge; dc, dorsal cornu; ds, dental sclerite; es, epistomal sclerite; dmh, dentate part of mouth hook; is, intermediate sclerite; lb, labrum; ls, ligulate sclerite; mh, mouth hook; pb, parastomal bar; ph, pharynx; r, rami; smh, stalk part of mouth hook; ss, subhypostomal sclerite; vb, ventral bridge; vc, ventral cornu; vp, vertical plate.



FIGURES 113–115. Cephaloskeleton of *C. splendidum*. From the top for each figure: dorsal, lateral and ventral view. 113. First instar. 114. Second instar. 115. Third instar. db, dorsal bridge; dc, dorsal cornu; ds, dental sclerite; es, epistomal sclerite; dmh, dentate part of mouth hook; is, intermediate sclerite; lb, labrum; ls, ligulate sclerite; mh, mouth hook; pb, parastomal bar; ph, pharynx; r, rami; smh, stalk part of mouth hook; ss, subhypostomal sclerite; vb, ventral bridge; vc, ventral cornu; vp, vertical plate.

Cephaloskeleton (Figs 109, 112, 115) similar to that of second instar. Mouth-hooks converging, dorsal apodeme blunt; tips of mouth hooks simple. Dental sclerite present. Subhypostomal sclerites as in the second instar. Ligulate sclerites short, simple. Epistomal sclerite narrow, convex, with paired lateral opening. Rami and parastomal bars slender. Dorsal bridge semi-lunular. Posterodorsal edge of dorsal cornu hyaline. Ventral cornu with triangular and sclerotised apodeme. Posterodorsal edge of ventral cornu sclerotised. Nine pharyngeal ridges present.

Puparium (Figs 116–121). Brownish to cinnamon, barrel-shaped, dorsal side convex, ventrally more or less flat; puparium opens through a dorsal operculum. Anterior end dorsoventrally flattened, with pseudocephalon and prothorax retracted up to the level of anterior spiracles. Integument corresponding to the first thoracic segment of the third instar, corrugated in the middle, but with lateral parts smooth and strongly sclerotised (appear almost dark in light microscope). Anterior spiracles dark pigmented except for the bright papillae. Posterior end of puparium tapering. Ambulatory lobes retracted. Posterior spiracles directed upward.



FIGURES 116–121. Puparia of *Lauxania* and *Calliopum* species. From the left for each figure: dorsal, lateral and ventral view. 116. *L. minor*. 117. *L. cylindricornis*. 118. *C. aeneum*. 119. *C. aeneum*, operculum, dorsal view. 120. *C. simillimum*. 121. *C. splendidum*.

Lauxania minor

Egg (Figs 2, 4). 0.59–0.65 mm. Dorsal side with straight, occasionally fusing ridges and perforated chorion. Ventral side with undulated and often fusing ridges, chorion covered by smooth layer.

First larval instar. Length 0.67–1.51 mm. Six teeth on distal part of mouth hook (Fig 5). Length of cephaloskeleton: 0.17–0.24 mm (Fig. 109).

Second larval instar. Length 1.48–2.98 mm. No unique character was found. Length of cephaloskeleton: 0.28 –0.39 mm (Fig. 110).

Third larval instar. Length 2.91–4.44 mm. Posterior border of first thoracic segment with broad smooth area (Fig. 16), posterior border of second and third thoracic segment with sparse comb spines (Fig. 19). Anterior spiracles with 9 papillae, the upper-most papilla curved back (Fig 17).

Integument of abdominal segments with large smooth area behind anterior creeping welt on dorsal and ventral sides. Two tubercules located inside the dorsal smooth area. Two smaller tubercules more laterally, outside the dorsal smooth area (Fig 20).

Length of cepahlopharyngeal skeleton: 0.56–0.61 mm. Cepahlopharyngeal skeleton (Fig. 111) markedly compressed dorsoventrally (approximately 0, 11 mm high). Dorsoventral axis of mouth hooks almost parallel. Intermediate sclerite slightly bent from lateral view. Ventral cornua not pressed inward on dorsal margin between vertical plate and apodeme of ventral cornu.

Puparium (Fig. 115). Length 2.94–3.24 mm. From lateral view, the puparium is somewhat dropshaped, with the acute anterior end. Crevices create small medial ovals on dorsal side.

Lauxania cylindricornis

Egg (Figs 1, 3). Length 0.60–0.71 mm. Chorion of egg with small clusters of perforated windows. Posterior tubercle surrounded by ring of perforated chorion. Chorion of mycropylar pole tufted.

First larval instar (Fig.6). Length: 0.70–1.50 mm. Length of cephaloskeleton: 0.19–0.24 mm (Fig. 106). Five teeth on distal part of mouth hook (Figs 7, 37). Two digitiform projections of ventral organ (Fig. 8) appear to be longer compared to *L. minor.*

Second larval instar (Figs 9–12). Length: 1, 07–3, 34 mm. Length of cephaloskeleton: 0.30–0.40 mm (Fig. 107). Probably without reliable differentiating characters.

Third larval instar. Length: 3.28–4.55 mm. The first thoracic segment almost completely covered by spines dorsally (Figs 26, 29). The second thoracic segment with anterior comb spines, middle spinous area and broad smooth area on posterior margin. The third thoracic segment with similar pattern, but note the hairs on posterior margin (Fig. 29).

Integument of abdominal segments almost completely villous both from dorsal and ventral side (Figs 30, 32). Two pairs of dorsal tubercules not surrounded by smooth area (Fig. 30). Anterior spiracles with 9–10 papillae, the upper-most papilla smaller (Fig. 27).

Length of cephaloskeleton: 0.62–0.68 mm. Cepahloskeleton (Fig 39) not compressed in dorsoventral axis. Dorsoventral axis of mouth hooks meets in acute angle, tips of mouth hooks slightly diverging. Intermediate sclerite straight from lateral view. Pharynx with pigmented transversal strip posteriorly, but in different specimens it can be variously developed. Dorsal edge of ventral cornua pressed inwards between apodeme and vertical plate. Pharynx relatively deep in posterior half.

Puparium. Length 2.97–3.93 mm. Surface more hairy than in *L. minor*, corresponding to the integument of the third instar. Processes of anal division more well developed than in *L. minor* (Fig. 116).

Characteristics of preimaginal stages of genus *Calliopum*

External morphology and morphology of the cephaloskeleton very much resembles that of *Lauxania*. Larvae are generally larger, which corresponds to larger body of adult *Calliopum*.

Egg (Figs 35–46). Always more or less differentiated dorsal and ventral side. Ventral side is simple, with longitudinal ridges and short transverse ribs. Dorsal side has fluffy, perforated chorion, ridges overdeveloped and ribs suppressed comparing to the ventral side. The chorion can be folded, creating longitudinal channels.

First larval instar (Figs 47–60). Four teeth in distal part of mouth hook. Further differences from *Lauxania* were not found (Figs 47–60).

Second larval instar (Figs 61–74). Thoracic segments with comb spines only in anterior half. Integument sparsely covered by spines laterally. Second instar larvae rather uniform and are not further described.

Third larval instar. First thoracic segment covered by comb spines anteriorly, up to the level of second (posterior) pair of dorsal tubercules, as in *L. minor* (e.g. Fig. 84). The second and third thoracic segments each with anterior comb pines, middle area with several rows of spines and a posterior smooth area. Anterior spiracles with 7–9 papillae. The shape of the cephaloskeleton resembles that of *L. cylindricornis*. Dorsoventral axis of mouth hooks almost parallel and tips of mouth hooks do not diverge. The pharynx is shallow compared to *L. cylindricornis*. Rather unexpectedly, the skeleton of *L. cylindriconris* is on average longer than in *Calliopum* (*L. cylindriconris* is much smaller than any studied species of *Calliopum*), but this may be an artefact. We found no differences in the morphology of the cephaloskeleton among species of *Calliopum* with the exception of *C. elisae*.

Puparium (Figs. 118–121). Usually longer than 4 mm (larger than in *Lauxania*). Other characters very similar.

Calliopum aeneum

Egg. Length 0.66–0.72 mm. Relatively thin. Ventral part of egg with simple longitudinal ridges. Dorsal part with deep longitudinal channels (Figs 35–37).

First larval instar. Length 0.97–1.96 mm (Figs 47, 48). Length of cephaloskeleton: 0.19–0.21 mm.

Second larval instar (Figs 61, 62). Length 1.90–3.33 mm. First row of cirri coalescing towards the mouths. Length of cephaloskeleton: 0.32–0.36 mm.

Third larval instar (Figs 83–89). Length 2.6–4.80 mm. Anterior spiracle with 7 papillae (Fig. 76). Dorsal side of abdominal segments completely villous. Ventral side with small smooth area surrounding the posterior creeping welts. Ventral posterior creeping welts partly spinous. Length of cephaloskeleton 0.54–0.61 mm.

Puparium (Figs 118, 119). The only acquired puparium was 5.0 mm long. Processes of anal division markedly longer than in other species. Crevices create on dorsal surface small, medial ovals, as in *L. minor.* Puparium markedly dorsoventrally flattened, but this may not be a stable character.

Calliopum elisae

Egg. Length 0.68–0.72 mm. Chorion slightly differentiated in dorsal and ventral part. Dorsal side is perforated and with simple ridges, no folding of chorion occurs (Figs 38–40).

First instar larva (Figs 49–50). Length 1.0–1.86 mm. Length of cephaloskeleton 0.18–0.21 mm.

Second larval instar (Figs 63, 64). Length 1.80–3.50 mm. Length of cephaloskeleton 0.30–0.36 mm.

Third larval instar (Figs 77, 90–95). Length 3.46–4.83 mm. Anterior spiracle with 9 papillae (Fig. 78). Integument of abdominal segments and anal division completely villous, even from ventral side. Length of cephaloskeleton 0.55–0.59 mm. Position of mouth hooks approximate to *L. cylindricornis* (tips of mouth hook slightly diverge).

No puparium was acquired.

Calliopum splendidum

Egg (Figs 41–43). Length 0.65–0.74 mm Chorion slightly differentiated in dorsal and ventral side. Dorsal side with more developed longitudinal ridges and regular longitudinal channels.

First larval instar (Figs 51, 52, 55–60). Length 1.12–2.16 mm. Length of cephaloskeleton (Fig. 113) 0.21–0.22 mm.

Second larval instar (Figs 65, 66, 69–74). Length 2.14–3.58 mm. Length of cephaloskeleton (Fig. 114) 0.37–0.39 mm.

Third larval instar (Figs 79, 101–106). Length 3.53–4.30 mm. Anterior spiracles with 7 papillae, the two marginal papillae are curved back (Fig 80). Integument of abdominal segments and anal division villous, small smooth areas are retained behind dorsal tubercules in first four segments and partly between segment borders. Extensive smooth areas are on the ventral side behind anterior creeping welt. The only species of *Calliopum* with comb spines on the posterior half of the first thoracic segment. Length of cephaloskeleton (Fig. 115) 0.61–0.63 mm.

Puparium (Fig. 121). Length 3.61–3.89 mm. Indistinguishable from *C. simillimum*. Smaller size can be result of unfavourable rearing conditions, as adult flies are comparably large to *C. simillimum*.

Calliopum simillimum

Egg (Figs 44–46). Length 0.70–0.78 mm. Dorsal and ventral side sharply delimited, ventral side only slightly smaller. Longitudinal ridges of ventral side low, with numerous transverse ribs. Dorsal side with large longitudinal ridges. Longitudinal channels are distinct.

First larval instar (Figs 53, 54). Length 0.93–2.08 mm. Length of cephaloskeleton 0.19–0.21 mm.

Second instar larva (Figs 81, 82). Length 1.74– 3.57 mm. Length of cephaloskeleton 0.30–0.37 mm.

Third larval instar (Figs 96–100). Length 2.98–4.85 mm. Anterior spiracles with 8–9 papillae, the two marginal papillae are curved back (Fig. 82). Second and third thoracic segment are almost completely spinous dorsally. Abdominal segments densely spinous, dorsal spines organized in transverse rows and overlap in a roof-like manner. Smooth area behind the dorsal abdominal tubercules and also on ventral side. Lateral parts of abdominal segments and the anal division densely spinous, but spines are rather “shaggy”. Length of cephaloskeleton 0.54–0.57 mm.

Puparium (Fig. 120). Length 4.20–4.39 mm. Only puparia reared from larvae collected in nature were measured.

Discussion

Eggs. Every species has a characteristic egg chorion. However, it is not possible to ascribe certain type of egg to certain genera. *Calliopum elisae* and *Lauxania* spp. have egg with simple longitudinal ridges. The remaining species of *Calliopum* have variously developed longitudinal channels.

Larvae of studied flies express typical lauxaniid traits, as formulated by Miller & Foote (1976): the facial mask has several rows of cirri, the presence of a pair of ambulatory lobes, the distal part of the Malpighian tubules are filled with white matter.

Generally, from the first to the third instar, the integument vestiture becomes more complex, the number of cirri rows rises and the number of mouth hook accessory teeth declines. Other morphological structures are best seen as unique features of individual instars, with the first instar being morphologically distinct from the second and third.

The first instar has many unique features. Most of them resemble those of the first instar of *Minettia longipennis* (Semelbauer & Kozánek 2011) e.g. the same basic form of the facial mask (five rows of simple cirri, first row sclerotised) and cephaloskeleton (two-pointed mouth-hooks with teeth on distal part, strong parastomal bars, missing rami, intermediate sclerite week and adherent to subhypostomal sclerite, pharynx covered with fine dendroid structure). The cephaloskeleton is very similar among the studied species. It is possible to recognise three taxa according to the number of teeth of the mouth hook: 6 for *L. minor,* 5 for *L. cylindricornis* and 4 for *Calliopum*.

According to Miller & Foote (1976), the first instar has at most two indistinct rows of spinules (= cirri). SEM images clearly show five rows of cirri. Tiny and translucent cirri can be easily overlooked under light microscopy. On the other hand, the first row of cirri is amber-coloured and thus easily spotted under light microscopy.

First instars of *Fannia canicularis* (Linnaeus) (Fanniidae) show a similar pattern in the structure of the cephaloskeleton—differing notably from that of the second and third instar (Grzywacz et al. 2011). The cephaloskeleton of the first instar of the studied species and of *F. canicularis* share, e.g. weak sclerotisation, parastomal bars stronger than intermediate sclerite and bottom of pharynx bearing fine dendroid structure (Grzywacz et al. 2011). The mouth hooks of second instar *F. canicularis* bear a serrated distal part similar to that of the first instar of lauxaniid, sarcophagid and calliphorid flies (Szpila & Pape 2007, Szpila & Pape 2008, Grzywacz et al. 2011).

Pharyngeal ridges are characteristic for the saprophagous life-habit (Keilin 1915, Dowding 1967) and are well developed in the second and third instars of all the studied species. Absence of pharyngeal ridges in the first instar remains unexplained, as phytosaprophagous *Quametopia terminalis* (Loew) and *Q. clintonia* Roháček & Barber (Anthomyzidae) have four clearly developed pharyngeal ridges in the first instar (Roháček & Barber 2011). Images of the first instars of kleptoparasitic *Beludzhia phylloteliptera* Rohdendorf and *Dolichotachina marginella* (Wiedemann) (Miltograminae, Sarcophagidae) (Szpila & Pape 2007) show a similar dendroid structure at the bottom of the pharynx, as in lauxaniids. This modification of the pharynx probably does not correspond to the mode of life.

The second instar. An accessory tooth on the mouth hook is a common condition in second larval instars of Lauxaniidae (Miller & Foote 1976). There is no clear difference between studied species in the external morphology or morphology of the cephaloskeleton. Second instars of *Lauxania* spp. differ in shape of the ligulate sclerite, but comparison of several specimens shows that it is a variable structure.

The third instar has the most differentiating characteristics among species. The spinulation of the first thoracic segment of *Calliopum* has the same pattern as in *L. minor* (partly with the exception of *C. splendidum*), but the spinulation of the second and third thoracic segments is reminiscent of *L. cylindricornis*. The cephaloskeleton of *Calliopum* is somewhat intermediate between *L. cylindricornis* and *L. minor* in terms of habitus and by the position of mouth hooks *Calliopum* recalls *L. minor.*

The third instar is described also for many Sciomyzoidea (Pont & Meier 2002, Kirk-Spriggs 2010). The facial mask of *Catacamilla cavernicola* Papp (Camillidae), many Drosophilidae and Sepsidae species consists of flattened outgrowths that are fringed at the posterior edge and resembling the soft outgrowth around the mouth of *Lauxania* and *Calliopum*. Cephaloskeleton is similar to *Lauxania* and *Calliopum* in overall shape (Pont & Meier 2002).

The puparium. Unlike puparia of lauxaniids described by Miller & Foote (1976), puparia of *Lauxania* and *Calliopum* spp. are not covered with calcareous matter. Studied species certainly have calcareous glands, though they are less developed. Both *Calliopum* and *Lauxania* have very similar puparia. Only *L. minor* and *C. aeneum* have distinctive puparia. The remaining species are unrecognisable according to the shape of puparium.

There are no consistent characters that adequately separate the genera *Lauxania* and *Calliopum* in the immature stages. But it is possible to characterise the two species (representing different subgenera) of *Lauxania* covered here. Species of *Calliopum* differ particularly in the density and arrangement of spines. But this character is of limited practical use for identification as it is not friendly to light microscopy.

The morphology of the cephaloskeleton allows recognition of only three taxa: *L. minor*, *L. cylindricornis* and *Calliopum* spp., but these are recognisable only in the first and third instars. Our results support conclusions of Papp & Shatalkin (1998), Pérusse & Wheeler (2000) and Merz (2001) that the systematics of genus *Lauxania* is not convincing and that it may not be monophyletic.

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