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Title	Larval morphology of the genus Hydrocassis Fairmaire (Coleoptera: Hydrophilidae)
Author(s)	Minoshima, Yûsuke; Hayashi, Masakazu
Citation	Journal of Natural History, 45(45-46), 2757-2784 https://doi.org/10.1080/00222933.2011.602805
Issue Date	2011-10-20
Doc URL	https://hdl.handle.net/2115/50319
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Type	journal article
File Information	JNH45-45-46_2757-2784.pdf

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Larval morphology of the genus *Hydrocassis* Fairmaire (Coleoptera: Hydrophilidae)

Yûsuke Minoshima [a], Masakazu Hayashi [b]

[a]*Systematic Entomology, Graduate School of Agriculture, Hokkaido University, Sapporo, 060–8589 Japan. E-mail: m-yusuke@res.agr.hokudai.ac.jp;* [b]*Hoshizaki Green Foundation, Okinoshima 1659-5, Sono-chô, Izumo-shi, Shimane Pref., 691–0076 Japan. E-mail: hgf-haya@green-f.or.jp*

Larval morphology of the genus *Hydrocassis* Fairmaire (Coleoptera: Hydrophilidae)

The larval morphology of the genus *Hydrocassis* Fairmaire, 1878 is described on the basis of three species of the genus: second instar of *H. jengi* Satô, 1998, all instars of *H. lacustris* (Sharp, 1884) and second and third instars of *H. uncinata* Ji et Schödl, 1998; the former two belong to the *H. scapulata* species group and the latter to the *H. scaphoides* species group. Primary chaetotaxy of the larval head of *Hydrocassis* is described based on the first instar larvae of *H. lacustris*. Larval morphology of all genera of Sperchopsini with known larvae is summarised based on descriptions and figures from the literature, which are compared with *Hydrocassis*: larval morphology of *Hydrocassis* is similar to that of *Sperchopsis*, and the presumably closely related genus *Ametor* is distinguishable from *Hydrocassis* by characters of larval morphology. A key to the Sperchopsini genera on the basis of the larval characters is provided.

Keywords: Coleoptera, Hydrophilidae, Sperchopsini, larva, *Hydrocassis*, *H. jengi*, *H. lacustris*, *H. uncinata*, chaetotaxy.

Introduction

The tribe Sperchopsini is a small group of the family Hydrophilidae, which includes five genera and 24 species (Hansen 1999, Short and Hebauer 2006, Liu et al. 2008). Members of the tribe occur in all major biogeographic regions except Africa, Antarctica and Oceania, and generally inhabit running waters (e.g. Spangler 1961, Hansen 1991, Archangelsky 1997). The genus *Hydrocassis* Fairmaire, 1878 from eastern Palaearctic and Oriental Regions is the most species rich in the tribe and comprises of 16 species assigned to three species groups (Hansen 1999,

Short and Hebauer 2006, Liu et al. 2008). Monophyly of the tribe is supported by the morphology of adults as well as immature stages (Hansen 1991, Archangelsky 2004), but the phylogenetic relationships among the genera have not been solved so far.

The larval morphology of all genera of the tribe Sperchopsini have been studied except for *Cylomissus* Brown, 1903. The latter genus includes one species from New Zealand and its larvae remain unknown (Table 1). Even though the genus *Hydrocassis* includes two-thirds of all species of the tribe Sperchopsini, the only information about its larval morphology concerns a common Japanese species, *H. lacustris* (Sharp, 1884) and is moreover not very detailed (Morioka 1955, Hayashi 2009). Larval morphology was shown to provide many informative characters useful both for taxonomy and phylogeny, which is also the case of the primary chaetotaxy of the larval head (e.g. Emden 1957, Solodovnikov 2007, Fikáček et al. 2008, Meier and Lim 2009). A nomenclature system for the chaetotaxy of hydrophilid larvae has been developed recently (Fikáček 2006, Fikáček et al. 2008, Byttebier and Torres 2009).

In this paper, we describe the larval morphology and chaetotaxy of the genus *Hydrocassis* based on three species of the genus: *Hydrocassis lacustris* (Sharp, 1884) and *H. jengi* Satô, 1998 from Japan, and *H. uncinata* Ji et Schödl, 1998 from Laos. Furthermore, we compare the larval morphology of *Hydrocassis* with that of other genera of the tribe and present a generic identification key to known Sperchopsini larvae.

Materials and methods

We examined immature stages of three *Hydrocassis* species (Table 2). Larvae were fixed in 80 or 70% ethanol in the field or laboratory. Dissected specimens were mounted on microscopic slides or preserved in glycerol, other specimens were preserved in 80% ethanol. Observations and dissections were carried out using Olympus SZ40 and SZX12 binocular microscopes, and Olympus BX40 and Zeiss Axiophot compound light microscopes. Illustrations were made with

the aid of a drawing tube attached to Nikon SMZ-800 binocular microscope and Olympus BX40 compound light microscope. Line drawings were prepared using the Paint tool SAI software (Systemax Inc., Tokyo, Japan) and Adobe Photoshop ® (Adobe Systems Inc., San Jose, USA). Photographs of larvae were taken with Nikon D100 digital camera or Nikon DS-Fi1 microscope digital camera attached to a Nikon SMZ-800 binocular microscope.

The head capsule was carefully removed with tweezers. For large specimens, bodies were divided between abdominal segments 4 and 5. Specimens were soaked in 10% KOH solution for one day at room temperature. They were subsequently rinsed with 80% ethanol, and were stained in lactic acid containing acid fuchsine and warmed in the liquid for about 120 minutes at 40–50 °C. Then, stained samples were rinsed with 80% ethanol, and dehydrated in 99% ethanol. Dissected parts were mounted on microscope slide, or HS-slide (Higgins-Shirayama slide; Shirayama et al. 1993) (Kanto Rika, Japan) with glycerol or Euparal (Chroma-Gesellschaft, Köngen, Germany) for examination. Some specimens, preserved in small glass tubes with glycerol, were pinned with data label.

Morphological terminology generally follows Archangelsky (1997), Archangelsky et al. (2005), Richmond (1920), and Spangler (1991). For the primary chaetotaxy of larval head we refer to Fikáček et al. (2008) and Byttebier & Torres (2009); for abbreviations of sensilla and their localization see Fikáček et al. (2008). Sometimes, secondary sensilla form a group with primary sensilla. In this case, the group of sensilla whose homology could not be defined separately were marked with number(s) of primary sensilla situated within the group and a question mark (e.g. 5–6? or 5?, see Figures 5D–E, 7E).

Following abbreviations are used throughout the paper: EUMJ: Ehime University Museum, Matsuyama, Ehime, Japan (M. Sakai, H. Yoshitomi); HGFC: Hoshizaki Green Foundation, Izumo, Shimane, Japan (M. Hayashi); L1, L2, L3, L?: first, second, third instars, and unknown instars; SEHU: Systematic Entomology, Hokkaido University, Sapporo, Hokkaidô, Japan (M. Ôhara).

Systematics

Family **HYDROPHILIDAE** Latreille, 1802

Subfamily **HYDROPHILINAE** Latreille, 1802

Tribe **SPERCHOPSINI** Hansen, 1991

Key to the genera of the larvae of the tribe Sperchopsini (excludes the genus Cylomissus, for all known instars)

1 Nasale with two teeth; mandibles with two inner teeth; inner surface of stipes with more than nine rather stout setae; dorsal sclerite of abdominal segment 8 subdivided into two halves; Neotropical region. (Only third instar larvae are known) ..... *Anticura* Spangler

- Nasale with four or five teeth (Figures 2C, 4C, 10C); mandibles with three inner teeth (Figure 7B–C); inner surface of stipes with five to eight rather stout setae; dorsal sclerite of abdominal segment 8 not subdivided medially (Figure 6D); Palaearctic, Nearctic and Oriental regions. 2

2 Clypeolabrum distinctly asymmetrical; epistomal lobes with stout setae; frontal lines not fused at base. (Only third instar larvae are known) ..... *Ametor* Semenov

- Clypeolabrum almost symmetrical or slightly asymmetrical (Figure 6C); epistomal lobes with rather slender setae (Figure 6C); frontal lines fused at base (Figure 6B). 3

3 Inner surface of stipes with 7–8 setae; Nearctic region. ..... *Sperchopsis* LeConte

- Inner surface of stipes with 5 setae (Figures 3C, 5D); eastern Palaearctic and Oriental regions. .. .. *Hydrocassis* Fairmaire

Genus ***Hydrocassis*** Fairmaire, 1878

(Figures 1–13)

Species examined

Hydrocassis jengi Satô, 1998 (L2), *Hydrocassis lacustris* (Sharp, 1884) (L1–3), *Hydrocassis uncinata* Ji & Schödl, 1998 (L2–3).

Diagnosis

Larvae of *Hydrocassis* can be distinguished from those of other known genera of the tribe Sperchopsini by the following combination of character states: 1) frontal lines fused at base (Figures 2A, 6B); 2) nasale with four to five teeth (Figures 2C, 4C, 6C, 10C); 3) clypeolabrum almost symmetrical (Figures 2C, 6C); 4) epistomal lobes with rather slender setae (Figure 4C); 5) mandibles with three inner teeth (Figures 5B–C); 6) inner surface of stipes with five setae in all instars (Figures 3C, 5D, 7D); 7) dorsal sclerite of abdominal segment 8 not subdivided medially (Figure 4D).

Description of general morphology

Body rather slender, widest between abdominal segments 3 and 4 (Figure 1). Lateral sides of meso- and metathorax and abdominal segments 1 to 8 with lateral projections on each segment (Figures 4A, 6A, 10A). Nine pairs of spiracles, one on anterolateral portion of mesothorax and eight on abdomen; mesothoracic and first seven abdominal spiracles non-functional, biforous; last pair annular, large and functional, enclosed within spiracular atrium.

Head. Head capsule subquadrate (Figures 4B, 6B, 10B). Frontal lines almost V-shaped, fused at base of head capsule; coronal line short. Dorsal and lateral surfaces of head capsule bearing dense, small, strong to rather strong, tooth-like cuticular projections (e.g. Figures 8C, 9B). Six stemmata on each anterolateral corner of head capsule. Clypeolabrum slightly

asymmetrical. Nasale with four to five teeth, median tooth small or absent (Figures 6C, 10C). Epistomal lobes rounded, almost symmetrical, projecting about as far as nasale or slightly lower than nasale; inner part of each lobe with spine-like cuticular projections.

Antenna: 3-segmented, rather short and stout (first instar, Figure 3A) to moderately long and slender (third instar, e.g. Figure 7A). Antennomere 1 the longest, longer than antennomeres 2 and 3 combined (third instar) to almost about as long as antennomere 2 (first instar). Antennomere 3 the shortest, small. Surface of antenna with small, spin-like cuticular projections (e.g. Figure 9C).

Mandibles symmetrical, with three inner teeth, two distal ones large, basal one small (e.g. Figures 5B–C).

Maxilla, counting cardo, 6-segmented, distinctly longer than antenna (e.g. Figures 3C–D, 5D–E). Cardo large, irregularly shaped, subtriangular to subquadrate. Stipes the longest, longer than palpomeres 1–4 combined, with one spine-like cuticular projection on apex of inner surface, and narrow and dense cuticular projections in basal part of inner surface, between sensilla MN7 and MN8. Maxillary palpus 4-segmented; palpomere 3 the longest, palpomere 2 the shortest; palpomere 1 the widest, palpomere 4 the narrowest; palpomere 1 incompletely sclerotised on dorsal surface, bearing small hair-like cuticular projections on anterior part of dorsal surface of intersegmental membrane between palpomeres 1and 2 (e.g. Figure 7D); inner process sclerotised; dorsolateral surface of intersegmental membrane of palpomeres 2 and 3 bearing fine hair-like cuticular projections; palpomere 3 slightly longer than palpomeres 1 and 2 combined, narrower than palpomere 2.

Labium well developed (Figures 2B, 3E–F, 7F–G). Submentum fused to head capsule, large, subpentagonal, wider than mentum (Figure 2B). Mentum trapezoidal in dorsal view, with short, stout tooth-like cuticular projections on dorsal surface except on median part. Prementum subquadrate, bearing fine hair-like cuticular projections on dorsolateral part of intersegmental membrane between prementum and labial palpi. Ligula shorter than palpi, mostly sclerotised.

Labial palpi moderately short, slender; palpomere 1 small; palpomeres and intersegmental membrane between palpomere 1 and 2 densely covered with fine hair-like cuticular projections (Figure 5F).

Thorax. Prothorax trapezoidal, wider than head capsule. Proscutum formed by one large plate divided by fine sagittal line, covered with short setae (Figure, 9D). Presternum subpentagonal (Figure 12A), with long, almost complete sagittal line on sclerite. Mesothorax with three dorsal sclerites on each side; medioanterior sclerite small, narrow; anterolateral sclerite small; posterior sclerite large, subdivided by fine sagittal line, and with transverse ridge on anterior part; one small tubercle outside of dorsal sclerite; four strong lateral projections on each side visible from dorsal view. Metathorax with two dorsal sclerites on each side; anterior sclerite large, subdivided by sagittal line; posterior sclerite narrow, smaller than anterior one, behind tubercle; lateral sides of posterior sclerites with two large projections; four strong to rather weak lateral projections on each side visible from dorsal view. Legs (Figures 12B–D): rather short, barely visible in dorsal view, 5-segmented, bearing numerous stout setae.

Abdomen. Abdomen 10-segmented, tapering towards posteriad, segments 8 to 10 forming spiracular atrium. Segments 1 to 7 similar in size and shape, each subdivided by transverse folds and bearing several tubercles (Figure 4E). Segment 1 with three lateral projections on each side dorsally, and with one pair of small dorsal sclerites medioanteriorly, the sclerites larger than those on segments 2 to 7, and three transverse rows of tubercles behind the sclerites; sclerites larger than those on segments 2 to 7. Segments 2 to 6 almost similar to segment 1. Segment 7 with one pair of small dorsal sclerites medioanteriorly, with three transverse rows of tubercles, third row of the tubercles with two tubercles on each side, outer one projected laterad.

Spiracular atrium (e.g. Figure 4D): Segment 8 with a large, oval dorsal plate, rather densely covered with short setae, posterior edge of the plate weakly rounded. Segment 9 trilobed, largely sclerotised on dorsum, partially covered by segment 8, with a pair of short, one-segmented urogomphi; urogomphi with three very long setae; procercus incompletely sclerotised, with two

setae. Segment 10 reduced.

Description of primary chaetotaxy of head

(based on first instar larvae of *H. lacustris*; slide preparations of eight specimens were examined) Frontale with 52–54 sensilla (Figures 2A, C). Central part with three pairs of sensilla (FR1–3) divergent posteriad; FR1 trichoid, short seta, situated close to frontal line; FR2 pore-like, situated mesally of line connecting FR1 and FR3; FR3 trichoid, moderately stout seta, situated more anteriorly and slightly more mesally than FR2. FR4–6 situated posteromesally to antennal socket, forming a triangular group; FR4 pore-like; FR5 rather short seta; FR6 rather long seta. FR7 rather short seta situated on inner margin of antennal socket. FR8 long seta situated posteriorly to two median teeth of nasale. Nasale with a group of eight setae (gFR1) (Figure 2C); six setae rather short, stout, intercalated between teeth; one short seta situated ventrally on inner margin of each large median tooth. Each epistomal lobe with a group of seven to eight moderately long, stout setae (gFR2), inner three slightly shorter than outer ones. FR15 pore-like, placed posteriorly to median setae of gFR1. FR9–10 and FR14 situated mesally to antennal socket; FR9 pore-like, located between FR10 and FR12; FR10 rather long, trichoid seta, situated posteriorly to FR9; FR14 pore-like, located laterally of FR9–10 and FR12, close to FR7. FR11 placed in inner part of epistomal lobe, anteriorly to FR9–10; FR11 pore-like, situated posterolaterally to outer seta of gFR1; FR13 pore-like, placed between FR11 and FR9; FR12 short seta, located laterally of line connecting FR11 and FR13.

Parietale with 30 sensilla each (Figures 2A–B). Dorsal surface with a group of sensilla (PA1–5) located posteriorly at midwidth, forming a slightly irregular longitudinal row; PA3 pore-like, situated slightly anteriorly to PA2; PA1–2 and PA4–5 short setae. PA6 pore-like, situated posteromesally, close to joint of frontal and coronal lines. PA7 moderately long seta close to frontal line, situated laterally of line connecting PA6 and PA8. PA8 moderately long seta situated between frontal line and inner stemma of anterior row. PA9 long seta, situated on outer

edge of antennal socket. PA10 pore-like, located between two inner-most stemmata. PA11 rather long seta, situated anteromesally to outer stemma of posterior row. PA12 and PA13 rather long, trichoid setae situated approximately at midlength of parietale, behind PA10–11 and stemmata. Anterolateral corner of epicranium with three sensilla (PA19–21); PA19 pore-like, situated more dorsally than others; PA20 rather short, trichoid seta, situated between PA19 and PA21; PA21 long, trichoid seta. Anterior one-third of lateroventral surface of parietale with four sensilla (PA14–17); the sensilla forming a slightly irregular transverse row; PA14 long seta; PA15 pore-like, situated close to PA14; PA16 long seta, situated between PA15 and PA17; PA17 pore-like; PA15–17 situated more ventrally than PA14. PA18 long seta, situated at midlength of lateral surface of parietale, posteriorly to PA15–16, more ventrally than PA14. PA22–25 located ventrally in along anterolateral portion of parietale; long seta PA22 and pore-like sensillum PA23 situated close to anterolateral corner; PA24–25 pore-like, close to inner margin of mandibular acetabulum; PA24 pore-like, located between PA23 and PA25, close to PA25. Median part of parietale with three sensilla (PA26–28), PA26 long seta, situated more anteriorly than remaining sensilla, PA27 pore-like, situated medially of line connecting PA26 and PA28; PA28 rather long seta, located more posteriorly than remaining sensilla. One pore-like sensillum (PA29) situated in basal one-third of parietale on ventral side behind PA28. PA30 pore-like, situated ventrally in about basal one-fourth of parietale.

Antenna (Figure 3A). Antennomere 1 with five pore-like sensilla (AN1–5); AN1 situated in basal one-third; AN2 situated in distal one-third; AN3 located apically on inner face, indistinct; AN4 located subapically on outer face; AN5 located subapically on ventral surface. Antennomere 2 with one pore-like sensillum (AN6) situated dorsally in distal two-fifths. Five setae (AN7–11) situated on intersegmental membrane between antennomeres 2 and 3; AN7–8 small, stout, situated on outer face of antenna next to sensorium (SE1); AN9 small, situated mesally of AN7–8. AN10–11 on inner face of antenna; AN10 long and trichoid, AN11 short, both setae close to each other. SE1 small, much shorter than antennomere 3, about as long as

AN7 and AN8. Antennomere 3 with one pore-like additional sensillum placed dorsally close to distal margin of sclerite, and six sensilla on apical membranous area (gAN): two long setae, two minute conical setae, and two minute setae.

Mandible (Figure 3B) bearing two setae (MN1 and MN5) and four pore-like sensilla (MN2–4 and MN6) situated on dorsal to lateral surface. MN1 rather long on outer face. MN2–4 forming a triangular group at midlength on dorsal surface; MN2 more closely attached to MN1 than to remaining sensilla; MN3 situated mesally of line connecting MN2 and MN4, MN4 on outer face. MN5 short seta, placed on outer margin in distal one-fourth of mandible. MN6 pore-like, very small, situated subapically on inner face.

Maxilla (Figures 3C–D). Cardo with one moderately long, trichoid seta ventrally (MX1). Stipes with a row of five setae (MX7–11) situated dorsally along inner surface; MX7 moderately short, shorter than MX8–11, situated basally; MX8–11 rather stout, spiniform, with subapical tooth, equidistant from each other. Ventral surface of stipes with two pore-like sensilla (MX2 and MX3) situated in basal 0.3 and distal 0.4 respectively; one pore-like sensilla (MX4) and two long trichoid setae (MX5 and MX6) situated subapically on outer surface of sclerite; MX4 situated posteriorly to MX5 and MX6, MX5 between MX4 and MX6. Palpomere 1 with one rather stout, spiniform seta (MX16) situated basally on inner face, and with one pore-like sensillum (MX12) and two setae (MX13–14) situated lateroventrally along distal margin of sclerite; MX12 situated more dorsally than MX13 and MX14; MX13 long seta, between MX12 and MX14; MX14 moderately long seta. Two pore-like sensilla (MX15 and MX17) on membrane below inner appendage, MX17 dorsal, MX 15 ventral. Appendage with one long and four minute setae apically (gAPP). Palpomere 2 with two pore-like sensilla (MX18 and MX19) and one minute seta (MX27); MX18 situated lateroventrally on anterior margin of sclerite; MX19 situated apically on inner face of intersegmental membrane between palpomeres 2 and 3; MX27 situated basally on outer face of sclerite. Palpomere 3 with four sensilla (MX20–23); MX21–22 rather short seta and pore-like sensillum respectively, both situated subapically at

midwidth of ventral surface of sclerite; MX22 located posteriorly to MX21; pore-like sensillum (MX20) and moderately long seta (MX23) situated apically on outer face of sclerite; MX23 dorsal, MX20 ventral. Palpomere 4 with proximal rather long seta (MX24) on inner face of sclerite, and with digitiform sensillum (MX25) and pore-like sensillum (MX26) situated subapically on outer face, MX25 dorsal, MX26 ventral. Apical membranous area of palpomere 4 with 6 minute setae (gMX).

Labium (Figures 2B, 3E–F). Submentum with two pairs of setae (LA1–2); LA1 long, on lateral corners; LA2 moderately short, on anterior corners (Figure 2B). Mentum with two pairs of sensilla (LA3–4) situated ventrally in distal one-fifth of sclerite; LA3 rather long seta, located more mesally than pore-like sensillum LA4. Dorsal surface of prementum with one pair of pore-like sensillum (LA8) and small seta (LA9); LA8 situated subbasally at midwidth of sclerite; LA9 on lateral part of membrane between prementum and palpi. Ventral surface of prementum with three pairs of sensilla (LA5–7); LA5 rather short seta, situated proximo-laterally; long seta LA6 and pore-like sensillum LA7 situated laterally on distal margin of sclerite; LA6 situated more mesally than LA7. Long seta (LA10) situated dorsally at base of ligula. Ligula with two pairs of pore-like sensilla (LA11–12); LA11 situated ventrally close to midlength of ligula; LA12 situated dorsally in apical membranous area. Labial palpomere 1 with a minute seta (LA13) on base of ventral surface of palpomere. Pore-like sensillum LA14 situated dorsally on intersegmental membrane between palpomeres 1 and 2. Palpomere 2 with one pore-like sensillum (LA15), situated subapically on outer face; apical membranous area with several small setae (gLA).

Variation

Hydrocassis shows a weak intra-specific variation (e.g. Figures 6C, 10C) in the following characters: 1) Number of teeth of nasale: in some specimens the small median tooth is reduced, therefore in this case, the number of teeth on nasale is four; 2) Number of setae of epistomal lobe

(gFR2): it ranges from six to nine based on the specimens examined in this study.

Hydrocassis scapulata group

Hydrocassis jengi Satô, 1998

Figures 1B, 4–5, 12A–B

Material examined

Japan: 1 L2 (SEHU), Kinsakubaru, Amami-Ôshima I., Kagoshima pref., 30.iv.1999, no collector data; 1 L2 (EUMJ), Akatsuchi-yama, Uken-son, Amami-Ôshima I., Kagoshima pref., 2.v.1999, no collector data.; 1 L2 (SEHU), upstream of Naon-kawa R., Yamato-son, Amami-Ôshima I., Kagoshima pref., 30.vii.2008, J. Fujiwara leg.

Description of general morphology.

Second instar (slide preparations of two specimens were examined). Colour (Figure 1B). Dorsal surface of head light brown, lighter in median and anterior parts of frontale and around stemmata, with two pairs of dark brown spots medioposteriorly; light brown ventrally. Pronotum light yellowish brown, median portion darkened; meso- and metathoracic terga and abdominal segments light yellowish brown to dark brown, lighter medially and laterally, thus looking as two dark longitudinal lines along the body; sclerotised parts darker than median part of abdomen; dorsal sclerite of abdominal segment 8 dark brown in medioposterior part. Ventral surface of thorax and abdomen greyish white to yellowish white, proscutum and legs darkened.

Head. Posterior half to two-thirds of head capsule bearing dense, small but strong tooth-like cuticular projections on dorsal and lateral surfaces behind stemmata (Figure 4B). Nasale with four teeth. Epistomal lobes not projecting further than nasale, each lobe with dense, rather short to short spine-like cuticular projections on inner margin (Figure 4C).

Antenna (Figure 5A): Ventral and lateral surfaces of antennomeres 1 and 2 covered with

fine hair-like cuticular projections, the projections on antennomere 2 more dense than those on antennomere 1. Antennomere 1 the longest, longer than antennomeres 2 and 3 combined. Antennomere 2 about 0.6 times as long as antennomere 1.

Abdomen. Segment 1 with three transverse rows of tubercles behind the sclerites (Figure 4E); the first row with four rather large tubercles on each side, outer one larger than inner three; second row with four tubercles on each side, inner three more closely situated than outer one, the size of the tubercles from median to lateral one,: rather large (L), L, rather L, L; third row with three large tubercles on each side, outer one projecting laterally. Arrangement of tubercles on segments 2 to 5 similar to that on segment 1 but first row with five tubercles, tubercles somewhat larger than those on segment 1: L, rather L, L, L, L. Segment 6 similar to segments 2 to 5 but tubercles of first row smaller than those on segments 2 to 5; inner four rather large, outer one large; outer two closely located. Segment 7 with three transverse rows of tubercles, the first row with five tubercles on each side, outer two closely located: rather L, very small (S) (hardly recognisable, absent on left side in specimen examined this study), rather L, rather L, L; second row with four tubercles, inner three closely located: S, L, very S (hardly recognisable, absent on left side in specimen examined for this study), L; third row with two tubercles each side, outer one projecting laterally.

Description of chaetotaxy of head

Second instar. Frontale: Lateral part bearing numerous rather short secondary setae behind sensilla FR4–6 (e.g. Figure 9B). Each epistomal lobe with group of six to seven moderately long setae (gFR2), inner two slightly shorter than outer setae.

Parietale bearing many secondary sensilla, on dorsal and lateral parts.

Mandible (Figures 5B–C) with several small secondary setae on lateral part; basal part of mandible with seven rather short secondary setae, three on dorsal, four on ventral face.

Maxilla (Figures 5D–E): Stipes with 10–11 long secondary setae on outer face; one of them

very long, close to MX5–6.

Labium (Figures 5F–G): Mentum with nine stout secondary setae in each anterolateral corner; a trichoid secondary seta situated ventrally, close to and outside of LA4.

Habitat

Running water. A larva was found in the root of a tree in the bank of a stream (Naon-kawa River) (J. Fujiwara, personal communication).

Identification

Hydrocassis jengi is the only species of the genus distributed in the Amami Islands, southeastern Japan. We identified the larval instar by presence or absence of secondary sensilla and comparing the size of their head capsule with those of *H. lacustris* and *H. uncinata*, of which the larvae and adults of each species are almost of the same size.

Hydrocassis lacustris (Sharp, 1884)

Figures 1A, 2–3, 6–9, 12C–D, 13A, C

Material examined.

Japan: 1 L? (SEHU), Yunosato, Shiriuchi-chô, Hokkaidô, 19.vi.2008 H. Yoshitomi leg; 1 L1, 2 L3 (EUMJ, SEHU), Yazawa-sawa, Kuroiso, Tochigi pref., 25.vii.1994, M. Satô leg.; 1 L1 (EUMJ), Shiga-kôgen, Yamanouchi-machi, Nagano pref., 4–5.viii.1978, no collector data; 12 L1, 4 L2 (reared from egg case) (SEHU), small stream near Abe-tôge, Umegashima, Aoi-ku, Shizuoka-shi, Shizuoka pref, alt. 1380 m, 35°18.81´ N, 138°20.96´ E, 22.vi.2008 (egg case collected), Y. Minoshima leg.; 1 L3 (EUMJ), Dando-uradani, 15.vii.1998, no collector data; 1 L3 (EUMJ), Seryô-tôge, Kyôto, 6.viii.1980, no collector data; 1 L1, 1 L2, 3 L3 (HGFC, SEHU), Shinji-gawa R., Matsue-shi, Shimane pref., 5.v.2007, M. Hayashi leg.; 1 L3 (SEHU), Mt.

Ôyorogi-san, Kadosaka, Iinan-chô, Shimane pref., 31.vii.2007, M. Hayashi leg.

Description of general morphology

Third instar (slide preparations of three specimens were examined). Colour (Figure 1A). Dorsal surface of head light brown, lighter in median and anterior parts of frontale and around stemmata, with two pairs of dark brown spots on medioposterior portion; light brown on ventral surface. Pronotum light brown, median portion darkened, the size of dark portion variable, sometimes looking like in *H. jengi* (Figure 1B); meso- and metathoracic terga and abdominal segments dark brown, darker than head, slightly lighter medially and laterally, thus looking as two dark longitudinal lines along the body; dorsal sclerite and tubercles of abdominal segments darker than those on other abdominal portions; dorsal sclerite of abdominal segment 8 dark brown in median portion. Ventral surface of thorax and abdomen light brown, proscutum and legs darkened.

Head. Posterior 0.5 to 0.6 of head capsule bearing dense, small but strong tooth-like cuticular projections on dorsal and lateral surfaces behind stemmata (Figure 9B). Nasale usually with five teeth, median tooth very small, sometimes absent, then nasale with four teeth. Epistomal lobes projecting about as far as nasale, right lobe with dense rather long spine-like cuticular projections on inner margin; left lobe with dense, short spine-like cuticular projections on inner margin.

Antenna: Antennomeres 1 and 2 covered with fine hair-like cuticular projections (Figures 7A, 9C). Antennomere 1 the longest, longer than antennomeres 2 and 3 combined. Antennomere 2 about half as long as antennomere 1.

Maxilla (Figures 7D–E): Dorsal surface of maxillary palpomeres 1–3 bearing sparse to rather dense fine hair-like cuticular projections.

Labium (Figures 7F–G): Ligula slightly shorter than palpi.

Abdomen. Segment 1 with three transverse rows of tubercles behind the sclerites (Figure

6E); the first row with five tubercles on each side, size of the tubercles from median to lateral one: large (L), small (S), rather L, S, L; second row with four tubercles on each side, inner three more closely situated than outer one: rather L, L, rather L, L; third row with three large tubercles on each side, outer one projecting laterally. Arrangement of tubercles on segments 2 to 5 similar to that on segment 1 but the tubercles of first and second transverse rows somewhat larger than those on segment 1: rather L, rather S, rather L, rather L, L in first row; rather L, L, L, L in second row. Segment 6 similar to segments 1 to 5 but first row composed of four rather large tubercles. Segment 7 with three transverse rows of tubercles, first row with four tubercles on each side, outer two closely situated: rather L, rather L, rather L, L; second row with four tubercles, inner three closely located: S, L, S, L; third row with two tubercles on each side, outer one projecting laterally.

Second instar (slide preparations of two specimens were examined). Similar to third instar, more weakly sclerotised than in third instar.

Head. Antenna moderately long, rather slender. Antennomere 2 about 0.5 to 0.6 times as long as antennomere 1.

Maxilla: Dorsal surface of maxillary palpomeres 1 and 2 sparsely covered with fine hair-like cuticular projections.

Abdomen. Abdominal transverse rows of tubercles smaller than in third instar.

First instar (slide preparations of eight specimens were examined). Similar to third instar, more weakly sclerotised than in second instar.

Head (Figure 2). Frontal lines almost reaching to antennal socket.

Antenna (Figure 3A) rather short and stout, bearing fine hair-like cuticular projections on ventral surface of antennomere 2. Antennomere 1 wider than antennomere 2, as long as antennomere 2.

Abdomen. Abdominal transverse rows of tubercles smaller than in second instar; inner two pairs of tubercles of the second row on segments 1 to 5 reduced, hardly recognisable.

Description of chaetotaxy of head

Second instar. Frontale: Central part bearing numerous, rather short secondary setae (e.g. Figure 9B). Each epistomal lobe with a group of seven to eight moderately long, stout setae (gFR2), inner two slightly shorter than outer ones.

Parietale bearing many secondary sensilla, on dorsal and lateral parts (e.g. Figure 9A–B).

Mandible with several small secondary setae on lateral part; basal part of mandible with four to five short secondary setae (e.g. Figures 7B–C).

Maxilla: Stipes with 10–12 long secondary setae on outer face; one of them very long, close to MX4–6 (e.g. Figures 7D–E).

Labium: Mentum with 9–12 stout secondary setae each anterolateral corner; a trichoid secondary seta on ventral close to and outside of LA4 (e.g. Figures 7F–G).

Third instar. Frontale Central part bearing many, rather short and stout secondary setae (Figure 9B). Each epistomal lobe with a group of seven to eight moderately long, stout setae (gFR2), inner two slightly shorter than outer ones.

Parietale bearing numerous, rather short and stout secondary sensilla each, on dorsal and lateral parts (Figure 9A–B).

Mandible (Figures 7B–C) with rather sparse, small secondary setae on lateral part; basal part of mandible with five short secondary setae, three on dorsal and lateral, two on ventral.

Maxilla (Figures 7D–E): Stipes with 13–15 long secondary setae on outer margin.

Labium (Figures 7F–G): Mentum with 8–12 stout secondary setae on each anterolateral corner.

Habitat

Running water (Figure 13A). Egg cases were found on the underside of rocks or leaves on the margins of a stream (Figure 13C); larvae usually hide behind rocks or leaves.

Identification

Hydrocassis lacustris is the only species of the genus distributed from Hokkaidô to Kyûshû Islands. Therefore, we identified the larvae collected from Honshû as *H. lacustris*. Instars of the larvae collected in the field were identified by sorting the specimens according the size of their head capsule and by comparison with the larvae of the known instar reared from egg cases collected in the field.

Remarks

Morioka (1955) published a description of the larva of "*Hydrocyclus* sp." based on the specimens collected in Shiga and Tochigi Prefectures, Honshû. This larva corresponds in all characters with our material of *H. lacustris*. Later, Yoshimura (1959) described the larva collected in Nara Prefecture, Honshu as "*Hydrocyclus* sp. B". Based on his description, his material differs substantially from *Hydrocassis* and belongs most probably to the genus *Sternolophus* Solier, 1834.

Hydrocassis scaphoides group

Hydrocassis uncinata Ji et Schödl, 1998

Figures 1C, 10–11, 13B

Material examined.

Laos: 1 L2, 2 L3 (SEHU), upstream of Nam Xan R., NW Mt. Phou Samsoun, Xieng Khouang prov., alt. 2050 m, N19°08.42´, E103°47.13´, 13.v.2008, Y. Minoshima leg. (MiYu-L-08-010); 2

L3 (SEHU), upstream of Nam Mo R., NE Mt. Phou Samsoun, Xieng Khouang prov., alt. 2200 m, N19°08.47´, E103°48.46´, 15.v.2008, Y. Minoshima leg. (MiYu-L-08-015); 1 L2 (SEHU), N Mt. Phou Samsoun (small stream), Xieng Khouang prov., alt. 2210 m, N19°08.73´, E103°48.24´, 15.v.2008, Y. Minoshima leg. (MiYu-L-08-016).

Description of general morphology

Third instar (slide preparations of three specimens were examined). Colour (Figure 1C). Dorsal surface of head light brown, darker in median part, with two pairs of dark brown spots on medioposterior portion; ventral surface slightly lighter than dorsal surface. Pronotum light brown, medioanterior portion darkened, dark portion spotted, rather variable in their positions; meso- and metathoracic terga and abdominal segments light yellowish brown, lighter than head, slightly lighter medially and laterally; mesotergum with two dark areas on median part; dorsal sclerites and tubercles of abdominal segments somewhat darker than other abdominal portions; dorsal sclerite on abdominal segment 8 dark brown in median portion. Ventral surface of thorax and abdomen light yellowish brown, proscutum and legs darkened.

Head. Posterior 0.5 to 0.6 of head capsule bearing dense, small tooth-like cuticular projections on dorsal and lateral surfaces behind stemmata (Figure 10B). Nasale usually with five teeth, median tooth very small (Figure 10C), sometimes absent, then nasale with four teeth. Epistomal lobes projecting about as far as nasale, right lobe with numerous short spine-like cuticular projections on inner margin; left lobe with dense, fine spine-like cuticular projections on inner margin (Figure 10C).

Antenna: Antennomere 2 covered with fine hair-like cuticular spines on ventral and inner surfaces (Figure 11A). Antennomere 2 about 0.6 times as long as antennomere 1.

Abdomen. Segment 1 with three transverse rows of tubercles behind sclerites (Figure 10E); first row with five tubercles on each side, size of the tubercles from median to lateral one: Large (L), Small (S), rather L, S, L; second row with four tubercles on each side, inner three more

closely situated than outer one: S, L, rather L, L; third row with three large tubercles on each side, outer one projecting laterally. Arrangement of tubercles of segments 2 to 5 similar to that of segment 1 but the tubercles of first and second transverse rows somewhat larger than those in segment 1: L, rather S, L, rather L, L in first row; rather L, L, rather L, L in second row. Segment 6 similar to segments 1 to 5 but tubercles somewhat smaller than those on segments 1 to 5: rather L, S, rather L, rather L, L in first row; rather S, rather L, rather S, L in second row. Segment 7 with three transverse rows of tubercles, first row with three tubercles on each side: rather L, S, L, lateral two more closely located; second row with three tubercles: S, rather L, L, median two more closely situated; third row with two tubercles on each side, outer one projecting laterally.

Second instar (slide preparations of two specimens were examined). Similar to third instar, more weakly sclerotised than in third instar.

Head. Antenna moderately long, rather slender. Antennomere 2 about 0.7 times as long as Antennomere 1.

Abdomen. Abdominal transverse rows of tubercles smaller than in third instar.

Description of chaetotaxy of head

Second instar. Frontale: Lateral part bearing numerous rather short secondary setae, behind FR4–6 sensilla (e.g. Figure 9B). Each epistomal lobe with a group of seven moderately long, stout setae (gFR2), inner three slightly shorter than outer ones.

Parietale bearing many secondary sensilla, on dorsal and lateral areas (e.g. Figure 9B).

Mandible with several small secondary setae on lateral part; base of mandible with five rather short secondary setae (e.g. Figure 11B–C).

Maxilla: Stipes with 13–14 long secondary setae on outer margin; one of them very long, close to MX 4–6 (e.g. Figure 11D–E).

Labium: Mentum with six to eight stout secondary setae in each anterolateral corner (e.g. Figure 11F–G).

Third instar. Frontale: Each epistomal lobe with a group of seven to nine moderately long, stout setae (gFR2), median three slightly shorter than lateral ones (Figure 10C).

Parietale bearing numerous, rather short secondary sensilla, on dorsal and lateral areas (e.g. Figures 9A–B).

Mandible (Figures 11B–C) with several small secondary setae on lateral part; basal part of mandible with five rather short secondary setae, three close to primary cranial articulations of mandibles.

Maxilla: Stipes with 11 long secondary setae on outer margin (Figures 11D–E).

Labium: Mentum with 10–11 stout secondary setae on each anterolateral corner (Figures 11F–G).

Habitat

Lotic water (Figure 13B). Larvae were found in leaf packs in the banks of a stream.

Identification

The larval specimens were collected from mountain stream of near the top of Phou (Mount) Samsoun, northern Laos. The first author surveyed the locality for about one week and was able to collect only one species of Sperchopsini, i.e. *H. uncinata*. Therefore, we identified the larvae as *H. uncinata*. Instars were identified by sorting larvae according the size of their head capsule and comparing the size of their head capsule with those of *H. lacustris*, which larvae as well as adults are almost of the same size.

Conclusion

Morphological comparison of allied genera of Hydrocassis

In this section, we compare the larval morphology among genera of Sperchopsini based on our results and published data (Table 3). Larva of *Cylomissus* remains unknown. The larvae of all genera are rather similar to each other in most characters, the only exception is the genus *Anticura* which differs from all remaining Sperchopsini genera in the following characters: 1) nasale with two teeth; 2) inner surface of stipes with numerous stout, rather short setae; 3) dorsal sclerite of abdominal segment 8 divided into two sclerites. On the other hand, larvae of *Hydrocassis* are most similar to the larvae of the genus *Sperchopsis* and may only be distinguished by the number of setae on inner face of stipes (5 in *Hydrocassis*, 7–8 in *Sperchopsis*). In contrast, the genus *Hydrocassis* is easily distinguishable from *Ametor* by the larval morphology, although Schödl & Ji (1995) indicated a close relationship of these genera and pointed out that there is the only adult character which clearly distinguishes the genus *Hydrocassis* from *Ametor*, i.e. the position of corona of the aedeagus. Larva of *Hydrocassis* is clearly distinguishable from *Ametor* by the following characters: 1) epistomal lobes with stout setae; 2) clypeolabrum asymmetrical; 3) frontal lines not fused at base. However, all information on larval stages of *Ametor* are based on the widely distributed *Ametor scabrosus* (Horn, 1873), which is easily distinguishable from the species of *Hydrocassis* by adult morphology. More extensive research on the larvae of *Ametor* is, therefore, necessary to solve the relationships of *Hydrocassis* and *Ametor* and confirm their separate generic states.

Larval morphology within Hydrocassis

Schödl & Ji (1995) subdivided the genus *Hydrocassis* into three species groups based on one adult morphological character: the shape of parameres. The *Hydrocassis* species treated in the present paper belong to two of these species groups: *Hydrocassis lacustris* and *H. jengi* into the *H. scapulata* group, *H. uncinata* into the *H. scaphoides* group. Larval morphology does not

provide good characters to distinguish both species groups. The only character in which the species groups differ are the projections of abdominal segments: the projections are larger and stronger in *H. uncinata* than in *H. jengi* and *H. lacustris* (Figure 1). However, the size and shape of these projections are more or less continuous quantitative characters and their using as diagnostic is therefore limited. Moreover, there are no good characters which clearly separate the *Hydrocassis* species treated in this paper from each other. Of all studied characters, only the distribution and the size of tubercles on abdominal segments show slight differences among *Hydrocassis* species (Figures 4E, 6E, 10E) and this character may be therefore useful for species identification and for phylogenetic studies. The examination of more materials would be necessary to evaluate the significance of this character and confirm the homology of tubercles not only in *Hydrocassis*, but also in other genera of Sperchopsini.

Acknowledgements

We thank Masahiro Sakai and Hiroyuki Yoshitomi (EUMJ) for giving us opportunity to examine the museum collection of the Ehime University Museum, and Jun'ichi Fujiwara (Laboratory of Insect Science, Kobe University) for a valuable specimen of *Hydrocassis jengi* and information of habitat of the species. We also thank Masahiro Ôhara and Kazunori Yoshizawa (SEHU), Miguel Archangelsky (LIESA, Universidad Nacional de la Patagonia San Juan Bosco), Martin Fikáček (National Museum, Prague) and Hiroyuki Yoshitomi (EUMJ) for their kind advice and critical reading of the draft of the manuscript, and the two anonymous reviewers for their constructive criticisms. The first author thanks Hiroyuki Wakahara and his family for their kind support during the Laotian field work. This study was partly supported by Grant-in-Aid for JSPS Fellows to Y. Minoshima.

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Figure captions

Figure 1. Habitus of *Hydrocassis* larvae, dorsal and ventral views. (A) *H. lacustris* Sharp, third instar; (B) *H. jengi* Satô, second instar; (C) *H. uncinata* Ji et Schödl, third instar.

Figure 2. Primary chaetotaxy of head capsule of *H. lacustris* Sharp, first instar. (A) Dorsal view; (B) ventral view; (C) detail of anterior margin of head capsule, dorsal view.

Figure 3. Primary chaetotaxy of head appendages of *H. lacustris* Sharp, first instar. (A) right antenna, dorsal view; (B) left mandible, dorsal view; (C) left maxilla, dorsal view; (D) left maxilla, ventral view; (E) labium, dorsal view; (F) labium, ventral view. Black square indicates additional sensilla.

Figure 4. *Hydrocassis jengi* Satô, second instar, dorsal view. (A) whole larva; (B) head; (C) clypeolabrum; (D) spiracular atrium; (E) tubercular pattern of thoracic and abdominal segments. Abbreviations: T3: metathorax; A1, 2, 6, 7: abdominal segments 1, 2, 6, and 7.

Figure 5. Head appendages of *H. jengi* Satô, second instar. (A) right antenna, dorsal view; (B–C) mandibles, dorsal view; (D) right maxilla, dorsal view; (E) right maxilla, ventral view; (F) labium, dorsal view; (G) labium, ventral view. Black square indicates additional sensilla.

Figure 6. *Hydrocassis lacustris* Sharp, third instar, dorsal view. (A) whole larva; (B) head; (C) clypeolabrum; (D) spiracular atrium; (E) tubercular pattern of thoracic and abdominal segments. Abbreviations see Figure 4.

Figure 7. Head appendages of *H. lacustris* Sharp, third instar. (A) right antenna, dorsal view; (B–C) mandibles, dorsal view; (D) left maxilla, dorsal view; (E) left maxilla, ventral view; (F) labium, dorsal view; (G) labium, ventral view. Black square indicates additional sensilla.

Figure 8. SEM photographs of *H. lacustris* Sharp, first instar. (A) head, dorsal view; (B) head, lateral view; (C) head capsule, dorsal view; (D) head appendages, dorsal view.

Figure 9. SEM photographs of *H. lacustris* Sharp, third instar, dorsal view. (A) head; (B) head capsule; (C) head appendages; (D) pro- and mesothorax.

Figure 10. *Hydrocassis uncinata* Ji et Schödl, third instar, dorsal view. (A) whole larva; (B) head; (C) clypeolabrum; (D) spiracular atrium; (E) tubercular pattern of thoracic and abdominal segments. Abbreviations see Figure 4.

Figure 11. Head appendages of *H. uncinata* Ji et Schödl, third instar. (A) right antenna, dorsal view; (B–C) mandibles, dorsal view; (D) left maxilla, dorsal view; (E) left maxilla, ventral view; (F) labium, dorsal view; (G) labium, ventral view. Black square indicates additional sensilla.

Figure 12. Thoracic structures of *Hydrocassis* species. (A) prosternum of *H. jengi*, second instar, ventral view; (B) mesothoracic leg of *H. jengi*, second instar, anterior view; (C) metathoracic leg of *H. lacustris*, first instar, anterior view; (D) mesothoracic leg of *H. lacustris*, third instar, anterior view.

Figure 13. Habitats (Figures A–B) and egg case (Figure C) of *Hydrocassis* species. (A) collecting locality of *H. lacustris* Sharp, Japan: small stream near Abe-tôge, Umegashima, Aoi-ku, Shizuoka-shi, Shizuoka pref, alt. 1380m, 22 June 2008; (B) collecting locality of *H. uncinata* Ji et Schödl, Laos: upstream of Nam Xan River, NW Mt. Phou Samsoun, Xieng Khouang province, alt. 2050m, 13 May 2008; (C) egg case of *H. lacustris* Sharp, same locality as Figure A.

Table

Table 1. State of knowledge of the immature stages of the tribe Sperchopsini and source of information. Abbreviations: E: egg case, eggs; L1, L2, L3, larva of first, second, or third instar; L?, larval instar unknown; P: pupa.

Genus	Original identification	Described stages	Reference
Ametor	*Hydrobius scabrosus*	L3, P	Richmond 1920 (briefly mentioned)
	Ametor scabrosus	L?, P	Spangler 1962
	Ametor scabrosus	L3, P	Archangelsky 1997
Anticura	*Anticura flinti*	L3, P	Spangler 1979
	Anticura flinti	L3, P	Archangelsky 1997
Hydrocassis	*Hydrocyclus* sp.	L?	Morioka 1955
	Hydrocassis lacustris	E, L?	Hayashi 2009 (photo, diagnosis)
Sperchopsis	*Hydrobius tesselatus* [!]	L?	Richmond 1920 (briefly mentioned)
	Sperchopsis tessellata	E, L1, L3, P	Spangler 1961
	Sperchopsis tessellata	E, L3?, P	Archangelsky 1997
Cylomissus	unknown		

Table 2. Selected taxa and instars examined in this paper. Identification: A, reared from egg case collected in the field and associated with adults; B, larvae collected in the field and associated with adults; L1, L2, L3, larva of first, second, or third instar.

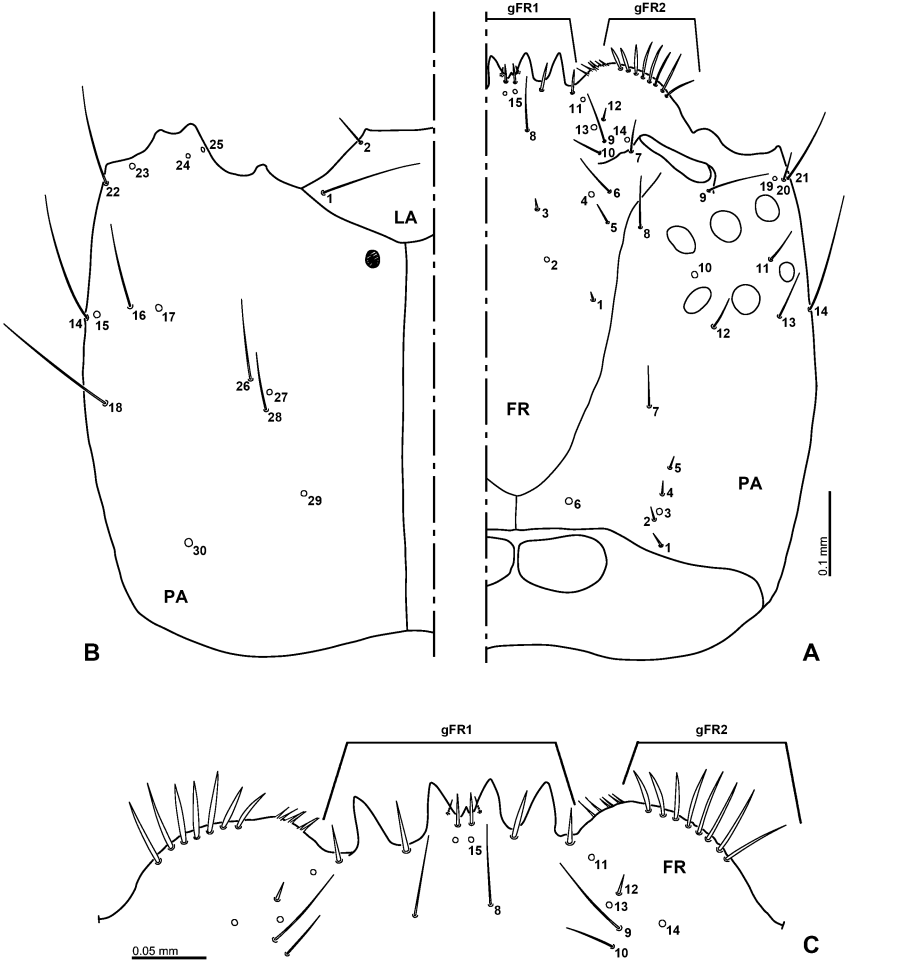
Species	L1	L2	L3
Hydrocassis jengi		B	
Hydrocassis lacustris	AB	AB	B
Hydrocassis uncinata		B	B

Table 3. Comparison of larval morphology of the genera of Sperchopsini based on third instar larva. Abbreviations: AU: Australasia; NA: Nearctic; NT: Neotropical; NZ: New Zealand; OR: Oriental; PA: Palaearctic. See Table 1 for references.

	Sperchopsis	*Hydrocassis*	*Ametor*	*Anticura*	*Cylomissus*
Distribution	NA	PA, OR	NA, PA	NT	AU (NZ)
Egg-case	Bag-shaped, cup	covered with silk	unknown	unknown	unknown
Frontal lines	fused at base	fused at base	not fused at base	fused at base	unknown
Clypeolabrum	slightly asymmetrical	almost symmetrical	asymmetrical	symmetrical	unknown

Nasale	with 5 teeth	with 4 or 5 teeth	with 4 teeth	with 2 teeth	unknown
Epistomal lobe	with (rather) slender setae	with rather slender setae	with stout setae	with slender setae	unknown
Number of setae on epistomal lobe	7–8	7–9	5	8 (6+2)	unknown
Inner teeth of mandible	3	3	3	2	unknown
Inner margin of stipes	with 7–8 setae	with 5 setae	with 5 setae	with a row of numerous setae	unknown
Dorsal plate of abdominal segment 8	not divided	not divided	not divided	divided to two sclerites	unknown

A
3.0 mm
B
2.0 mm
C
3.0 mm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A
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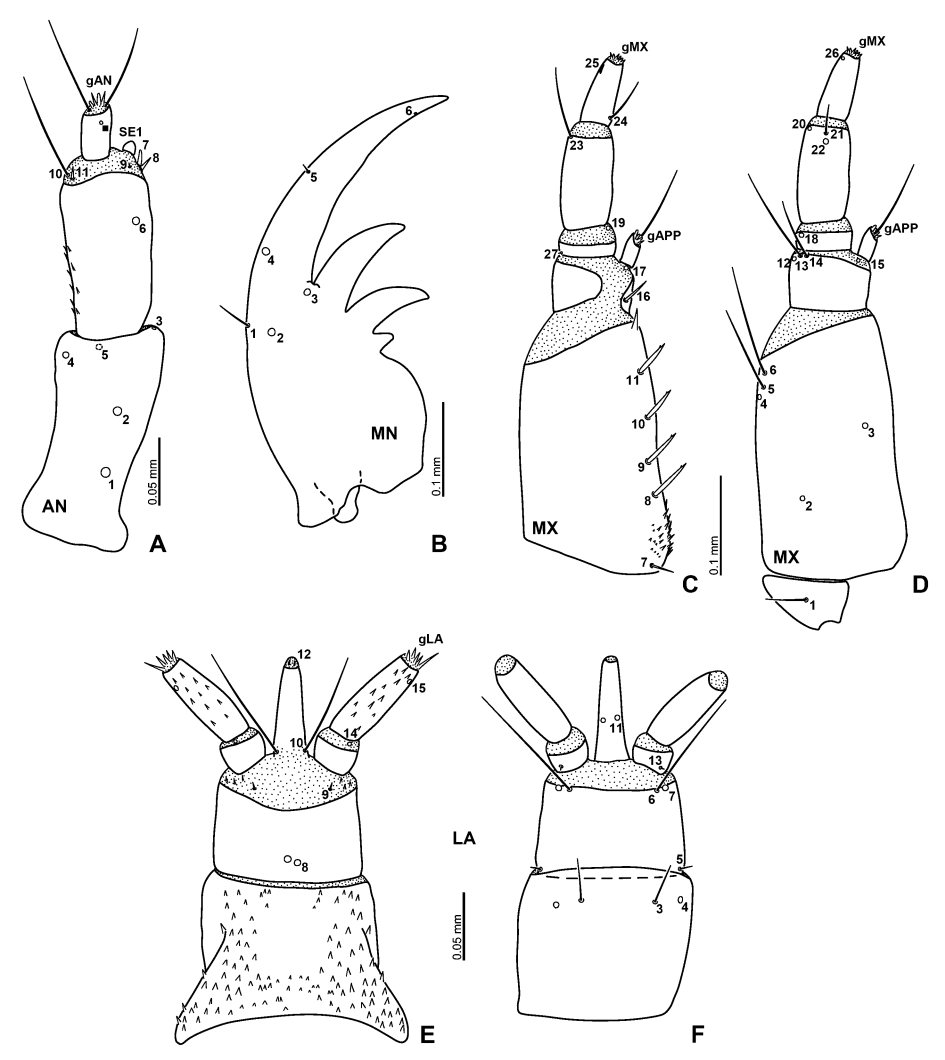
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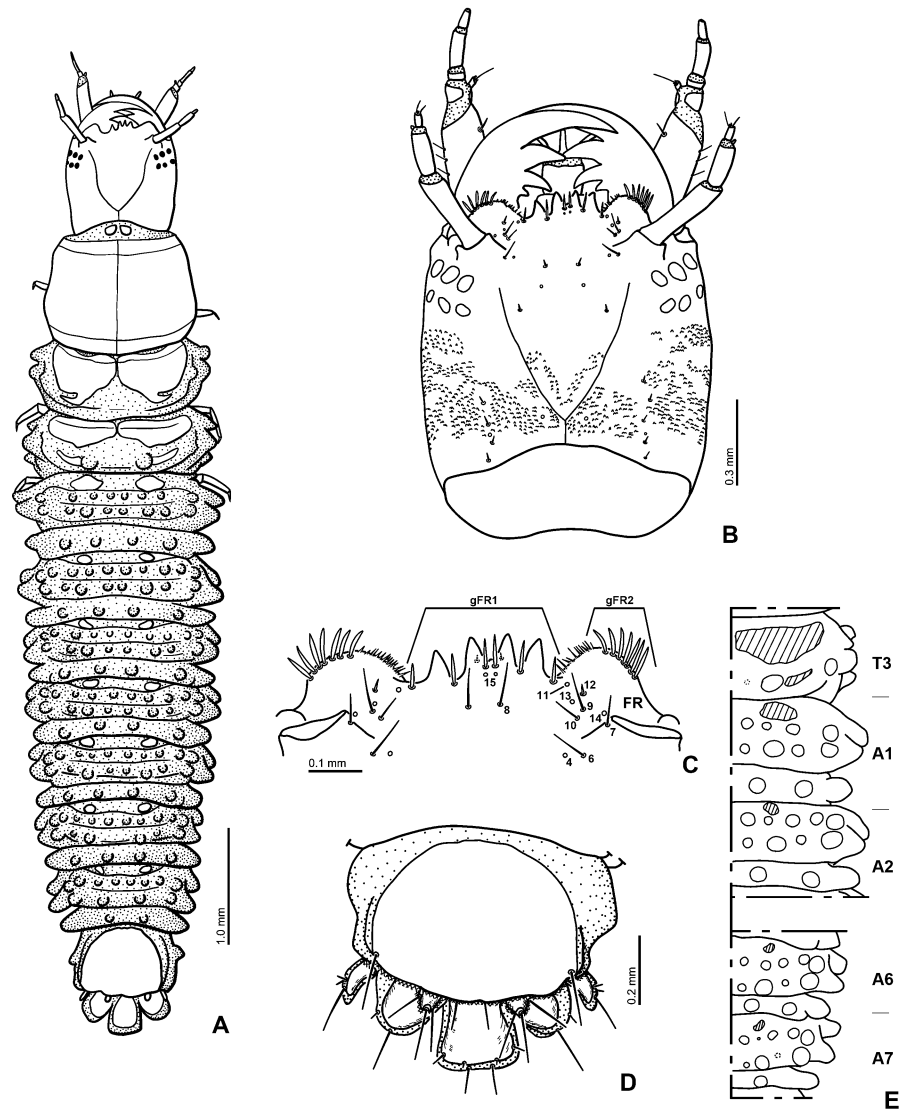
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gFR1
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11
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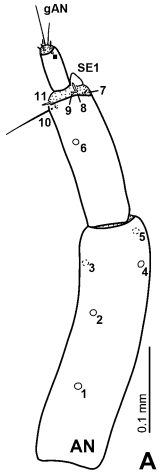
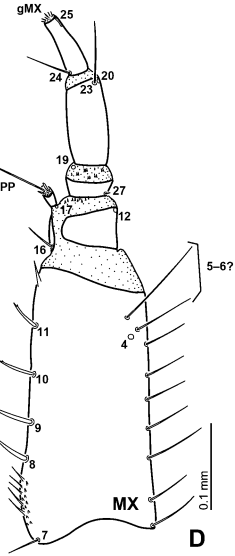
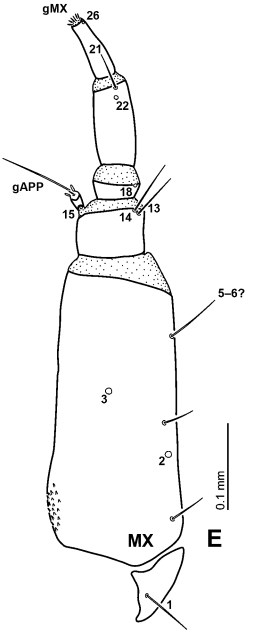
7
4
6
0.1 mm
C
D
0.2 mm
T3
A1
A2
A6
A7
E


gAN
SE1
11
7
9
8
10
6
5
3
4
2
1
AN
0.1 mm

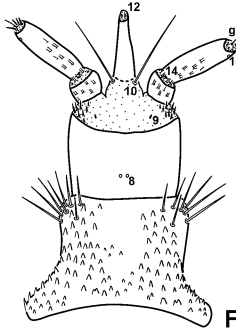
A


gMX
25
24
23
20
19
gAPP
27
17
12
16
5–6?
11
4
10
9
8
7
MX
0.1 mm

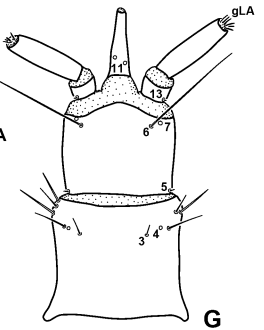
D


gMX
26
21
22
gAPP
18
15
14
13
5–6?
3
2
MX
1
0.1 mm

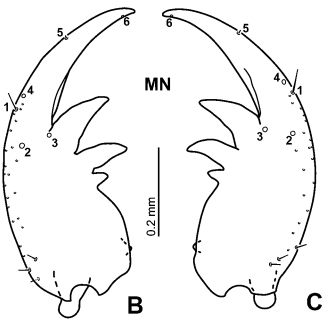
E


12
gLA
15
14
10
9
11
LA
8
0.1 mm

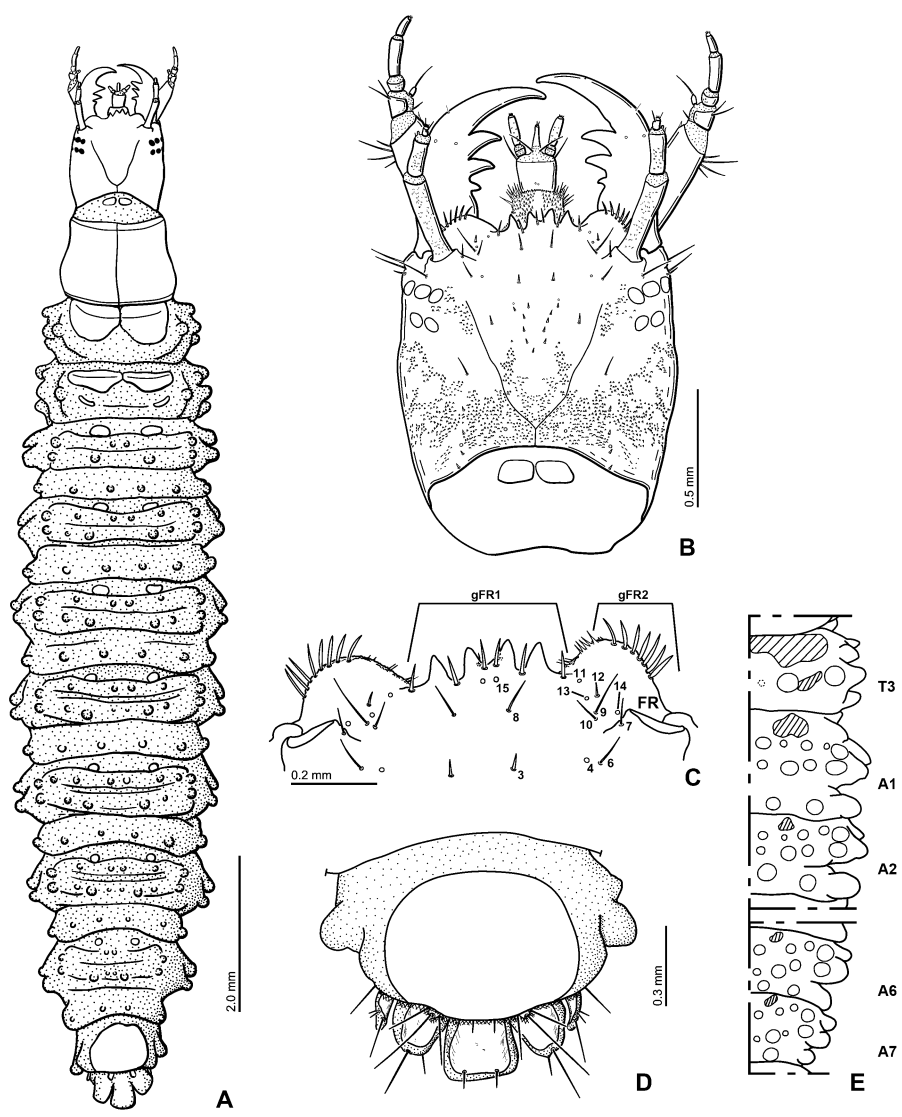
F

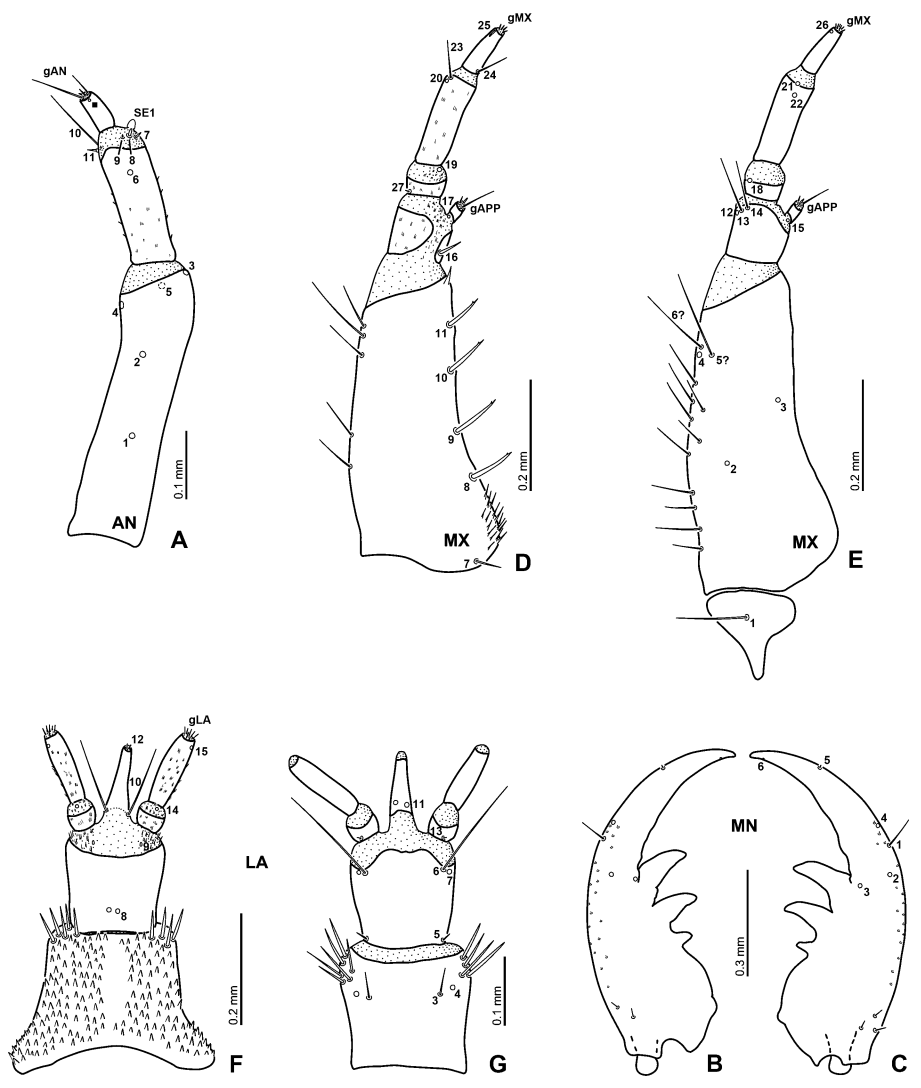

gLA
11
13
6
7
5
3
4

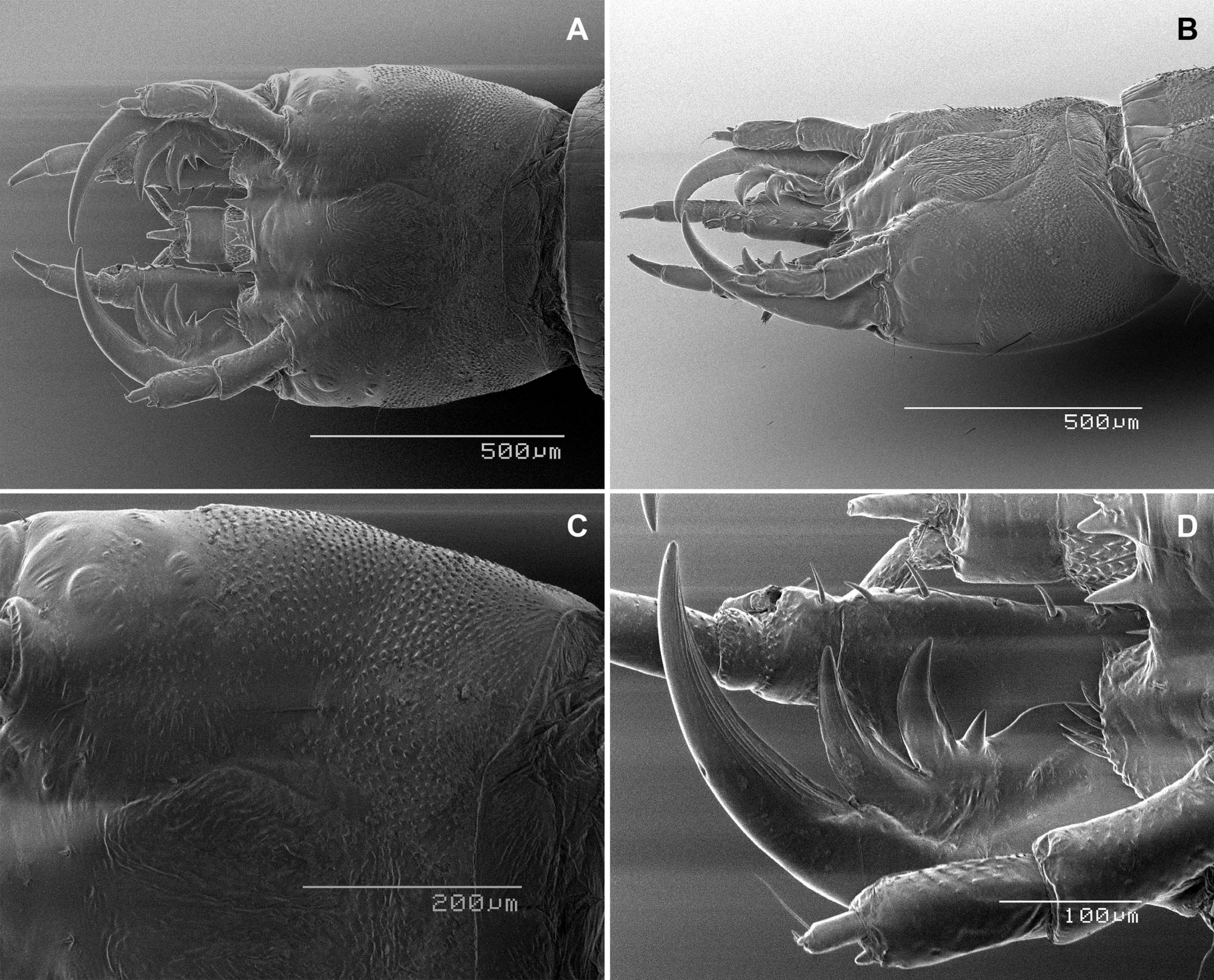
G

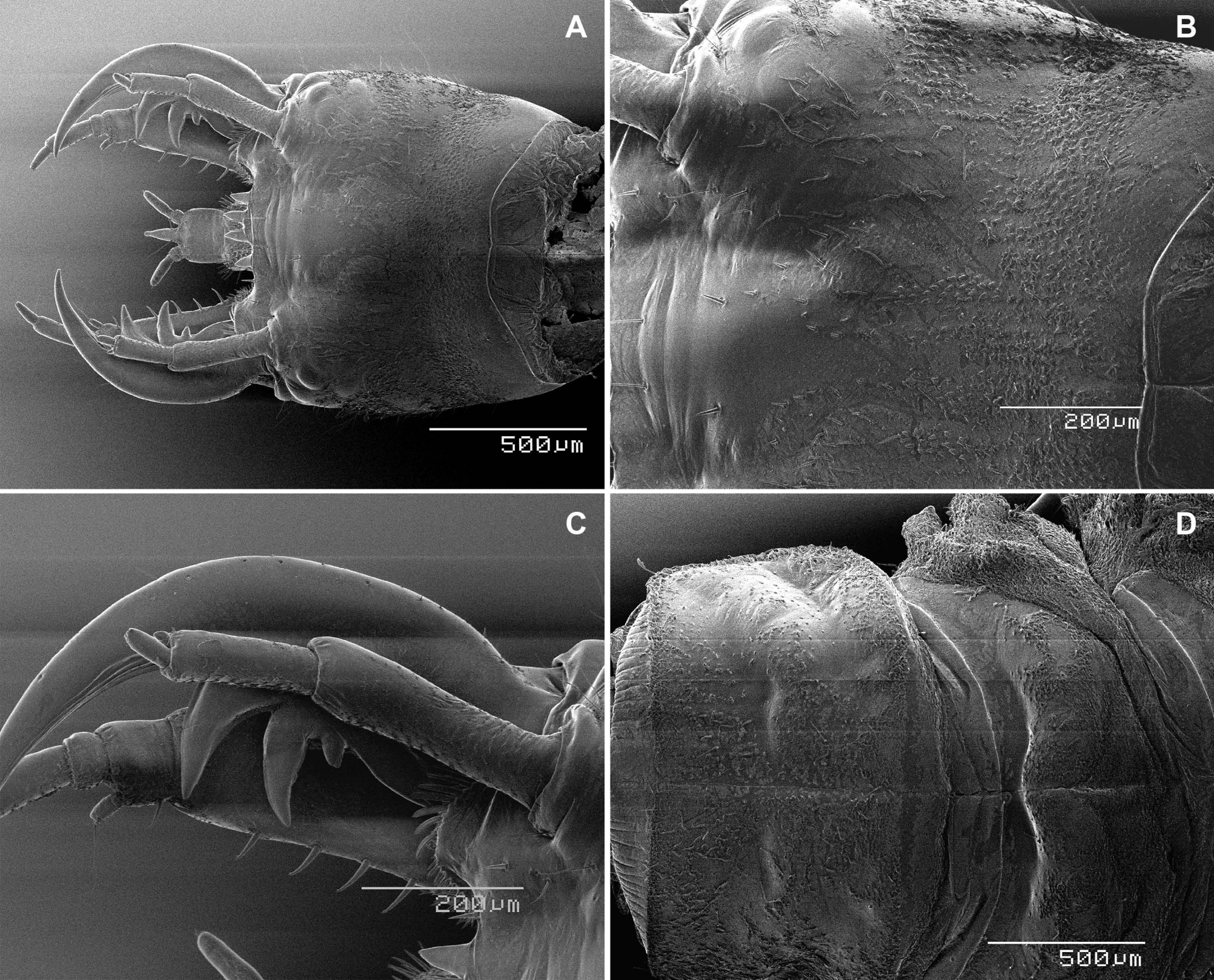

MN
5
6
1
4
3
2
0.2 mm

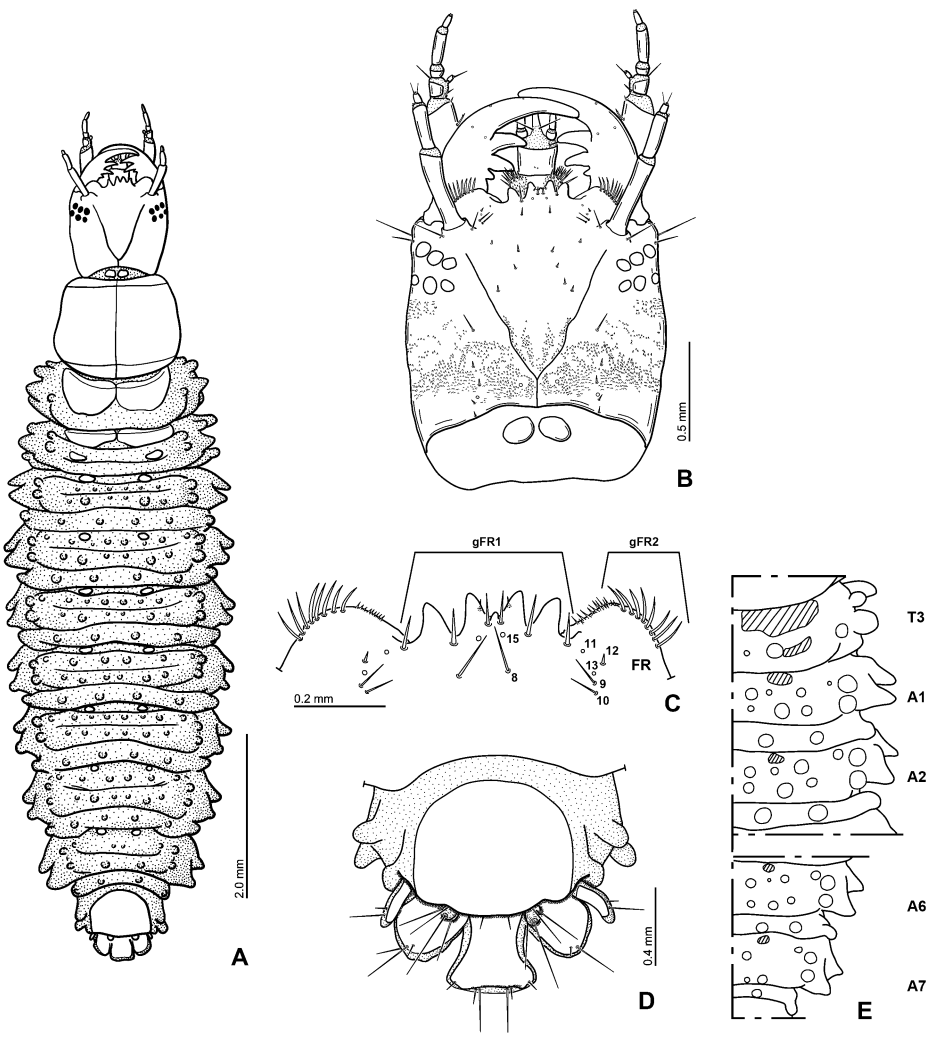
B C

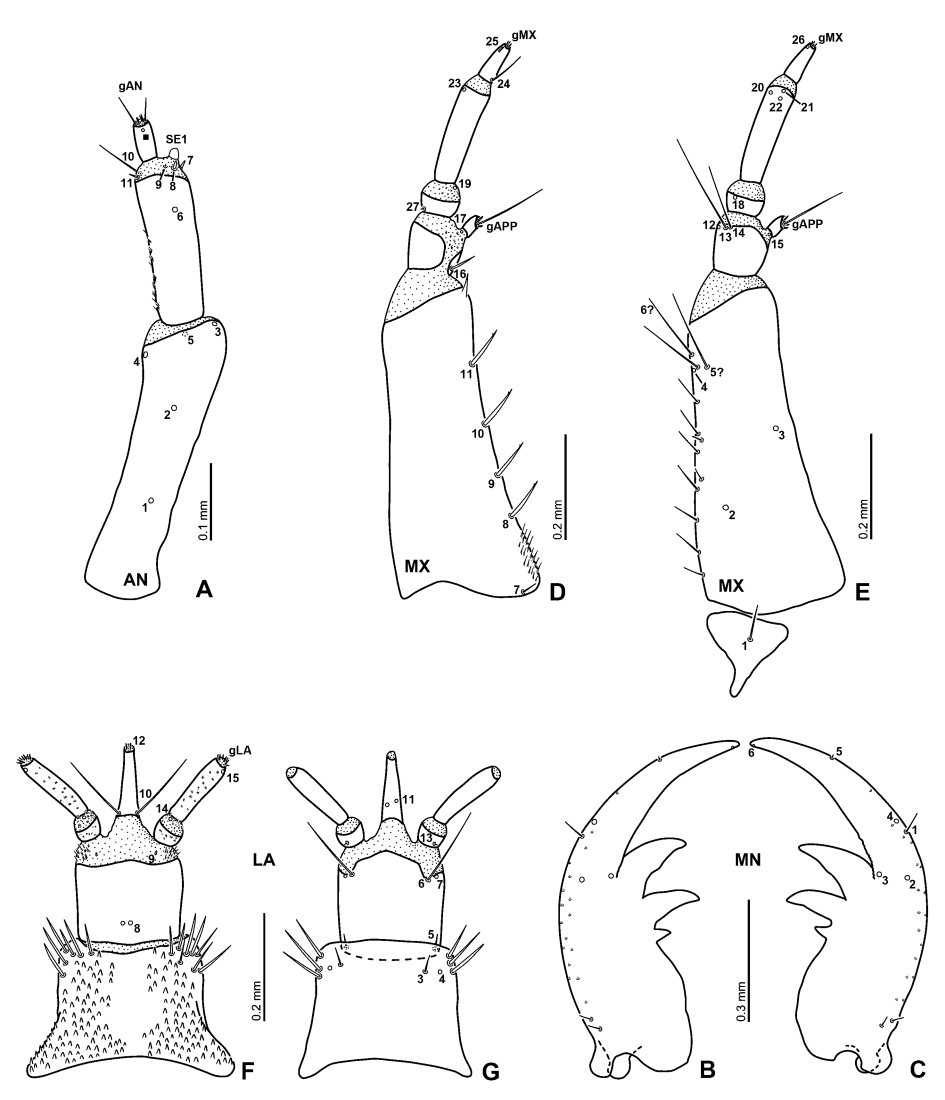

2.0 mm
A
0.5 mm
B
gFR1
gFR2
FR
15
11
12
13
14
9
10
8
7
0.2 mm
3
4
6
C
0.3 mm
D
T3
A1
A2
A6
A7
E

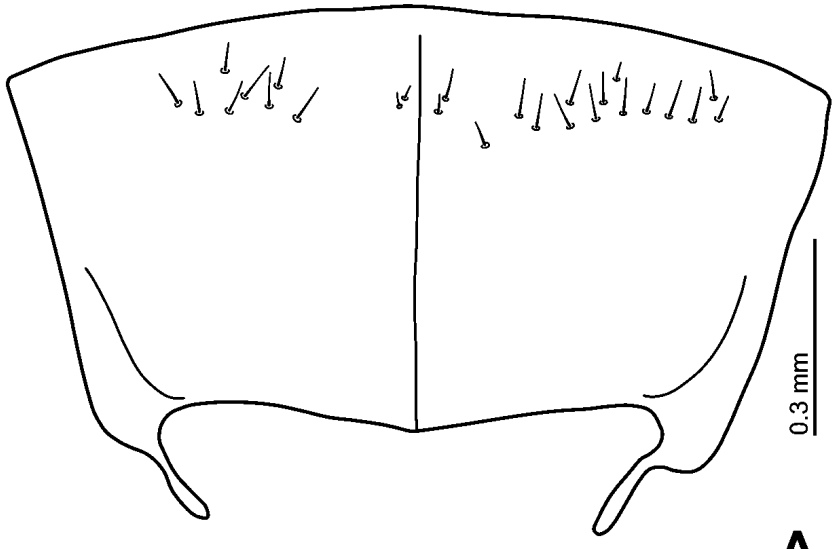

gAN
SE1
10
7
11
9
8
6
3
5
4
2
1
0.1 mm
AN
A
gMX
25
23
20
24
19
27
17
gAPP
16
11
10
9
8
7
0.2 mm
MX
D
gMX
26
21
22
18
12
13
14
gAPP
15
6?
4
5?
3
2
0.2 mm
MX
E
gLA
12
15
10
14
8
8
0.2 mm
LA
F
11
13
6
7
5
3
4
0.1 mm
G
MN
6
5
4
1
2
3
0.3 mm
B
C


A
500µm
B
500µm
C
200µm
D
100µm

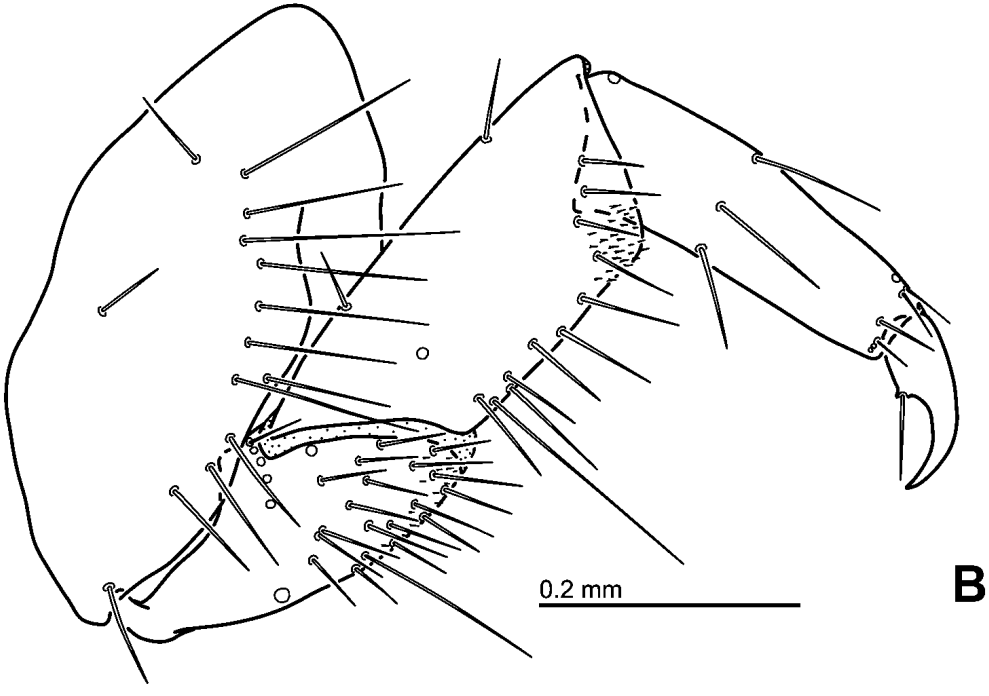

A
500µm
B
200µm
C
200µm
D
500µm


A
2.0 mm
B
0.5 mm
gFR1
gFR2
15
11
12
13
9
8
10
FR
0.2 mm
C
D
0.4 mm
T3
A1
A2
A6
A7
E

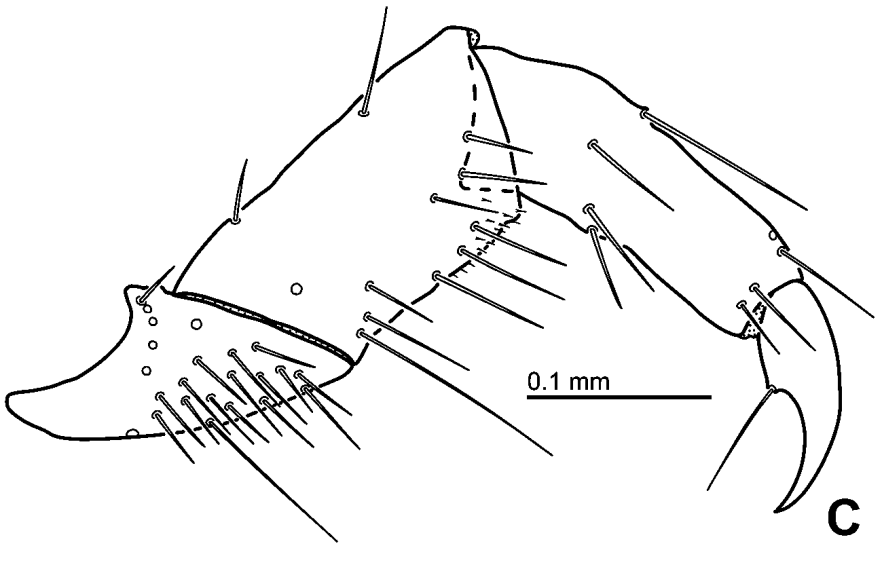

gAN
SE1
10
7
11
9
8
6
5
3
4
2
1
0.1 mm
AN
A
25
gMX
23
24
19
27
17
gAPP
16
11
10
9
8
7
0.2 mm
MX
D
26
gMX
20
22
21
18
12
13
14
gAPP
15
6?
5?
4
3
2
0.2 mm
MX
E
1
12
gLA
15
10
14
9
8
0.2 mm
F
LA
11
13
6
7
5
3
4
G
6
5
4
1
3
2
0.3 mm
MN
B
C



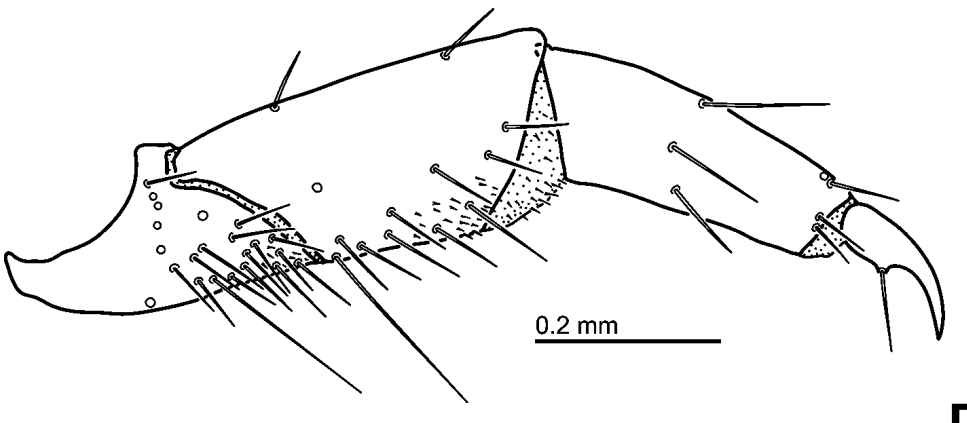
A



B



C



D

A

B

C