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Miniaturisation effects in larvae and adults of *Mikado* sp. (Coleoptera: Ptiliidae), one of the smallest free-living insects

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ABSTRACT

We present the first morphological study of larvae and adults of *Mikado* sp. – one of the smallest known beetles and free-living insects (body length of adult is 390–455 μm). Morphological and developmental consequences of miniaturisation in *Mikado* and insects in general are discussed. We used histological sectioning, scanning electron microscopy, laser confocal microscopy and 3D-computer reconstruction. For the first time we report that according to the morphometric data of *Mikado* sp., at least some ptiliid beetles have three larval stages. We studied the muscular system of adults and larval stages. It is shown that ptiliid beetles have nearly the complete set of muscles found in larger staphyliniform beetles. Developmental and size dependent changes in the relative volume of different organs are addressed. All organ systems change allometrically in the development of *Mikado* sp. as well as in comparison with larger representatives of Ptiliidae and closely related groups of beetles, such as Staphylinidae. We conclude that the factors limiting miniaturisation are the size of the neural system, associated with the number and size of neurons, the mass of the skeleton, the egg size (free-living insects), and consequently the volume of the reproductive system.

1. Introduction

Miniaturisation is one of the fundamental trends in insect evolution (Chetverikov, 1915). As a result, the size of many insects is comparable to that of unicellular organisms. The smallest free-living insects belong to Ptiliidae. The body length of some members of the group is merely 300 μm and therefore distinctly below the size of an *Amoeba proteus*. Some ptiliid species belong to the smallest metazoans.

Such an important parameter as body size, especially that approaching the lower limit, largely determines the morphology, physiology, and biology of a species. The anatomical traits related to miniaturisation have been considered in detail for many vertebrates (Hanken, 1983a,b, 1985; Schmidt-Nielsen, 1984; Hanken and Wake, 1993; Roth et al., 1988, 1990, 1994, 1995, 1997; Alexander, 1996; Kaas, 2000; Yeh, 2002; Faisal et al., 2005), whereas only fragmentary data are available for insects (Beutel and Haas, 1998; Grebennikov and Beutel, 2002; Beutel et al., 2005; Polilov, 2005, 2007, 2008; Grebennikov, 2008). The main goal of this work is a detailed study of adult and larval anatomy *Mikado* sp. and it is aiming at a deeper understanding of miniaturisation limitations and mechanisms.

For many vertebrates, allometric changes in morphology due to body size decrease were shown (Schmidt-Nielsen, 1984; Roth et al., 1994, 1995). Significant allometric changes due to miniaturisation were also reported for several insect species, but only for the adult stage (Rensch, 1948; Polilov, 2007, 2008). Changes in relative size of body structures and organs in early developmental stages were never studied. The detailed examination of postembryonic developmental changes in miniaturised ptiliid beetles and a description of the first instar larval morphology may help to extend and specify hypotheses concerning the question by what and how insect miniaturisation is limited.

The present contribution continues a series of the authors' studies devoted to the structure of Ptiliidae and the phenomenon of miniaturisation in insects (Grebennikov and Beutel, 2002; Polilov, 2005, 2008).

2. Materials and methods

2.1. List of taxa examined

Mikado sp. (Coleoptera: Ptiliidae), undescribed species; adults and different larval instars; collected in Vietnam (2007) by the first author.

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This species belongs to *Mikado;* this genus indeed is monophyletic and has the following characteristic features: elongate shape of head, shape and width of mesosternal process, shape and size of pro- and metacoxa, type of spermatheca, wing setal formula. This genus consists of 4 species: *M. japonicus* Mattews, 1989, *M. agilis* (Deane, 1930), *M. parva* (Deane, 1931), *Medetera parvicornis* (Deane, 1932). Our species is similar to *M. japonicus* and *M. parva* but is separated from *M. japonicus* by the shape of the spermatheca and from *M. parva* by body size and colour. Original species descriptions of *Mikado* (especially Deane's species) are very incomplete and without the study of type material it is impossible to identify the species and without revision we cannot describe a new species.

For comparative purposes, specimens of adults and larvae of *Acrotrichis montandoni* (Allibert, 1844) and *Ptilium myrmecophilum* (Allibert, 1844), adults of *Porophilla mystacea* Polilov, 2008, *Primorskiella anodonta* Polilov, 2008, *Nanosella* sp. (Ptiliidae), *Hydraena* sp. (Hydraenidae), *Atheta* sp., *Aleochara* sp. and *Staphylinus* sp. (Staphylinidae) were studied.

2.2. Histology

Adults and larvae of *Mikado* sp. were fixed in 2% glutaraldehyde solution on 0.1 M cacodilat buffer pH 7.2. They were embedded in Araldite, cut at 1 μm with a Microm microtome (HM 360), and stained with toluidine blue. The serial sections were photographed with a PixeLINK PL-A622C digital camera on a Zeiss Axioskop.

2.3. Three-dimensional reconstruction (3D)

Cross-section series were used for 3D reconstructions. The images were aligned with AutoAligner 6.4 software. Based on the obtained image stacks, structures of adults and larvae were reconstructed with Bitplane Imaris 5.7. All discrete structures were outlined manually and automatically transferred into surface objects by Imaris software. The data files were then transferred to Alias Maya 2008, in order to use the smoothing function, the specific display, and rendering options of this software. The volume of organs and the body (without legs, wings, antenna and palps) was calculated based on 3D models.

2.4. Scanning electron microscopy (SEM)

After cleaning, the specimens were dried at the critical point (Hitachi HCP-1) and coated with gold (Hitachi IB-3). Pictures were taken with a Jeol JSM-6380.

2.5. Confocal laser scanning microscopy (CLSM)

For CLSM, specimens were mounted between two cover glasses in a drop of glycerine. Image stacks were created with a Zeiss LSM 510 using the auto fluorescence at 488 nm of argon laser.

2.6. Muscular nomenclature

The muscles are named following Kéler (1963) (head and abdomen) and Larsén (1966) (thorax). The numbers introduced for thoracic muscles by Beutel and Haas (2000) are used.

3. Results

3.1. Larva

3.1.1. Differences between instars (Figs. 1 and 2)

The larval morphometry shows 3 non-overlapping clusters of measurements that suggests that *Mikado* sp. has 3 larval stages (Fig. 2). The main differences in their morphology are described and discussed below.

Fig. 1. *Mikado* sp., SEM. (A) First instar larva; (B) last instar larva; (C) adult. Scale bar: 100 μm.

3.1.2. First instar larva (Figs. 1 and 3–6)

General appearance. Body length 390–450 μm ($M = 430$, $n = 6$). Body slender, elongate, parallel-sided and cylindrical, slightly curved ventrally. Integument smooth, unpigmented, weakly sclerotised. Legs normally developed. Urogomphi absent. Abdomen with ten segments.

Head capsule (Figs. 3A and 4). Head prognathous. Width 48–53 μm ($M = 52$, $n = 6$). All sutures and stemmata absent. Tentorium without dorsal arms. Anterior and posterior arms well developed, together forming nearly parallel rod-like structures without definable border between both elements (referred to as tentorial arms in the following). Tentorial bridge strongly curved and located in prothorax.

Labrum (Fig. 3A). Fused with head capsule, nearly vertical. Musculature. M 7 (M. labroepipharyngalis), M 9 (M. frontoepipharyngalis) absent.

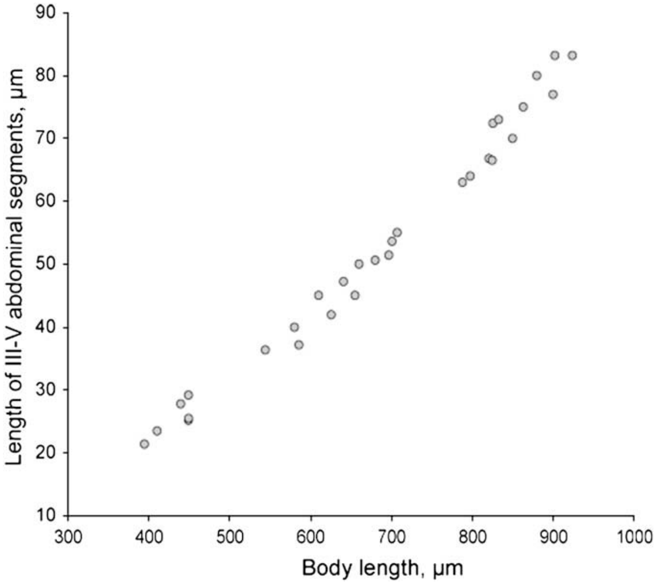

Fig. 2. Morphometry of larvae of *Mikado* sp.

Antenna (Figs. 3A and 4). Short, 3-segmented. Antennomere I very short, without sensilla. Antennomere II with large sensorium and several trichoid sensilla. Antennomere III with several apical trichoid sensilla. Musculature. M. tentorioscapalis anterior, posterior (M 1, 2), origin (O): anterior tentorial arm, insertion (I): base of antennomere I. M 4 (M. tentorioscapalis medialis) absent.

Mandible (Figs. 3A and 4). Short and compact, without retinaculum and movable appendage. Mola present. Musculature. M. 11 (M. craniomandibularis internus): the largest muscle of the head, O: dorsolateral and lateral areas of posterior head capsule, I: adductor tendon. M 12 (M. craniomandibularis externus): moderately large, O: lateral areas of posterior head capsule, I: abductor tendon.

Maxillae (Figs. 3A and 4). Composed of cardo, stipes, galea with fringed apex, and 3-segmented palp. Lacinia not recognizable as a discrete structure. Musculature. M 15 (M. craniocardinalis), O: ventrolaterally from head capsule, I: laterally on cardinal base. M 17 (M. tentoriocardinalis), O: tentorial arm, I: ventral surface of cardo. M 18 (M. tentoriostipitalis): one of the largest muscles of the head, O: tentorial arm, anterad of and very close to M 17, I: ventral surface of stipes. M 19 (M. craniolacinialis), O: laterally from posterior head capsule, I: dorsally on base of galea.

Labium (Figs. 3A and 4). Submentum fused with gula and mentum fused with submentum. Palp segmentation indistinct. Prementum markedly developed, with two strong setae. Musculature. M 28 (M. submentopraementalis) absent. M 29 (M. tentoriopraementalis inferior), O: tentorial arm, I: laterally on prementum. M 30 (M. tentoriopraementalis superior), O: tentorial arm, I: dorsolaterally on prementum.

Epipharynx (Figs. 4 and 5A,B). Musculature. M43 (M. clypeopalatalis), O: frontoclypeal region, I: dorsolaterally on epipharynx.

Hypopharynx (Figs. 4 and 5A,B). Hypopharyngeal sclerome not found. Musculature. M 41 (M. frontohypopharyngalis), O: frons, I: laterally on posterior edge of epipharynx. M 42 (M. tentoriohypopharyngalis), absent, O: tentorial bridge, I: medially on hind margin of hypopharynx. Transverse hypopharyngeal muscle absent.

Pharynx (Figs. 4 and 5A,B). The pharynx is moderately wide and approximately quadrangular in cross-section. Musculature. M 45 (M. frontobuccalis anterior), O: anterior part of frontal region, I: dorsolaterally on pharynx. M 46 (M. frontobuccalis posterior), O: frons, posterad of origin of M 41, I: dorsolaterally on pharynx. M 48 (M. tentoriobuccalis anterior), O: tentorial bridge, I: medially on hind margin of hypopharynx. M 50 (M. tentoriobuccalis posterior), O: tentorial bridge, I: ventrally on anterior pharynx. M 52 (M. tentoriopharyngalis): absent. Ring musculature present.

Cerebrum and suboesophageal ganglion (Figs. 4 and 5C,D). Elongate cerebrum completely shifted to postcephalic body, posteriorly reaching abdominal segment II. Tritocerebrum asymmetric. Suboesophageal ganglion located in prothorax and fused with prothoracic ganglion. Cerebrum and suboesophageal ganglion form secondary connection posterad of tentorial bridge.

Cephalic glands. Not developed.

Prothorax (Fig. 4). Slightly longer than meso- and metathorax, cylindrical. Cuticle unpigmented, weakly sclerotised, all sclerites indistinct. Profurca present. Legs well developed. Musculature. Dorsal muscles: M1 (M. pronoti primus), O: protergum, I: dorsally on cervical membrane. M 2 (M. pronoti secundus), O: medially on first phragma, I: dorsally on postoccipital ridge. M 3 (M. pronoti

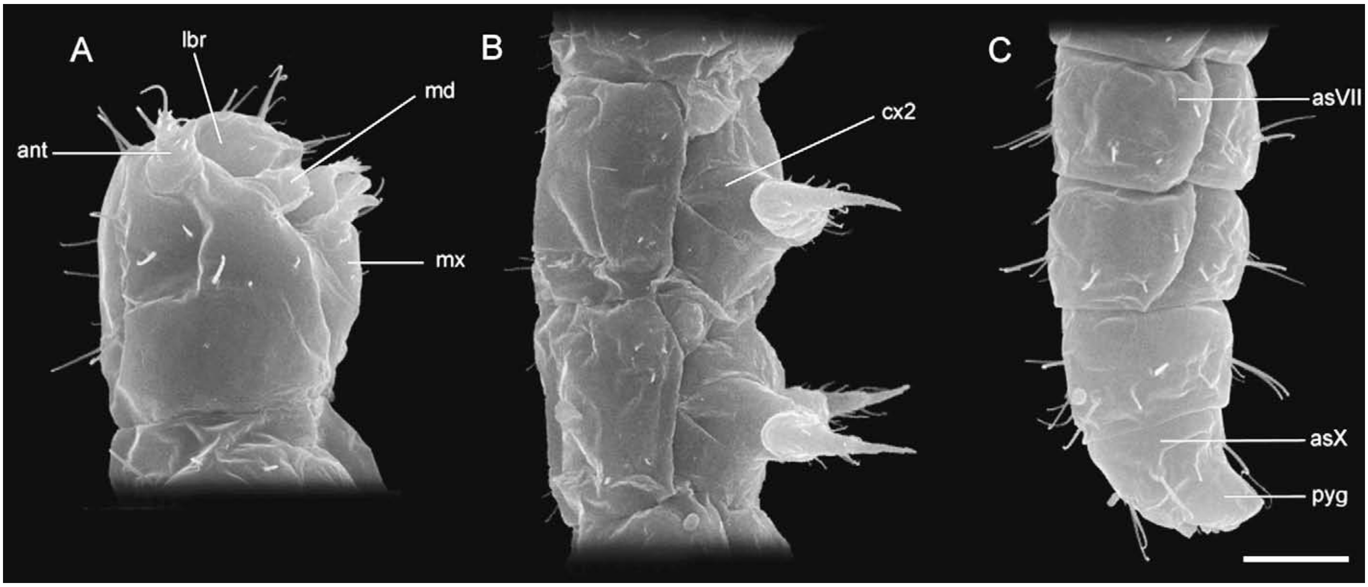

Fig. 3. First instar larva of *Mikado* sp., lateral view, SEM. (A) Head; (B) meso- and metothorax; (C) abdominal segments VI–IX. Abbreviations: ant, antenna; as, abdominal segment; cx2, mesocoxa; lbr, labrum; md, mandible; mx, maxilla; pyg, pygopod. Scale bar: 20 μm.

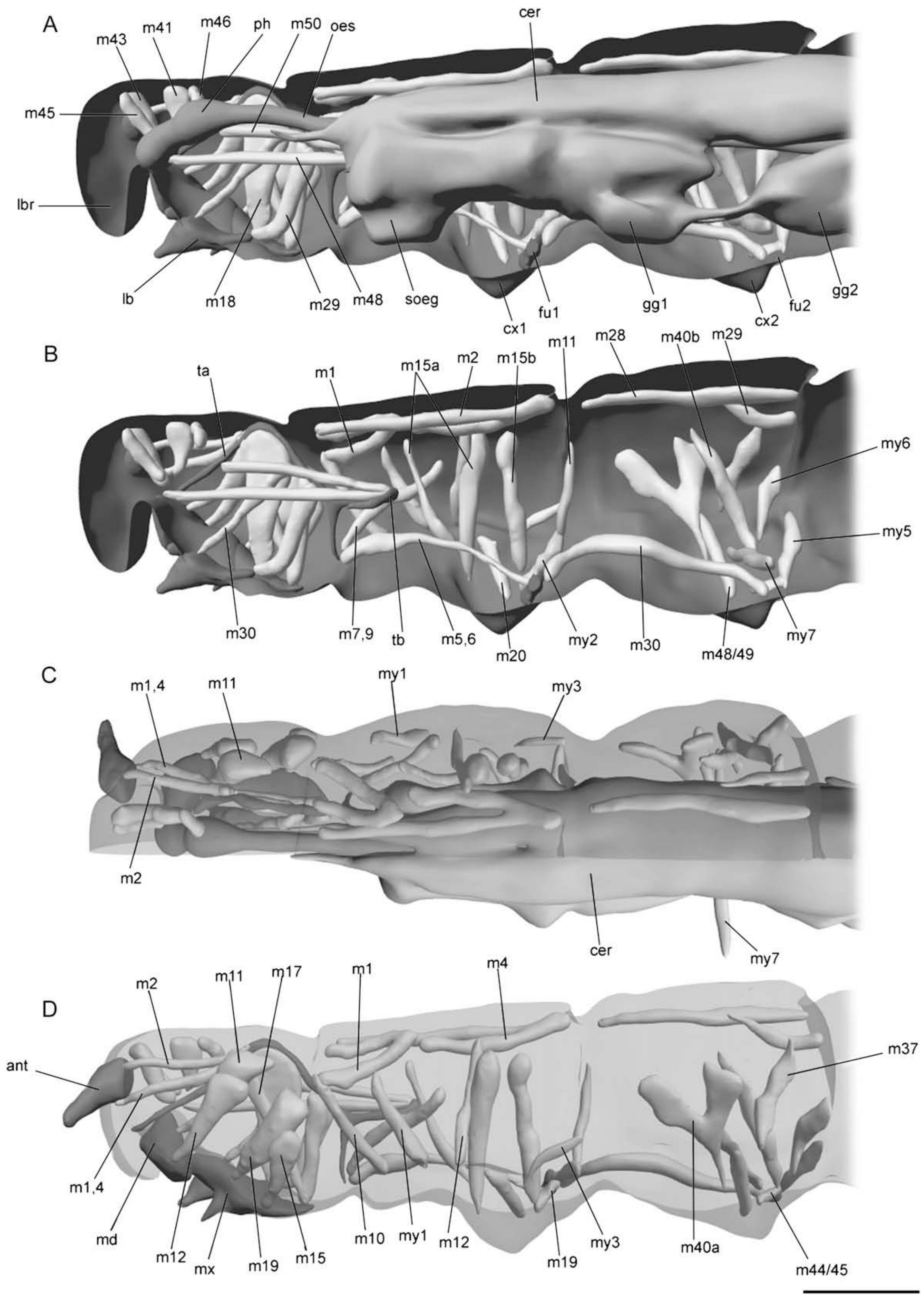

Fig. 4. First instar larva of *Mikado* sp., head, pro- and mesothorax, 3D. (A, B, D) Lateral view; (C) dorsal view. Abbreviations: ant, antenna; cer, cerebrum; cx1,2, pro- and mesocoxa; fu1,2, pro- and mesofurca; gg1,2, pro- and mesothoracic ganglia; lbr, labrum; m28, 29, M. mesonoti primus, secundus; m30, M. mesosterni primus; m40, M. notocoxalis; m44/45, M. furcacoxalis; m48/49, M. episterno- or epimero-trochanteralis; md, mandible; mx, maxilla; oes, oesophagus; ph, pharynx; soeg, suboesophageal ganglion; ta, tentorial arms; tb, tentorial bridge. For musculature of head and prothorax, see text. Scale bar: 20 μm.

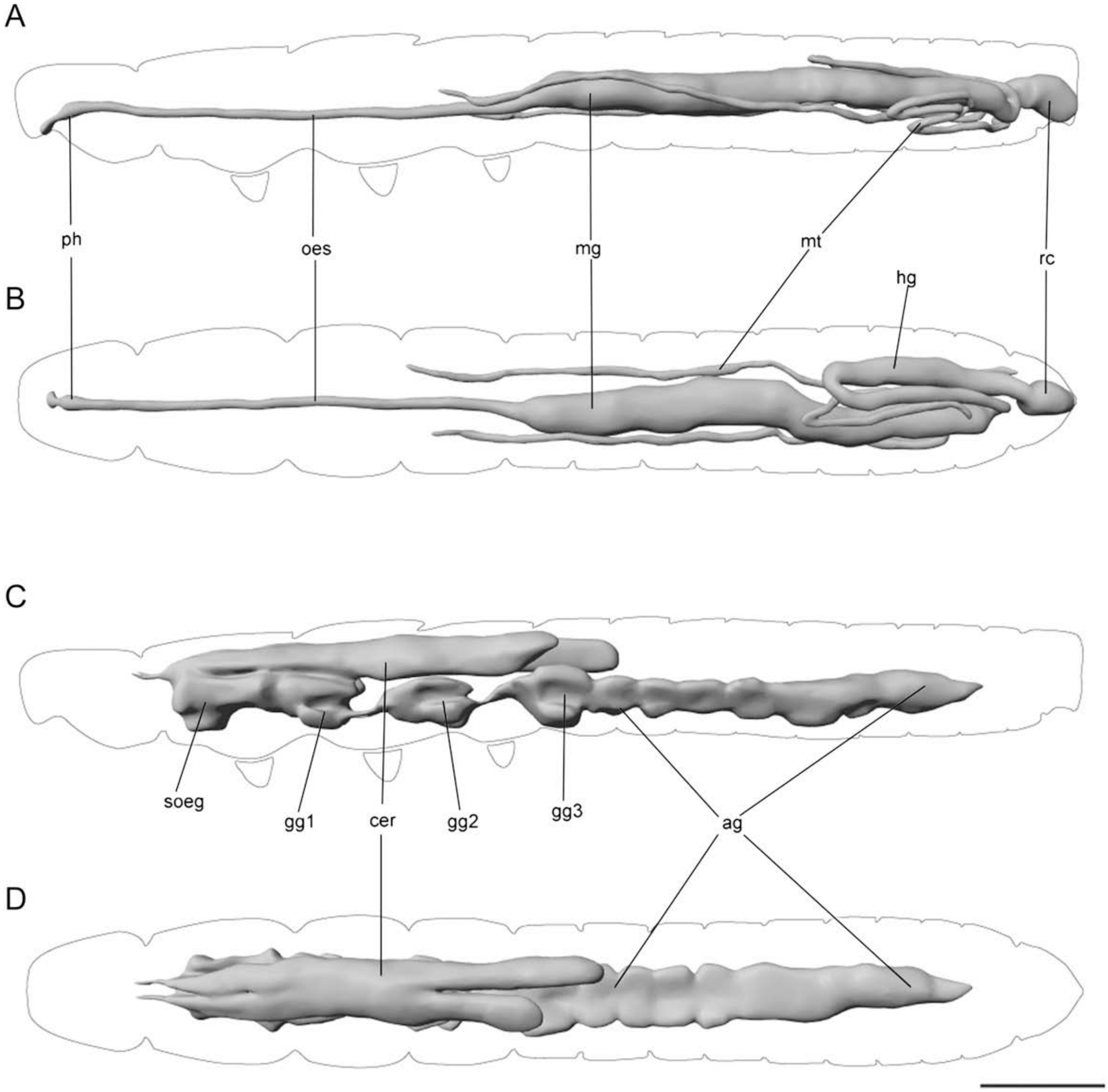

Fig. 5. Digestive (A, B) and nervous (C, D) systems of first instar larva of *Mikado* sp. (A, C) Lateral view; (B, D) dorsal view. Abbreviations: ag, abdominal ganglia; cer, cerebrum; gg1,2,3 pro-, meso- and metathoracic ganglia; hg, hind-gut; mg, mid-gut; mt, malpighian tubules; oes, oesophagus; ph, pharynx; rc, rectum; soeg, suboesophageal ganglion. Scale bar: 50 μm.

tertius) absent or fused with M 2. M 4 (M. pronoti quartus), O: first phragma, together with M2; I: protergum. Ventral muscles: M 5 (M. prosterni primus), O: profurca, I: ventrolaterally on postoccipital ridge. M 6 (M. prosterni secundus), absent or fused with M 5. Dorsoventral muscles: M 7 (M. dorsoventralis primus, O: pronotum, I: ventrolaterally on postoccipital ridge. M 8 (M. dorsoventralis secundus; MI7b), absent. M 9 (M. dorsoventralis tertius), absent or fused with M 7. M 10 (M. dorsoventralis quartus), O: prosternum, I: laterally on postoccipital ridge. M 11 (M. dorsoventralis quintus), O: first phragma, I: posterior part of propleura. Lateral muscles: M 12 (M. noto-pleuralis), O: laterally from protergum, I: propleura. Legs muscles. M 14 (M. noto-trochantinalis): not identified, probably absent. M 15a (M. noto-coxalis anterior), O: protergum, laterad of attachment area of M 4, I: anterior coxal margin. M 15b (M. noto-coxalis posterior), O: posterior protergum, I: posterior coxal margin. M 16, 17 (Mm. episterno/epimerocoxalis): absent. M 18 (M. sterno coxalis): not identified, probably absent. M 19 (M. furca coxalis): thin muscle, O: profurca, I: laterally on coxal base. M 20 (M. pleuratrochanteralis), O: propleura, I: trochanteral tendon. Three muscles with unclear homology: MY1 (Speyer, 1922: 10c?; Grebennikov and Beutel, 2002: y), O: anterior protergum, I: anterior sternum. MY2 (Speyer, 1922: 4f?), O: profurca, I: laterally between pro- and mesothorax. MY3 (Speyer, 1922: 10g?), O: not clearly identified, probably posterior protergum, I: not clearly identified, probably posterior sternal or pleural region. Intrinsic leg muscles not examined. Prothoracic ganglion see suboesophageal ganglion.

Mesothorax (Figs. 3B and 4). Slightly shorter than prothorax. Structure, legs and musculature similar to mesothorax except for cervical muscles and one transverse muscle with unclear homology between coxae (MY7). Mesothoaracal ganglion located in posterior part of mesothorax.

Metathorox (Figs. 3B and 4). Overall structure, legs and musculature similar to mesothorax.

Abdominal segments I–VIII (Figs. 3C and 6A,C,E). Segments similar in shape, cylindrical, shorter than thoracic segments. Cuticle unpigmented, weakly sclerotised, sclerites indistinct. Musculature: dorsal longitudinal muscles: dim (Mm. dorsales interni mediales), O: anterior phragma, I: posterior phragma. dil (Mm. dorsales interni laterales), O: anterior phragma, I: posterior phragma. del (Mm. dorsales interni lateralis) O: tergum, I: posterior phragma. Ventral longitudinal muscles: vi (Mm. ventrales interni), O: anterior

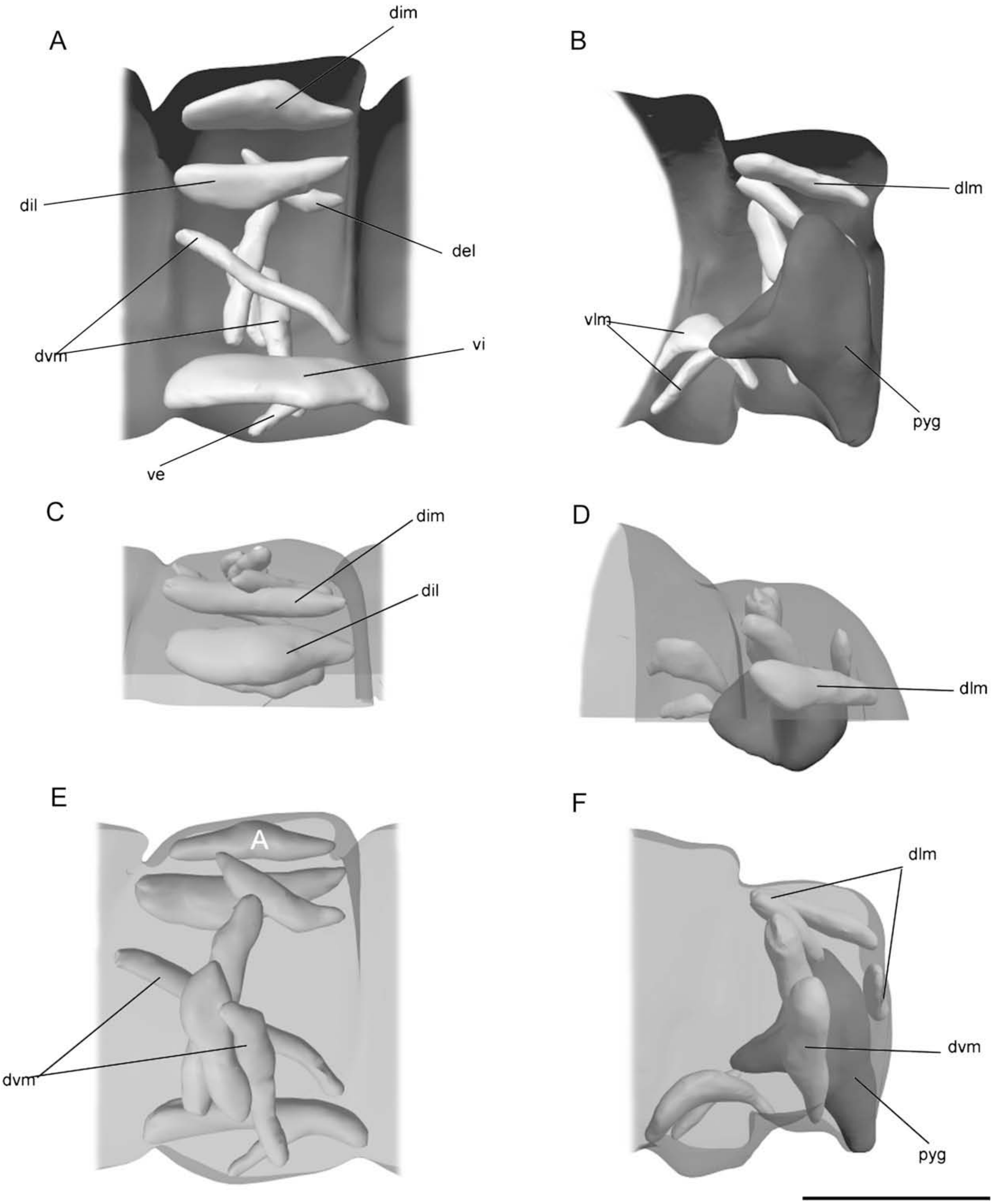

Fig. 6. First instar larva of *Mikado* sp., abdomen, 3D. (A, C, E) Abdominal segment II; A.-M. Dorsales interni medialis; (B, D, F) segments IX–X. Abbreviations: as, abdominal segment; pyg, pygopod. For musculature, see text. Scale bar: 20 μm.

margin of segment, I: posterior margin of segment. Ve (Mm. ventrales externi), O: sternum, I: posterior margin of segment. Dorsoventral muscles (dvm): three urotergosternal muscles. The precise homology is unclear. Abdominal ganglia: VI–VIII form complex.

Abdominal segments IX–X (Figs. 3C and 6B,D,F). Segments IX well developed, slightly narrower than VIII. Urogomphi absent. Segment X large, slightly conical. Segment X with protruding cuticular structure with unclear homology (referred to as pygopod in the following; Dybas, 1976: anal vesicle; Grebennikov and Beutel, 2002: pygopod). Musculature. Segment IX: dorsal longitudinal muscles (dlm), ventral longitudinal muscle (vlm), dorsoventral muscle (dvm). Segment X: one dorsal longitudinal muscles (dlm). Muscles of urogomphi absent. Pygopod with two dorsal and one ventral longitudinal muscles with origin at anterior border of segment IX. Anal hooks absent.

Postcephalic gut (Figs. 4 and 5A,B). Oesophagus long, approximately round in cross-section, difficult to distinguish from posterior pharynx; with thin intima and ring muscles. Midgut straight and long. Hindgut long, forming a loop; round in cross-section with ring muscles. Rectum short, wider, than hindgut and wall much thicker.

Malpighian tubules (Fig. 5A,B). Four free malpighian tubules. One pair directed forward, the others forming several loops.

Circulatory system and fat body. Circulatory system strongly reduced, without heart and cephalic aorta or other parts delimited by an endothelium; space for haemolymph circulation strongly reduced. Fat body filling out large parts of body cavity, largely displacing haemolymph. Fat body consisting of variously shaped cells with large inclusions.

Tracheal system. Strongly reduced. Spiracles present only between pro- and mesotorax. Abdominal spiracles and abdominal tracheae absent.

3.1.3. *Last instar larva (Figs. 1 and 7–11)*

General appearance. Body length 790–920 μm ($M = 840$, $n = 12$). Similar to first instar, but body strongly elongate.

Head capsule (Figs. 7B,D, 8B–E and 9). Width 55–61 μm ($M = 56$, $n = 12$). All structures of head capsule similar to first instar larva.

Labrum (Fig. 7B,D). Divided from head capsule by thin fold. Musculature similar to first instar.

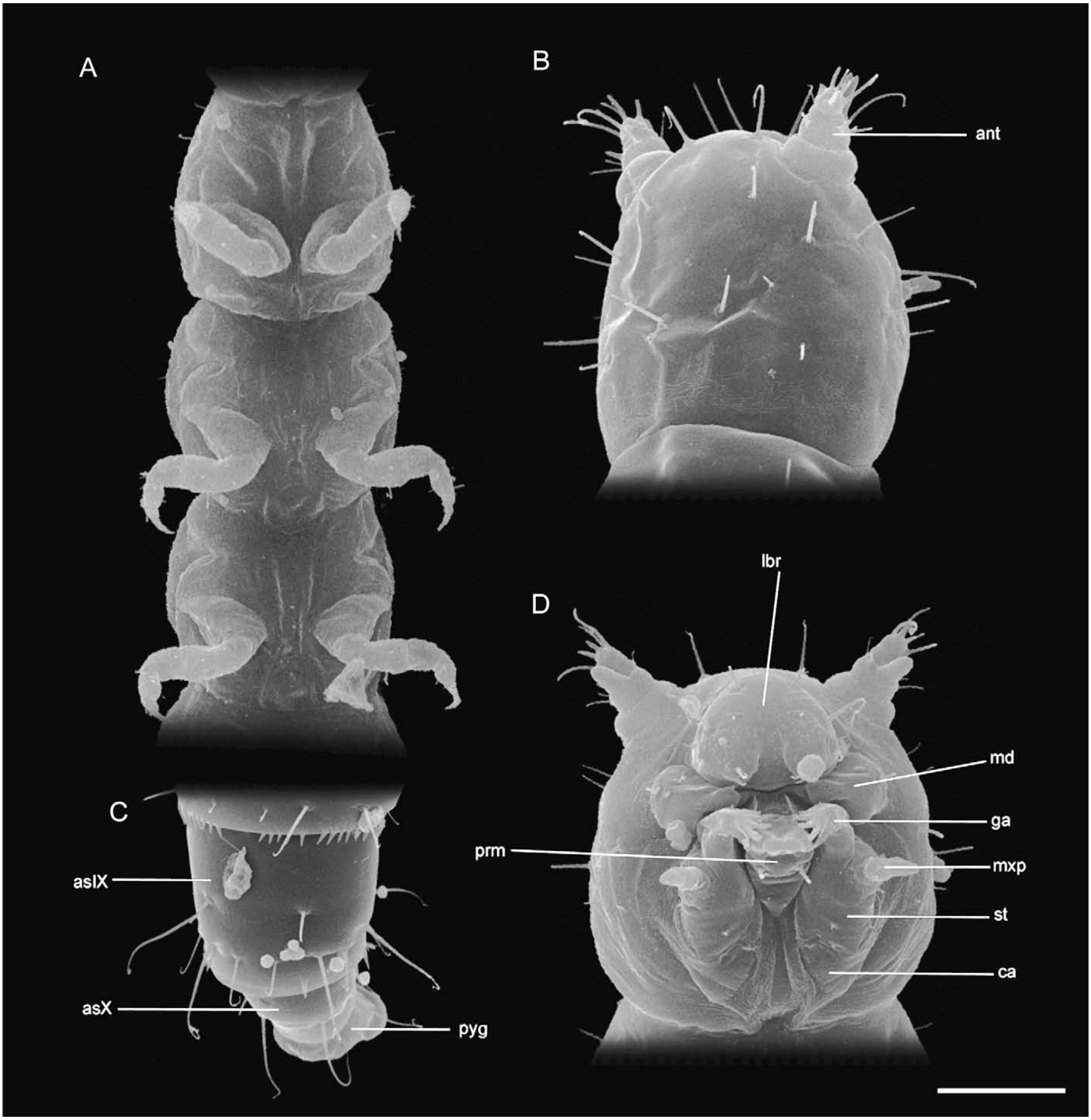

Fig. 7. Last instar larva of *Mikado* sp., lateral view, SEM. (A) Head; (B) meso- and metothorax; (C) abdominal segments VI–IX. Abbreviations: ant, antenna; as, abdominal segment; ca, cardo; ga, galea lbr, labrum; md, mandible; mxp, maxillary palp; prm, prementum; pyg, pygopod; st, stypes. Scale bar: A, C, 40 μm; B, D, 20 μm.

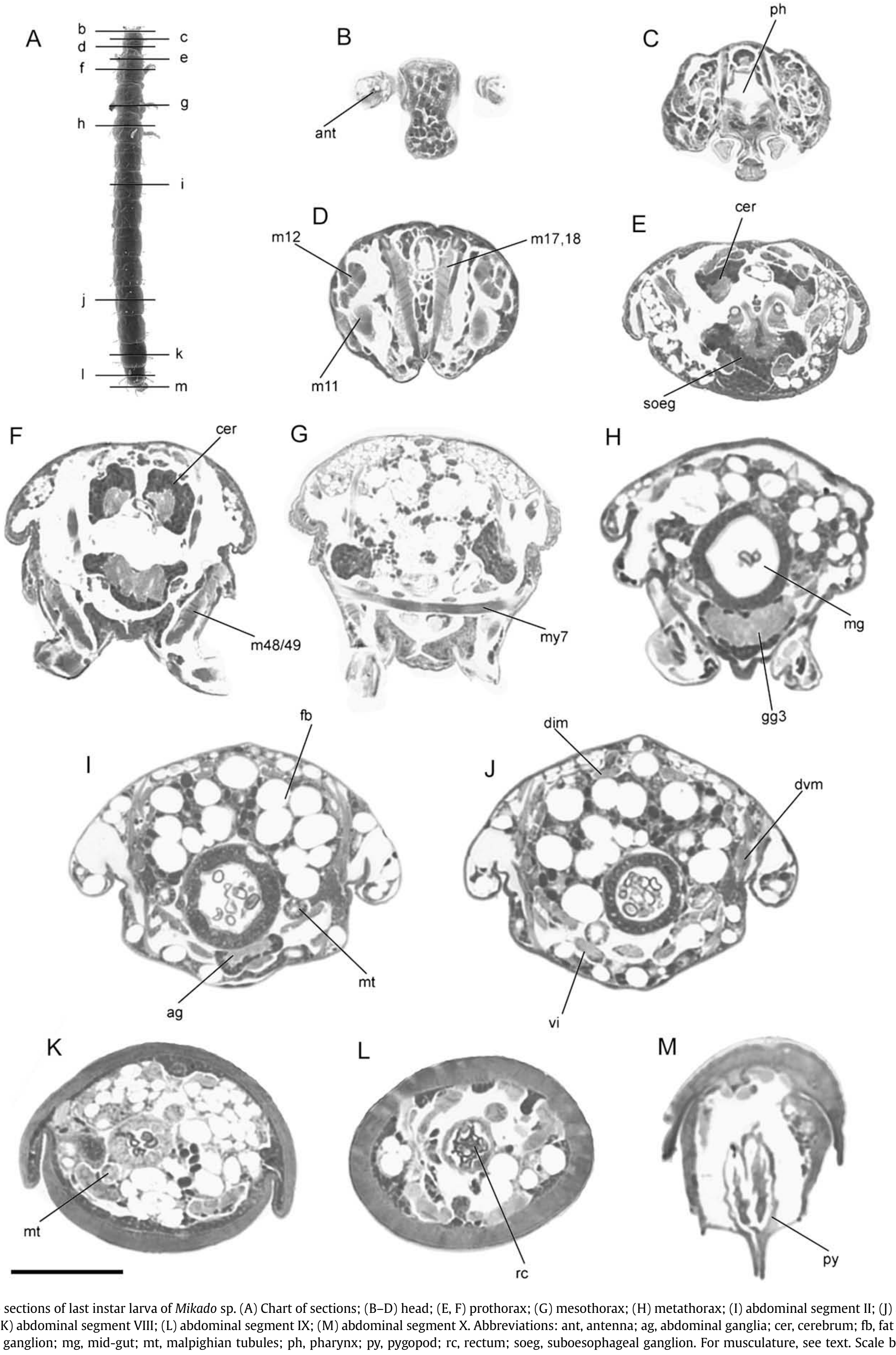

Fig. 8. Cross- sections of last instar larva of *Mikado* sp. (A) Chart of sections; (B–D) head; (E, F) prothorax; (G) mesothorax; (H) metathorax; (I) abdominal segment II; (J) abdominal segment VI; (K) abdominal segment VIII; (L) abdominal segment IX; (M) abdominal segment X. Abbreviations: ant, antenna; ag, abdominal ganglia; cer, cerebrum; fb, fat body; gg3, metathoracic ganglion; mg, mid-gut; mt, malpighian tubules; ph, pharynx; py, pygopod; rc, rectum; soeg, suboesophageal ganglion. For musculature, see text. Scale bar: 20 μm.

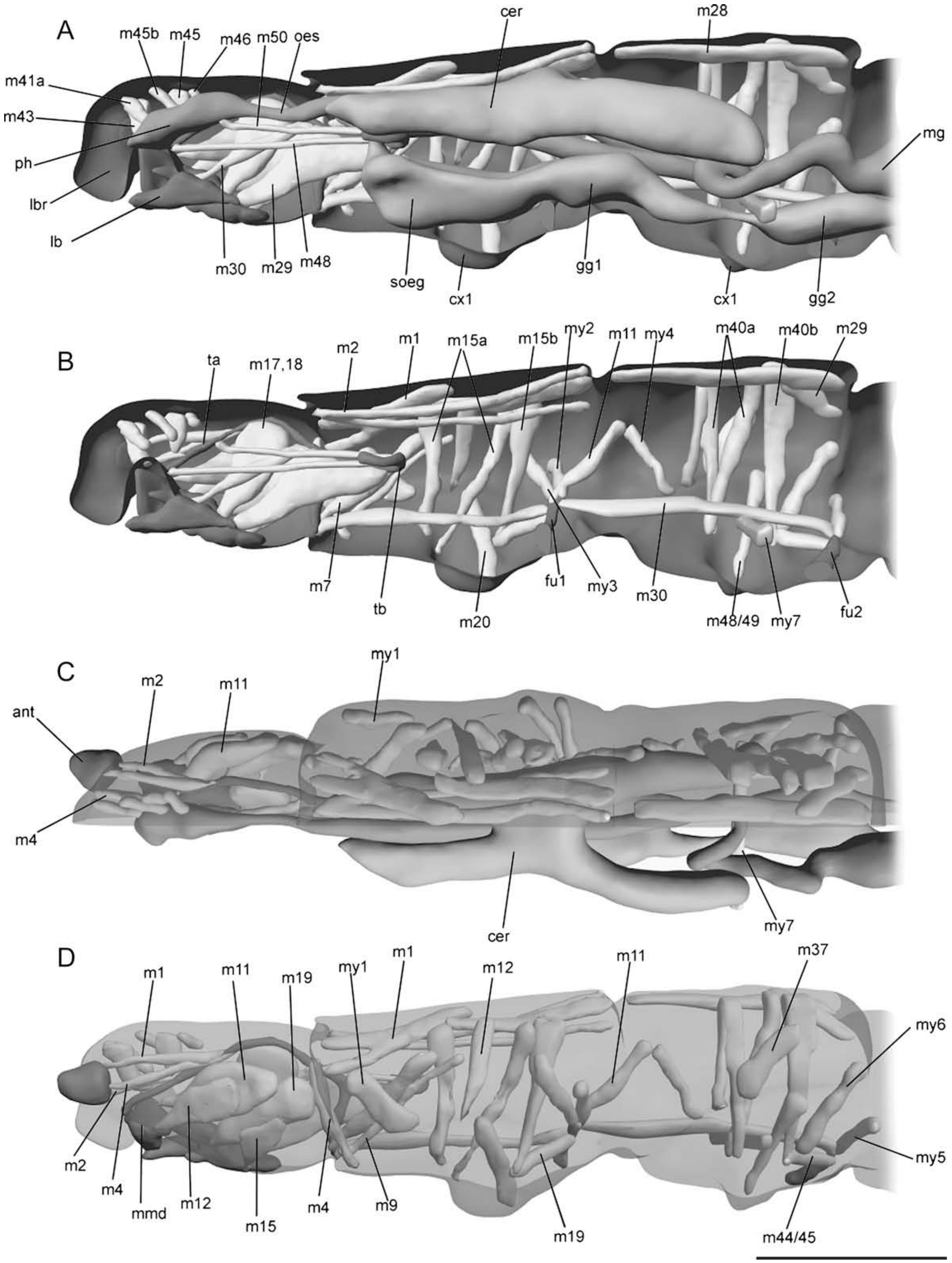

Fig. 9. Last instar larva of *Mikado* sp., head, pro- and mesothorax, 3D. (A, B, D) Lateral view; (C) dorsal view. Abbreviations: ant, antenna; cer, cerebrum; cx1,2, pro- and mesocoxa; fu1,2, pro- and mesofurca; gg1,2, pro- and mesothoracic ganglia; lbr, labrum; m28, 29, M. mesonoti primus, secundus; m30, M. mesosterni primus; m40, M. notocoxalis; m44/45, M. furcacoxalis; m48/49, M. episterno- or epimero-trochanteralis; mmd, mandible; mg, midgut; oes, oesophagus; ph, pharynx; soeg, suboesophageal ganglion; ta, tentorial arms; tb, tentorial bridge. For musculature of head and prothorax, see text. Scale bar: 50 μm.

Antenna (Figs. 7B,D and 9). Similar to first instar, but M 4 (M. tentorioscapalis medialis) well developed, O: anterior tentorial arm, I: base of antennomere I.

Mandible, *maxillae*, *labium*, *epipharynx*, *hypopharynx* and *pharynx* (Figs. 7B,D and 9). Structure and musculature similar to first instar, but differing in the following features: M17 and M18 not separated. M 41 (M. frontohypopharyngalis): composed of two subcomponents.

Cerebrum and suboesophageal ganglion (Figs. 8, 9 and 10C,D). Elongate cerebrum completely shifted to pro- and mesothorax.

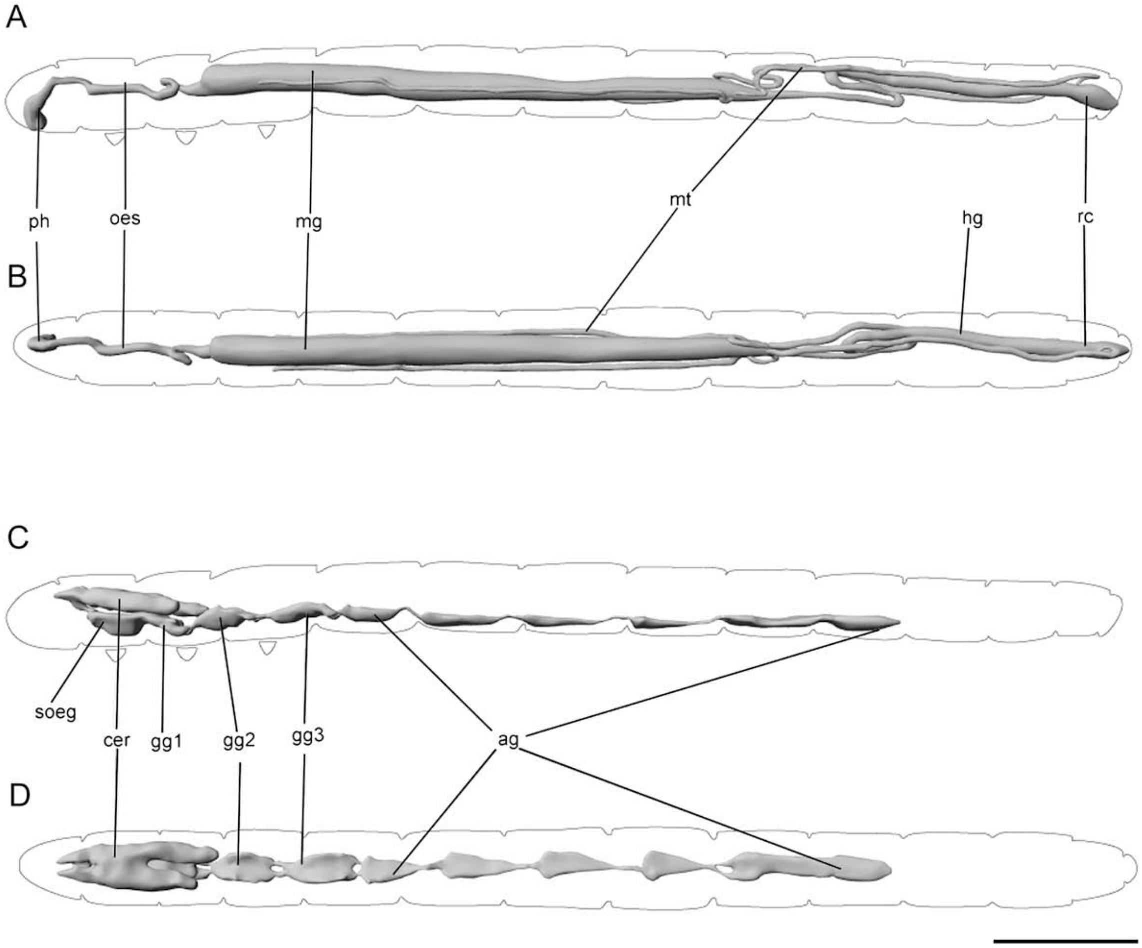

Fig. 10. Digestive (A, B) and nervous (C, D) systems of last instar larva of *Mikado* sp. (A, C) Lateral view; (B, D) dorsal view. Abbreviations: ag, abdominal ganglia; cer, cerebrum;; gg1,2,3 pro-, meso- and metathoracic ganglia; hg, hind-gut; mg, mid-gut; mt, malpighian tubules; oes, oesophagus; ph, pharynx; rc, rectum; soeg, suboesophageal ganglion. Scale bar: 100 µm.

Tritocerebrum symmetrical. Suboesophageal ganglion located in prothorax and fused with prothoracic ganglion. Cerebrum and suboesophageal ganglion without secondary connection posterad of tentorial bridge.

Cephalic glands. Not developed.

Prothorax (Figs. 7A, 8F and 9). Slightly longer than meso- and metathorax, cylindrical. Cuticle unpigmented, weakly sclerotised, all sclerites indistinct. Profurca present. Legs well developed. Musculature. Similar to first instar, but differing in the following features: M 3 (M. pronoti tertius), O: medially on first phragma, I: dorsally on postoccipital ridge. M 9 (M. dorsoventralis tertius), O: pronotum, I: ventrolaterally on postoccipital ridge.

Mesothorax and metathorox (Figs. 7A, 8G,H and 9) Overall structure, legs and musculature similar to first instar.

Abdominal segments I–VIII (Figs. 7C and 11A,C, E). Segments similar in shape, cylindrical, longer than wide. Structure and musculature similar to first instar, except cuticule of segments VIII–X much thicker than that of segments I–VII. Abdominal ganglia: V–VIII form complex in segments V and VI.

Abdominal segments IX–X. Structure and musculature similar to first instar.

Postcephalic gut (Fig. 10A,B). Similar to first instar but oesophagus forming loop in mesothorax.

Malpighian tubules (Fig. 10A,B). Similar to first instar.

Circulatory system and fat body. Similar to first instar.

Tracheal system. Similar to first instar.

3.2. *Adult (Figs. 1 and 12–19)*

General appearance. Body oval, narrowed posteriorly, convex dorsally; coloration yellowish to brown. Body length 390–455 µm ($M = 430$, $n = 10$). Pronotum wider than long, widest at base. Elytra narrowed posteriorly. Legs normally developed.

Head capsule (Figs. 12A, 13B–E and 16). Head prognathous or slightly declined, but appearing hypognathous in a maximally flexed position. Width 157–176 µm ($M = 168$, $n = 10$). Cuticle smooth, light brown. All sutures absent. Clypeus and gula completely fused with rest of head capsule. Compound eyes large, rounded, protruding, consisting of 25–30 ommatidia each. Ocelli absent. Tentorium distinctly simplified. Dorsal arms and lamina tentoria absent. Anterior and posterior arms well developed, elongate, thin round in cross- section, forming undivided, rod-like, nearly parallel structures. Tentorial bridge slightly curved. At rest, labrum and mentum adjacent anteriorly, enclosing preoral cavity, mandibles and maxillae (preoral pocket), thus isolating them from external environment. Maxillae protrude from preoral pocket during feeding activities (filtering food and pushing it into preoral cavity).

Labrum (Figs. 12A and 14). Rounded anterolaterally. Moveably attached to head capsule. Anterior and lateral margin without bristles and spines. Musculature. M 7 (M. labroepipharyngalis): O. dorsal wall of labrum, I: ventral wall of epipharynx, M 9 (M. frontoepipharyngalis) absent.

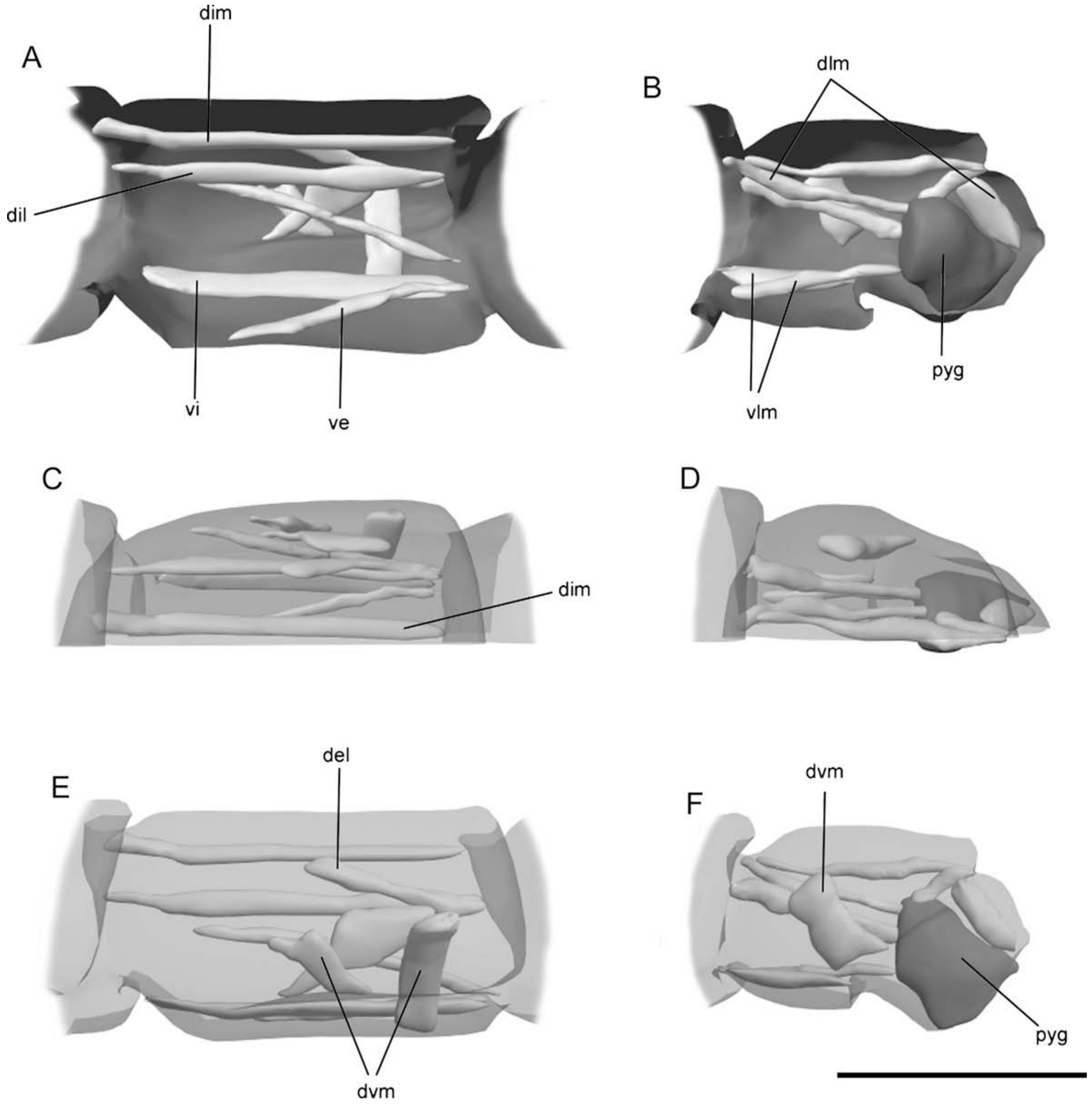

Fig. 11. Last instar larva of *Mikado* sp., abdomen, 3D. (A, C, E) II abdominal segment; (B, D, F) IX–X segments. Abbreviations: pyg, pygopod. For musculature, see text. Scale bar: 50 μm.

Antenna (Figs. 12A and 16). 11-segmented, with 2-segmented club. Musculature. M. tentorioscapalis anterior, posterior and medialis (M 1, 2, 4), O: anterior tentorial arm, I: base of antennomere I.

Mandible (Figs. 12A and 16). Distinctly retracted, asymmetric, short and compact. Prostheca well developed. Mola large, not firmly connected with rest of mandible, with several comparatively large teeth. Musculature. M. 11 (M. craniomandibularis internus): largest muscle of head, O: dorsolateral and lateral areas of posterior head capsule, I: adductor tendon. M 12 (M. craniomandibularis externus): moderately large, O: lateral areas of posterior head capsule, I: abductor tendon.

Maxillae (Figs. 12A and 16). Internally connected with labium. Composed of cardo, stipes, galea, lacinia and 4-segmented palp. Galea moderately long, with several regular rows of curved hairs. Lacinia with several teeth. Musculature. M 15 (M. craniocardinalis), O: ventrolaterally from posterior head capsule, I: ventrolaterally on cardinal base. M 17 (M. tentoriocardinalis), O: anterior tentorial arm, I: ventral surface of cardo. M 18 (M. tentoriostipitalis): one of the largest muscle of the head, O: anterior tentorial arm, very close to M 17, I: ventral surface of stipes. M 19 (M. craniolacinialis), O: posterolateral part of head capsule, I: base of lacinia.

Labium (Fig. 16). Submentum fused with gular area. Mentum large, movably connected with head capsule. Prementum strongly reduced, semimembranous. Ligula with rounded anterolateral edges, semimembranous. Palp very small, segmentation indistinct. Musculature. M 28 (M. submentopraementalis), absent. M 29 (M. tentoriopraementalis inferior), O: ventral part of posterior head capsule I: not clearly identified, probably on posterior margin of prementum. M 30 (M. tentoriopraementalis superior), O: ventral part of posterior head capsule, posterad of M 29, I: not clearly identified, probably on posterior margin of prementum.

Epipharynx (Figs. 15A,B and 16). Semimembranous, with well developed median longitudinal epipharyngeal bulge. Musculature. M 43 (M. clypeopalatalis), O: frontoclypeal region, I: dorsolaterally on epipharynx.

Hypopharynx (Figs. 15A,B and 16). Not clearly separated from mentum. Musculature. M 41 (M. frontohypopharyngalis), O: frons, I: laterally on epipharynx. M 42 (M. tentoriohypopharyngalis), absent. Transverse hypopharyngeal muscle absent.

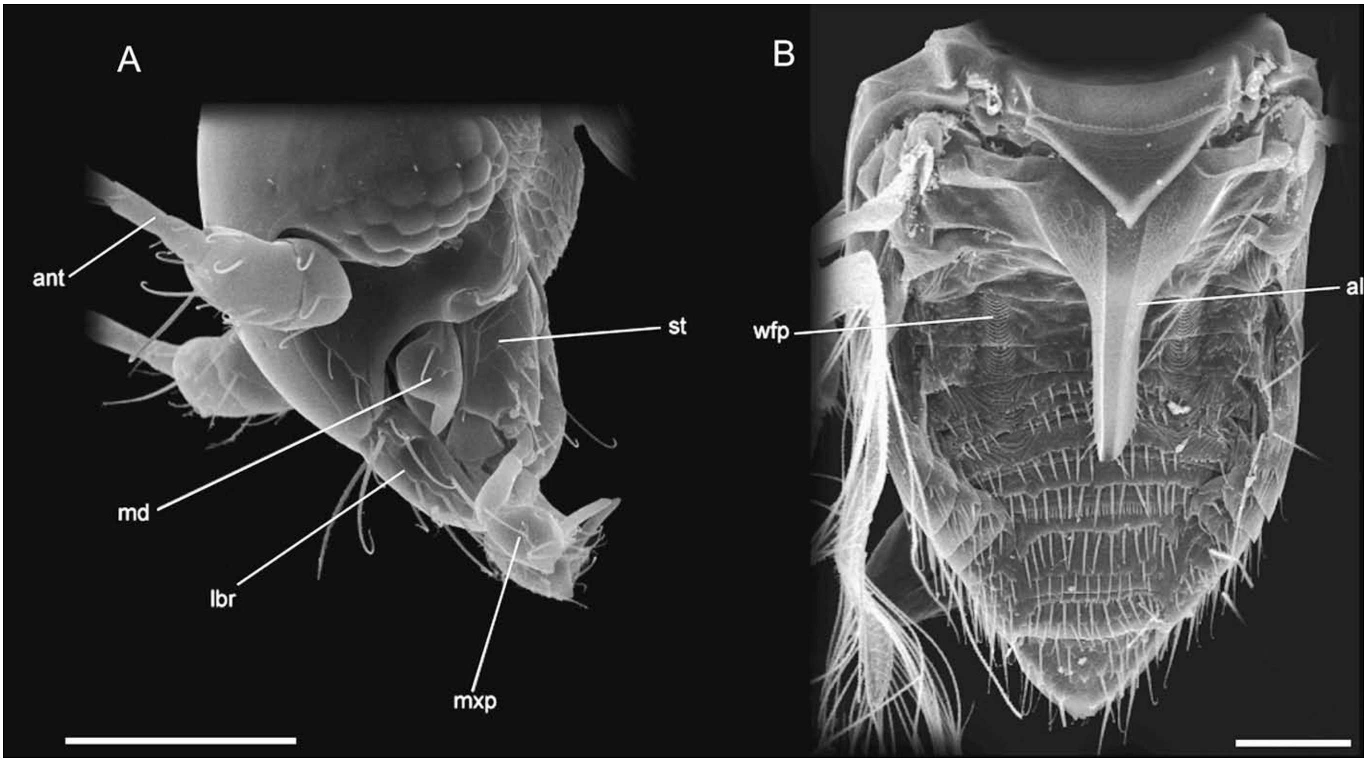

Fig. 12. Adult *Mikado* sp., SEM. (A) Head, lateral view; (B) thorax and abdomen, dorsal view. Abbreviations: al, alacristae; lbr, labrum; md, mandible; mxp, maxillary palp; st, stypes; wfp, wing folding patches. Scale bar: 50 μm.

Pharynx (Figs. 15A,B and 16). Moderately wide and approximately quadrangular in cross-section. Musculature. M 45 (M. frontobuccalis anterior), O: anterior part of frontal region, I: dorsolaterally on pharynx. M 46 (M. frontobuccalis posterior), O: frons, posterior to origin of M 41, I: dorsolaterally on pharynx. M 48 (M. tentoriobuccalis anterior), O: tentorial bridge, I: ventral side of posterior part of hypopharynx. M 52 (M. tentoriopharyngalis), O: tentorial bridge, I: ventral pharyngeal wall. M 67 (M. transversalis buccae), absent. M 68 (M. anularis stomodaei), present. M69 (M. longitudinalis stomodaei), absent.

Cerebrum and suboesophageal ganglion (Figs. 14 and 15C,D). Cerebrum located in posterior part of head and anterior part of prothorax. Suboesophageal ganglion located in posterior part of head and nearly fused with prothoracic ganglion. Cerebrum and suboesophageal ganglion form secondary connection posterad of tentorial bridge. Large central body, corpora pedunculata and protocerebral bridge distinctly recognizable. Optic lobes well developed. Neuron bodies 0.8–1.4 μm in diameter.

Cephalic glands. Not developed.

Prothorax (Figs. 13F, 14 and 17). Cervical membrane connecting head and prothorax without sclerotised areas, cervical sclerites absent. Pronotum large, light brown. Hypomeron well developed. Pleuron fused with trochantinus. Trochantinopleuron not visible externally; upper part forming apodemal plate. Prosternum very narrow. Notosternal suture absent. Procoxal cavities open posteriorly. Procoxae very large, cone-shaped. Trochanter obliquely attached to femur. Femur wide, slightly flattened. Tibia slender, with several spikes near apex. Tarsi with 2–3 indistinctly separated segments. Two simple claws present. Musculature. Dorsal muscles: M. 1 (M. pronoti primus), O: posteromedian pronotum; I: paramedially on postocciput, together with M. 2. M. 2 (M. pronoti secundus), O: medially on prophragma; I: postocciput, together with M. 1. M. 6 (M. pronoti quartus), strong; O: prophragma; I: medially on pronotum. Ventral muscles: M. 9 (M. prosterni primus), O: profurca; I: gular ridge. M. 10 (M. prosterni secundus), O: profurca; I: cervical region. Dorsoventral muscles: M. 12 (M. dorsoventralis primus), O: notum, laterad of M. 1; I: cervical region. M. 15 (M. dorsoventralis quartus), O: lateral area of prosternum; I: laterally on postocciput. M. 16 (M. dorsoventralis quintus), O: profurca; I: intersegmental membrane, anterad of mesanepisternum. Lateral muscles: M. 17 (M. notopleuralis), O: lateral part of notum; I: dorsal side of apodemal plate of trochantinopleura. M. 18 (M. pronoto-mesepisternalis), O: posterior pronotum, laterad of M. 1; I: intersegmental membrane, anterad of mesanepisternum. Leg muscles: M. 22 (M. noto-trochantinalis), strong; O: dorsolaterally on notum; I: membrane posterad of trochantin by means of a tendon. M. 23 (M. noto-coxalis), O: dorsolaterally on notum; I: anteriorly on lateral part of coxal edge. M. 24 (M. episterno-coxalis), O: apodemal plate of trochantinopleura; I: anterior rim of coxa, posterad of trochantin. M. 25 (M. epimero-coxalis), O: apodemal plate of trochantinopleura; I: posterior rim of coxa. M. 30 (Mm. furcacoxales), O: profurca; I: posteromesally on coxa. M. 31 (M. pleura-trochanteralis), O: apodemal plate of trochantinopleura; I: trochanter, by means of a tendon. Intrinsic leg muscles not examined. Protothoracic ganglion nearly fused with suboesophageal ganglion.

Mesothorax (Figs. 12B, 13G,H, 14 and 18). Dorsal side weakly sclerotised, except for mesoscutellar shield. Ventral side strongly sclerotised, ventrite, anepisternum and epimeron fused. Mesosternal process very wide. Mesofurca well developed, with indistinct anterior and lateral arms. Elytra completely concealing abdomen, with narrow epipleuron; inner surface without stridulatory file. Musculature. Dorsal muscles: M. 39 (M. mesonoti primus), O: prophragma, I: mesophragma. M. 40 (M. mesonoti secundus), O: median ridge of prophragma, I: lateral edge of mesophragma. Ventral muscles: M. 42 (M. mesosterni primus), O: profurca; I: mesofurca. Dorsoventral muscles: M. 45 (M. dorsoventralis), absent. Lateral muscles: M. 47 (M. noto-pleuralis),

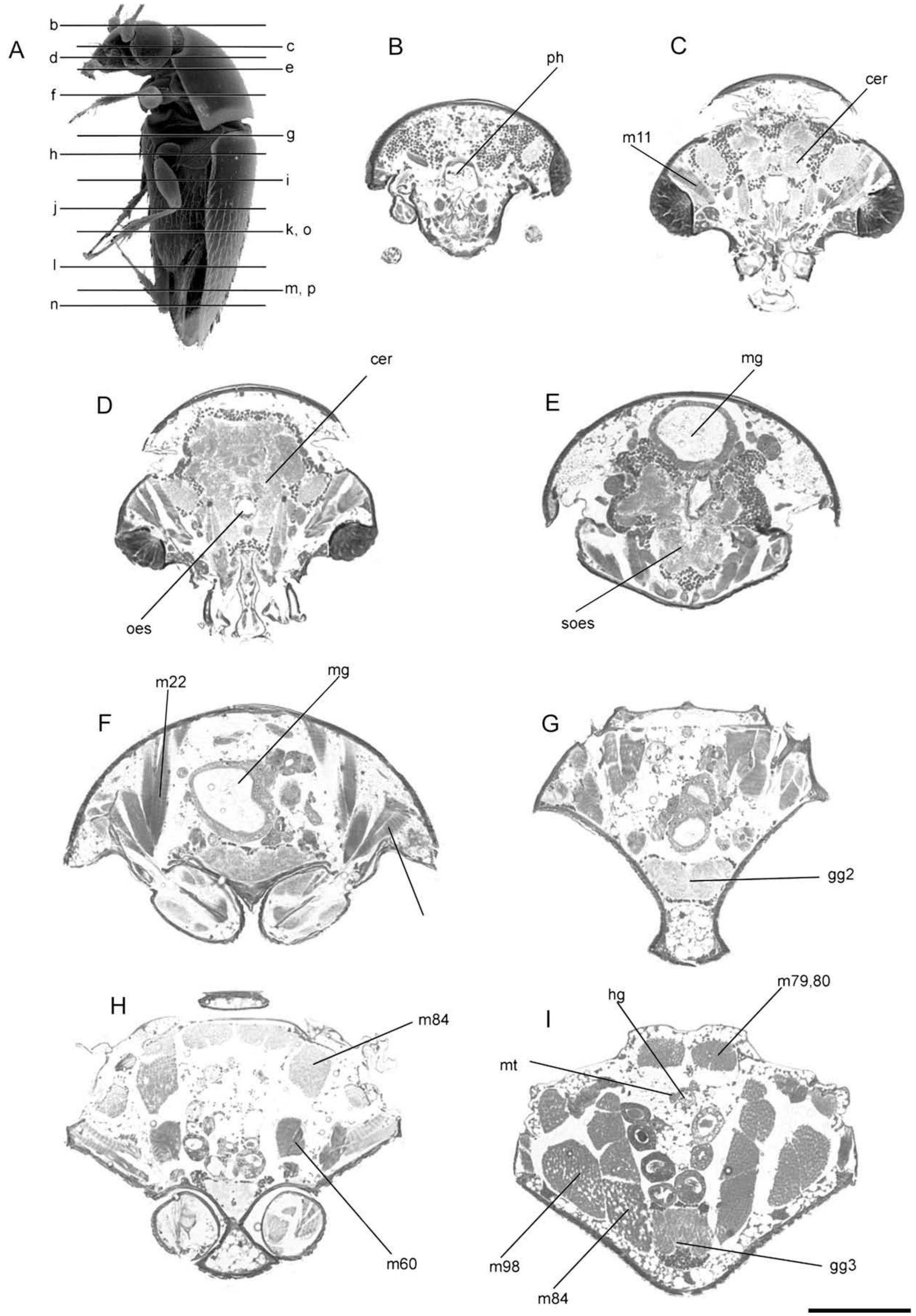

Fig. 13. Cross -sections of adult *Mikado* sp. (A) Chart of sections (B–N) male; (O, P) female; (B–D) head; (E, F) prothorax; (G, H) mesothorax; (I–K, O) metathorax; (L–N, P) abdomen. Abbreviations: al, alacristae; cer, cerebrum; cx3, metacoxa; egc, egg chamber; gg2, gg3, meso-; metathoracic ganglion; gl, gland; hg, hind-gut; mg, mid-gut; mt, malpighian tubules; pe, penis; oes, oesophagus; ov, ovariole; ph, pharynx; rc, rectum; St, spermatheca; soes, suboesophageal ganglion; te, testis. For musculature, see text. Scale bar: 100 μm.

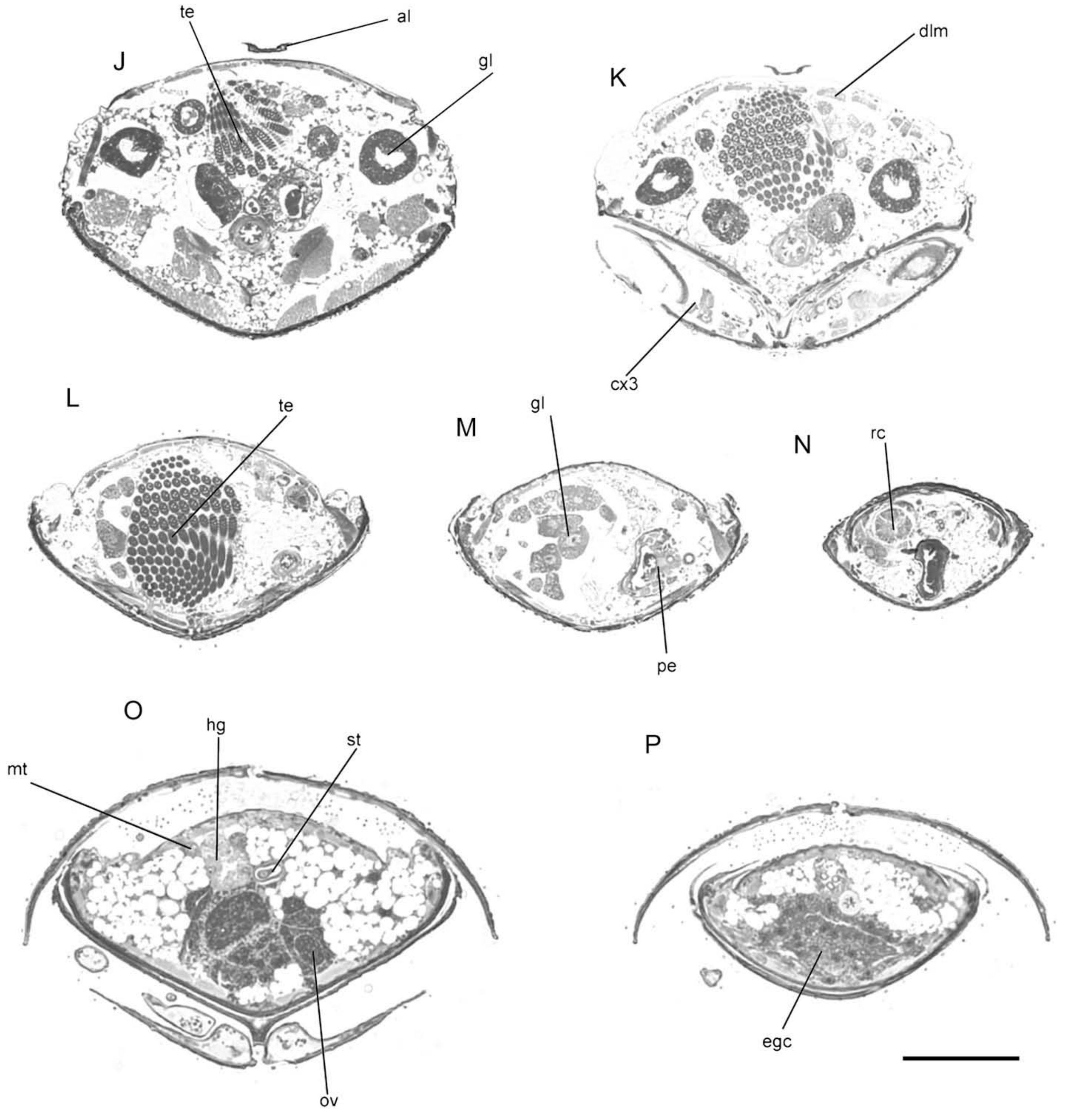

Fig. 13. (*continued*).

O: pleural arm; I: anterolaterally on mesonotum. M. 52 (M. epimerosubalaris) absent. M. 53 (M. pleura-alaris a), combined with following muscle, O: anepisternal region: I: third axillary by means of thin tendons. M. 54 (M. pleura-alaris b), see above (M. 53). M. 55 (M. furcapleuralis) absent. Leg muscles: M. 60 (Mm. notocoxales), strongly developed; O: prophragma; I: posterior rim of coxa. M. 61 (M. episterno-coxalis), O: anepisternal region; I: anterior rim of coxa with a tendon. M. 62 (M. coxabasalaris), O: basalare; I: together with M. 61. M. 64 (M. coxa-subalaris) absent. M. 65 (M. furca-coxalis anterior), O: mesofurca; I: anteriorly on mesocoxa. M. 67 (M. furca-coxalis posterior), O: mesofurca; I: anteriorly on mesocoxa. M. 68 (M. furca-trochanteralis) absent. M. 70 (M. episterno-trochanteralis), O: anepisternal region; I: trochantenal tendon. M. 71 (M. epimero-trochanteralis), O: basalare; I: together with M. 70. M. 72 (M. trochantero-basalaris), absent. Intrinsic leg muscles not examined. Ganglia of meso- and metathorax and abdomen ganglia form large, compact complex within metathorax.

Metathorax (Figs. 12B, 13I–K,O, 14 and 18). Ventrite fused with distinctly separated anepisternum and epimeron. Metanotum divided into prescutum, scutum, scutellum and postnotum; weakly sclerotised and pigmented except for scutellum. Alacristae and area

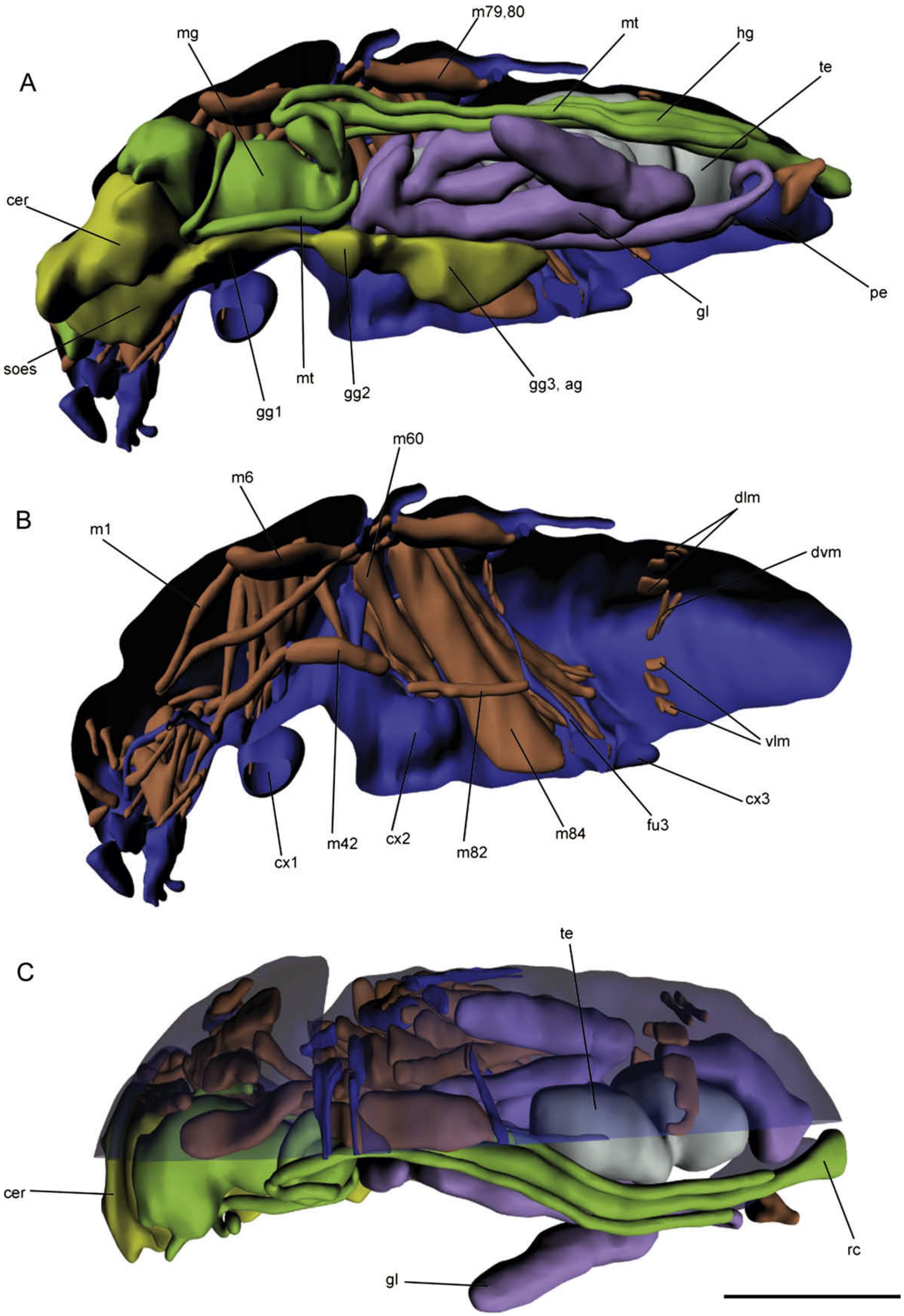

Fig. 14. Adult *Mikado* sp., male, 3D (skeleton, blue; musculature, brown; gut and malpighian tubules, green; nervous system, yellow; reproductive system, gray and violet). (A, B) Lateral view; (C) dorsal view. Abbreviations: ag, abdominal ganglia cer, cerebrum; cx1,2,3, pro-,meso- and metacoxa; fu3, metafurca; gg3, metathoracic ganglion; gl, glande; hg, hind-gut; mg, mid-gut; mt, malpighian tubules; pe, penis; rc, rectum; soes, suboesophageal ganglion; te, testis. For musculature, see text. Scale bar: 100 µm.

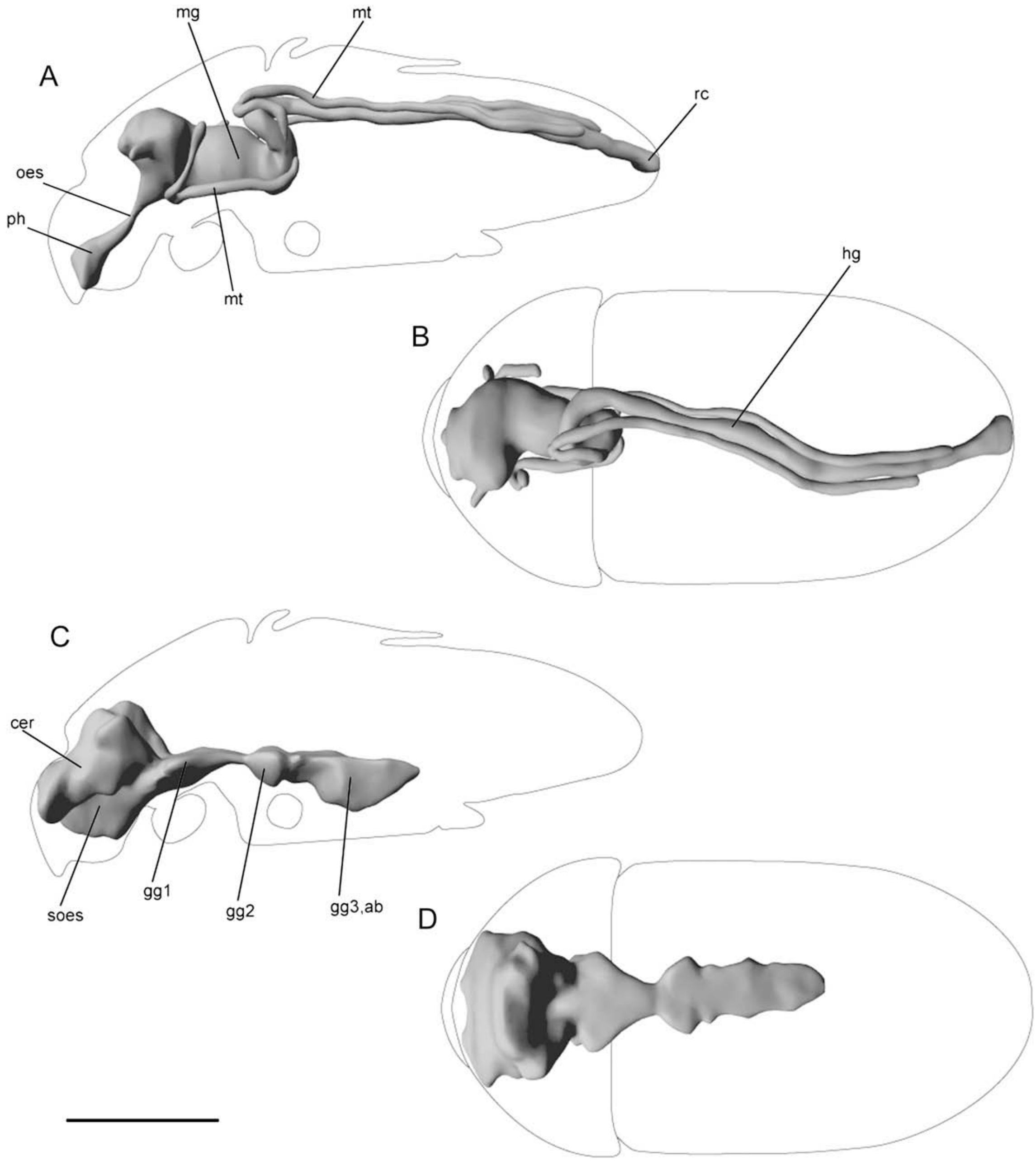

Fig. 15. Digestive (A, B) and nervous (C, D) systems of adult *Mikado* sp. (A, C) Lateral view; (B, D) dorsal view. Abbreviations: cer, cerebrum; gg1,2,3 pro-, meso- and metathoracic ganglia; hg, hind-gut; mg, mid-gut; mt, malpighian tubules; oes, oesophagus; ph, pharynx; rc, rectum; soes, suboesophageal ganglion. Scale bar: 50 μm.

enclosed by them strongly elongated, narrowed posteriorly. Mesofurca well developed, anterior and lateral arms indistinct. Metacoxae transverse, very large, adjacent medially, with large coxal plates. Wings with typical feather-like structure, with very narrow wing plate and fringe of hairs with serrated surface structure running along margin. Homology of wing venation unclear, two veins present, probably representing radius anterior and media posterior. Musculature. Dorsal muscles: M. 79 (M. metanoti primus) and M. 80 (M. metanoti secundus) not separated, O: mesophragma and scutum; I: metaphragma. Ventral muscles: M. 82 (M. metasterni primus), O: mesofurca; I: metafurca. Dorsoventral muscles: M. 84 (M. dorsoventralis primus), strongly developed, O: anterior metanotum and upper part of mesophragma; I: metaventrite. M. 85: M. dorsoventralis secundus, O: anterior apex of metafurca; I: metaphragma. M. 86: M. dorsoventralis tertius, O: apex of metafurca, together with M. 85; I: metaphragma. Lateral muscles: M. 90 (M. pleura-praealaris), O: prealar sclerite; I: pleural ridge. M. 93 (M. noto-basalaris), O: lateral margin of metanotum; I: upper side of muscle disc of basalare. M. 94 (M. epimero-subalaris) not identified, probably absent. M. 95 (M. pleura-alaris a), combined with following muscle, O: anterior part of pleural ridge, close to basalare; I: muscle disc of third axillary sclerite. M. 96 (M. pleuraalaris b), see M. 95. M. 97 (M. sternoepisternalis), O: pleural ridge; I: lateral margin of metaventrite. M. 98 (M. sterno-basalaris), large, O: metaventrite, laterad of M. 84; I: muscle disc of basalare. Leg muscles: M. 100 (M. noto-trochantinalis), O: metascutellum; I: process of trochantin. M. 101 (M. noto-coxalis anterior), O: metanotum, posterad of M. 84; I: posteriolateral metacoxal rim. M. 102 (M. noto-coxalis posterior), O: posterolateral part of metanotum; I: laterally on metacoxa by means of a tendon. M. 103 (M. episternocoxalis), O: anepisternum; I: laterally on anterior coxal margin. M. 104 (M. coxa-basalaris) absent. M. 105 (M. coxa-subalaris), large,

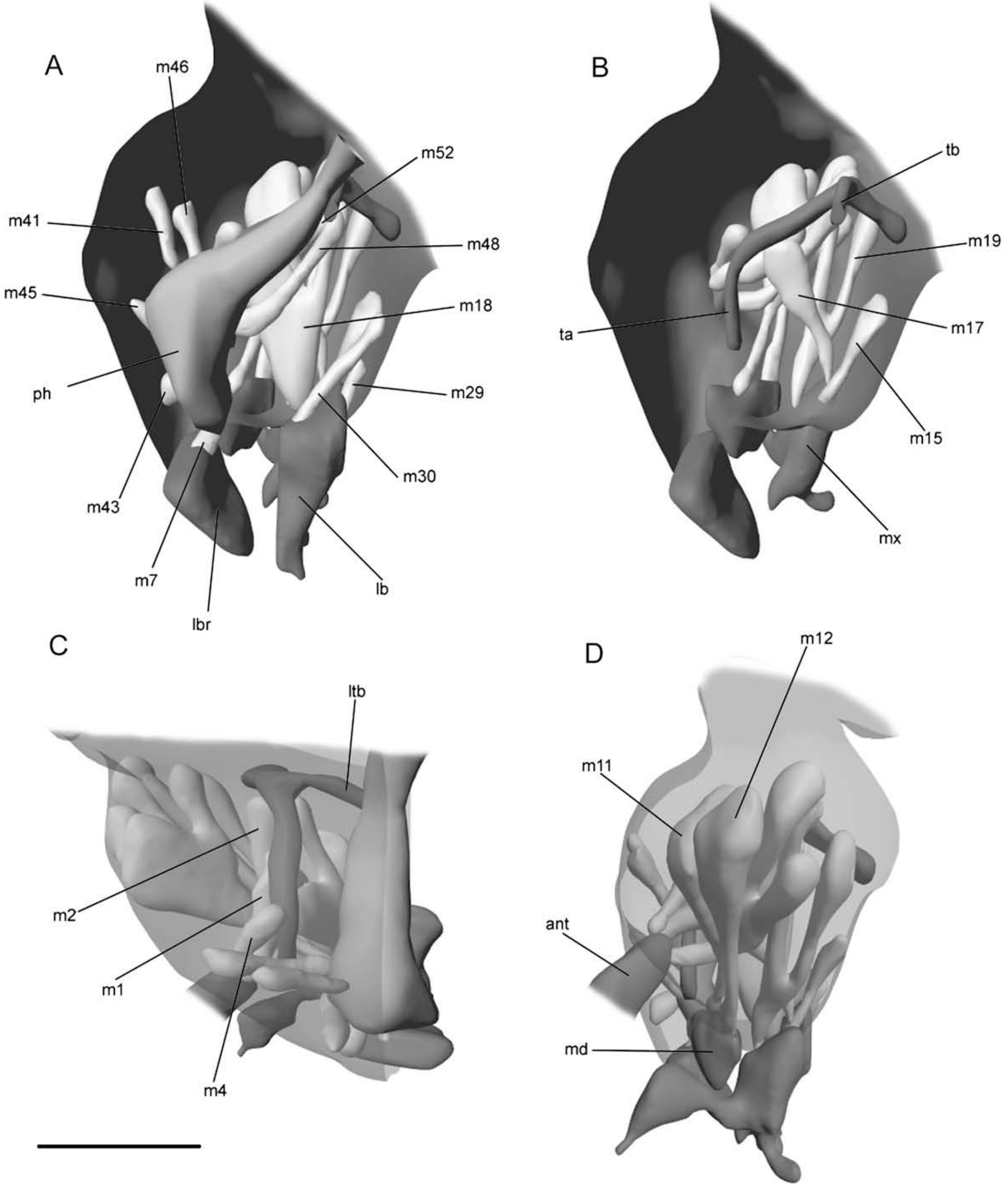

Fig. 16. Adult *Mikado* sp., head, 3D. (A, B, D) Lateral view; (C) dorsal view. Abbreviations: ant, antenna; lbr, labrum; md, mandible; mx, maxilla; ph, pharynx; ta, tentorial arms; tb, tentorial bridge. For musculature, see text. Scale bar: 100 μm.

O: metacoxa; I: subalare. M. 107 (M. furca-coxalis anterior), O: anterior side of metafurcal stalk; I: anteromesal coxal rim. M. 108 (M. furca-coxalis lateralis) absent. M. 109 (M. furcacoxalis posterior) not identified, probably absent M. 111 (M. noto-trochanteralis) absent. M. 113 (M. furca-trochanteralis), O: metafurca; I: trochanteral tendon. Intrinsic leg muscles not examined. Metathoracic ganglion see mesothoracic ganglion.

Abdomen (Figs. 12B, 13L–N,P and 14). Ten apparent tergites present, weakly sclerotised and unpigmented except for tergites VII–X. Wing folding patches present on tergites II–VI. Ventral side divided into six visible sclerotised sternites. Musculature (except genital apparatus): dorsal longitudinal muscles (Mm. dorsales), several parallel bundles, O: anterior phragma, I: posterior phragma. Ventral longitudinal muscles (Mm. ventrales), several parallel bundles, O: anterior margin of segment, I: posterior margin of segment. Dorsoventral muscles, two urotergosternal muscles. The precise homology is unclear. Abdominal ganglia: moved to metathorax and fused with meso- and metathoracic ganglion.

Postcephalic gut (Fig. 15A,B). Oesophagus narrow, approximately round in cross-section, difficult to distinguish from posterior pharynx, with thin intima and ring muscles. Midgut wide and very short; with numerous short diverticula, which are somewhat longer and directed anterad in the cardial area. Midgut epithelium formed by single layer of cells (8–12 similar cells visible in cross-sections). Hindgut long, with loop; round in cross-section, with ring muscles. Rectum short, noticeably wider than hindgut.

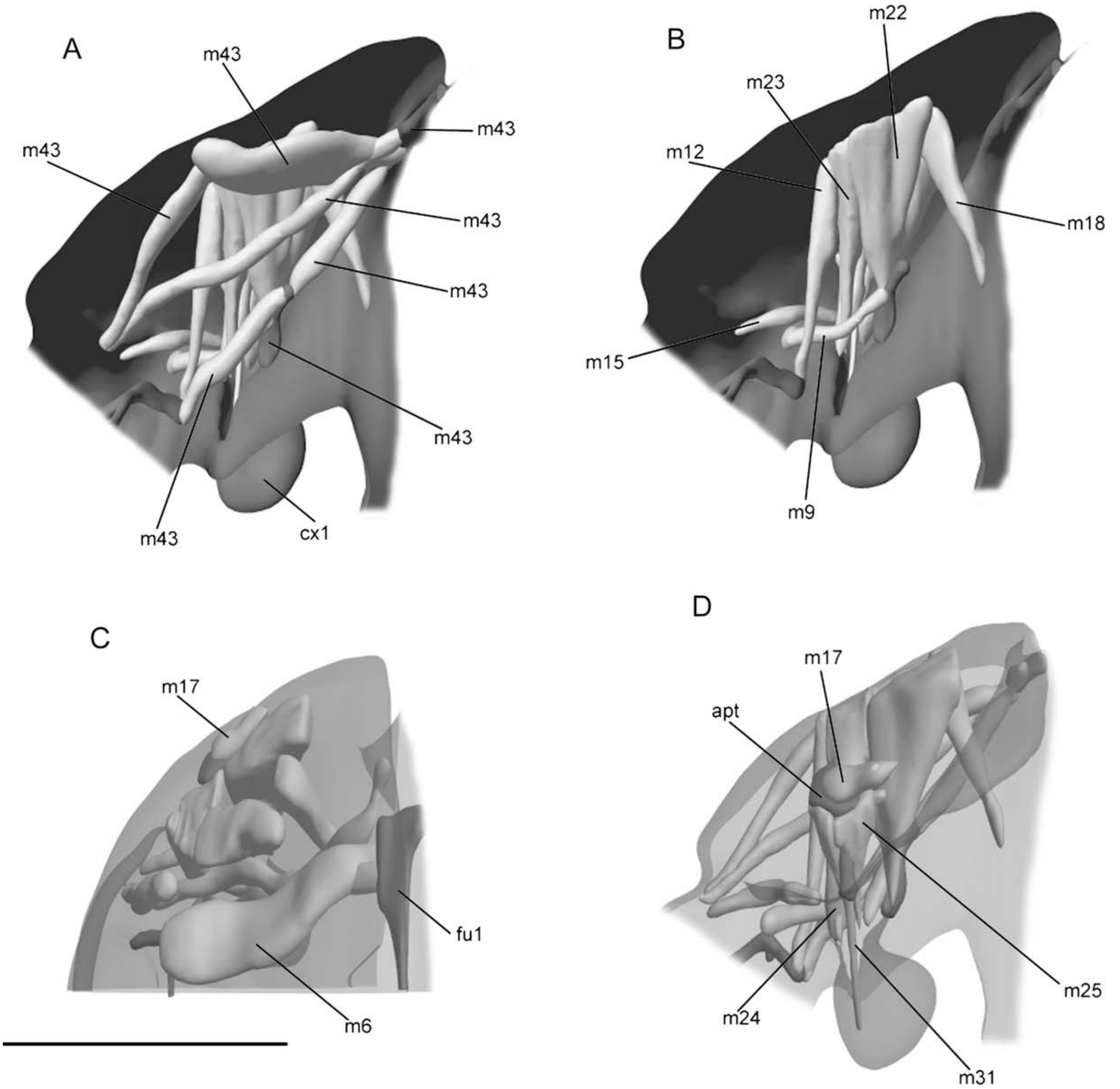

Fig. 17. Adult *Mikado* sp., prothorax, 3D. (A, B, D) Lateral view; (C) dorsal view. Abbreviations: apt, apodemal plate of trochantinopleura; cx1, procoxa; fu1, profurca. For musculature, see text. Scale bar: 100 µm.

Malpighian tubules (Fig. 15A,B). Four free malpighian tubules present. One pair directed forward and forming loop; other tubules also forming loop and directed backwards parallel to hindgut.

Circulatory system and fat body. Circulatory system strongly reduced, without heart and cephalic aorta; space for haemolymph circulation strongly restricted, almost completely filled out by fat body. Fat body consists of variously shaped cells.

Tracheal system. Strongly reduced, with few weakly branching tracheae originating from spiracles. Spiracles placed in membranous area between pro- and mesothorax, and tergites I–VIII. Longitudinal and transverse tracheal branches and air sacs absent.

Reproductive system (Fig. 19). Male reproductive system with unpaired spiral testis (on right side), sperm duct, accessory glands, and a copulatory apparatus. The latter shaped as slightly tapering tube and lacking parameres. Female reproductive system composed of several teleotrophic ovarioles originating on single oviduct (probably ovarioles from a single ovary, or both ovaries combined), receptaculum seminis (spermatheca), and accessory glands. Oviduct dilates into egg sac where egg development takes place.

4. Discussion

4.1. Effects of miniaturisation on structural features of Ptiliidae

All organ systems change allometrically in the individual development of *Mikado* sp. (Fig. 20) as well as in comparison with representatives of closely related groups of beetles such as Staphylinidae (Fig. 21). The way in which organs change depends on organ functions and also on the relative changes in physical features and effective forces.

Skeleton. In the adult's skeleton we find multiple fusions of sclerites (e.g., thoracic ventrites and pleural parts), absence of joints, simplifications of endoskeletal elements, and reductions in the number of tarsomeres, of segments of antennae and palpi. Similar changes are also found in other groups of beetles (Beutel et al., 1999; Grebennikov and Beutel, 2002). The larvae display extreme amalgamation of the skeleton – no well-defined sclerites are found and the cuticle is very thin and lacks pigmentation. Interestingly, the head of the extremely miniaturised first instar larva of Strepsiptera is even more simplified, whereas a distinctly

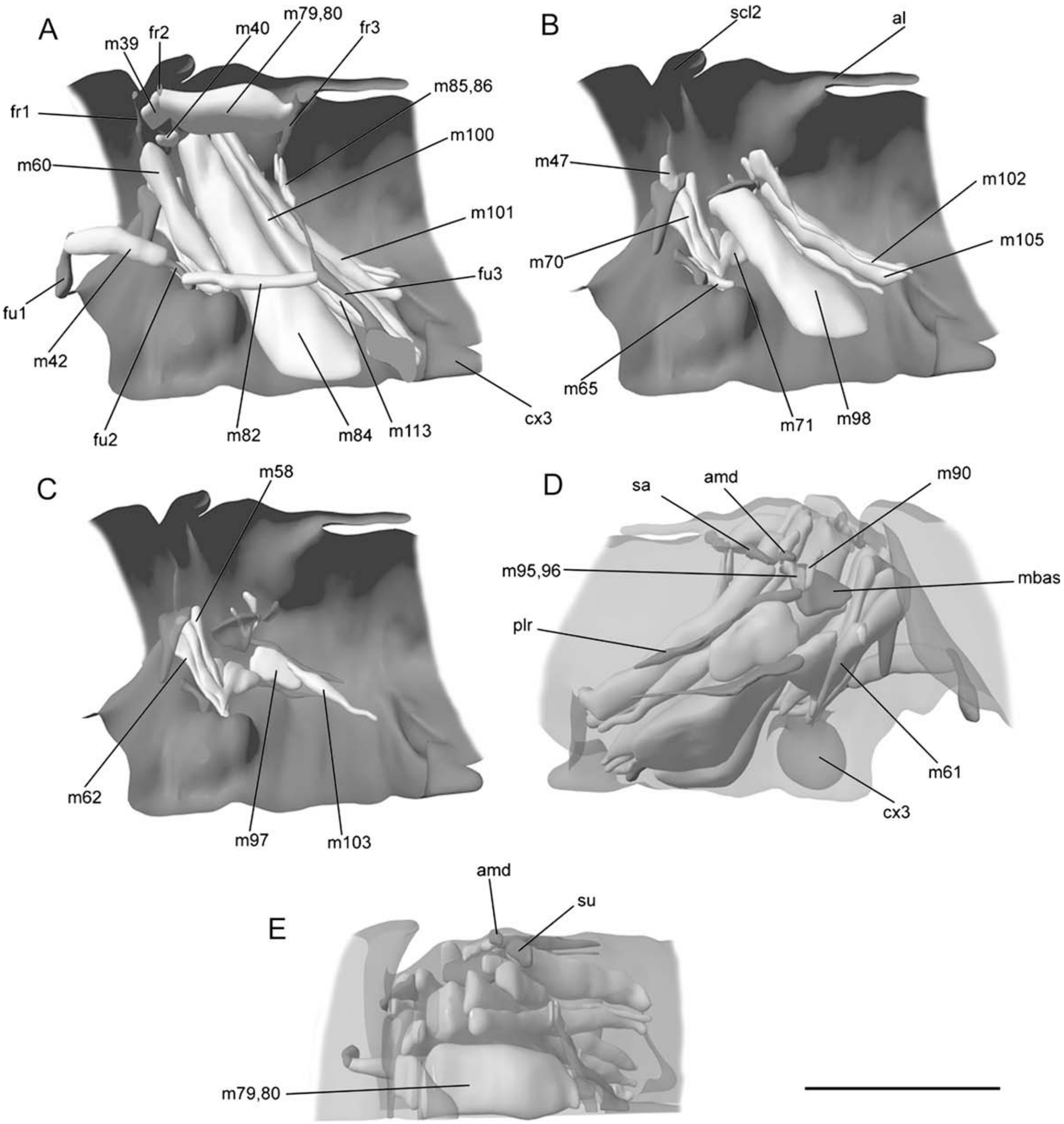

Fig. 18. Adult *Mikado* sp., pterothorax, 3D. (A, B, D) Lateral view; (C) dorsal view. Abbreviations: al, alacrista; amd, third axillary muscle disc; cx2, metacoxa; fr1,2,3, pro-, meso- and metaphragma; fu1,2,3, pro-, meso- and metafurca; mbas, muscle disc of basalare; plr, pleural ridge; su, sa, subalare; scl2, mesoscutellum. For musculature, see text. Scale bar: 100 μm.

lesser degree of modification is found in the skeleton of the thorax and abdomen (Pohl, 2000).

In adults of *Mikado* the relative volume of the skeleton increases compared to larger representatives of the family (Fig. 21A) and also the relative strength of the cuticle. This can be explained by the fact that the external skeleton has not only mechanical functions in the context of locomotion. It also serves as an important defensive device, and miniaturisation leads to a relative increase of the exposed surface area. The relative skeleton volume also increases in the successive immature stages (Fig. 20A). This can be explained with the thickening of the cuticle, which is likely to be essential to prevent desiccation during dispersal and movements among various substrates, which probably take place in the later larval stages. There are no data available about locomotor activities of ptiliid larvae, but our observation showed that first instar larvae very rarely leave the substrate. In contrast to this, the dispersal activities seem to increase in the later instars and especially in adults.

Muscles. As the strength of muscles depends on the cross-section area, the relative strength increases with the body size reduction (Gorodkov, 1984). Therefore it is possible that ptiliid beetles possess nearly the complete set of muscles compared to the condition found in larger relatives. In *Mikado* sp. we found 81 of a maximum total number of 103 muscles of the larval head and the head and thoracic segments of adults occurring in non-miniaturised representatives of closely related groups (Staphyliniformia). We observed only 6 unique modifications not found in larger larvae or adults of related taxa (Table 1). It is remarkable that first instar larva of Strepsiptera possess a very complex set of thoracic muscles despite the extremely small size, i.e. 94 muscles in the three segments (J. Osswald, pers. obs.). Miniaturisation decreases the number of muscle subunits and muscle fibres, what is especially evident in the abdominal segments, where in larger larvae the entire system is much more complex (Speyer, 1922; Beutel and Hörnschemeyer, 2002; Crome, 1957) and the muscles are composed of several types of different myofibrils (Speyer, 1922;

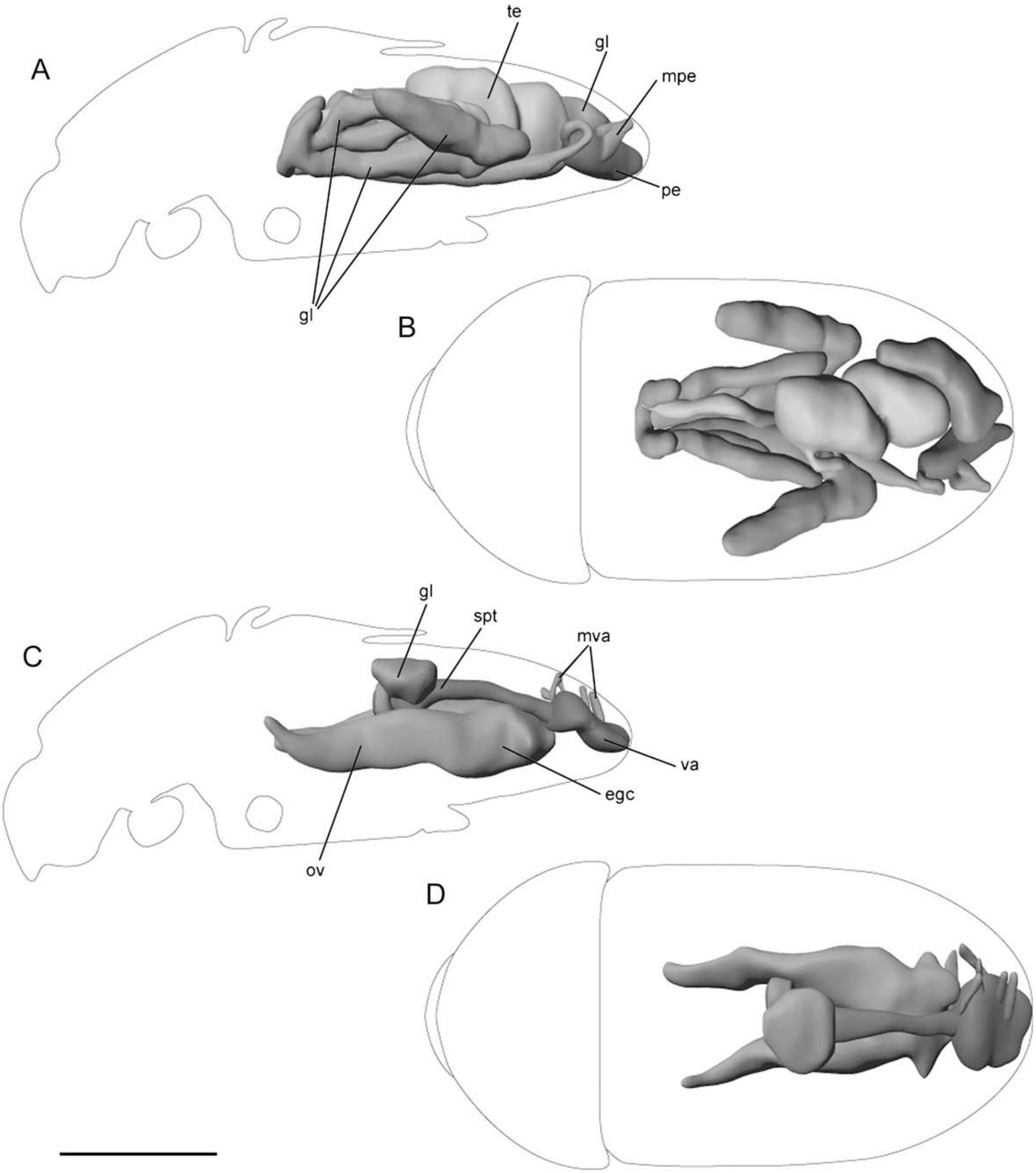

Fig. 19. Reproductive system of *Mikado* sp. (A, B) Male; (C, D) female. Abbreviations: egc, egg chamber; gl, gland; mpe, muscle of penis; mva, muscle of vagina; ov, ovariole; pe, penis; spt, spermatheca; te, testis; va, vagina. Scale bar: 50 μm.

Crome, 1957). The relative total volume of muscles decreases with the body size reduction in Ptiliidae. In the postembryonic development, the muscle volume is changing isometrically The relative muscle volume in adults is 2.5 times larger compared to the larval stages, which is likely due to the increased agility of adults and the presence of well-developed wing muscles.

Digestive and excretory systems. The relative volume of the metabolic organs is changing isometrically with the body size reduction in members of the families of Staphylinidae and Ptiliidae, and is decreasing from Staphylinidae to Ptiliidae. This is likely correlated with a relative increase of the effective areas. Their functioning depends on their surface area, which is relatively increasing with body size reduction (Fig. 21C). The relative volume of the metabolic organs is highest in the last larval instar, which is the metabolically most active stage. It has to accumulate a large amount of energy for the metamorphosis (Fig. 20C,D).

Heart and circulatory system. Due to the miniaturisation the circulatory system is greatly reduced in Ptiliidae. The heart and cephalic aorta are totally absent and the fat-body (corpus adiposum) occupies nearly the entire volume of the body cavity, thus greatly reducing the space for haemolymph circulation. These changes are made possible by size reduction as this increases capillary forces, which render impossible active haemolyph circulation, whereas diffusion is sufficient for the transport of substances between the organs. Similar changes are observed in the first instar larva of Strepsiptera (Pohl, 2000) and various miniaturised mites (Sylvere and Stein-Margolina, 1976). In the latter the haemolymph is totally replaced by parenchyma – tissues combining functions of the fat body and haemolymph.

Tracheal system. The tracheal system is largely reduced. Only a few tracheae connected to the spiracles are preserved in adults, and the number of spiracles is also reduced in the smallest ptiliid beetles

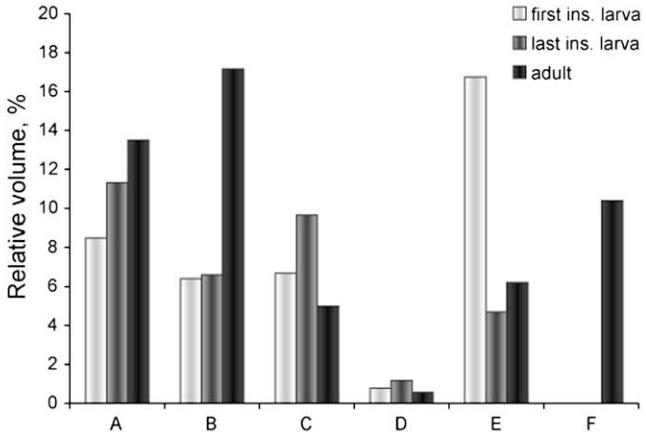

Fig. 20. Relative volume of organs of *Mikado* sp. in ontogenesis. (A) Skeleton; (B) musculature; (C) digestive system; (D) excretory system; (E) nervous system; (F) reproductive system. Single measurements shown for all systems except reproductive, for the latter the mean between male and female system is shown.

(Sörensson, 1997; Hall, 1999; Polilov, 2008). Larvae show even a greater degree of reduction – they posses only one pair of metathoracic spriacles and we assume that larvae at least partly rely on surface respiration. The first instar larva of Strepsiptera has a closed tracheal system and uses surface respiration too (Pohl, 2000). The simplification of the tracheal system is possible as the amount of oxygen transferred by diffusion is sufficient for the metabolism of very small organisms. We were unable to calculate the actual volume of the respiratory system due to the very small size of the tracheae, but due to the extreme degree of reduction, we assume that with the decrease of body size its relative volume is reduced as well.

Nervous system. In the central nervous system of ptiliid beetles a high degree of oligomerisation and concentration of ganglia is found. The larval brain is completely shifted to the thoracic segments, like in some other small larvae such as for instance first instars of *Micromalthus debilis* LeConte (Archostemata) or Strepsiptera (Beutel and Haas, 1998; Beutel and Hörnschemeyer, 2002; Beutel et al., 2005). The brain of adults of the majority of ptiliid beetles is located in the head, but in the smallest representatives of the family part of the cerebrum and the suboesophageal ganglion are displaced to the prothorax. This was never reported for adults of any other group of insects (e.g., Mymaridae; Polilov, 2007). The increased movabilty of the head of adults and different degrees of narrowing of the foramen occipitale normally prevent this dislocation of the brain. Neuron body size in *Mikado* and other greatly miniaturised members of Ptiliidae is much smaller than in medium sized or moderately small insects. In larger ptiliid beetles such as *Acrotrichis montandoni* (Allibert, 1844) it is about 2–4 μm, whereas in the smallest ptiliids like *Nanosella* sp. it is only 0.8–1 μm. A significant reduction of neuron size was shown for many miniaturised insects as for instance *Hydroscapha* (Myxophaga) or first instars of Strepsiptera (Beutel and Haas, 1998; Beutel et al., 2005). The nucleus occupies from 80 to 90% of the neuron body volume. Some rough estimations of neuron numbers in Ptiliidae indicate that members of this family possess 1–3 orders less than most other insects (Polilov, 2008). Despite the extremely small size and greatly reduced number of neurons, the neural system in ptiliids occupies a relatively larger portion of the body volume compared to larger insects, and with increasing body size reduction of the relative volume of the central neural system is increasing (Fig. 21D). The first instar larva displays the maximum relative volume of the neural system among all postembryonic developmental stages (Fig. 20E). Despite the extremely small size, the first-stage larvae possess the full set of sensory organs and a complex behaviour pattern as it is found in later instars. The relative volume of the nervous system of adults is somewhat increased compared to the pre-metamorphic larva. This is apparently correlated with the increased number of receptors (e.g., compound eyes, antennal sensilla) and a more complex behaviour (e.g., flight, mating).

Reproductive system. In the reproductive system of Ptiliidae we observed a complete reduction of one of the testes in males and a significant reduction of the ovariole number in females, and in some cases, possibly, a complete reduction of one of the ovaries. The relative volume of the internal genital organs and structures is greatly increased with size reduction (Fig. 21E). This allometry is carried to an extreme in the relative size of the spermatozoa. In the majority of ptiliids the sperm cells are about as long as the entire body and in some cases they are even much longer (Dybas and Dybas, 1981, 1987; De Marzo, 1992; Polilov, 2008). Nevertheless it should be noticed that males of some genera possess very short spermatozoa (De Marzo, 1992; Polilov, 2005). Females of Ptiliidae can only develop one egg simultaneously. It occupies up to one half of the female body volume (Dybas, 1966; Polilov, 2008; Grebennikov, 2008).

4.2. Factors limiting body size

The first assumptions on factors limiting size reduction in insects were made by Rensch (1948). Based on studies of some moderately small insects (above 1.5 mm) he suggested that insect miniaturisation is constrained by the presence and size of eyes, muscles, digestive tract, eggs and brain. The detailed study of some of the smallest insects allowed us to challenge some of his assumptions (Polilov, 2005, 2007, 2008). Eyes or eye size are apparently not a limiting factor. Compound eyes of normal proportions in relation to the body size occur in extremely small insects. It is only the number of ommatidia which is reduced. Whereas the larval eyes are absent in the immature stages of Ptiliidae, which live in the substrate, well developed stemmata are present in the extremely miniaturised first instar larvae of Strepsiptera (Pohl, 2000). The digestive system is changing nearly isometrically within the studied families, and as it was mentioned above, its relative efficiency is increasing with decreasing body size. The muscular system comprises a nearly full set of muscles (ca. 80% of the muscles occurring in all examined representatives of Staphyliniformia) despite extremely small size, and the relative volume is smaller in the smallest representatives of Ptiliidae due to the increase of the relative muscle strength with size reduction. Both systems are apparently not limiting factors. In contrast to that, the volume of the neural system and egg size seem to be indeed of major importance as miniaturisation-restricting factors.

Neuron size in ptiliid beetles is much lower than in any other insects examined and approaches the minimum value due to a limitation by the size of the nucleus. In this context, it should be noted that all representatives of Nanosellini studied demonstrated the highest value of chromatin compression, resulting in a distinct reduction of the nucleus volume (Polilov, 2008). The number of neurons in ptiliid beetles is much smaller than in most of the insects, but at the same time the relative volume of the neural system of first instar larvae of *Mikado* sp., is the highest known for any insect. An increase of the relative brain volume was also shown for some other small insects (Rensch, 1948; Beutel and Haas, 1998; Beutel et al., 2005) and vertebrates (Schmidt-Nielsen, 1984; Roth et al., 1990). The minimum neuron size is also limited by a molecular disturbance preventing the distribution of action potentials along the neuron when the axon diameter is less than 0.06 microns (Faisal et al., 2005). This allows us to conclude that the limit of neural system miniaturisation in beetles is associated with neuron number and size. Future studies on miniaturisation effects on the nervous system should be focused on first instar larvae, as this stage has the maximum relative volume in the individual postembryonic development.

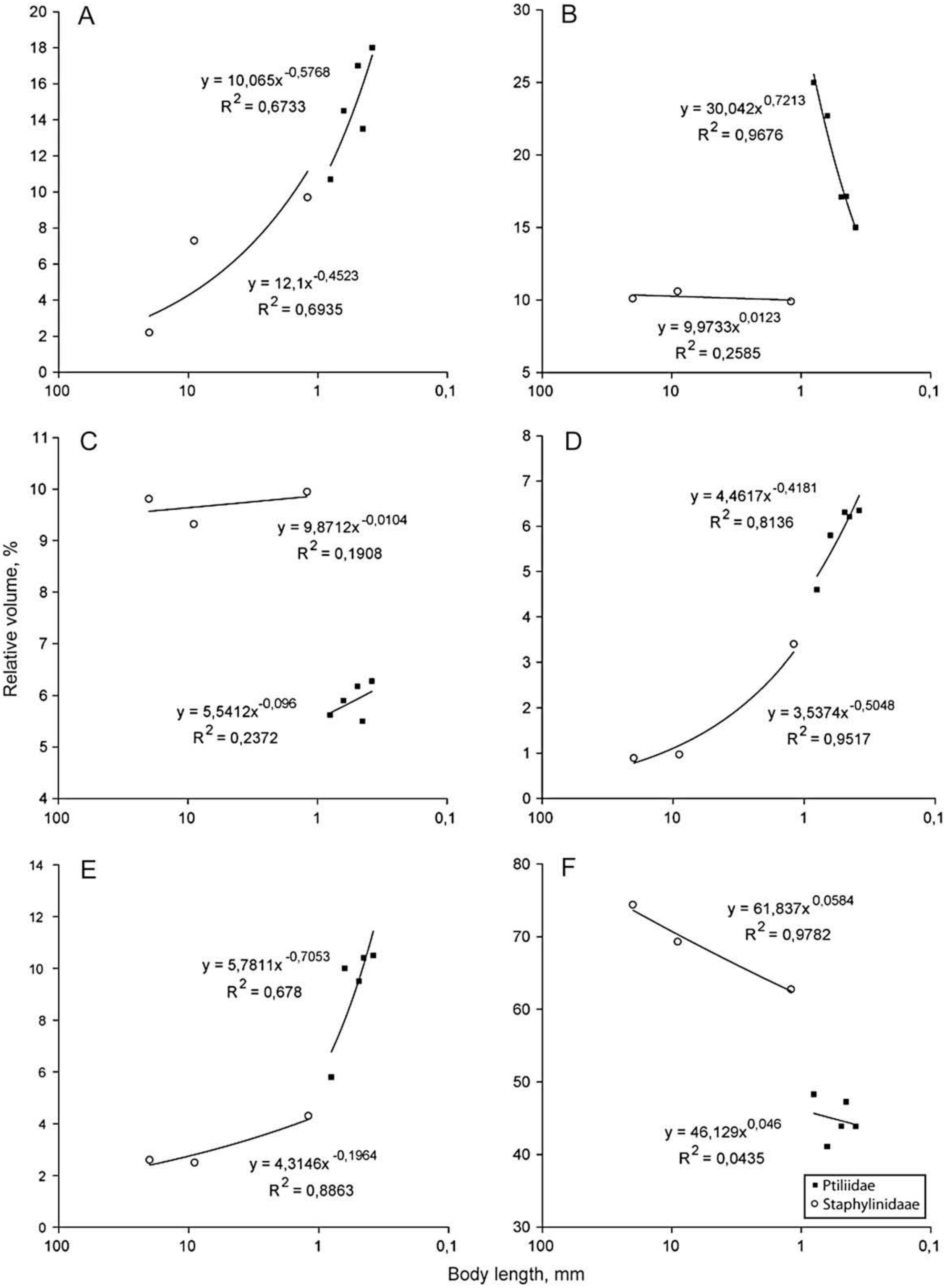

Fig. 21. Relative volume of organs of Staphylinoidea. (A) Skeleton; (B) musculature; (C) metabolic system; (D) nervous system; (E) reproductive system; (F) haemolymph and fat body. Single measurements shown for all systems except reproductive, for the latter the mean between male and female system is shown. Power regression is calculated. R, reliability approximation.

With body size reduction the relative size of eggs is increasing whereas their number is reduced (Rensch, 1948; Garcia-Barros, 2000, 2002). Ptiliidae can develop only one egg at a time and it occupies up to 1/2 of body length in females. It is conceivable that the egg size is the principal restriction of a further reduction of body size in Coleoptera. It is likely that the necessity to develop a disproportionally large egg leads to the increase of the relative volume of the reproductive system. This is not the case in parasitic Hymenoptera, where eggs contain a relatively small amount of nutrients. Their larvae develop within the host egg and this probably leads to significant progenesis (desembryonisation) (Ivanova-Kazas, 1961; Polilov, 2007). This allows adult parasitic

Table 1
Muscles of *Mikado* sp. and some Staphyliniformia (partly based on Beutel and Hass, 1998, 2000; Leschen and Beutel, 2001; Grebennikov and Beutel, 2002; Beutel and Komarek, 2004)

Taxon	Head muscles of larva																													
	1	2	4	7	9	11	12	15	17	18	19	28	29	30	41	43	44	45	46	48	50	52								
Mikado, first ins.	x	x	–	–	–	x	x	x	x	x	x	–	x	x	x	x	–	x	x	x	x	–								
Mikado, last ins.	x	x	x	–	–	x	x	x	x	x	x	–	x	x	x	x	–	x	x	x	x	–								
Ptinella, last ins.	x	x	x	x	–	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	x	x								
Hydraena	x	x	x	–	x	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	?	x								
Catops	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	?	x								
Tachinus	x	x	x	–	x	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	?	–?								
Staphylinus	x	x	x	–	–	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	?	x								
Sepedophilus	x	x	x	–	x	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	x	x								
Silpha	x	x	x	–	x	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	?	x								
Hydrochara	x	x	x	–	–	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	?	–								
Oryctes	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	x	x	x	x	x	?	x								
	Thoracic muscles of larva																													
	1	2	3	4	5	6	7	8	9	10	11	12	15	19	20	Y1	Y2	Y3	28	29	30	37	40	44/45	48/49	Y4	Y5	Y6	Y7	
Mikado, first ins.	x	x	–	x	a	a	b	–	b	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	–	x	x	x	
Mikado, last ins.	x	x	x	x	a	a	x	–	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Ptinella, last ins.	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	–	–	x	x	x	x	x	x	x	–	–	–	–	
	Head muscles of adult																													
	1	2	4	7	9	11	12	15	17	18	19	29	30	41	42	43	45	46	51	52	67	68	69							
Mikado	x	x	x	x	–	x	x	x	x	x	x	x	x	x	x	x	x	x	–	x	–	x	–							
Ochthebius	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	–	x	?	?	?							
Helophorus	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x							
	Thoracic muscles of adult																													
	1	2	6	9	10	12	15	16	17	18	22	23	24	25	30	31	39	40	42	45	47	52	53	55	60	61	62	64		
Mikado	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	–	x	–	x	–	x	x	x	–		
Ochthebius	–?	x	x	x	x	x	x	x	x	x	x	?	?	?	x?	x	x	x	x	–	x	?	?	–	?	x	–	–		
Creophilus	x	x	x	x	x	x	x	x	x	x	x	x	x	–	–	x	x	x	x	x	x	–	x	–	x	x	x	–		
Nicrophorus	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	–	x	–	x	x	x	x	–	–		
Hydrophilus	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	–	x	–	x	x	x	–		
Margarinotus	x	x	x	x	x	x	x	x	x	x	x?	x	x	–	x	x	x	x	x	x	x	–?	x	x	x	x	x	x		
Aphodius	x	x	x	x	x	x	x	x	x	x	–	x	x	x	–	x	x	x	x	x	x	x	x	x	x	x	x	–		
Cetonia	x	x	x	x	x	x	x	x	x	x	–	x	x	x	–	x	x	x	x	x	x	–	x	–	x	x	x	–		
	65	67	68	70	71	72	79	80	82	84	85	86	90	93	94	95	96	97	98	00	01	02	03	04	05	07	08	09	11	13
Mikado	x	x	–	x	x	x	c	c	x	x	x	x	x	x	?	a	b	x	x	x	x	x	x	–	x	x	–	?	–	x
Ochthebius	?	x	x	x	–	–	x	x	x?	x	x	x	x	x	x?	a	b	x	x	–	x	x	x	?	x	x	x	x	x	x
Creophilus	x	x	–	x	–	–	x	x	x	x	x	x	x	x	x	a	b	x	x	x	x	x	x	–	x	x	x	x	–	x
Nicrophorus	x	x	x	x	–	–	x	x	x	x	x	x	x	x	x	a	b	x	x	x	x	x	x	x	x	x	x	x	–	x
Hydrophilus	x	x	x	–	–	x	x	x	x	x	x	x	x	x	x	a	b	x	x	–	x	x	x	–	x	x	x	x	–	x
Margarinotus	x	x	x	?	–	x	x	x	x	x	x	–?	x	x	x	a	b	x	x	x	x	x	x	x	x	x	x	x	–	x
Aphodius	x	x	x	–	–	x	x	x	x	x	x	x	x	x	x	a	b	x	x	x	x	x	x	x	x	x	x	x	–	x
Cetonia	x	x	–	–	x	–	x	x	x	x	x	x	x	x	x	a	b	–	x	–	x	x	x	–	x	x	x	x	–	x

x, present; –, absent; a, b, c, parts of muscle.

hymenoptera to reach a smaller size than any insects with free-living larvae.

Despite the various modifications in skeletal structure the relative volume of the skeleton significantly increases and we can assume that skeleton size (and weight in particular) is also limiting further size reduction.

It can be concluded that the major factors limiting body size reduction in insects are the size of the neural system associated with the number and size of neurons, the mass of the skeleton, and in free-living insects the egg size, and consequently the volume of the reproductive system.

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