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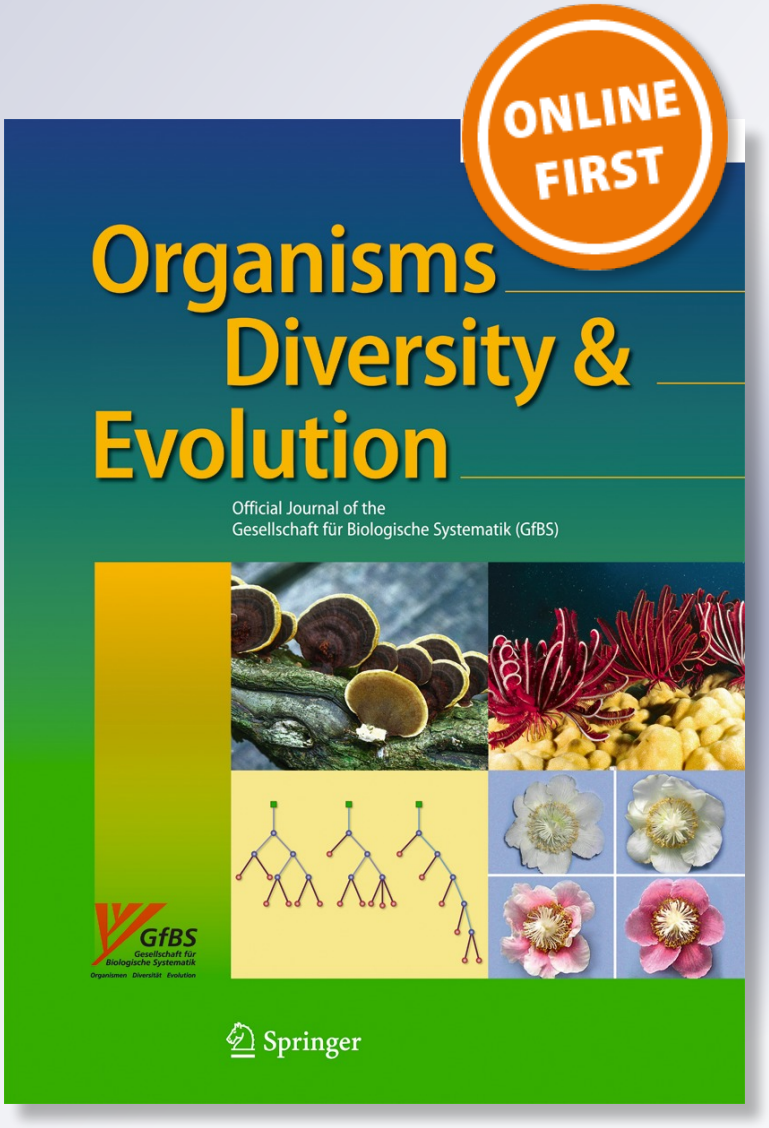

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ORIGINAL ARTICLE

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Immature stages of *Meiosimyza* Hendel 1925 and related genera (Diptera, Lauxaniidae)

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Abstract The immature stages of Holometabolan insects can provide valuable data both for taxonomy and phylogeny, but they are well known only for a negligible proportion of the described species. Scanning electron microscopy (SEM) analysis of all preimaginal stages of the lauxaniid species *Meiosimyza decempunctata* (Fallén 1820), *M. platycephala* (Loew 1847), *M. rorida* (Fallén 1820), *M. subfasciata* (Zetterstedt 1838), *Tricholauxania praeusta* (Fallén 1820) and *Aulogastromyia anisodactyla* (Loew 1845) is presented for the first time. Detailed images of eggs and all three larval instars are provided along with illustrations of the cephaloskeleton and photos of puparia. The egg is rather simple, white, with developed longitudinal ridges. The body integument of all larval instars is smooth and uniform across species. The cephaloskeleton is slender; the mouth hooks are parallel, subhypostomal sclerites are arched and fusing to a ventral bridge. The puparium is brownish, oval, without calcareous deposits. Generally, the morphology of larvae and puparia is very conservative. On the other hand, the eggs show considerable degree of interspecific variability.

Keywords Diptera · Lauxaniidae · *Aulogastromyia anisodactyla* · *Meiosimyza decempunctata* · *M. platycephala* · *M. rorida* · *M. subfasciata* · *Tricholauxania praeusta* · Egg · Larva · Cephaloskeleton · Puparium · Morphology · SEM

Introduction

Knowledge of immature insects generally lags behind the imagos. This applies particularly to Cyclorrhapha (Diptera); for example, Teskey (1981) estimates that roughly 5 % of Nearctic Cyclorrhapha has described larvae, though our knowledge of the immatures has certainly matured since that time. Flies with fairly commonly known immature stages are those with forensic, medical or economic importance. The poor knowledge of immature stages contradicts their potential in taxonomy and phylogenetics. Several researchers have shown that, e.g. the chorion bears informative phylogenetic characteristics (Meier and Hilger 2000, and references therein) and that it can differ even among closely related taxa (Jagadeeshan and Singh 2007; Kalantzi-Makri et al. 1999). The larvae have been argued to be useful in uncovering earlier splits in phylogeny. For more information, see the review by Meier and Lim (2009).

Lauxaniidae is a common family of acalyptrate flies, rich in species (Papp and Shatalkin, 1998), currently with up to 2,000 valid species (Gaimari in verb.). The larvae are generally phytosaprophagous; characteristic morphological traits include elongated cirri, one pair of ambulatory abdominal prolegs and often widened distal parts of Malpighian tubules (Miller and Foote 1976; Semelbauer and Kozánek 2011). A summary of knowledge about lauxaniid immature and adult morphology and biology can be found in Papp and Shatalkin (1998) and Gaimari and Silva (2010a). Adults can be acquired in high numbers and in a considerable diversity of species. Larvae can be reared easily in high numbers on rotting tree leaves (Miller 1977a, b, c); the life cycle is relatively short: it can take few weeks, in exceptional cases even less than 1 week, to get from the first to third instar (Semelbauer and Kozánek 2011). All of these facts argue for the suitability of lauxaniids for investigation of immature morphology.

The aim of this paper is to provide a detailed description of immatures of four *Meiosimyza* Hendel 1925 species: *M. decempunctata* (Fallén 1820), *M. platycephala* (Loew 1847), *M. rorida* (Fallén 1820) *M. subfasciata* (Zetterstedt 1838), *Tricholauxania praeusta* (Fallén 1820) and *Aulogastromyia*

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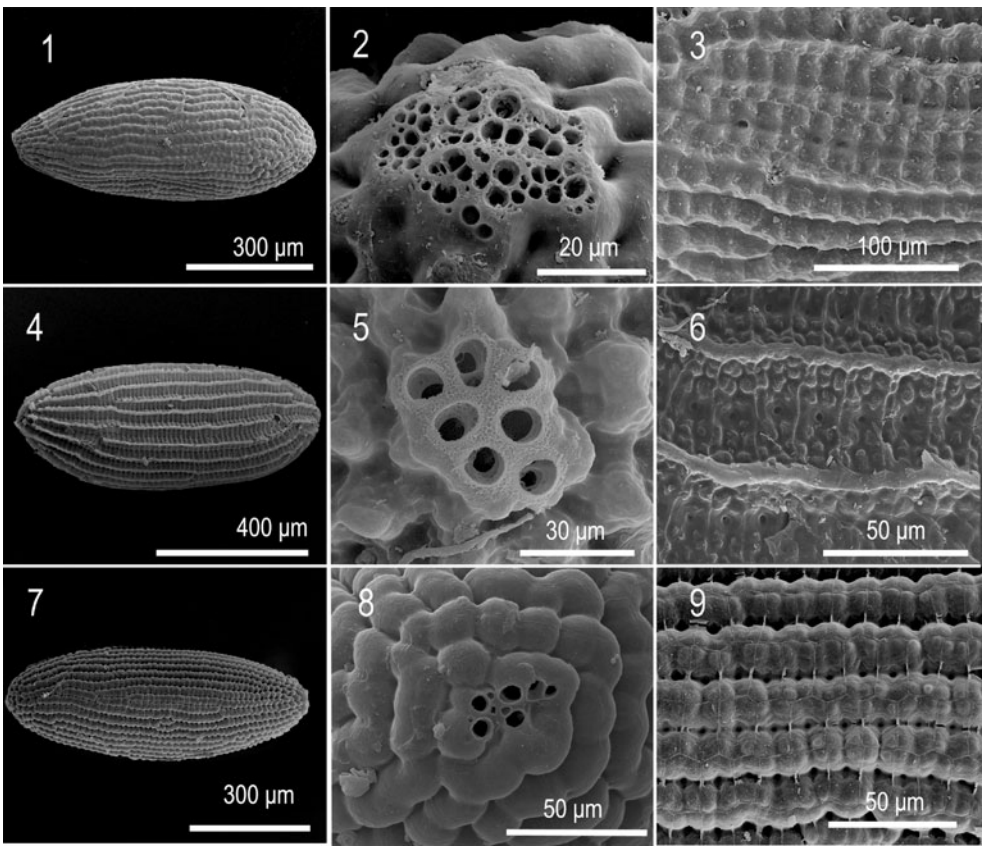
anisodactyla (Loew 1845). All these genera, together with genus *Eusapromyza* Malloch 1923 are believed to form a monophyletic group (Shatalkin 2000).

Materials and methods

Adult flies were obtained by sweeping vegetation at several localities in western and northern Slovakia. The key of Shatalkin (2000) and its English translation (Schacht et al. 2004) were used for determination. Nomenclature follows Shatalkin (2000). Both males and females were kept in plastic jars filled with moss (*Hypnum* sp.) to maintain suitable humidity. A mixture of honey and yeasts was used as an adult food source (Miller 1977b, 1977c). Eggs were laid on the moss singly by females. Eggs were transferred to glass dishes and kept at a temperature of 10±2 °C. Rotting leaves of wild cherry (*Cerasus avium*) were added to glass dishes as a larval food source immediately after the first larva appeared. In the winter of 2009 we also collected larvae from nature to acquire puparia. Living larvae were washed in a stream of liquid water from a vaporizer except for the tiny first instars. Larvae were put in water and slowly heated until the temperature reached 45 °C, consequently retaining an exposed pseudocephalon and a pair of extendable ambulatory lobes. Eggs, individual larvae and puparia were stored in 70 % ethanol. Preparation of larvae for SEM involved dehydration through 80 %, 90, and 99.5 % ethanol series and soaking in hexamethyldisilazane (HMDS) (Brown 1993). We removed redundant HMDS so that all larvae remained immersed and remaining HMDS was evaporated slowly by atmospheric pressure and room temperature. For eggs, the ethanol series was replaced by soaking directly in 99.5 % ethanol and then in HMDS. Larvae and eggs were coated with gold or a gold-palladium mixture. SEM pictures were taken with a Quanta 3D 200i. Cephaloskeletons and puparia were photographed by Nikon Coolpix 990 digital camera mounted on a Nikon Eclipse E600 microscope under Nomarski contrast. Cephaloskeletons were extracted mechanically; for the first instar we used the whole anterior half of the body. Soaking in 4 % KOH removed soft tissues. The line drawings were prepared directly from digital photographs in Adobe Photoshop, version 6.0.1 CE. Morphometrical data were taken in the program Micrometrics SE Premium—318-318004BF in combination with a Micrometrics camera



Figs. 1–9 **1** SEM images of eggs. *Left column* Whole egg, *middle column* posterior pole, *right column* detail of the egg chorion. **1–3** *Aulogastromyia anisodactyla*. **4–6** *Meiosimyza decempunctata*. **7–9** *M. platycephala*

mounted on a Zeiss binocular microscope Stemi 2000-C. Terminology follows Courtney et al. (2000) and Miller and Foote (1976a).

Results

Eggs and larvae of most instars were acquired in sufficient numbers. The eggs were laid singly, only *M. platycephala* occasionally laid eggs in small clusters. Puparia were obtained from larvae collected in nature and reared to the adult stage. We obtained puparia of all species except for *Meiosimyza subfasciata*. No puparium was acquired from laboratory rearing.

The egg

Always white, scaphoid from the lateral view and oval from the dorsal view, usually with strong ridges. Micropylar pole with depression surrounded by star-like collar; in some species with recognisable hatching line. Posterior pole with chorion of spongy appearance, with more or less distinct tubercle. The ventral side often covered by a thin layer, possibly an adhesive secreted by the female.

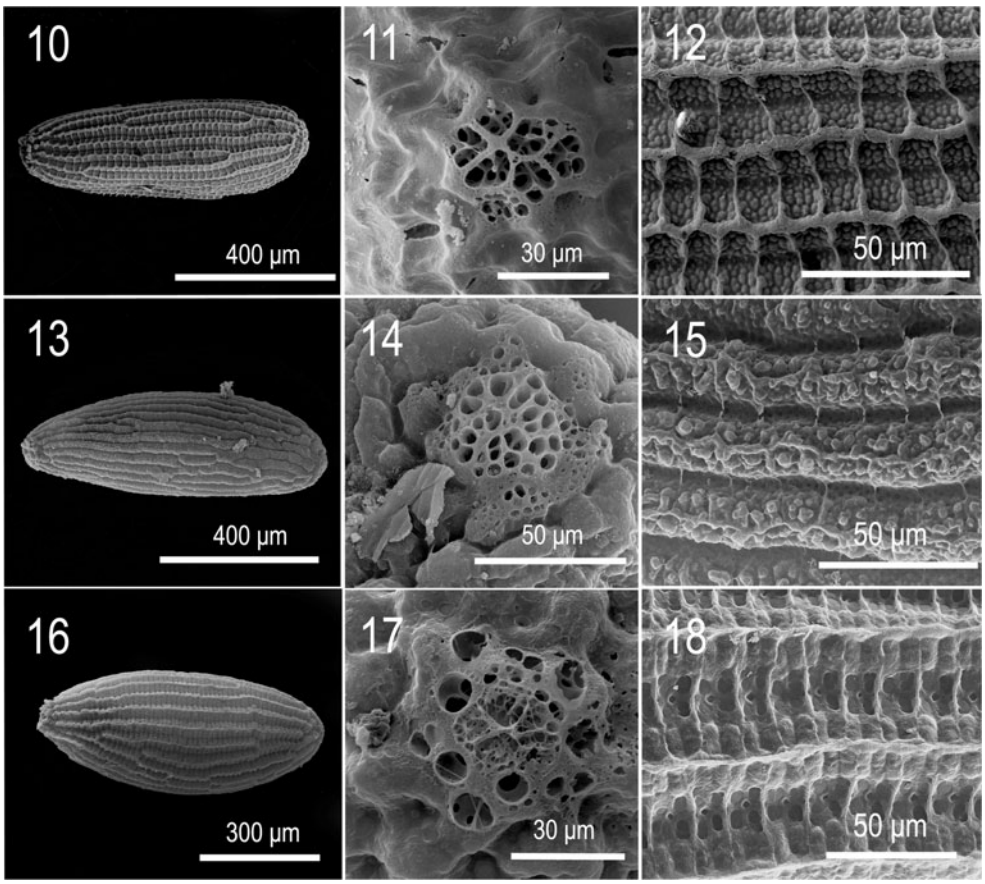
Aulogastromyia anisodactyla. (Figs. 1–3) Length 0.65–071 mm. The egg with dense and inflated ridges, on dorsal side the ridges not inflated. The posterior tubercle plane.

Meiosimyza decempunctata. (Figs. 4–6). Length 0.79–0.85 mm. The ridges prominent, the inter-ridge space concave with dense ribs, usually with small pit in the centre, the surface nodular. The posterior tubercle small with few approximately equally sized openings.

Meiosimyza platycephala. (Figs. 7–9). Length 0.75–0.81 mm. The eggs were laid in clusters. The chorion appears to consist of small bullets in longitudinal rows. Over the bullets is laid a fine net corresponding to the ridges and ribs in the other species. The posterior pole inconspicuous.

Meiosimyza rorida. (Figs. 10–12). Length 0.71–0.79 mm. The ridges and ribs well developed, the chorion finely nodulated.

Meiosimyza subfasciata. (Figs. 13–15). Length 0.78–0.83 mm. The ridges well developed, swelled. The chorion finely nodular. Over the ridges is indicated a fine net.



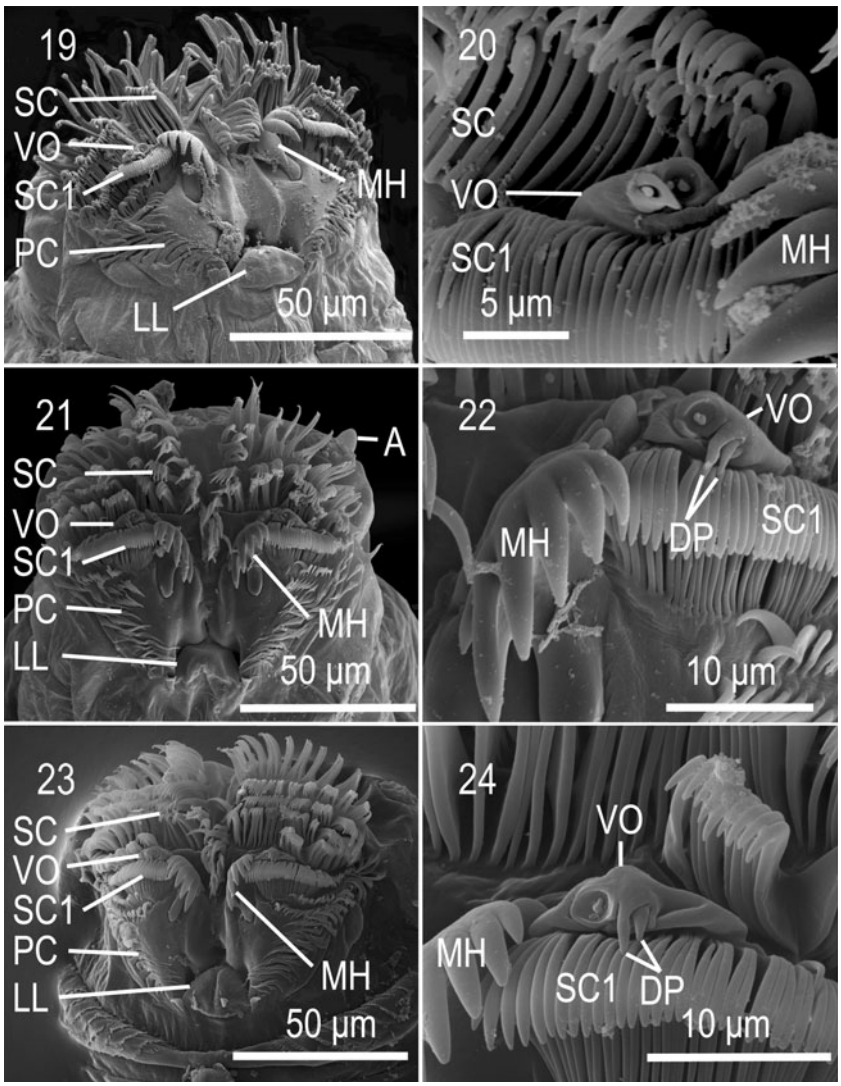
Figs. 10–18 The SEM images of the egg. *Left column* Whole egg, *middle column* posterior pole, *right column* detail of the egg chorion. **10**–**12** *Meiosimyza rorida*. **11** Detail of the egg chorion. **13**–**15** *M. subfasciata*. **16**–**18** *Tricholauxania praeusta*

Tricholauxania praeusta. (Figs. 16–18). Length 0.74–0.81 mm. The longitudinal ridges well developed, the ribs slightly indicated, the rectangles delimited by the ridges and ribs with a central pit. The chorion surface tuberculous. The posterior tubercle plane with spongy chorion.

External morphology of the larvae

The body is cylindrical. Integument translucent, glassy, shiny; distal parts of Malpighian tubules filled with white matter but not widened. All instars amphipneustic, but in the first instar anterior spiracles present as a small pore. The body differentiated into pseudocephalon, three thoracic segments, seven abdominal segments and the anal division. The segments of the body bear a number of sensilla not mentioned here, peg and pit sensilla usually dispersed throughout the integument. Comprehensive mapping of them is omitted here. We will only note that peg sensilla are usually located at the tips of body extremities, suggesting their mechanosensory function.

The pseudocephalon is bilobed, bearing antenna, maxillary palpus, cirri, ventral organ and labial lobe. Antenna simple, dome shaped, longer than wide. Maxillary palpus consists of a cluster of several sensilla (three sensilla coeloconica, four sensilla basiconica and two accessory sensilla). Cirri differentiated in two distinct types: the first localized more anteriorly, forms 5–7 transverse rows, stands upright, at base extended and laterally flattened, tips with sclerotised and ventrally curved papillae (in the first instar tips simple). The second type surrounds the mouth opening: it is flattened and adjoining to the face (e.g. Fig. 42). The first type we will refer to as "scraping cirri", the second as "peristomal cirri". Labial lobe with villous internal surface.



Figs. 19–24 The pseudocephalon and detail of the ventral organ, first instar. **19**, **20** *Aulogastromyia anisodactyla.* **21**, **22** *Meisoimyza decempunctata*. **23**, **24** *M. platycephala*. *DP* Digitiform processes, *LL* labial lobe, *MH* mouth hook, *SC* scraping cirri, *SC1* first row of scraping cirri, *VO* ventral organ

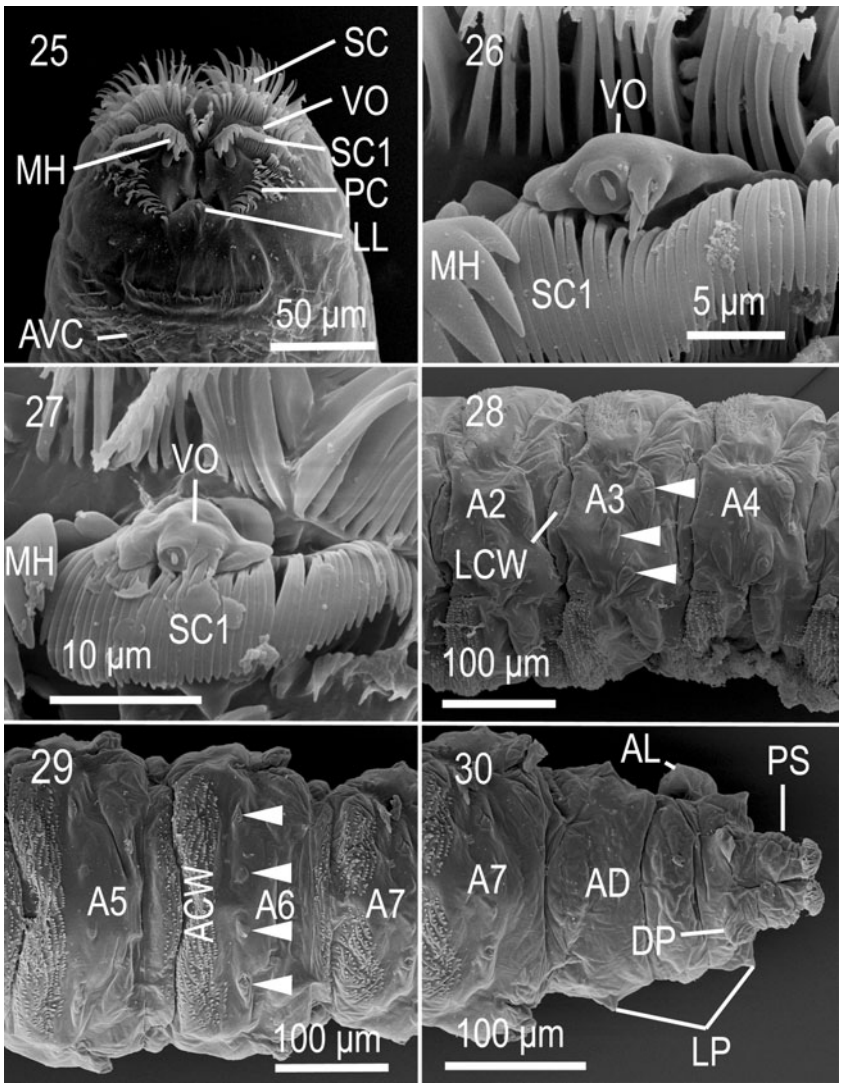
The thoracic segments cylindrical. The first thoracic segment has an anteroventral collar. Anteriorly, the thoracic segments can bear comb spines both on dorsal and ventral sides. Paired Keilin's organ is present. Dorsally, there are two pairs of small tubercles equipped with trichoid sensilla, the lateral pair supplemented by a peg sensilla.

The abdominal segments with several crevices and welts. One dorsal and one ventral spinous creeping welt per segment; creeping welts covered by comb spines or transversal rows of spines. Each side of the abdominal segments with 1–3 lateral tubercles and a lateral welt. The seventh and often the sixth abdominal segment dorsally smooth. Dorsally, in the middle of each segment, two pairs of small oval tubercles aligned in a transversal row, the lateral pair equipped by trichoid sensilla.

The anal division completely smooth except for the ventral spinous creeping welt. A pair of extendable ambulatory lobes surrounds the anus. Three pairs of processes present: two lateral and one dorsal. A small pad present behind the ambulatory lobes. Posterior spiracles placed on closely adjacent, basally inflated cylindrical sprouts; a spiracular plate with three spiracular slits located on ovoid elevations and with four peristigmatic tufts (first instar with two slits and three tufts).

The first instar. Cirri aligned in five transverse rows. The first row of scraping cirri chitinized, very compact and loosely attached to the mouth-hooks. The remaining cirri soft, slender, with hook-shaped tips. Medial rows of cirri-like processes running ventrally. Ventral organ lying on the first row of cirri, consisting of a dorsoventrally flattened lobe, digitiform projections and large sensilla (e.g. Fig. 28). Peristomal cirri simple and narrow, in paired oblique strips connecting the mouth opening and the first row of cirri.

Aulogastromyia anisodactyla. (Figs. 19, 20). Length 1.0–1.89 mm. Only two digitiform projections of the ventral organ present, the smaller one outgrowth from within the larger one.



Figs. 25–30 The SEM images of the first instar. **25**, **26** Pseudocephalon and the ventral organ of *Meiosimyza subfasciata*. **27** The ventral organ of *Tricholauxania praeusta*. **28**–**30** The body segments of *M. decempunctata*. **28** Lateral view of the abdominal segments. **29** Dorsal view of the abdominal segments. **30** Dorsal view of the last abdominal segment and the anal division. *AD* Anal division, *AL* ambulatory lobes, *AVC* antero–ventral collar, *A2–A7* second to seventh abdimonal segment, *DP* dorsal process, *LCW* lateral creeping welt, *LL* labial lobe, *LP* lateral process, *MH* mouth hook, *PC* peristomal cirri, *PS* posterior spiracles, *SC* scraping cirri, *SC1* first row of scraping cirri, *VO* ventral organ

Meiosimyza decempunctata. (Figs. 21, 22). Length 1.05–1.98 mm. Two digitiform projection of the ventral organ present.

Meiosimyza platycephala. (Figs. 23, 24). Length 1.03–1.58 mm. Two digitiform process of the ventral organ well developed, the third one indicated.

Meiosimyza rorida. Length 1.21–1.55 mm. Preparing of the first larval instar for SEM was unsuccessful.

Meiosimyza subfasciata. (Figs. 25, 30). Length 1.29–1.80 mm. The ventral organ with four digitiform projections.

Tricholauxania praeusta. Length 1.41–1.67 mm. The ventral organ with three digitiform projections.

The second instar. Cirri organized in 6–7 rows. The ventral organ cylindrical, located in the third row of cirri. The peristomal cirri triangular with fimbriated margins, organized in transversal and oblique rows. Anterior spiracles simple, ovate, with tiny holes at periphery.

Aulogastromyia anisodactyla. (Figs. 31–36). Length 1.92–2.95 mm. The seventh abdominal segment dorsally smooth.

Meiosimyza decempunctata. (Fig. 37). Length 2.38–3.25 mm. The seventh dorsal creeping welt slightly spinous.

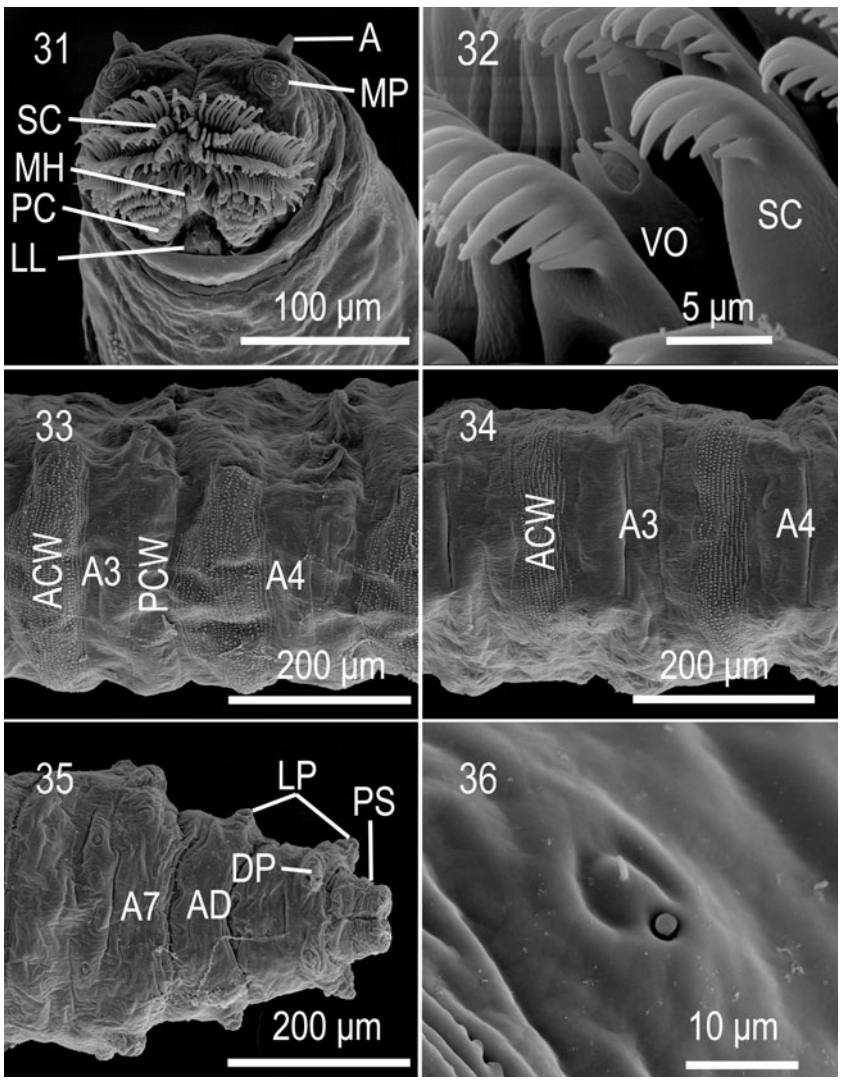
Meiosimyza platycephala. (Fig. 38). Length 1.96–2.78 mm. The seventh abdominal creeping welt dorsally smooth.

Meiosimyza rorida. Length 2.72–3.90 mm. The dorsal creeping welt of the seventh abdominal segment smooth.

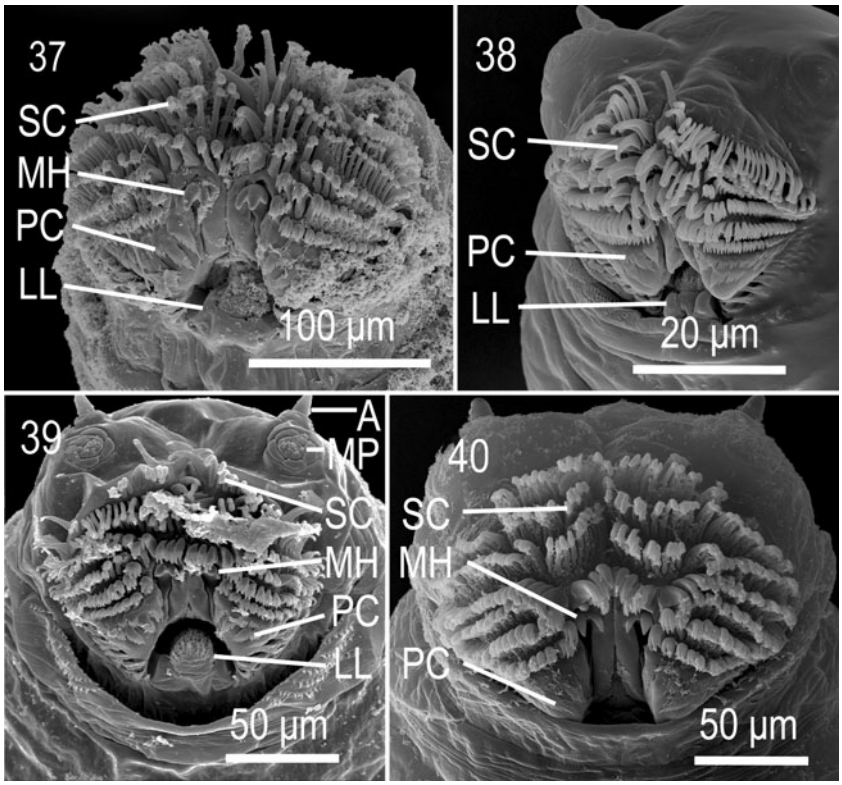
Meiosimyza subfasciata. (Fig. 39) Length 1.18–2.87 mm. The dorsal creeping welt of seventh abdominal segment smooth.

Tricholauxania praeusta. (Figs 40–46). Length 1.79–3.25 mm. The last abdominal dorsal creeping welt smooth.

The third instar. The organisation of facial mask is similar to the second instar, but with cirri in seven to eight rows. The vestiture of body segments as in the second instar. Anterior spiracles fan shaped with several papillae.



Figs. 31–36 *Aulogastromyia anisodactyla*, second instar. **31** The pseudocephalon, ventral view. **32** The ventral organ, lateral view. **33** The abdominal segments, ventral view. **34** The abdominal segments, dorsal view. **35** The anal division, dorsal view. **36** The lateral sensilla on the first thoracic segment, the latero–dorsal view. *A* Antenna, *ACW* anterior creeping welt, *AD* anal division, *A3–A7* third to seventh abdominal segment, *LL* labial lobe, *LP* lateral process, *MH* mouth hook, *MP* maxillary palpus, *PC* peristomal cirri, *PCW* posterior creeping welt, *PS* posterior spiracles, *SC* scraping cirii



Figs. 37–40 The pseudocephalon of the second instar, ventral view. **37** *Meiosimyza decempunctata*. **38** *M. platycephala*. **39** *M. subfasciata*, note the internal side of the labial lobe. **40** *Tricholauxania praeusta*. *A* Antenna, *LL* labial lobe, *MH* mouth hooks, *MP* maxillary palpus, *PC* peristomal cirri, *SC* scraping cirri

Aulogastromyia anisodactyla. (Figs. 47–52). Length 2.81–4.88 mm. The anterior spiracles with seven papillae. The seventh abdominal segment dorsally smooth.

Meiosimyza decempunctata. Length 2.85–4.75 mm. Preparing for SEM microscopy was not successful.

Meiosimyza platycephala. (Figs. 53–56). Length 2.97–4.86 mm. The seventh dorsal abdominal creeping welt smooth. Only one pair of lateral abdominal process present. The anterior spiracles with eight papillae.

Meiosimyza rorida. (Figs. 57–60). Length 2.90–4.71 mm. The seventh dorsal abdominal creeping welt smooth. The anterior spiracles with seven papillae.

Meiosimyza subfasciata. Length 2.49–5.06 mm. The anterior spiracles with seven papillae. The eighth abdominal segment dorsally smooth.

Tricholauxania praeusta. (Figs. 61–64). Length 4.18–4.56 mm. The anterior spiracles with seven papillae. The last dorsal abdominal creeping welt with few spines.

The cephaloskeleton

The first instar. The cephaloskeleton delicate, very distinct from the second and third instars. Mouth hooks divided into two parts. The distal part spatular with a row of six to seven teeth, always sclerotised. The proximal stalked part hyaline; attaches to the tips of the parastomal bars; dental sclerites unrecognisable. Intermediate sclerite and parastomal bars basally coalescent, the parastomal bars strong, the intermediate sclerite slender and apically coalescing to subhypostomal sclerites. Ligulate sclerites appear as simple vertical plates. Rami absent. The ventral bridge arched from the ventral view. The epistomal sclerite semilunar, with lateral windows; a narrow labrum borders the anterior margin of the epistomal sclerite. Cibarial ridges not developed, a light-fracturing structure resembling a tree developed instead.

Aulogastromyia anisodactyla. (Fig. 65). Length 0.24–0.25 mm. The posterodorsal margin of ventral cornua partly sclerotised.

Meiosimyza decempunctata. Length 0.21–0.23 mm. The dorsal margin of ventral cornua hyaline.

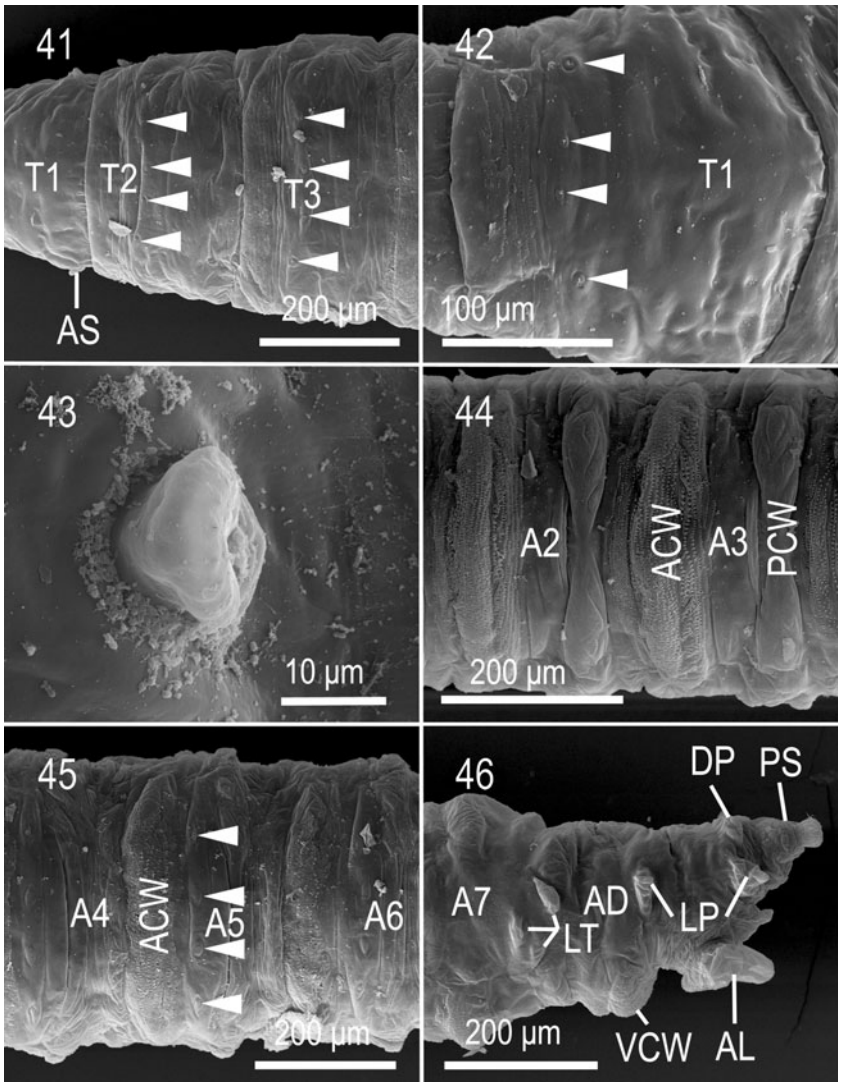
Meiosimyza platycephala. Length 0.22–0.23 mm. The dorsal margin of ventral cornua hyaline.

Meiosimyza rorida. Length 0.22–0.23 mm. The dorsal margin of ventral cornua hyaline.

Meiosimyza subfasciata. Length 0.23–0.25 mm. The dorsal margin of ventral cornua hyaline.

Tricholauxania praeusta. Length 0.26–0.28 mm. The mouth hooks with five to six teeth. The dorsal margin of ventral cornua hyaline.

The second instar. The basic organisation of the cephaloskeleton the same as in the third instar, but the tips of the mouth hook terminate in two to four teeth, the dorsal



Figs. 41–46 *Tricholauxania praeusta*, second instar. **41**. The thoracic segments and the pseudocephalon, dorsal view. **42**. The first thoracic segment, dorsal view. **43**. The anterior spiracle, lateral view. **44**. The abdominal segments, ventral view. **45**. The abdominal segments, dorsal view. **46**. The last abdominal segment and the anal division, lateral view. *ACW* Anterior creeping welt, *AD* anal division, *AL* ambulatory lobes, *A2–A7* second to seventh abdominal segment, *DP* dorsal process of anal division, *LP* lateral process of anal division, *LT* lateral tubercles of abdominal segments, *PCW* posterior creeping welt, *PS* posterior spiracles, *T1–T3* first to third thoracic segments. *Arrowheads* Dorsal tubercles of body segments.

apodeme of ventral cornu hyaline and the pharynx with only seven cibarial ridges.

Aulogastromyia anisodactyla. (Fig. 66). Length 0.44–0.45 mm. The mouth hooks ending in three teeth. The epistomal sclerite semilunar, broader than long, the posterior hyaline process trapezoid. The subhypostomal sclerites coalescing to ventral bridge.

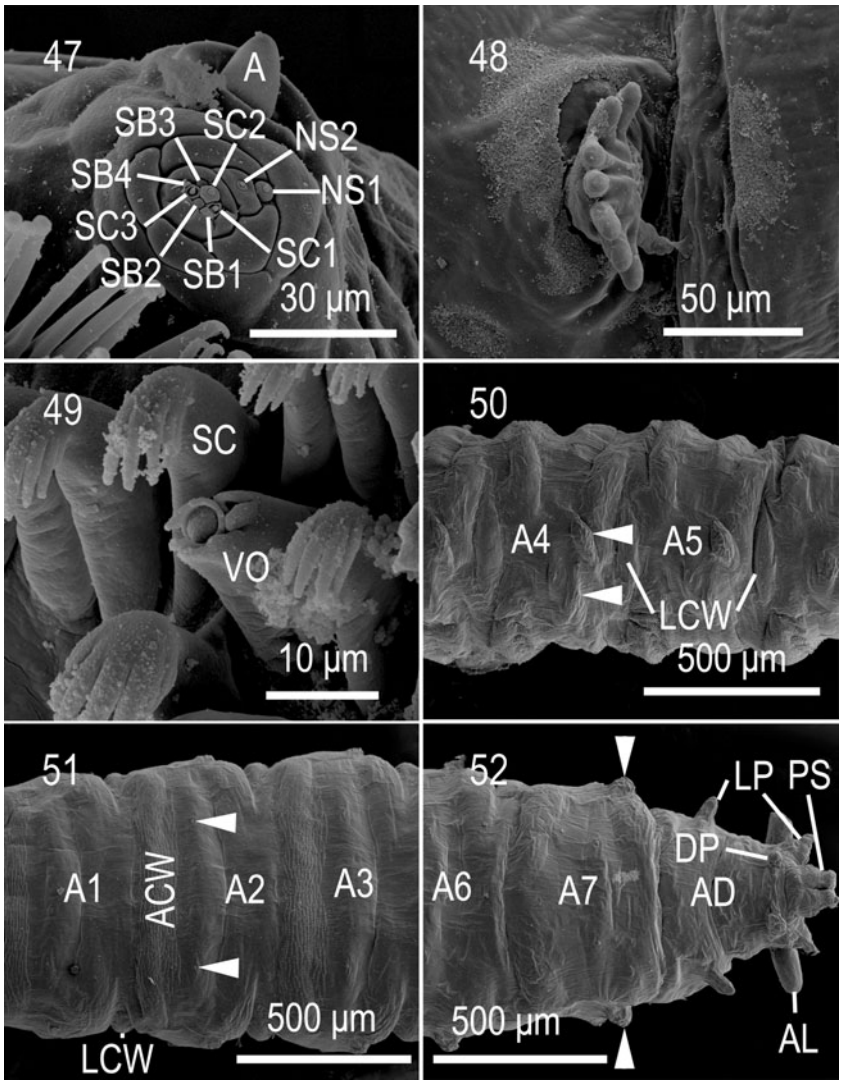
Meiosimyza decempunctata. Length 0.47–0.48 mm. The tips of mouth hooks with three apical teeth. The epistomal sclerite semilunar, posteriorly with trapezoid hyaline process. The attachment of subhypostomal sclerite to the ventral bridge only indicated.

Meiosimyza platycephala. (Fig. 67). Length 0.46–0.47 mm. The mouth hooks almost parallel, the distal part slender, two weak teeth indicated at apex. The epistomal sclerite semilunar with posterior trapezoid hyaline plate; two deep lateral incisions present (or sclerotization omitted). The subhypostomal sclerites arising from the ventral bridge.

Meiosimyza rorida. (Fig. 68). Length 0.39–0.46 mm. The mouth hooks with three to four apical teeth. The subhypostomal sclerites arising from the ventral bridge. The epistomal sclerite semilunar with posterior trapezoid hyaline plate.

Meiosimyza subfasciata. Length 0.42–0.45 mm. The mouth hooks ending in three teeth. The epistomal sclerite shorter than broad, the posterior hyaline plate trapezoid. The subhypostomal sclerite arising from the ventral bridge. The dorsal bridge longer than broad, the anterior margin rounded.

Tricholauxania praeusta. (Fig. 69). Length 0.43–0.48 mm. The mouth hooks ending in three teeth. The epistomal sclerite trapezoid, the lateral incisions striking from the dorsal view; the hyaline posterior plate trapezoid. The subhypostomal sclerites free. The rami long, the posterior tip almost reaches the anterior edge of the dorsal bridge. The dorsal bridge square-like.



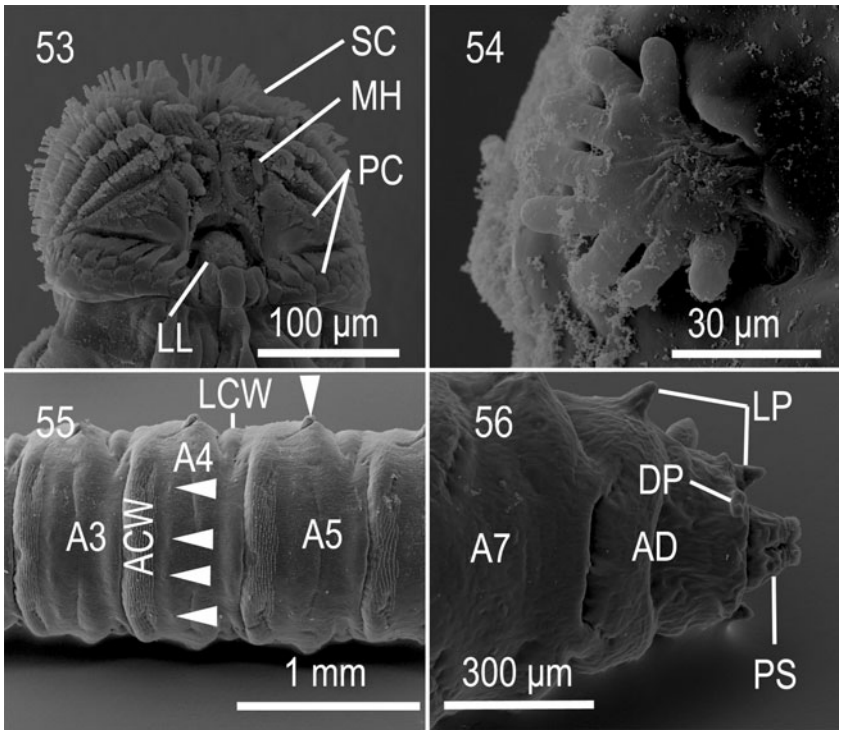
Figs. 47–52 *Aulogastromyia anisodactyla*, third instar. **47** The maxillary palpus and the antenna, ventral view. **48** The anterior spiracle, lateral view. **49** The ventral organ, latero–ventral view. **50** The abdominal segments, lateral view. **51** The abdominal segments, dorsal view. **52** The last abdominal segments and the anal division, dorsal view. *A* Antenna; *ACW* anterior creeping welt; *AD* anal division; *AL* ambulatory lobes; *A1–A7* first to seventh abdominal segment; *DP* dorsal process of anal division; *LCW* lateral creeping welt; *LP* lateral process; *NS1*, *NS2* first and second accessory sensilla; *SC* scraping cirri; *SC1–SC3* first to third sensilla coeloconica; *SB1–SB4* first to fourth sensilla basiconica; *PS* posterior spiracles; *VO* ventral organ

The third instar. The mouth hooks slightly converging. The base of the mouth hook flattened, contiguous to the tip of the intermediate sclerite; the dorsal apodeme directed medially; the distal part of the mouth hook dorsoventrally flattened and concave from the ventral side, tips always simple and amber-coloured. Dental sclerites present. The intermediate sclerite H-shaped from the ventral view, the bars strongly sclerotised and apically dorsoventrally flattened, the apex slightly bent ventrally; the ventral bridge shallow and with a descending posteriad; usually with a rounded cut-out on the posterior margin. The epistomal sclerite often semi-lunar from the dorsal view, convex, with paired lateral opening and lateral incisions touching the openings, and a posteriorly arising convex hyaline plate. Subhypostomal sclerites rod-like, arched, arising from the anterior edge of the ventral bridge or free. Ligulate sclerites appear as vertical converging plates. Rami simple, rod like, located medially to slender and rod like parastomal bars. The dorsal bridge mostly trapezoid. The dorsal cornua simple. The ventral cornua convex to straight and sclerotised along the dorsal margin; the dorsal apodeme of ventral cornua mostly sclerotised. The pharynx with nine cibarial ridges, with the inter ridge space pigmented.

Aulogastromyia anisodactyla. (Fig. 70). Length 0.77–0.83 mm. The epistomal sclerite oval from the dorsal view, shorter than broad; the posterior hyaline process rounded, continuous to sclerotised part of the epistomal sclerite. The subhypostomal sclerite coalescing to the ventral bridge. The dorsal bridge trapezoid, longer than wide.

Meiosimyza decempunctata. (Fig. 71). Length 0.79–0.85 mm. The ventral bridge narrow, simple. The epistomal sclerite shorter than long (sclerotised part) with trapezoid posterior hyaline process. The subhypostomal sclerite weekly attaches to the ventral bridge.

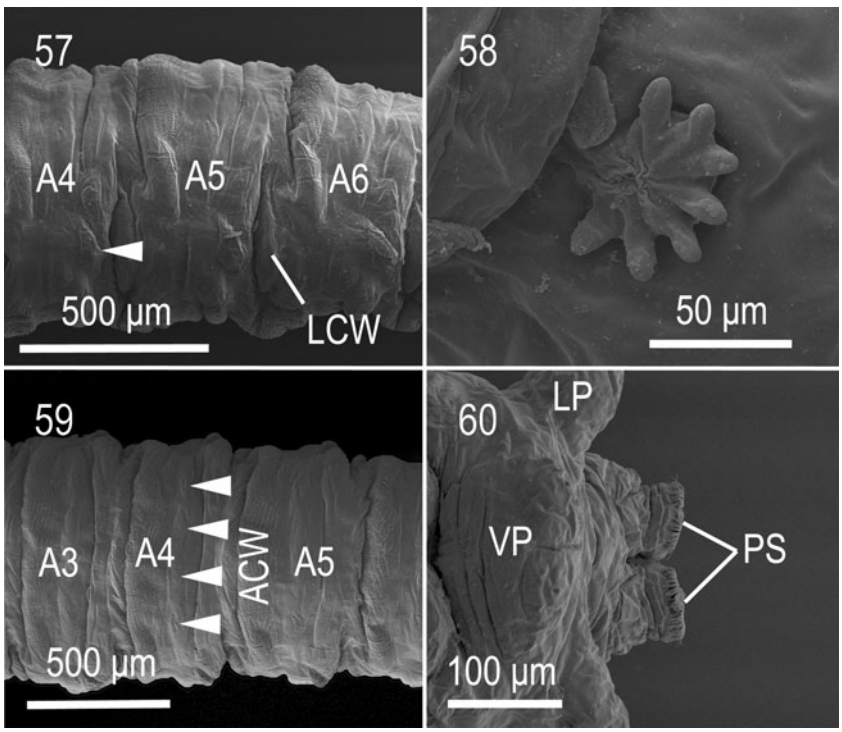
Meiosimyza platycephala. (Fig. 72). Length 0.86–0.93 mm. The mouth hooks slender, subparallel, a single window at base, the tips slightly converge; the ventral edge



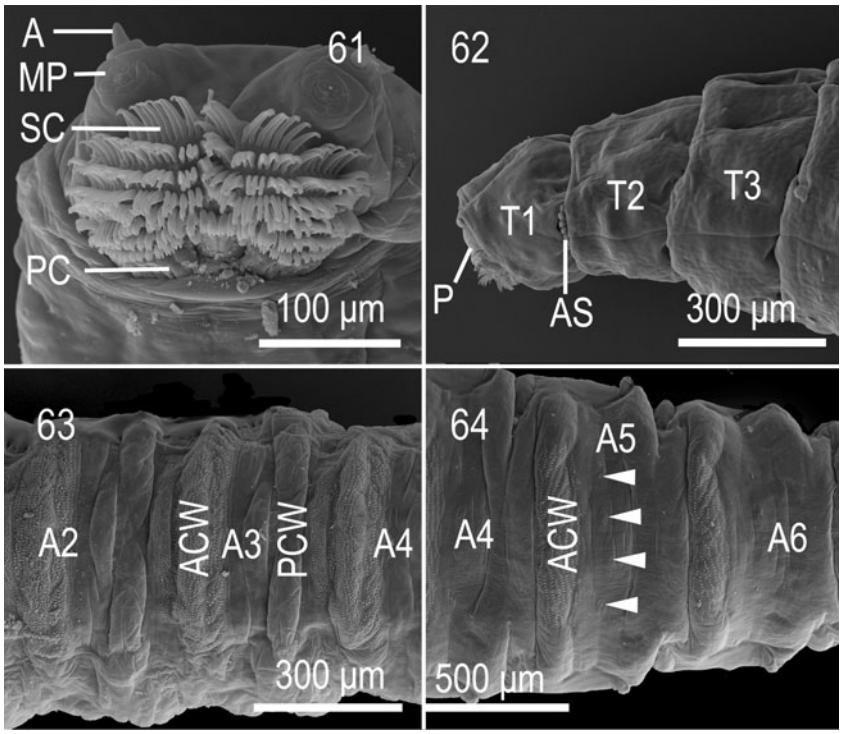
Figs. 53–56 *Meiosimyza platycephala*, third instar. **53** The pseudocephalon, ventral view. **54** The anterior spiracle, lateral view, the spiracle is forced to the body surface, probably during specimen manipulation. **55** Abdominal segments, dorsal view. **56** The last abdominal and the anal division, dorsal view. *A3–A7* Third to seventh abdominal segment, *ACW* anterior creeping welt, *AD* anal division, *LCW* lateral creeping welt, *DP* dorsal process of anal division, *LP* lateral processes, *MH* mouth hook, *PC* peristomal cirri, *PS* posterior spiracles

hyaline. The ventral bridge broad. The epistomal sclerite shorter than broad, almost rectangular, the anterior border convex, the posterior hyaline plate trapezoid. The subhypostomal sclerites not coalescing to the ventral bridge. The vertical plates relatively broader comparing to the remaining species.

Meiosimyza rorida. (Fig. 73). Length 0.65–0.71 mm. The mouth hooks almost parallel. The subhypostomal sclerites



Figs. 57–60 *Meiosimyza rorida*, third instar. **57** The abdominal segments, lateral view. **58** The anterior spiracle, lateral view, the spiracle was forced to surface probably during manipulation with specimen. **59** The abdominal segments, dorsal view. **60** Detail of the anal division showing small pad behind the ambulatory lobes and the posterior spiracles, note the two peg sensilla, ventral view. *ACW* Anterior creeping welt, *A3–A6* third to sixth abdominal segment, *LCW* lateral creeping welt, *LP* lateral process of anal division, *PS* posterior spiracles, *VP* ventral pad. *Arrowheads* Tubercles of abdominal segments



Figs. 61–64 *Tricholauxania praeusta*, third instar. **61** The pseudocephalon, ventral view. **62** The thoracic segments, lateral view. **63** The abdominal segments, ventral view. **64** The abdominal segments, dorsal view. *A* Antenna, *ACW* anterior creeping welt, *AS* anterior spiracle, *MP* maxillary palpus, *P* pseudocephalon, *PC* peristomal cirri, *SC* scraping cirri, *T1–T3* first to third thoracic segment

adherent to the ventral bridge. The epistomal sclerite semilunar, the posterior hyaline process trapezoid. The dorsal bridge trapezoid, slightly longer than broad.

Meiosimyza subfasciata. (Fig. 74). Length 0.65–0.87 mm. The mouth hooks converging. The epistomal sclerite shorter than broad, the anterior margin convex, the posterior hyaline process trapezoid. The subhypostomal sclerite basally coalescing to the ventral bridge. The dorsal bridge longer than broad. The cibarial filter pigmented.

Tricholauxania praeusta. (Fig. 75). Length 0.633–0.746 mm. The mouth hooks subparallel. The ventral bridge with rounded incision in the posterior margin. The epistomal sclerite semilunar, the posterior hyaline plate oval. The subhypostomal sclerites free. The dorsal bridge trapezoid, longer than broad. The pharynx slightly pigmented.

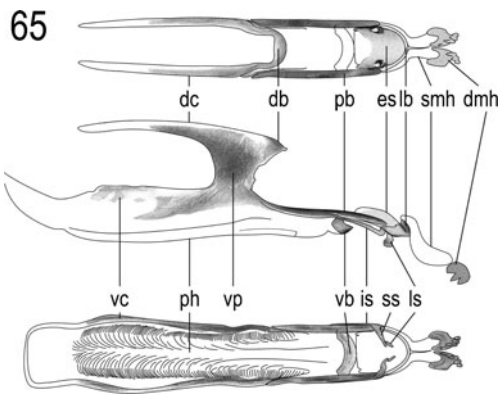


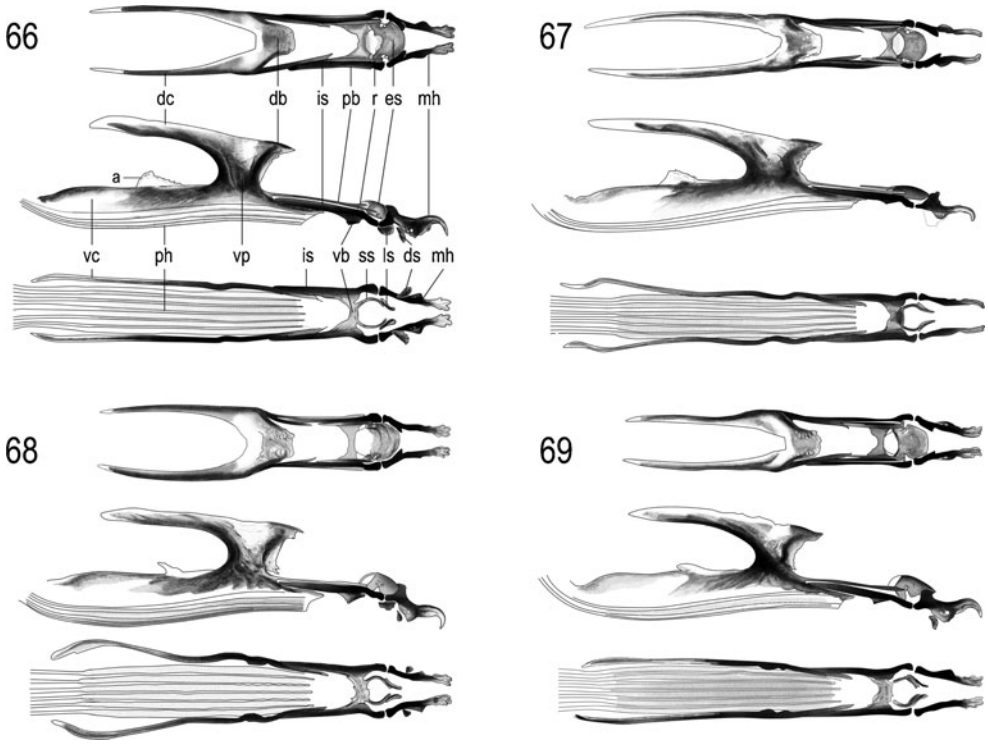
Fig. 65 The cephaloskeleton of the first instar of *Aulogastromyia anisodactyla*. From the top: dorsal, lateral and ventral view. *db* dorsal bridge, *dc* dorsal cornua, *es* epistomal sclerite, *lb* labrum, *is* intermediate sclerite, *ls* ligulate sclerite, *pb* parastomal bars, *ph* pharynx, *ss* subhypostomal sclerite, *vc* central cornua, *vp* vertical plate

The puparium

Brownish to cinnamon, lenticular from the dorsal view, moderately dorsoventrally flattened. Anterior end dorsoventrally flattened; the pseudocephalon and prothorax retracted up to the level of the anterior spiracles; the bases of spiracles and adjoining cuticle dark, but the papillae remain hyaline. The operculum trapezoid in dorsal view. The first thoracic segment corrugated in medial part. The abdominal segments are homogenous, laterally with sutures delimiting the lateral creeping welt of larva. The abdominal segments near rear end marginally flattened. The anal division conical, the ambulatory lobes retracted.

Aulogastromyia anisodactyla. (Figs. 76, 77). Length 3.71–4.02 mm ($n=4$); rather slender comparing to the other species. The lateral suturae, apical and lateral incisions of the operculum well developed. The medial part of the first thoracic segment polygonally corrugated, the following segments transversely wrinkled. The anal division with distinct processes.

Meiosimyza decempunctata. (Figs. 78, 79). Length 4.96 mm. ($n=1$); robust, dorsal side convex. The first part of the operculum corrugated, the second part transversally



Figs. 66–69 The cephaloskeletons of second instars, from the top: dorsal, lateral and ventral view. **66** *Aulogastromyia anisodactyla*. **67** *Meiosimyza platycephala*. **68** *M. rorida*. **69** *Tricholauxania praeusta*. *a* apodeme of ventral cornua; *db* dorsal bridge; *dc* dorsal cornua; *es* epistomal sclerite; *lb* labrum; *mh* mouth hook; *is* intermediate sclerite; *ls* ligulate sclerite; *pb* parastomal bars; *ph* pharynx; *ss* subhypostomal sclerite; *vc* central cornua; *vp* vertical plate

corrugated, the third and fourth parts finely transversally wrinkled anteriorly. The processes of the anal division only slightly indicated.

Meiosimyza platycephala. (Figs. 80, 81). Length: 3.35 mm ($n=1$). The apical incision slightly indicated; the corrugation of the first thoracic segment forms longitudinal wrinkles on the anterior margin, the polygonal corrugation extends to the second thoracic segment. The anal division short, the posterior spiracles shortened, processes of the anal division slightly indicated.

Meiosimyza rorida. (Figs. 82, 83). Length 3.45–3.76 mm. The operculum trapezoid, the apical incision well developed. The first thoracic segment with polygonal corrugation in the medial part. Processes of the anal division slightly indicated.

Meiosimyza subfasciata. No puparium was acquired.

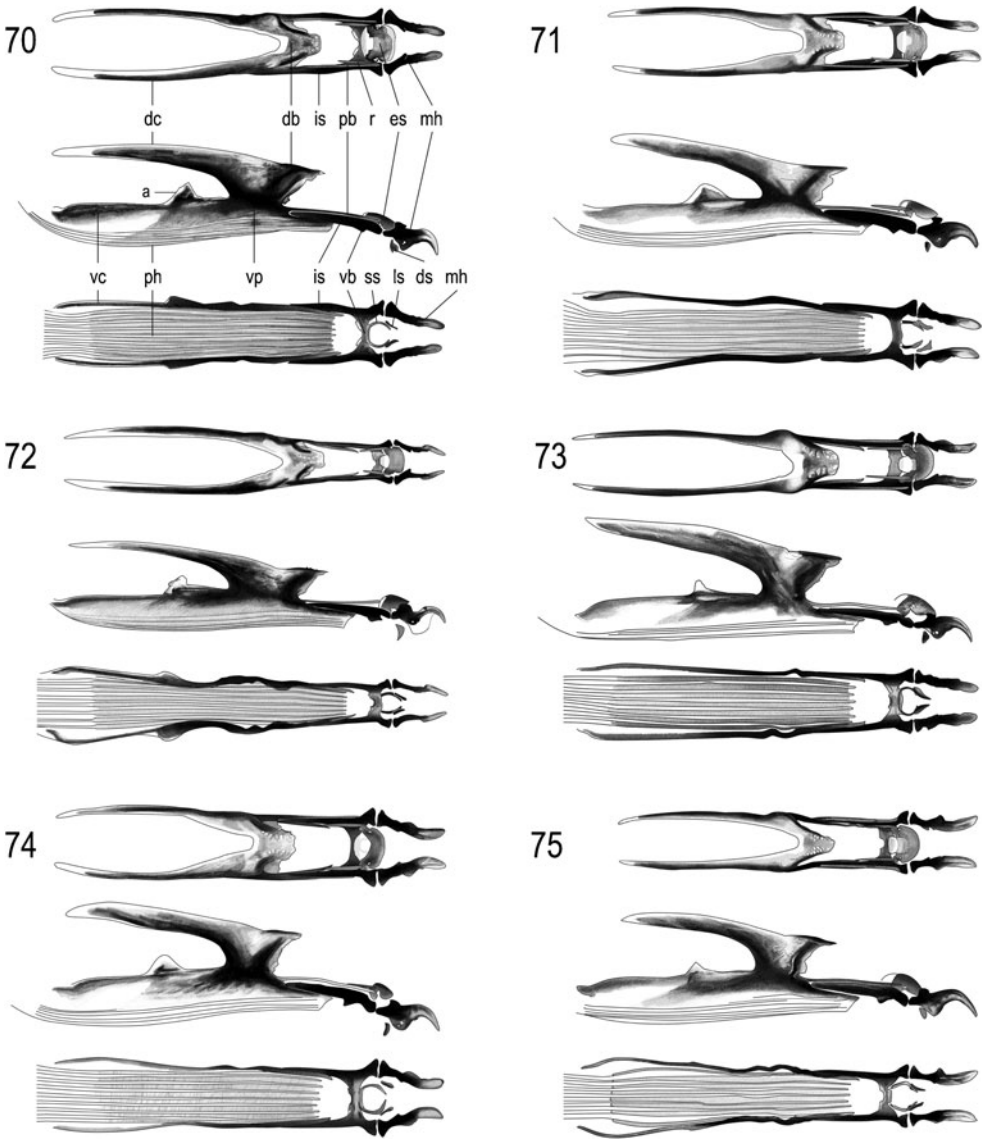
Tricholauxania praeusta. (Figs. 84, 85). Length 3.72–4.24 mm ($n=2$). The apical incision well developed; the first thoracic segment with polygonal corrugation in the medial part, the lateral parts smooth. The processes of anal division well developed.

Discussion

The eggs show typical lauxaniid characters with the well-developed ridges and spongy posterior tubercle (Miller and Foote 1976; Gaimari 2004; Gaimari and Silva 2010). The sculpture of the egg chorion is rather diverse comparing to the external morphology of larvae. We can divide the eggs of studied species in two types: the first one has sharp ridges and tuberculous chorion (*M. decempunctata*, *M. rorida* and *T. praeusta*) while the second has rather longitudinal rows of bullets instead of a ridge (*A. anisodactyla* and *M. platycephala*). *M. subfasciata* appears somewhat intermediate, as the ridges are swelled and the form of posterior pole much resembles that of *A. anisodactyla* and *M. platycephala*. However, *M. affinis* (Zetterstedt 1847) and *M. laeta* (Zetterstedt 1838) (unpublished results) also have swelled ridges near the posterior pole; in addition they have small pits in the inter-ridge space, exactly as *M. decempunctata*. Rather surprisingly, the egg of *T. praeusta* is almost identical to that of *M. decempunctata*.

The external morphology of larvae is very uniform: all species are rather smooth, giving a glassy shining appearance to the living larvae, some differences occur in the spine pattern of the last abdominal segments, but these differences may arise from the low number of specimens studied. Some interspecific differences exhibit the ventral organ of the first instar.

The ventral lobe behind the ambulatory lobes is probably homologous to the ventral pair of processes of the anal division of *Lauxania, Calliopum* and *Minettia longipennis* as suggested by the presence of peg sensilla (Semelbauer and Kozánek 2011, 2012). Another distinct feature is the villous internal surface of labial lobe, developed also in species of *Lauxania* and *Calliopum* (Semelbauer and Kozánek 2012).



Figs. 70–75 The cephaloskeletons of the third instars, from the top: dorsal, lateral and ventral view. **70** *Aulogastromyia anisodactyla*. **71** *Meiosimyza decempunctata*. **72** *M. platycephala*. **73** *M. decempunctata*. **74** *M. subfasciata*. **75** *Tricholauxania praeusta*. *a* apodeme of ventral cornua; *db* dorsal bridge; *dc* dorsal cornua; *es* epistomal sclerite; *lb* labrum; *mh* mouth hook; *is* intermediate sclerite; *ls* ligulate sclerite; *pb* parastomal bars; *ph* pharynx; *ss* subhypostomal sclerite; *vc* central cornua; *vp* vertical plate

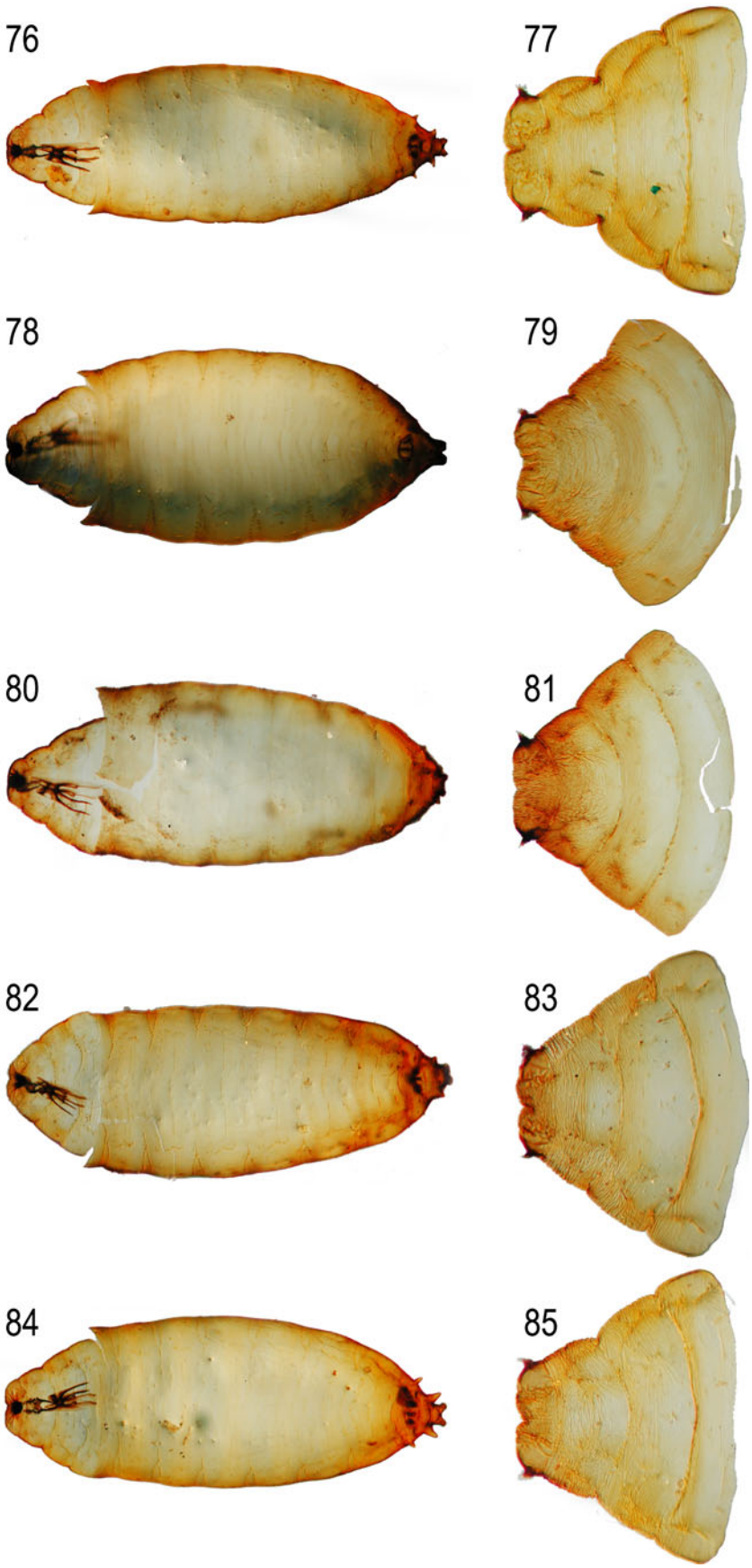
Though the hairs are not visible in every figure due to retracted pseudocephalon, they are clearly recognisable in the light microscope. The view through light microscope reveals that villous bulb of labial lobe freely connects to tips of ligulate sclerites. The hairs of labial lobe probably serve as a brush and help to ingest food. The remaining features are uniform with the other lauxaniids (Semelbauer and Kozánek 2011, 2012).

The cephaloskeleton exhibits basically the same organisation, as in other lauxaniid species (Semelbauer and Kozánek 2011; Miller and Foote 1976). The first instar is typical in having five to seven teeth and hyaline stalked part of the mouth hooks. The cibarial filter is not developed in its typical form (Dowding 1967), as in later instars. The shape of the cephaloskeleton is fully uniform among all studied species.

The second and third instars have arched subhypostomal sclerites fusing to the ventral bridge, character missing in remaining studied species (Miller and Foote 1976; Semelbauer and Kozánek 2011). Interspecific differences are poorly developed. Only *M. platycephala* can be recognised easily according to weakly developed mouth hooks, in the second instar with only two apical teeth. *A. anisodactyla* can also be recognised relatively easily by shape of the epistomal sclerite.

The puparium is not covered by the calcareous matter, as is typical in several other lauxaniids (Semelbauer and Kozánek 2011; Miller and Foote 1976). The puparia of *Calliopum* and *Lauxania* can be recognised easily according to the dark marking on the first thoracic segment. The interspecific differences are poorly developed.

We conclude that the larval instars together with puparia are very conservative and provide only limited number of characters, e.g. for species determination. Contrary to the larvae, eggs are rather variable what is in accordance with previous knowledge (Meier and Lim 2009).



Figs. 76–85 The puparia (*left column*) and respective operculum (*right column*). **76** *Aulogastromyia anisodactyla*, ventral view. **77** *Aulogastromyia anisodactyla*, operculum. **78** *Meiosimyza decempunctata*, ventral view. **79** *Meiosimyza decempunctata*, operculum. **80** *M. platycephala*, dorsal view. **81** *M. platycephala*, operculum. **82** *M. rorida*, ventral view. **83** *M. rorida*, operculum. **84** *Tricholauxania praeusta*, ventral view. **85** *Tricholauxania praeusta*, ventral view

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