Acta Zoologica (Stockholm) **86**: 119–134 (April 2005)

Comparative morphology of the first instar of three species of *Metopia* Meigen (Diptera: Sarcophagidae, Miltogramminae)

K. Szpila[1] and T. Pape[2]

[1]Nicolas Copernicus University, Institute of Ecology and Environmental Protection, Department of Animal Ecology, Gagarina 9, PL-87-100 Toruń, Poland; [2]Zoological Museum, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

Keywords:
cephaloskeleton, comparative morphology, first instar, larva, *Metopia*, Miltogramminae, scanning electron microscopy

Accepted for publication:
11 March 2005

Abstract

Szpila, K. and Pape, T. 2005. Comparative morphology of the first instar of three species of *Metopia* Meigen (Diptera: Sarcophagidae, Miltogramminae). — *Acta Zoologica* (Stockholm) **86**: 119–134

The first instar larva is described for three species of the kleptoparasitic miltogrammine genus *Metopia* Meigen: *M. campestris* (Fallén), *M. argentata* Macquart and *M. argyrocephala* (Meigen). Using a combination of light microscopy and scanning electron microscopy, the morphology of the cephaloskeleton as well as the general external morphology are extensively documented, and the phylogenetic implications are discussed. Like other species of Miltogramminae, the first instar of species of *Metopia* possesses a strong labrum and well-developed mouth-hooks. Some other features found in *Metopia* spp. are rare in the Miltogramminae, such as a serrated ventral surface of the tip of the mouth-hook and the lack of a posterior spiracular cavity. A few larval features apparently unique for species of *Metopia* have so far been documented: base of mouth-hook with a lateral arm-like extension and abdominal segments with transverse furrow ventrally. The body is equipped with longitudinal cuticular ridges on all segments, which may be a subfamily ground-plan autapomorphy. Marked morphological and behavioural differences are documented between the first instar of *M. argentata* and that of *M. argyrocephala*, the adult females of which are otherwise difficult to separate.

K. Szpila, Nicolas Copernicus University, Institute of Ecology and
Environmental Protection, Department of Animal Ecology, Gagarina 9,
PL-87-100 Toruń, Poland. E-mail: szpila@biol.uni.torun.pl

Introduction

The kleptoparasitic species of the flesh fly subfamily Miltogramminae are characterized by a remarkable diversity in the external morphology of the first instar (Thompson 1921; Szpila and Pape, in press). Too few genera and species have been documented to make firm generalizations about the utility of first instar morphology in higher level phylogeny, generic circumscriptions and species identification, although a picture of significant potential is emerging. Very few data have been published on the morphology of the preimaginal stages of miltogrammine flesh flies, and most data relate to the cephaloskeleton (see review by Ferrar 1987). Thompson's descriptions and figures of the larvae of 12 species of Miltogramminae were a pioneering study (Thompson 1921), but the morphological documentation was restricted by the techniques available (i.e. light microscope) and by being limited to material from dissections of dried female specimens. In the case of first instars dissected from dry museum material, only the more sclerotized structures such as the cephaloskeleton and spinulation can be credibly described. Moreover, it is sometimes difficult to assess the degree of development of the first instars obtained in this way (Schumann 1974; Szpila 2004).

The present paper is a redescription of the morphology of the first instar of three species of *Metopia* Meigen: *M. argentata* Macquart, *M. argyrocephala* (Meigen) and *M. campestris* (Fallén), using both scanning electron microscopy (SEM) and light microscopy to show the potential for species-level identification and the prospects for phylogeny. The genus *Metopia* Meigen contains 38 described species from all biogeographical regions, and the genus is well defined in the adult stage by the unique arrangement in all included species of two proclinate and two reclinate fronto-orbital bristles in

Table 1 Numbers of first instars of *Metopia* Meigen obtained and their localities

Species	Location/UTM code	Specimen	Number of larvae
M. argentata	Zbocza Płutowskie reserve/CE20	‘M11’	9
M. argentata	Zbocza Płutowskie reserve/CE20	‘M12’	14
M. argentata	Grudziądz/CE42	‘Mros10’	11
M. argentata	Grudziądz/CE42	‘Mros11’	15
M. argentata	Grudziądz/CE42	‘Mros12’	8
M. argentata	Grudziądz/CE42	‘Mros13’	7
M. argentata	Grudziądz/CE42	‘Marg1 2004’	12
M. argyrocephala	Toruń airfield/CD37	‘M1’	17
M. argyrocephala	Toruń airfield/CD37	‘M2’	21
M. argyrocephala	Toruń airfield/CD37	‘M7’	23
M. argyrocephala	Toruń airfield/CD37	‘M9’	34
M. argyrocephala	Toruń airfield/CD37	‘M13’	13
M. argyrocephala	Toruń airfield/CD37	‘M16’	16
M. argyrocephala	Toruń airfield/CD37	‘M17’	14
M. argyrocephala	Toruń airfield/CD37	‘M1 2003’	22
M. argyrocephala	Zbocza Płutowskie reserve/CE20	‘M3’	16
M. argyrocephala	Zbocza Płutowskie reserve/CE20	‘M15’	14
M. argyrocephala	Zbocza Płutowskie reserve/CE20	‘M20’	28
M. campestris	Czarny Bryńsk/DD19	‘Mcamp1’	11
M. campestris	Czarny Bryńsk/DD19	‘Mcamp2’	10
M. campestris	Czarny Bryńsk/DD19	‘Mcamp3’	14
M. campestris	Czarny Bryńsk/DD19	‘Mcamp4’	14

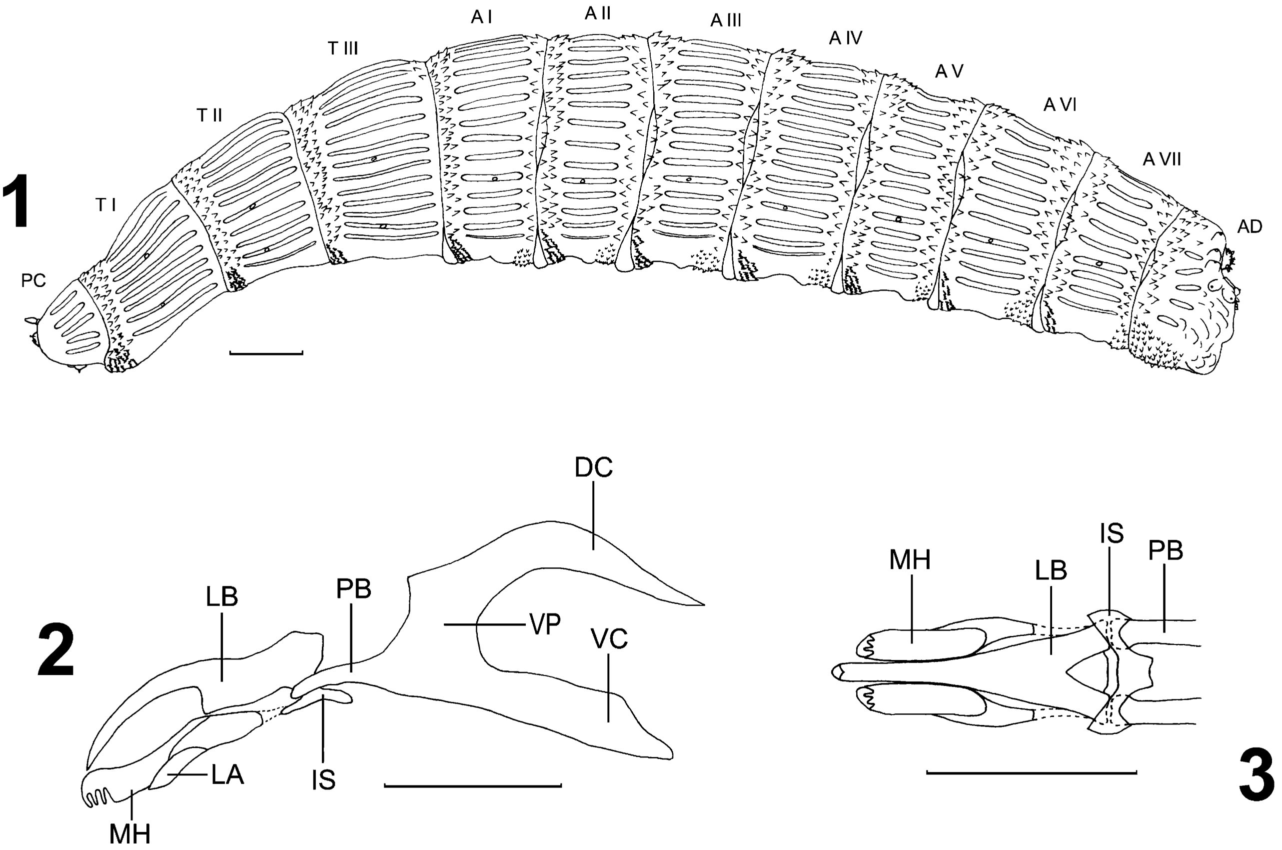
partly overlapping rows (Pape 1996). Species of *Metopia*, like many other Miltogramminae, are kleptoparasites of a wide range of solitary wasps and bees (Aculeata: Vespidae, Sphecidae, Pompilidae, Crabronidae, Halictidae, Apidae). The only known exception is *M. sinensis* Pape, which is a predator of *Liphistius* spiders (Schwendinger and Pape 2000). The host range for Palaearctic *Metopia* has been summarized by Pape (1987a) and Povolný and Verves (1997), and for Nearctic *Metopia* by Spofford and Kurczewski (1990, 1992).

The three species dealt with in the present paper are common and widespread. *Metopia argyrocephala* occurs in the Neotropical, Holarctic and Oriental regions, *M. campestris* in the Holarctic and Oriental regions, while *M. argentata* is restricted to the Palaearctic and Oriental regions (Pape 1996). *Metopia campestris* and *M. argyrocephala* are frequently encountered in sandy habitats with psammophilic vegetation. They prefer such habitats as midland dunes, forest edges, fallows and sandbars in river valleys. Both species are typical kleptoparasites with hosts apparently restricted to the Apoidea and Pompilidae. *Metopia argentata* is possibly more specialized, with only one species of pompilid wasp, *Batozonellus lacerticida* (Pallas 1771), recorded as host of this fly (Rohdendorf and Verves 1980). Larviposition behaviour of *Metopia* has been thoroughly studied for only two species, the East Palaearctic *M. sauteri* (Townsend) and *M. campestris* mentioned above (Endo 1980; Wcislo 1986). According to the classification of Spofford and Kurczewski (1990), *Metopia* spp. are typical ‘hole searchers’, with the female seeking out the host nest using visual cues during a characteristic searching flight. After finding the hole in the ground, the female checks it for presence of the wasp and stored prey using olfactory cues. If the nest is provisioned, the fly larviposits, and in most cases the first instar will actively seek out the wasp’s food supplies. This short prefeeding period of the first instars is possibly the reason for the great morphological diversity of this instar in the Miltogramminae (Szpila and Pape, in press).

Materials and methods

Larval material was obtained by keeping wild-caught females under laboratory conditions. Females were collected at four sites in northern Poland (Table 1) and identified using the keys of Pape (1987a) and Povolný and Verves (1997), either directly or through the males obtained from rearing, but see the discussion below for further detail on the identification of females of *M. argentata*. To obtain larvae, each freshly caught female was kept in a 50 mL plastic vial with a finely perforated cover and with a layer of filter paper in the bottom. During the following days, females, if gravid, spontaneously and repeatedly larviposited, and the first instars were immediately collected and processed as explained below. After the death of the females, additional larvae were extracted from their abdomen by carefully cutting the membrane between segments VI and VII and gently squeezing the abdomen. All females were subsequently pinned and labelled (deposited in coll. Szpila).

Larvae were killed by soaking in hot water (just after boiling, about 95 °C) to avoid deformation and stored in 70% alcohol. In case of offspring of the females from the *argyrocephala* species-group (sometimes separated as subgenus



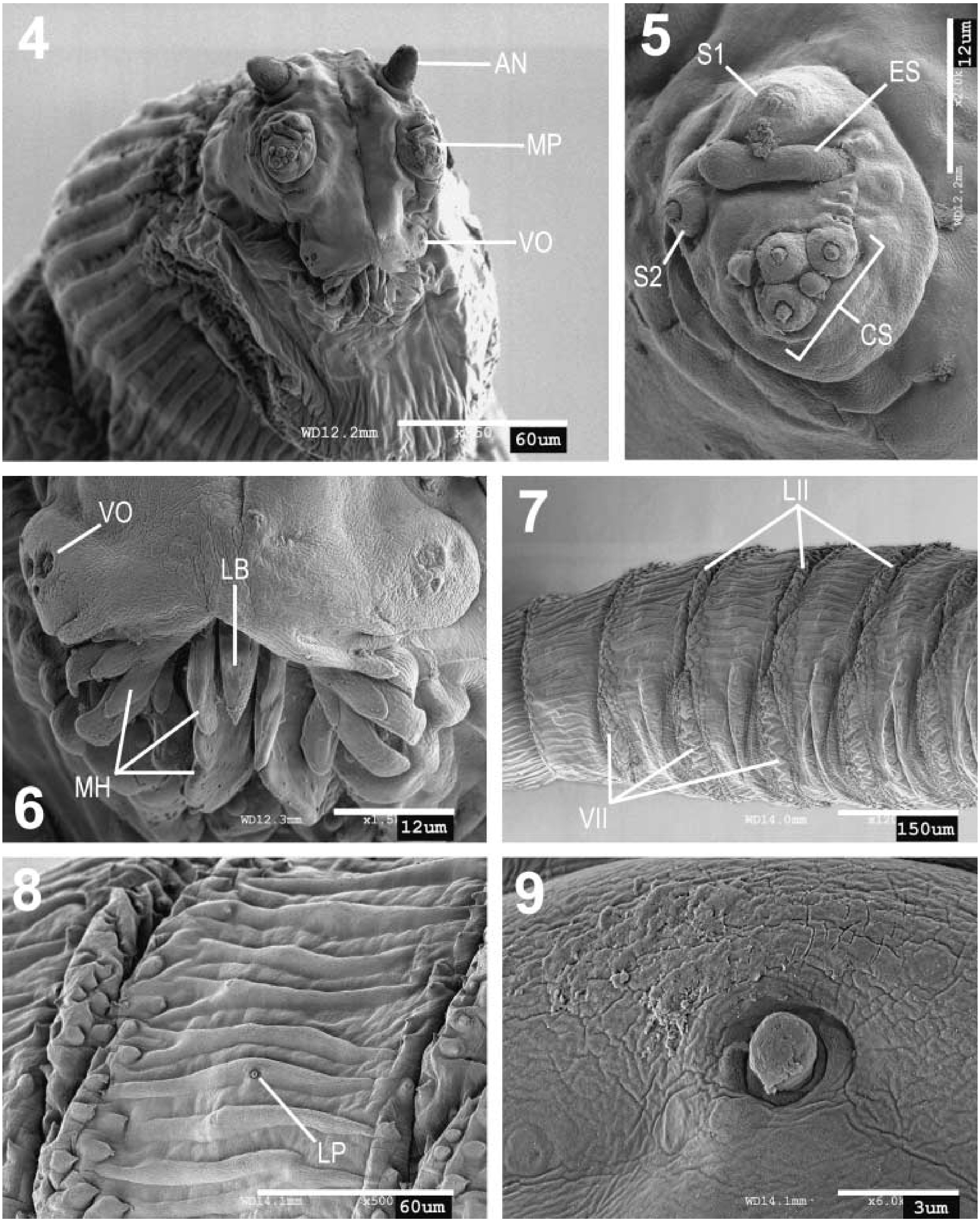
Figs 1–3—First instar of *Metopia argentata* (Macquart 1850). —**1.** Whole body, lateral view. —**2.** Cephaloskeleton, lateral view. —**3.** Cephaloskeleton, ventral view. Scales = 0.1 mm. Abbreviations: AI–AVII = abdominal segments, AD = anal division, DC = dorsal cornua, IS = intermediate sclerite, LA = lateral arm of mouth-hook, LB = labrum, MH = mouth-hook, PB = parastomal bar, PC = pseudocephalon, TI–TIII = thoracic segments, VC = ventral cornua, VP = vertical plate.

Metopia (s. str.), e.g. Rohdendorf 1971) a number of larvae were offered food in an attempt to rear adult males required for exact species identification. Freshly killed flies from various families (Calliphoridae, Syrphidae, Therevidae) were used for feeding. Preserved larvae were slide-mounted in Hoyer's medium for light microscopy on concave (Figs 1, 3, 16–18, 32 and 34) or flat slides (other figures). Preparation for SEM involved dehydration through 80, 90 and 99.5% ethanol and critical-point drying in CO_2 (for JSM-6300) or soaking for 2 × 30 min in hexamethyldisilazane (HMDS) followed by air-drying under a fume hood (for Hitachi S-4300). In both cases the larvae were eventually coated with gold. Illustrations were produced from photographs made using a digital Axiocam HRc camera mounted on a Zeiss Axioskop 2 plus microscope. SEM pictures were taken with a Jeol Scanning Microscope JSM-6300 (Figs 26, 27 and 31) and a Hitachi S-4300 Scanning Electron Microscope (other SEM pictures).

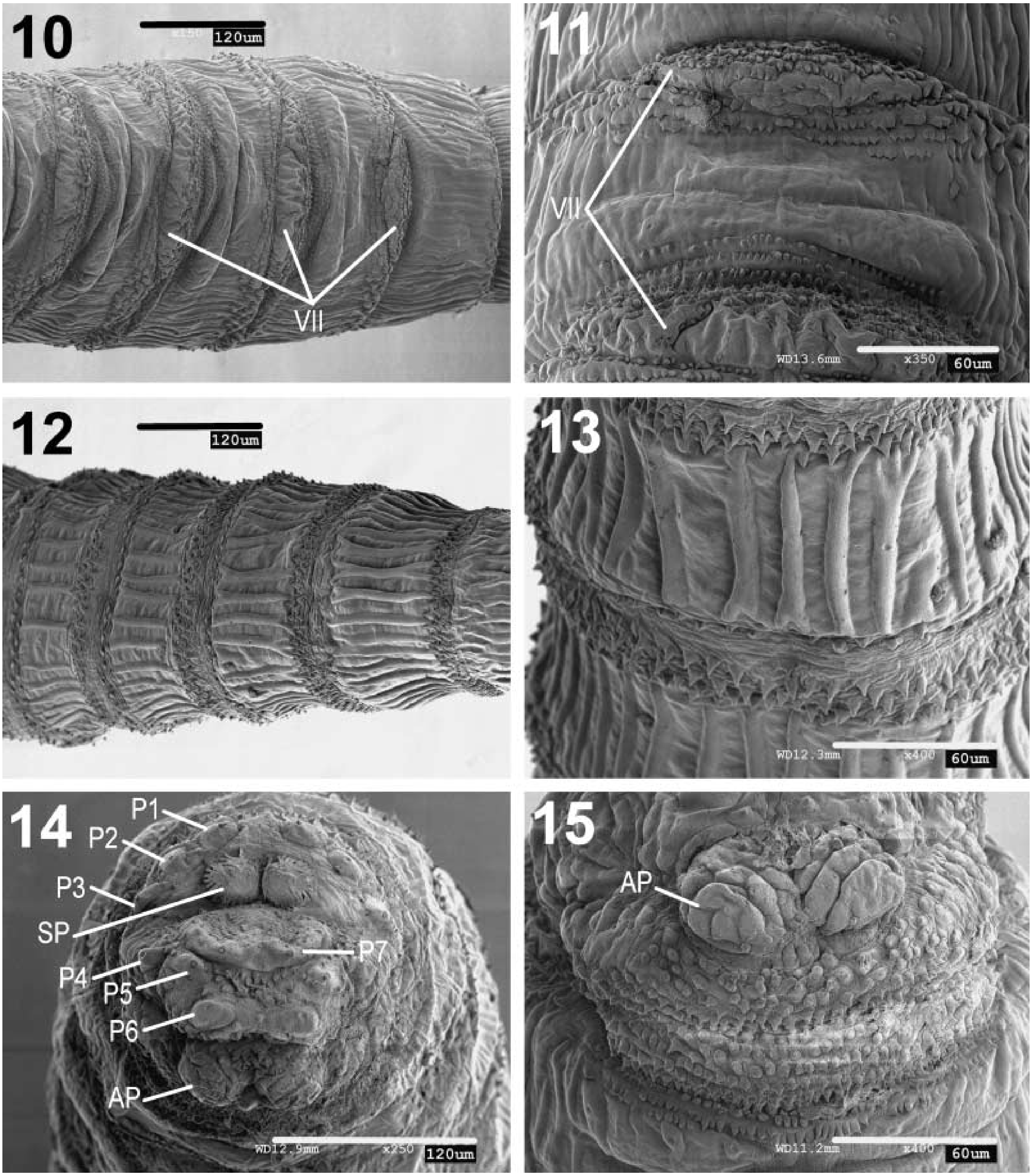
The terminology follows Courtney *et al.* (2000) with a few modifications proposed by Szpila and Pape (in press), but it should be noted that alternative terminology exists, e.g. Cobb (1999) and Oppliger *et al.* (2000).

Results

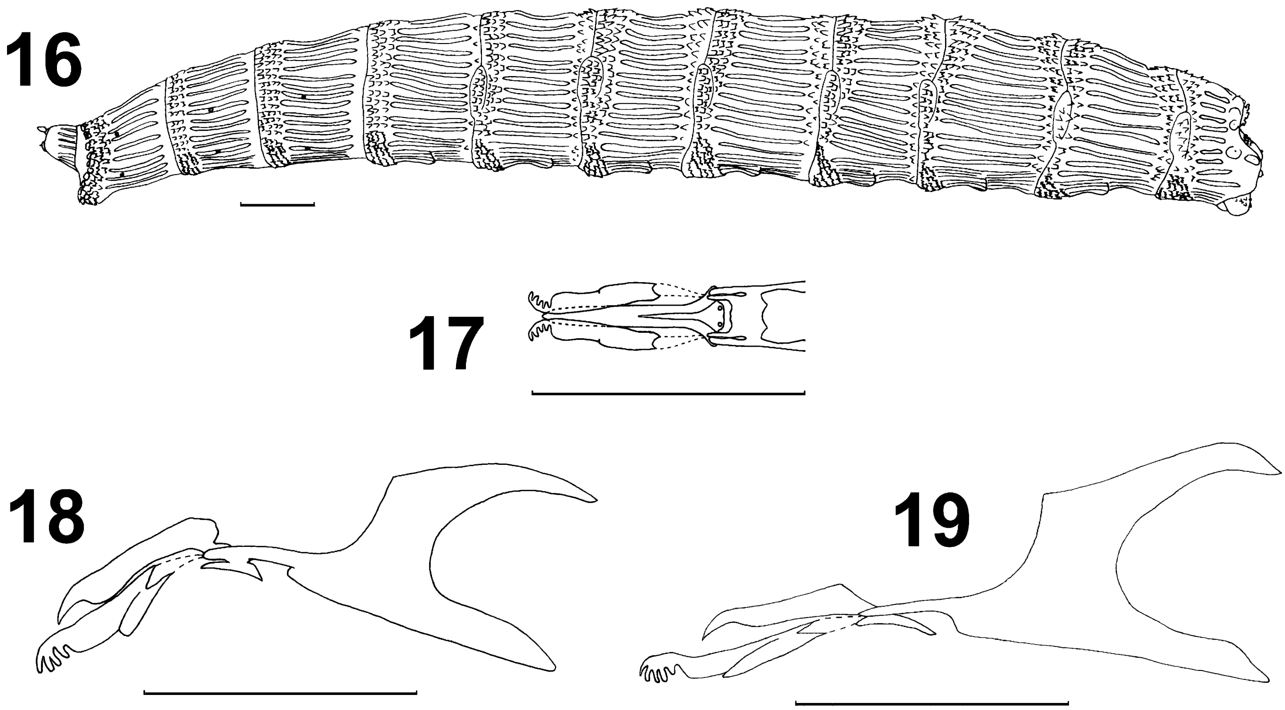
A total of 343 first instars were obtained from four females of *M. campestris*, seven of *M. argentata*, and 11 of *M. argyrocephala* (Table 1). The rearing attempts of *M. argentata* were unsuccessful as larvae did not develop. (Only one larva out of brood 'M11' developed to the second instar, and this larva was observed attacking and killing at least one other larva. In contrast, 34 larvae of *M. argyrocephala* completed development and none were unsuccessful.) Female identification was therefore performed from adult morphology alone, and it was noted that in addition to the lack of the median marginal bristles on abdominal tergite 1+2, which is the only (but not fully reliable) character given by Pape (1987a), the frons of female *M. argentata* is more protruding than in *M. argyrocephala*. Males of both *M. argentata* and *M. argyrocephala* were abundant in the study areas, while no males of



Figs 4–9—First instar of *Metopia argentata* (Macquart 1850). —**4**. Anterior end, antero-lateral view. —**5**. Maxillary palpus. —**6**. Mouth opening. —**7**. TI–AIII, lateral view. —**8**. AI, lateral view. —**9**. Lateral papilla. Abbrevations: AN = antennal socket sensillum, ES = elongated sensillum, LB = labrum, LII = lateral intersegmental insertion, LP = lateral papillae, MH = mouth-hooks, CS = clustered maxillary palpus sensilla, S1 = middle separated maxillary sensillum, S2 = dorsal separated maxillary sensillum, VII = ventral intersegmental insertions, VO = ventral organ.



Figs 10–15—First instar of *Metopia argentata* (Macquart 1850). —**10**. TIII–AIII, ventral view. —**11**. AI, ventral view. —**12**. TII–AII, dorsal view. —**13**. TIII, dorsal view. —**14**. Anal division, posterior view. —**15**. Anal division, ventral view. Abbrevations: AP = anal papillae, P1 = dorsal papillae, P2 = subdorsal papillae, P3 = supralateral papillae, P4 = infralateral papillae, P5 = subventral papillae, P6 = ventral papillae, P7 = supraventral papillae, SP = spiracles, VII = ventral intersegmental insertions.



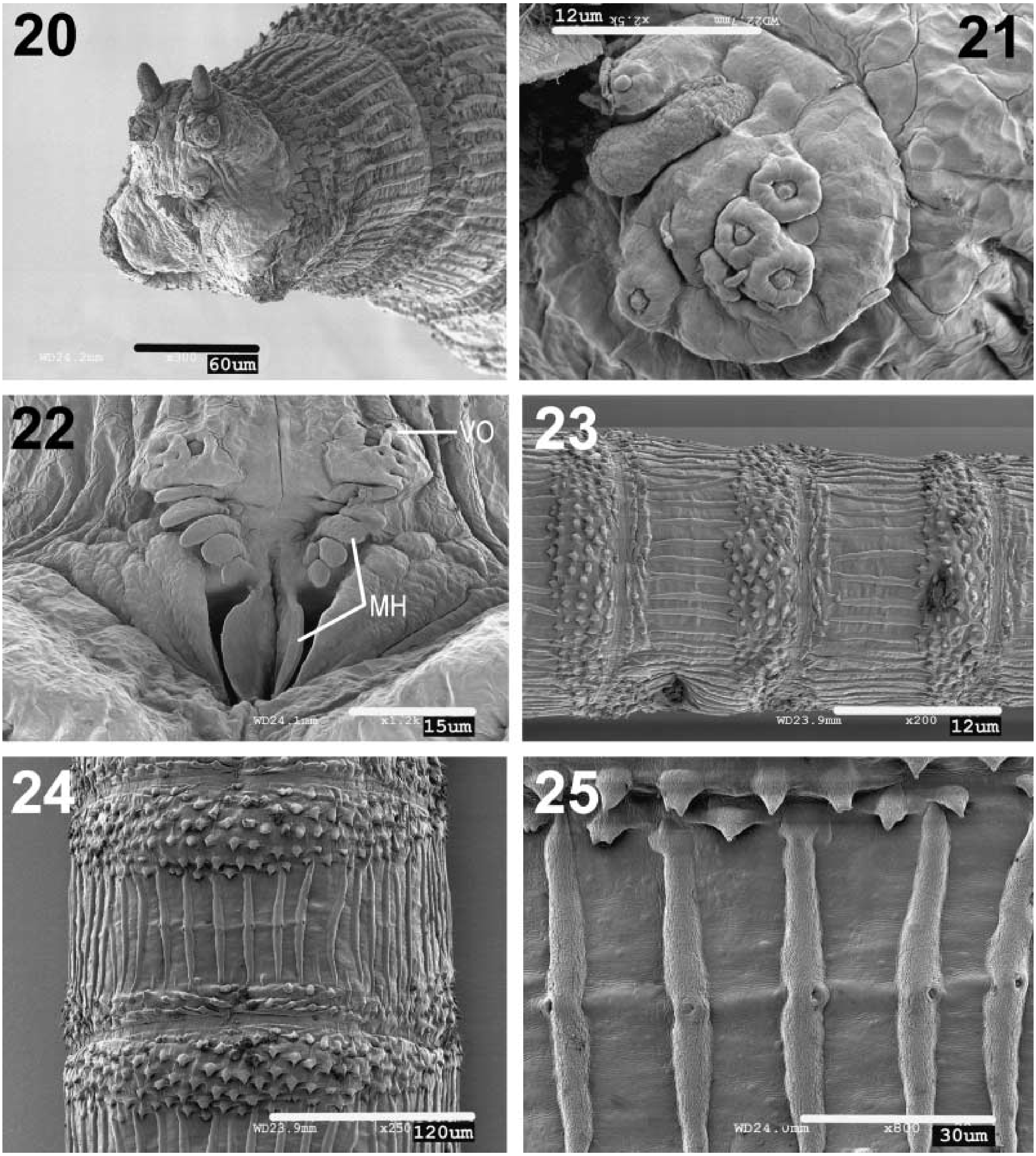
Figs 16–19—First instar of *Metopia argyrocephala* (Meigen 1824). —**16.** Whole body, lateral view. —**17.** Cephaloskeleton, ventral view. —**18.** Cephaloskeleton, lateral view. —**19.** Cephaloskeleton, lateral view. Scales = 0.1 mm.

M. staegerii Rondani were caught, which may be considered as support for the species identification. The latter species is sometimes separated along with the other two species of *Metopia* in the subgenus *Metopia* (s. str.), the females of which are very similar.

The body of the first instar in *Metopia* follows the general pattern for the Calyptrata in being divided into a bilobed pseudocephalon, three thoracic segments (termed TI–TIII below), seven abdominal segments (AI–AVII), and the anal division (AD) (Fig. 1). The entire body surface is covered by longitudinal cuticular ridges (Figs 1, 16 and 32), the thickness and number of which vary depending on species. Each of the pseudocephalic lobes has an antennal peg situated in a distinct socket on the dorsal surface (Figs 4, 20 and 35) (dorsal organ of Cobb 1999 and Oppliger *et al.* 2000). The cuticle behind the antenna is wrinkled by short, longitudinal ridges (Fig. 35). The maxillary palpus (terminal organ of Cobb 1999 and Oppliger *et al.* 2000) is located on the anterior surface of the pseudocephalic lobe and has the form of a flattened protuberance clearly distinguished from the ambient surface of the pseudocephalon (Figs 5, 21 and 36). In the central part of the maxillary palpus is a cluster of four distinct peg sensilla (MPS), three set in swollen sockets and one non-socketed (Fig. 5). Another non-socketed sensillum is situated above the MPS cluster, and this sensillum is strongly elongated and more or less spherically thickened at the tip. Two additional socketed peg sensilla (S1, S2, 'separated sensilla', apparently non-maxillary in origin (Courtney *et al.* 2000)) are arranged along the dorsal periphery of the maxillary palpus. Above and laterally to the mouth opening are the sensilla of the ventral organ (Figs 4, 6, 22 and 38). No oral ridges are present.

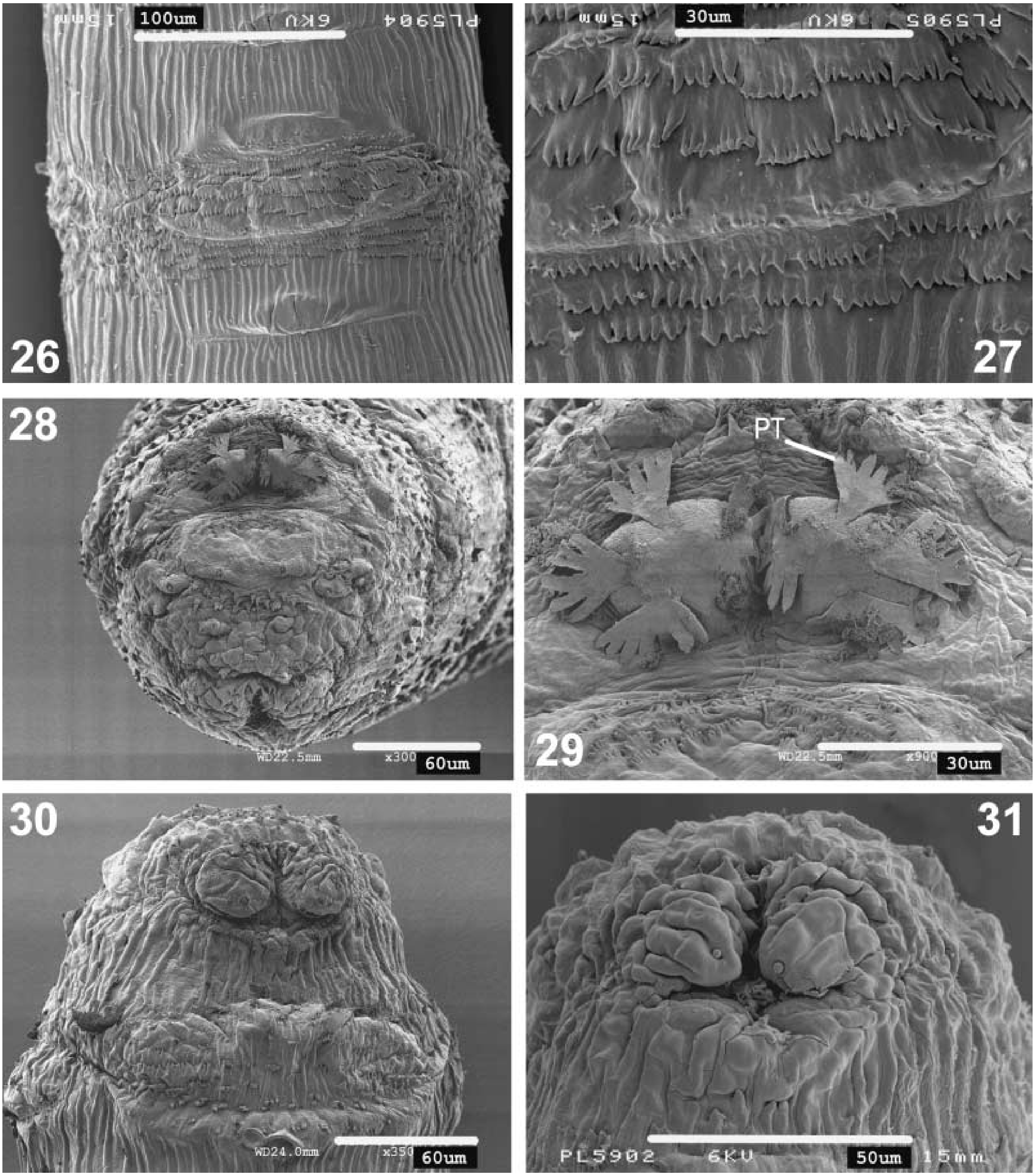
The cephaloskeleton consists of an unpaired labrum, paired mouth-hooks, an unpaired intermediate sclerite, paired parastomal bars, and paired vertical plates each with ventral and dorsal cornua (Figs 2 and 3). The labrum is strongly sclerotized and pointed. The basal part of the labrum is joined to the anterior part of the parastomal bars. The apical part of labrum has the form of a downward-curved pointed hook. The mouth-hooks are moderately sclerotized and may be equipped with a lateral arm-like extension at the base (Figs 2, 18, 19, 33, 34 and 45–48). The apical part of each mouth-hook is equipped with several teeth (Figs 2, 6, 18, 19, 22, 33, 34, 38 and 45–48). The weakly sclerotized intermediate sclerite is located between and ventral to the parastomal bars (Fig. 3). The vertical plate is wider than the dorsal cornua (Figs 2, 18, 19 and 33). Dorsal and ventral cornua are of a similar length and height. No dorsal bridge is present.

Segments TI–TIII are equipped with spinose bands (creeping welts) only anteriorly (Fig. 1), and the remaining segment surface is arranged in numerous longitudinal cuticular ridges. Spines on dorsal and lateral surfaces are individually separated from each other (Figs 13, 24 and 42), while the spines on the ventral surface are arranged in small

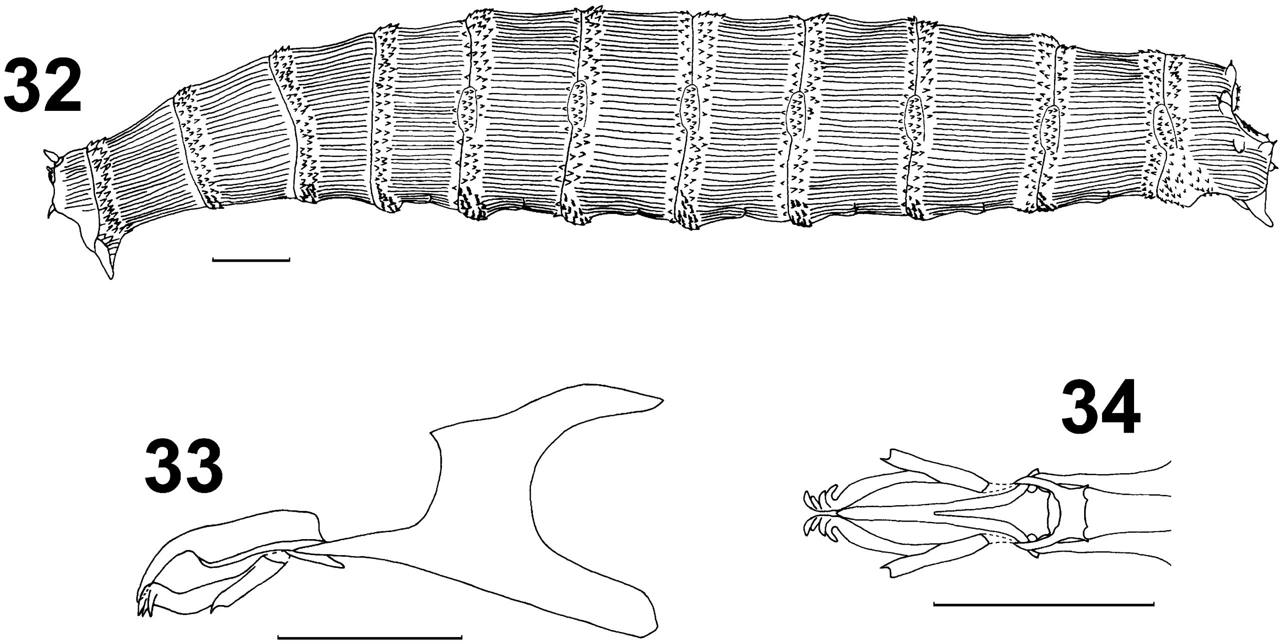


Figs 20–25—First instar of *Metopia argyrocephala* (Meigen 1824). —**20**. Anterior end, antero-lateral view. —**21**. Maxillary palpus. —**22**. Mouth opening. —**23**. AII–III, dorsal view. —**24**. AII, dorsal view. —**25**. AII, dorsal view.

clusters or short rows where spines are fused basally (Figs 11 and 27). A pair of typically developed Keilin's organs and two lateral papillae occur on each of TI–TIII. Segments AI–AVII are armed with both anterior and posterior spinose bands (Figs 1, 16 and 32). The anterior bands have three to five rows of spines on all abdominal segments, with numbers of rows decreasing toward the end of the body. The posterior bands have a single row of spines on AI and AII (sometimes



Figs 26–31—First instar of *Metopia argyrocephala* Meigen, 1824. —**26**. AII, ventral view. —**27**. AII, ventral view. —**28**. Anal division, posterior view. —**29**. Anal divison, spiracles. —**30**. Anal division, ventral view. —**31**. Anal division, anal opening. Abbrevations: PT = peristigmatic tuft.



Figs 32–34—First instar of *Metopia campestris* (Fallén 1810). —**32.** Whole body, lateral view. —**33**. Cephaloskeleton, lateral view. —**34**. Cephaloskeleton, ventral view. Scales = 0.1 mm.

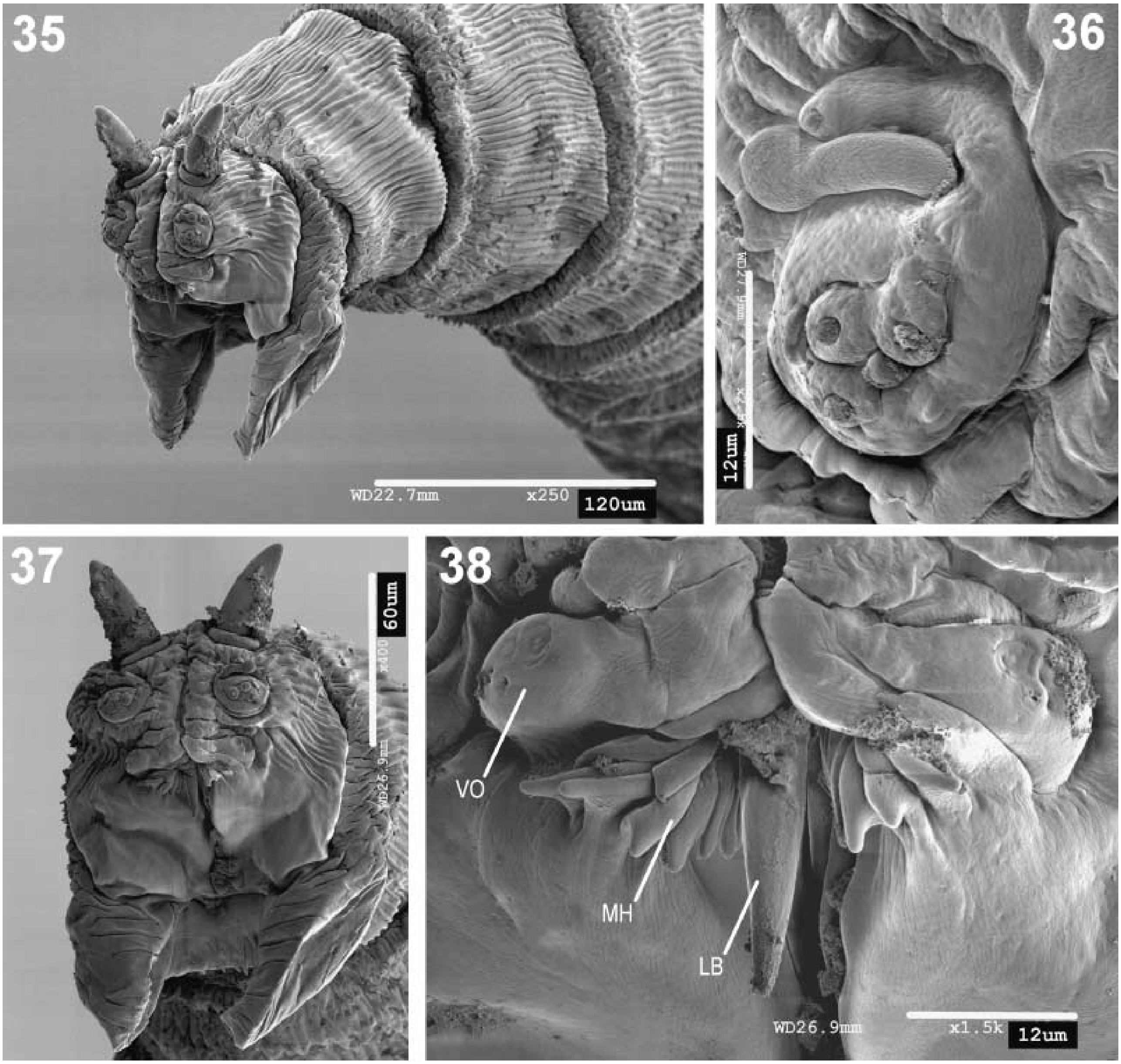
discontinuous) and the number of rows increases to four or five towards the anal division. The lateral surface of AI–AVII presents distinct intersegmental insertions covered by spines (Fig. 7), and unpaired intersegmental insertions occur also on the ventral surface (Figs 7, 11, 26, 39 and 40). Each of segments AI–AVII are equipped ventrally with a transverse furrow (Figs 11, 26 and 39) and dorsally with a few sensilla situated on the cuticular ridges (Fig. 25). Seven pairs of clearly visible papillae (P1–P7) are present on the anal division, with papillae P1, P3, P5 and P7 bearing an elongated apical sensillum (Figs 14, 28 and 43). Anal papillae are developed either as rather flat, dome-like structures as in *M. argentata* and *M. argyrocephala* (Figs 15 and 31), or they are carried on protuberances as in *M. campestris* (Figs 43 and 44). The antero-ventral surface of the anal division presents several rows of spines, the shape and arrangement of which show infraspecific variation (Figs 15, 30 and 44). Each of the posterior spiracles are surrounded by four peristigmatic tufts, with the median ones often reduced (Figs 14, 29 and 43).

Metopia argentata *Macquart 1850, Figs 1–15, 45, 46*

Antennae are relatively short, 1.5–2.0 times ($n = 10$) as long as the diameter at base (Fig. 4). Labrum is strongly sclerotized with a very long apical tooth, its length constituting at least half the length of the labrum (Fig. 2). The mouth-hooks are equipped with a lateral arm at the base (Figs 2 and 46), although this is not clearly visible in ventral view, and the serrations along the tip are weakly sclerotized (Fig. 45). Near the base of the elongated sensillum of the maxillary palpus are a few additional small sensilla (Fig. 5). Cuticular ridges are broad and sparsely arranged, and the ridges on the ventral surface of AI–AVII are indistinct (Figs 10 and 11). On the ventral surface of AI–AVII are unpaired intersegmental insertions that are covered by spines in their anterior parts (Figs 10 and 11). Anterior and posterior to the tranverse furrow there are two distinct transverse protuberances (Fig. 11). On the surface of the posterior one are a few sensilla. Numerous separately arranged spines are distributed on the antero-ventral surface of the anal division (Fig. 15). These spines cover the entire surface from the anterior edge of the division to the anal opening. Anal papillae are dome-like (Fig. 15).

Metopia argyrocephala *(Meigen 1824), Figs 16–31, 47*

Antennae are moderately long, 2.0–2.5 times ($n = 10$) as long as the diameter at base (Fig. 20). Labrum is moderately sclerotized with a short apical tooth, its length constituting at most one-third the length of the labrum (Figs 18 and 19). Each of the mouth-hooks is equipped with a distinct lateral arm at the base (Figs 18 and 19), and the serrations along the tip are weakly sclerotized. Cuticular ridges are moderately broad and sparsely arranged (Figs 24 and 25), but ridges are distinctly developed on the ventral surface of AI–AVII (Fig. 26). The ventral intersegmental insertions are covered by clustered spines in their anterior parts (Figs 26 and 27). Anterior to the transverse furrow is a flattened protuberance

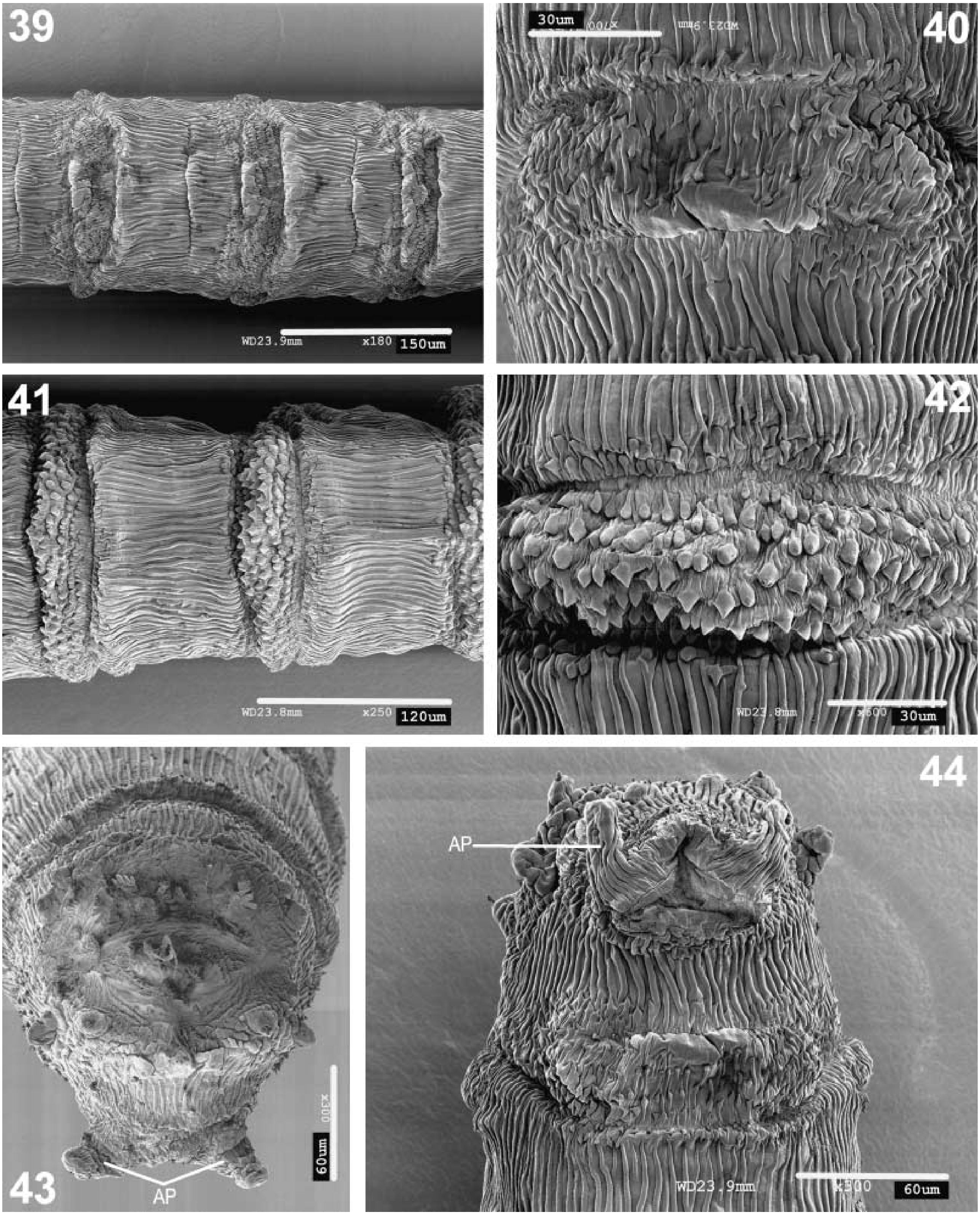


Figs 35–38—First instar of *Metopia campestris* (Fallén 1810). —**35**. Anterior end, antero-lateral view. —**36**. Maxillary palpus. —**37**. Pseudocephalon and TI, anterior view. —**38**. Mouth opening.

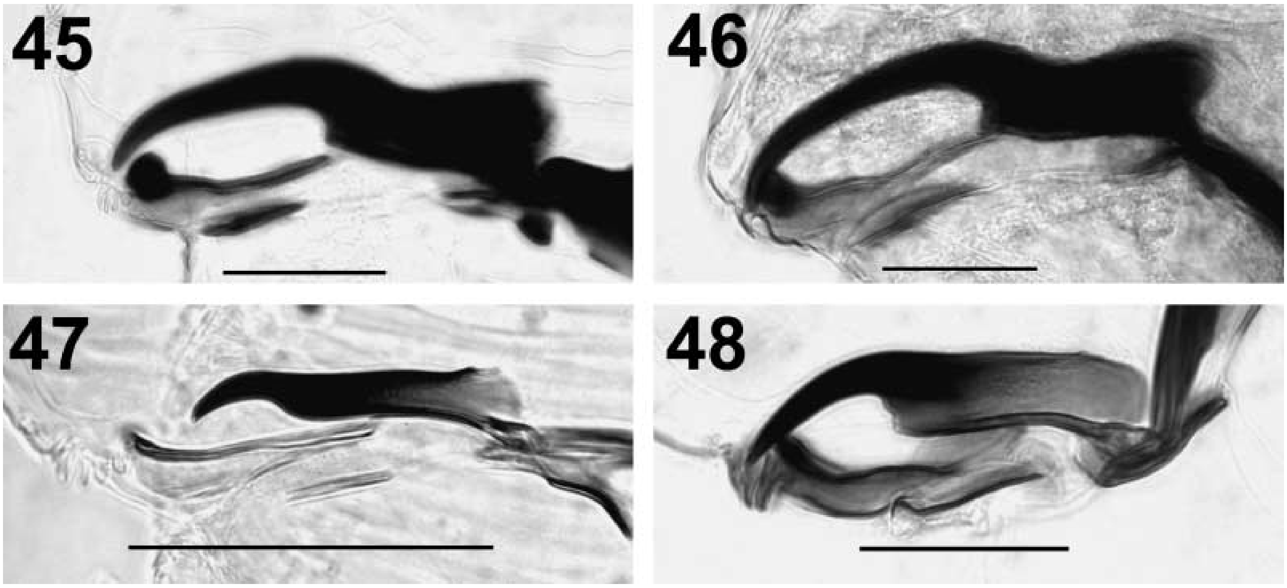
cut by a longitudinal crevice (Fig. 26). A moderate number of spines are clustered in small groups on the antero-ventral surface of the anal division (Fig. 30). Anal papillae are dome-like (Figs 30 and 31).

Metopia campestris *(Fallén 1810), Figs 32–44, 48*

Antennae are large and elongated, 3.0–3.5 times (*n* = 10) as long as the diameter at base (Fig. 35). Labrum is strongly sclerotized with a long apical tooth, its length constituting at least one-third (and at most half) the length of the labrum (Figs 33 and 48). Each of the mouth-hooks is equipped with a lateral arm at its base (Figs 33, 34 and 48), and the serrations along the tip are strongly sclerotized. Cuticular ridges are narrow and densely arranged, with ridges clearly developed ventrally on AI–AVII (Figs 39–42). On the antero-ventral edge of TI are two very large, elongated protuberances (Fig. 35, 37). The ventral unpaired intersegmental insertions are almost entirely covered by spines (Figs 39 and 40). Spines on the antero-ventral surface of the anal division are rather sparse and do not reach the anal opening (Fig. 44). Anal papillae are situated on raised protuberances (Figs 43 and 44).



Figs 39–44—First instar of *Metopia campestris* (Fallén 1810). —**39**. AII–III, ventral view. —**40**. AII, ventral view. —**41**. AII–III, dorsal view. —**42**. AII, dorsal view. —**43**. Anal division, posterior view. —**44**. Anal division, ventral view.



Figs 45–48—Anterior part of cephaloskeleton of first instar of *Metopia* (Meigen 1803). —**45**. *M. argentata* (Macquart 1850). —**46**. *M. argentata* (Macquart 1850). —**47**. *M. argyrocephala* (Meigen 1824). —**48**. *M. campestris* (Fallén 1810). Scales = 0.05 mm.

Discussion

The external and cephaloskeletal morphology of the first instar of *Metopia* shows considerable promise not only for species-level identification of otherwise similar taxa but also as a rich source of phylogenetically informative characters. Compared to that of *Apodacra pulchra* Egger, which is the only other miltogrammine taxon documented with SEM (Szpila and Pape, in press), the first instar of *Metopia* may appear less specialized by lacking major modifications of the pseudocephalon, and the lateral papillae on AI–AVII are not drawn out to form long, bristle-like sensilla. Other differences concern the anal division, where the species of *Metopia* studied do not possess a spiracular cavity, and all papillae (P1–P7) are well developed. The posterior spiracles are often diagnostic at the species level in the Calyptrata (Ferrar 1987), but they appear much more homogeneous in the Miltogramminae. On the other hand, the shape of the mouth-hooks and the arrangement and shape of ridges, swellings and intersegmental insertions in *Metopia* spp. indicate extensive modifications from the miltogrammine groundplan. Thompson (1921) described the first instar of four *Metopia* spp., two based on North American specimens: *M. lateralis* (Macquart) and '*M. leucocephala*' (= *M. argyrocephala*, see below) and two based on European specimens: *M. campestris* and '*M. argyrocephala*' (= *M. argentata*, see below). The only other descriptive work on *Metopia* immatures is that of Townsend (1942; figs 326, 327), who provided an illustration of the first instar cephaloskeleton of an '*Anicia* or ally' and of *M. meridiana* Townsend, but the illustrations are too sketchy to allow for meaningful comparisons with those of Thompson (1921) and the present paper. Thompson relied on identifications made by contemporary specialists, and when the larvae he dissected from a European female determined by Mario Bezzi as *M. argyrocephala* differed from those of an American female identified as *M. leucocephala* (identification authority not given), Thompson simply decided to maintain the names in spite of the general notion of conspecificity of these two nominal taxa. Thompson's illustration of '*M. argyrocephala*' (his fig. 114) undoubtedly identifies this larva as *M. argentata* based on the size and proportions of the labrum. Thompson mentioned that the larvae analysed by him were premature ('pas mûres', p. 424), and he argued this by describing the low degree of sclerotization of the cephaloskeleton and the rather membranous labrum. However, except for a few descriptive notes on the mouth-hooks, no further morphological details were discussed by Thompson for this species.

The present description of *M. argyrocephala* agrees sufficiently well with the one provided by Thompson under the name '*M. leucocephala*'. The apical part of each mouth-hook is equipped with several teeth along the ventral margin, and although Thompson described (p. 423) and figured (his fig. 105) the mouth-hooks as truncated anteriorly, he also provided a more detailed figure (his fig. XXXVIb), which is an almost perfect match with the present study. Furthermore, the long lateral arm of the mouth-hook is clearly indicated. Only small differences are found in the shape of the labrum and the intermediate sclerite, and when Allen (1926) studied 'several uterine maggots' of North American *M. argyrocephala*, he also found them to agree with the description by Thompson (1921) of *M. leucocephala* 'in all but a few minor points'

(p. 49). Townsend (1892, 1942), however, considered the North American *M. argyrocephala* as a distinct species, *M. luggeri* Townsend, which has been maintained by W.L. Downes (e.g. Spofford *et al*. 1989).

Probably the most important discrepancy relates to the interpretation of the described structures. For all the species of *Metopia* studied, Thompson tentatively interpreted the apical teeth of the mouth-hooks, which are visible above the mouth opening flanking the tip of the labrum, as part of the oral ridges (e.g. for *M. campestris*: 'l'éventail dont les côtes ont l'aspect d'une série de petites dents', p. 422). The results of this paper show that oral ridges are absent in first instars of *Metopia*. It is interesting that Thompson considered the occurrence of oral ridges only for larvae of *Metopia*, which have the mouth-hooks with serrated tips. Other first instar miltogrammine larvae, which have mouth-hooks with a simple apical tooth (e.g. *Miltogramma punctata* Meigen), are described by Thompson as being devoid of oral ridges.

Thompson's description of the cephaloskeleton of *M. campestris* differs from the present description in a few details of the mouth-hook, but the exact shape of the distal part of the mouth-hook is generally difficult to observe because of its weak sclerotization and the associated transparency ('la dent du crochet étant transparente et difficile à voir', p. 423). Thompson stated that each mouth-hook has a simple apical tooth, but according to our observations the tip is actually serrated in a way very similar to the other species of *Metopia*. However, as indicated above, Thompson mentioned the occurrence of 'l'éventail', which here is taken to constitute a strong indication that he actually observed the serrated apical part of the mouth-hooks. Also, Thompson did not notice the lateral arm of the mouth-hook, which is clearly visible in the material of *M. campestris* studied in this paper (Figs 33, 34 and 48). The reason for this apparent oversight may again be the weak sclerotization as in the case of the apical part of the mouth-hook. Remaining parts of the cephaloskeleton described by Thompson (1921) are in good agreement with what is presented in the present paper, and especially the shape of labrum presents a perfect match.

The adult (male) morphology of *M. campestris* differs in many respects from that of the two other species described here (Rohdendorf 1971; Pape 1987a; Povolný and Verves 1997), and this has been used as an argument for a subgeneric separation (e.g. Rohdendorf 1955; Venturi 1960). The first instar of *M. campestris* is therefore expected to be easy to distinguish from first instars of *M. argyrocephala* and *M. argentata*, with the most pronounced differences being the large antero-ventral protuberances on T1; the longer antennae; and the densely arranged, narrow cuticular ridges of the body. Documentation from more species is needed to test if these differences will support the phylogenetic hypothesis inherent in the subgeneric classification. Very interesting and perhaps more surprising are the rather large differences between the first instars of the two apparently closely related species, *M. argentata* and *M. argyrocephala*, the females of which cannot be reliably separated (Rohdendorf 1955; Pape 1987a; Povolný and Verves 1997). The character matrix given in Table 2 thus points to several quite distinct differences.

During laboratory breeding, even behavioural differences were observed between larvae of these species. The larva of *M. argyrocephala*, after being placed in a vial, will crawl quickly toward the food supply (dead flies). In vials without a food supply, a larva crawls unceasingly around in an unsystematic or disorganized way. Larvae of *M. argentata* did not crawl, even in the presence of a food supply, but would remain in one place, anchored on the posterior part of their body, performing circular or number eight-shaped movements with the anterior part of the body, and with the apical part of the labrum distinctly exposed. A similar behaviour has been described for larvae of Rhinophoridae, some Calliphoridae, and some Tachinidae (Zumpt 1965; Bedding

Table 2 Most important morphological differences of the first instar of *Metopia argentata*, *M. argyrocephala* and *M. campestris*

	Feature						
Species	Antenna	Labrum	Serrations on tip of mouth-hook	TI	Cuticular ridges, shape	Anal division, ventral spinulation	Anal division, anal papilla
M. argentata	moderately long (2.0–2.5× as long as diameter at base)	short apical tooth	weakly sclerotized	without any specialized structures	broad	sparse (but spines arranged in small sets), not reaching the anal opening	flat, dome-like
M. argyrocephala	relatively short (1.5–2.0× as long as diameter at base)	long apical tooth	weakly sclerotized	without any specialized structures	broad	numerous spines cover the entire surface from the anterior margin of the division to the anal opening	flat, dome-like
M. campestris	large and elongated (3.0–3.5× as long as diameter at base)	long apical tooth	strongly sclerotized	antero-ventral edge with two large protuberances	narrow	sparse, not reaching the anal opening	elongated

1973; Pape and Arnaud 2001), all of which are waiting for direct contact with their host. Another difference observed in the larval feeding behaviour was that *M. argyrocephala* larvae would start feeding inside the dead body of their mother, while this never happened for *M. argentata*.

Significant interspecific morphological differences between early preimaginal instars of species with similar adult morphology have been reported also for other oestroid flies (Bedding 1973; Szpila 2003, 2004). These examples concern mainly parasitic taxa with a first instar, which has to either actively search or wait in ambush for a suitable host. This is in contrast to saprophagous oestroids (Calliphoridae: Calliphorinae, Chrysomyiinae, Lucilinae; Sarcophagidae: Sarcophaginae), where the first instars in general are almost inseparable, sometimes even at the subfamily level.

The general morphology of the cephaloskeleton of *Metopia* does not differ much from that of other Miltogramminae (Thompson 1921; Allen 1926; Ferrar 1987; Verves 1993), but some significant differences are found in the shape of the mouth-hooks. An unusual feature of *Metopia* mouth-hooks is the lateral arm ('pièce chitineuse accessoire' of Thompson 1921, p. 424), which is present in *M. lateralis* and all three species documented in the present paper. This feature has not been reported for any other miltogrammine species or in any member of the Sarcophagidae and Calliphoridae. The feature is a possible synapomorphy uniting at least a part of the species of *Metopia*. Another noteworthy feature is the conspicuous ventral serrations apically on the mouth-hook (Figs 2, 3, 6, 17–19, 22, 33, 34, 36, 45, 47 and 48). Serrations on the apical part of the mouth-hooks are very common among saprophagous species of Calliphoridae (Keilin 1915; Lopes 1983; Leite and Guevara 1993; Sukontason *et al.* 2003a,b; Szpila & Pape, unpublished data) and present in all Rhinophoridae (Bedding 1973; Pape and Arnaud 2001). In the Sarcophagidae, however, a serrated ventral margin of the tip of the first instar larval mouth-hook is quite rare (Lopes 1983; Ferrar 1987). Very small, spine-like dents appear along the dorsal margin of the mouth-hook in *Oxysarcodexia angrensis* (Lopes) (Lopes 1943; figs 31, 34; Lopes and Leite 1987; fig. 2), but the first instar sarcophagine mouth-hook appears otherwise to be very uniform (Lopes 1983). In the Miltogramminae, apart from the present species of *Metopia*, a serrated tip has only been documented for *Senotainia albifrons* (Rondani) (Verves 1990). Other species of *Senotainia* with described larval stages, *S. conica* (Fallén), *S. tricuspis* (Meigen), *S. litoralis* Allen, *S. rubriventris* Macquart, *S. trilineata* (Wulp) and *S. vigilans* Allen possess a simple tooth at the end of the mouth-hook (Allen 1926; Ferrar 1987; Verves 1990). *Metopia lateralis*, as figured by Thompson (1921; fig. XXXVII), has the mouth-hook apically equipped with only a single, strong, recurved tooth, but Thompson mentions the presence of 'l'éventail' (p. 424), which as already discussed is good evidence for the presence of serrations along the apico-ventral part of the mouth-hooks. *Metopia lateralis* is a member of a monophyletic New World subset of *Metopia* sometimes separated in the (sub)genus *Allenanicia* Townsend (Shewell 1987; Pape 1987b), which would seem to indicate that a serrated tip of the first instar mouth-hook is an autapomorphy for a clade containing most or all species of *Metopia*.

The longitudinal cuticular ridges covering most of the body have been reported for many miltogrammine species (Thompson 1921; Szpila and Pape, in press), and their presence may be considered a ground-plan autapomorphy for the subfamily, although at least one exception is the first instar of *Sphenometopa* Townsend, where the longitudinal ridges are replaced by rounded cuticular swellings, which cover almost the entire surface of the body (Thompson 1921; figs XXXIIIa, XXXIXd).

The ventral furrow on the abdominal segments appears to be absent from other species of Miltogramminae (Szpila and Pape, in press and unpublished results), but it occurs in a few species from the two other sarcophagid subfamilies examined by us (*Sarcophaga* Meigen and *Sarcophila* Rondani) as well as in saprophagous Calliphoridae (e.g. *Calliphora* Robineau-Desvoidy) (Szpila & Pape, unpublished results).

The position of the posterior spiracles of at least the third instar larva in a recession or cavity is usually considered as an autapomorphic feature for the family Sarcophagidae (Rognes 1986; Pape 1996, 1998). Shewell (1987, p. 1161) provided the descriptive statement: 'Posterior spiracular field sunken in cavity, especially in later instars', and there have been some doubts on how to interpret the sparse data from first instars. Verves (1990) interpreted the lack of a spiracular cavity in some first instar Miltogramminae as a plesiomorphic condition retained from saprophagous ancestors, following the traditional view of the origin of Sarcophagidae proposed by many earlier authors (e.g. Zumpt 1965; Rohdendorf 1967). However, the first instar of several species of Miltogramminae possess a typical spiracular cavity as illustrated by Szpila and Pape (in press) for *Apodacra pulchra*. Ferrar (1987, p. 320) noted that the posterior spiracles of the miltogrammine first instar are 'set in a pit in most but not all species'. Even without a well-supported sister group for the Sarcophagidae, the most parsimonious explanation will be to consider recessed posterior larval spiracles as a ground-plan feature for the family Sarcophagidae, and the lack of a posterior spiracular pit, as in the first instar of the three species of *Metopia* described here, is accordingly best accounted for as a secondary modification. A full assessment of the exceptions to the recessed posterior spiracles will require morphological documentation of the first instar of many more genera as well as a cladogram for mapping the character.

As already mentioned, our knowledge on the morphology of the preimaginal instars of Miltogramminae is still fragmentary, and conclusions regarding the incorporation of this information into generic circumscriptions will still be premature. However, the sparse data available are sufficient for the hypothesis that *M. campestris*, *M. argentata* and *M. argyrocephala* share the following features, which are probably apomorphic relative to the miltogrammine ancestor: base of mouth-hook with lateral arm, tip of mouth-hook serrated, abdominal segments with transverse furrow ventrally, and posterior spiracular cavity not developed. Settling the exact level at which each of these character states are autapomorphic will have to await further morphological studies with a much better taxonomic coverage.

Acknowledgements

Part of the SEM work was supported by a grant to the senior author from HighLat resources (HPRI-CT-2001-00125) through the European Commission's Access to Research Infrastructure action under the program Improving Human Potential. We are grateful to Dr R. Meier, National University of Singapore, for commenting on the manuscript, to Mr T. Konefał, State Plant Health and Seed Inspection Service Central Laboratory, Toruń, for access to equipment for digital photography of light microscope slides, to Mrs E. Grafström, Swedish Museum of Natural History, Stockholm, for technical help with part of the photomicroscopy, and to Mr R. Kaliszuk, Nicolas Copernicus University, Toruń, for help with digital processing of the SEM pictures.

References

Allen, H. W. 1926. North American species of two-winged flies belonging to the tribe Miltogrammini. – *Proceedings of the United States National Museum* **68**: 1–106.

Bedding, R. A. 1973. The immature stages of Rhinophorinae (Diptera: Calliphoridae) that parasitise British woodlice. – *Transactions of the Royal Entomological Society of London* **125**: 27–44.

Cobb, M. 1999. What and how do maggots smell? – *Biological Reviews* **74**: 425–459.

Courtney, G. W., Sinclair, B. J. and Meier, R. 2000. Morphology and terminology of Diptera larvae. In Papp, L. and Darvas, B. (Eds): *Contributions to a Manual of Palaearctic Diptera (with Special Reference to Flies of Economic Importance): General and Applied Entomology*, Vol. 1, pp. 85–161. Science Herald Press, Budapest.

Endo, A. 1980. The behaviour of a Miltogrammine fly *Metopia sauteri* (Townsend) (Diptera, Sarcophagidae) cleptoparasitizing on a spider wasp *Episyron arrogans* (Smith) (Hymenoptera, Pompilidae). – *Kontyû* **48**: 445–457.

Ferrar, P. 1987. A guide to the breeding habits and immature stages of Diptera Cyclorrhapha. – *Entomonograph* **8**: 1–907.

Keilin, D. 1915. Recherches sur les larves de Diptéres cyclorhaphes. Cycle evolutif de *Pollenia rudis* Fabr., parasite *d'Allolobophora chlorotica* Sav. Biologie comparée des larves de Diptères. – *Bulletin Scientifique de la France et de la Belgique* **49**: 15–198.

Leite, A. C. R. and Guevara, J. D. E. 1993. Scanning electron microscopy of the larval instars of *Cochliomyia hominivorax*. – *Medical and Veterinary Entomology* **7**: 263–270.

Lopes, H. S. 1943. Contribuição ao conhecimento das larvas dos Sarcophagidae com especial referência ao esqueleta cefálico (Diptera). – *Memórias do Instituto Oswaldo Cruz* **38**: 127–163.

Lopes, H. S. 1983. The importance of the mandible and clypeal arch of the first instar larvae in the classification of the Sarcophagidae (Diptera). – *Revista Brasileira de Entomologia* **26**: 293–326.

Lopes, H. S. and Leite, A. C. R. 1987. Studies on some features of the first instar larvae of *Oxysarcodexia* (Diptera, Sarcophagidae) based on scanning electron microscope observations. – *Revista Brasileira de Biologia* **46**: 741–746.

Oppliger, F. Y., Guerin, P. M. and Vilmant, M. 2000. Neurophysiological and behavioural evidence for an olfactory function for the dorsal organ and a gustatrory one for the terminal organ in Drosophila melanogaster larvae. – *Journal of Insect Physiology* **46**: 135–144.

Pape, T. 1987a. The Sarcophagidae (Diptera) of Fennoscandia and Denmark. – *Fauna Entomologica Scandinavica* **19**: 1–203.

Pape, T. 1987b. Revision of Neotropical *Metopia* Meigen (Diptera: Sarcophagidae). – *Systematic Entomology* **12**: 81–101.

Pape, T. 1996. Catalogue of the Sarcophagidae of the world (Insecta: Diptera). – *Memoirs of Entomology International* **8**: 1–558.

Pape, T. 1998. Family Sarcophagidae. In Papp, L. and Darvas, B. (Eds): *Contribution to a Manual of Palaearctic Diptera: Higher Brachycera*, Vol. 3, pp. 649–678. Science Herald Press, Budapest.

Pape, T. and Arnaud, P. H. Jr. 2001. *Bezzimyia* – a genus of New World Rhinophoridae (Insecta, Diptera). – *Zoologica Scripta* **30**: 257–297.

Povolný, D. and Verves, Yu. G. 1997. The flesh-flies of Central Europe (Insecta, Diptera, Sarcophagidae). – *Spixiana Supplement* **24**: 1–260.

Rognes, K. 1986. The systematic position of the genus *Helicobosca* Bezzi with a discussion of the monophyly of the calyptrate families Calliphoridae, Rhinophoridae, Sarcophagidae and Tachinidae. – *Entomologica Scandinavica* **17**: 75–92.

Rohdendorf, B. B. 1955. [The species of genus *Metopia* Mg. (Diptera, Sarcophagidae) from USSR and neighbouring countries.]. – *Entomologicheskoe Óbozrenie* **34**: 360–373. [In Russian.]

Rohdendorf, B. B. 1967. [The directions of historical development of Sarcophagidae (Diptera).]. – *Trudy Paleontologicheskogo Instituta Akademii Nauk SSSR* **116**: 1–92. [In Russian.].

Rohdendorf, B. B. 1971. 64 h. Sarcophaginae. In Lindner, E. (Ed.): *Die Fliegen der Palaearktischen Region 11 [Lieferung 285]*, pp. 129–176. E. Schweizerbart'sche-Verlagsbuchhandlung, Stuttgart.

Rohdendorf, B. B. and Verves, Yu. G. 1980. On the fauna of Sarcophagidae (Diptera) of the Mongolian People's Republic. III. – Miltogrammatinae. – *Insects of Mongolia* **7**: 445–518. [In Russian with English subtitles.]

Schumann, H. 1974. Revision der palaearktischen *Bellardia*-Arten (Diptera, Calliphoridae). – *Deutsche Entomologische Zeitschrift* **21**: 231–299.

Schwendinger, P. J. and Pape, T. 2000. *Metopia sinensis* (Diptera, Sarcophagidae), an unusual predator of *Liphistius* (Araneae, Mesothelae) in northern Thailand. – *Journal of Arachnology* **28**: 353–356.

Shewell, G. E. 1987. *Sarcophagidae*. In McAlpine, J. F. (Ed.): *Manual of Nearctic Diptera*, **2**, pp. 1159–1186. Agriculture Canada, Ottawa.

Spofford, M. G. and Kurczewski, F. E. 1990. Comparative larvipositional behaviours and cleptoparasitic frequencies of Nearctic species of Miltogrammini (Diptera, Sarcophagidae). – *Journal of Natural History* **24**: 731–755.

Spofford, M. G. and Kurczewski, F. E. 1992. Counter-cleptoparasitic behaviours of species of Sphecidae (Hymenoptera) in response to Miltogrammini larviposition (Diptera, Sarcophagidae). – *Journal of Natural History* **26**: 993–1012.

Spofford, M. G., Kurczewski, F. E. and Downes, W. L. Jr. 1989. Nearctic species of Miltogrammini (Diptera: Sarcophagidae) associated with species of *Aculeata* (Hymenoptera: Vespoidea, Pompiloidea, Sphecoidea, Apoidea). – *Journal of the Kansas Entomological Society* **62**: 254–267.

Sukontason, K. L., Sukontason, K., Lertthamnonghtam, S., Kuntalue, B., Thijuk, N., Vogtsberger, R. C. and Olson, J. K. 2003a. Surface ultrastructure of *Chrysomya rufifacies* (Macquart) larvae (Diptera: Caliphoridae). – *Journal of Medical Entomology* **40**: 259–267.

Sukontason, K. L., Sukontason, K., Piangjai, S., Boonchu, N., Chaiwong, T., Vogtsberger, R. C., Kuntalue, B., Thijuk, N. and Olson, J. K. 2003b. Larval morphology of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) using scanning electron microscopy. – *Journal of Vector Ecology* **28**: 47–52.

Szpila, K. 2003. First instar larvae of nine West-Palaearctic species of *Pollenia* Robineau-Desvoidy, 1830 (Diptera, Calliphoridae). – *Entomologica Fennica* **14**: 193–210.

Szpila, K. 2004. First instar larvae of five West Palaearctic species of *Bellardia* Robineau Desvoidy, 1863 and *Onesia* Robineau-Desvoidy, 1830 (Diptera, Calliphoridae). – *Studia Dipterologica* **11**: 301–312.

Szpila, K. and Pape, T. The first instar larva of *Apodacra pulchra* (Diptera: Sarcophagidae, Miltogramminae). – *Insect Systematics and Evolution* (in press).

Thompson, W. R. 1921. Recherches sur les diptères parasites. I – Les larves des Sarcophagidae. – *Bulletin Biologique de la France et Belge* **54**: 313–463.

Townsend, C. H. T. 1892. Notes on North American Tachinidae, with descriptions of new genera and species. — Paper V. – *Canadian Entomologist* **24**: 64–70, 77–82.

Townsend, C. H. T. 1942. Manual of Myiology. Part XII. General considerations of the Oestromuscaria, pp. 1–364, pls 84. Charles Townsend and Filhos, Itaquaquecetuba, São Paulo.

Venturi, F. 1960. Sistematica e geonemia dei Sarcofagidi (escl. *Sarcophaga* s.l.) italiani (Diptera). – *Frustula Entomologica* **2**: 1–124.

Verves, Yu. G. 1990. A key to Sarcophagidae (Diptera) of Mongolia, Siberia and neighbouring territories. – *Nasekomye Mongolii* **11**: 516–616. [In Russian with English subtitles.]

Verves, Yu. G. 1993. Palaearctic species of the genus *Craticulina* (Diptera, Sarcophagidae). – *Vestnik Zoologii* **[1993]**: 9–17. [In Russian with Ukrainian and English summaries.]

Wcislo, W. T. 1986. Host determination by a cleptoparasitic fly, *Metopia campestris* (Fallén) (Diptera: Sarcophagidae: Miltogramminae). – *Journal of Kansas Entomological Society* **59**: 82–88.

Zumpt, F. 1965. *Myasis in Man and Animals in the Old World*, p. 267. Butterworths, London.