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Two new species of *Amphinemura* (Plecoptera, Nemouridae) from the southern Qinling Mountains of China, based on male, female and larvae

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Abstract

Two new species of the genus *Amphinemura*, *A. albicauda* **sp. n.** and *A. dingoidea* **sp. n.** from the southern Qinling Mountains, Foping County of Shaanxi Province, western China, are described based on both sexes and the larval stage. The new species are compared with related taxa, and the *A. sinensis* species group is defined for an Oriental lineage of the genus. *Amphinemura sinensis* (Wu, 1926) and *A. unihamata* (Wu, 1973) are reported from Shaanxi for the first time, and the hitherto unknown female of *A. unihamata* is described. A distribution map of the Amphinemurinae known from Qinling Mountains is given.

Keywords

Amphinemura albicauda, *A. dingoidea*, Amphinemurinae, new records, new species, Shaanxi, Stoneflies

Introduction

The subfamily Amphinemurinae belongs to the stonefly family Nemouridae. It is the second largest group in China (DeWalt et al. 2018, Yang and Li 2018, Yang et al. 2015). The most species rich genus, *Amphinemura* Ris, 1902, includes 85 described Chinese species (DeWalt et al. 2018). The monograph by Yang et al. (2015) treats all the species described by Klapálek (1912), Wu (1926, 1935, 1938, 1949, 1962, 1973), Martynov (1928), Shimizu (1997, 1998), Zhu and Yang (2002, 2003), Yang et al. (2004, 2005), Li and Yang (2005, 2006, 2007a, 2008a, b, c, d, e, 2011), Li et al. (2005), Wang et al. (2006, 2007), Du and Wang (2007), and Du et al. (2007b). Since then, Li et al. (2013, 2016b, 2017a, b, 2018), Ji and Du (2014), Ji et al. (2014), and Mo et al. (2017) have described an additional 17 species of *Amphinemura* from China.

Recent research has highlighted the high diversity of stonefly fauna of the poorly investigated Qinling Mountains, which range from the Qinghai-Tibet Plateau to the North China Plain and separate northern and southern China (Ji et al. 2014; Li and Murányi 2015; Li et al. 2016a, 2018; Li and Mo 2018). In April 2018, we made a short collecting trip to the southern Qinling Mountains, in the vicinity of Foping, Shaanxi. In the present paper, we describe two new species of *Amphinemura* collected during that trip, along with some further contributions to the Amphinemurinae, including the definition of a new Oriental species group where one of the new species belongs. We also present a distribution map of the Amphinemurinae hitherto known from the Qinling Mountains.

Material and methods

The material studied was collected along stream banks by hand, or by using a beating sheet or an aquatic net. All material is deposited in the Entomological Museum of China Agricultural University, Beijing (CAUC), the Henan Institute of Science and Technology, Xinxiang (HIST), and the Collection of Smaller Insect Orders, Department of Zoology, Hungarian Natural History Museum, Budapest (HNHM), as indicated in the text. The specimens are preserved in 75% ethanol. The morphological terminology follows that of Baumann (1975) and Zwick (2010). Specimens were examined with the aid of a Leica M420 dissecting microscope and the color images and illustrations were made with the aid of a Leica S8APO and a Keyence LHX5000 digital microscope.

Results and discussion

Amphinemura albicauda **sp. n.**
http://zoobank.org/2F1C3751-1EE0-4237-9B4D-7FB10A427220
Figs 1–14, 31, 32, 35

Diagnosis. Male: tergum IX with short spines and long setae, epiproct with closely spaced lateral processes having sharp and out-curved apex, paraproctal outer lobe long

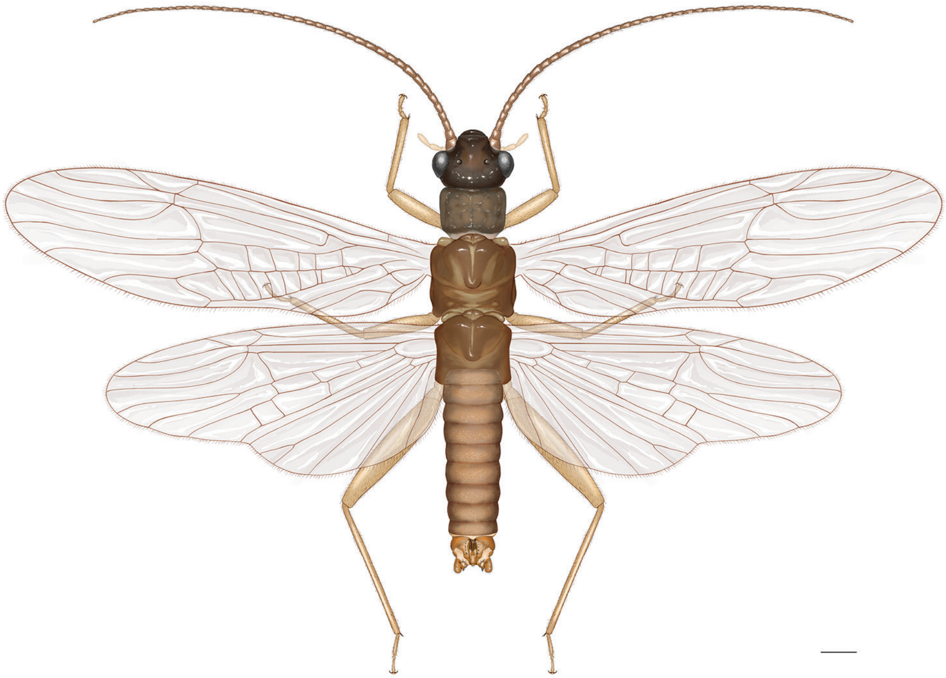
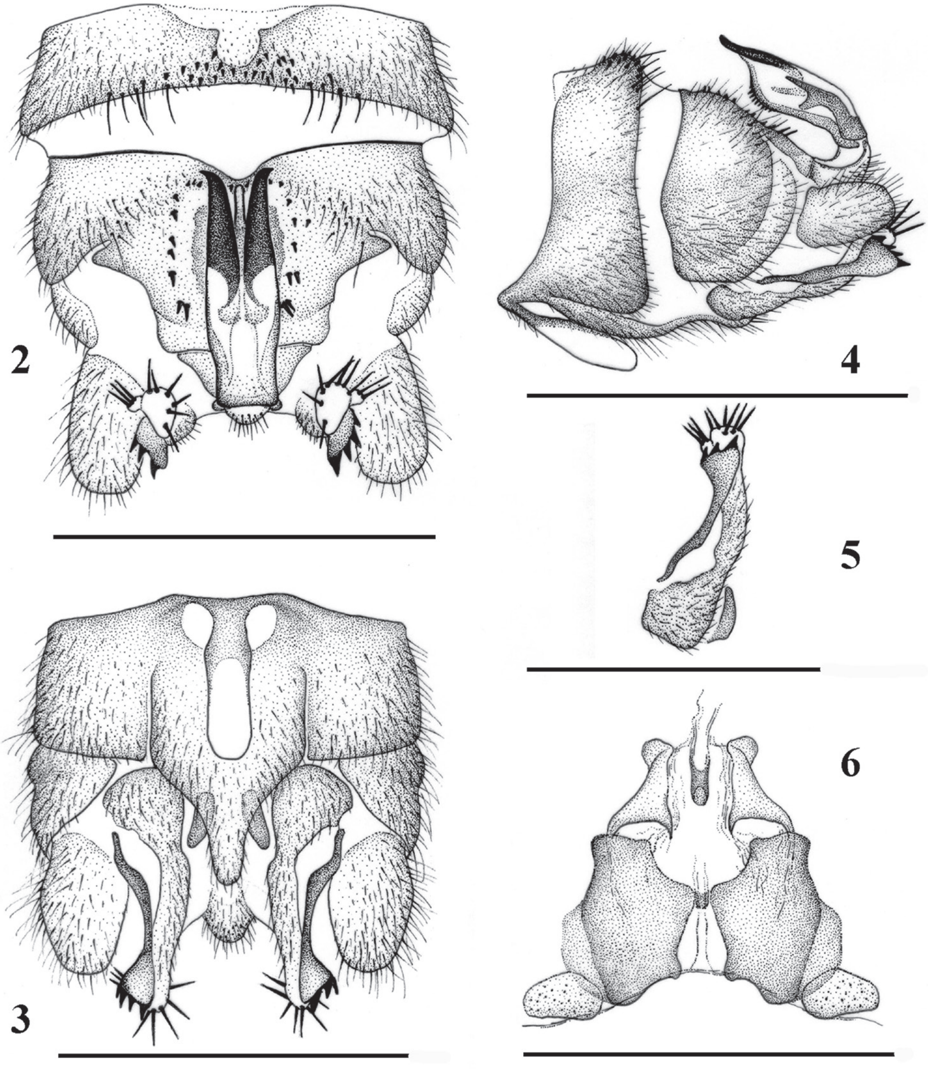


Figure 1. *Amphinemura albicauda* sp. n., habitus of male holotype adult. Scale bar: 0.5 mm.

and armed with large apical teeth, median lobe apically bilobed. Female: subgenital plate subquadrate and slightly lobed, inner sclerite with ear-shaped lobes. Larva: general color brown but cerci contrasting white and hairy, legs with distinct swimming hairs.

Description. Adult habitus (Fig. 1): General color light brown to brown. Head and antennae brown, palpi light brown. Thorax brownish, pronotum with distinct rugosities. Legs light brown. Wing membranes grayish, veins brown. Abdomen brown with darker terminalia.

Male (Fig. 2–5): Forewing length 6.4–6.6 mm. Tergum IX (Fig. 2) moderately sclerotized, with 11–13 short mesal spines and 4 or 5 paramedial long hairs along mid-posterior margin. Sternum IX with claviform vesicle, distal ½ membranous (Fig. 3). Hypoproct subquadrate at base, apical ½ tapering, apex tubular (Fig. 3). Tergum X weakly sclerotized, concavity beneath epiproct narrow with 10–12 small to medium-sized spines along lateral sides (Figs 1, 2). Cercus slightly sclerotized, stout and short. Epiproct (Figs 1, 2, 4) ca 3.5 times longer than wide, distal portion trifurcate but median process nearly unpigmented and hardly detected in dorsal view (Fig. 2). The lateral processes horn-shaped and closely located, with apex sharp and out-curved; median process originates from ventral sclerite and shorter than lateral ones, subapically forming distinct rounded ridge in lateral view (Fig. 4). Paraproct trilobed (Figs 2–5): inner lobe triangular, short and well sclerotized, mostly hidden by hypoproct; median lobe long and tubular, apical portion up-curved, apex membranous with 6 or 7 long marginal spines, outer margin with an additional small lobe bearing 3 long spines of



Figures 2–6. *Amphinemura albicauda* sp. n., terminalia of the adult paratypes. **2** Male terminalia, dorsal view **3** Male terminalia, ventral view **4** Male terminalia, lateral view **5** Male paraproct, oblique lateral view **6** Female inner genitalia, dorsal view. Scale bar: 0.5 mm.

same size; outer lobe extends along with median lobe but shorter, well sclerotized, dilated apex with 3 or 4 lateral, large teeth along outer margin.

Female (Fig. 14): Forewing length 7.8 mm. Sternum VII produced in a broad semicircular pregenital plate covering anterior half of subgenital plate; the plate is mostly pale with brownish posterior margin. Sternum VIII forms sclerotized subquadrate subgenital plate with narrowed anterior portion, medial notch is being broad but shallow medially; inner portion of the posterior lobes with small, sometimes indistinct secondary lobes.

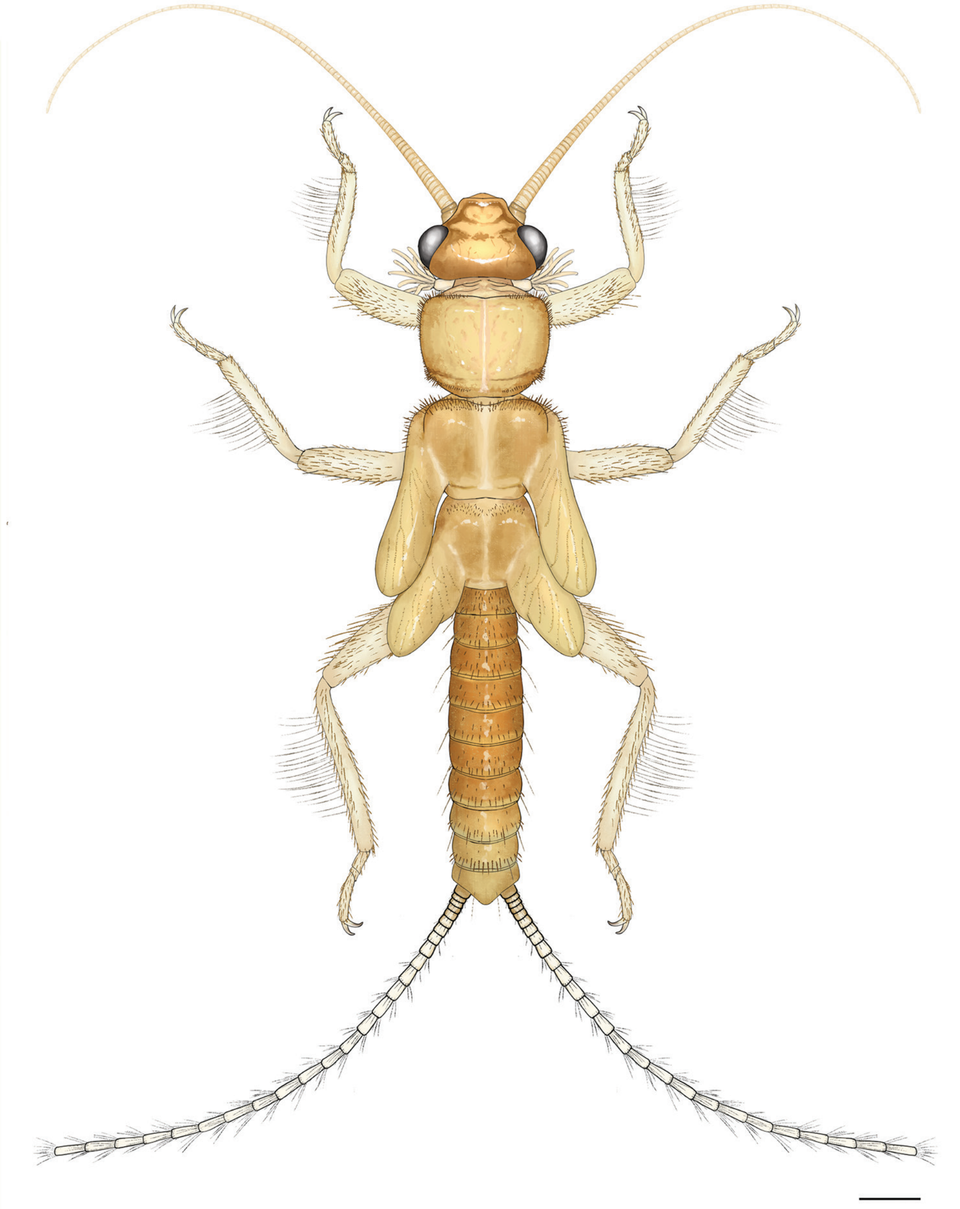
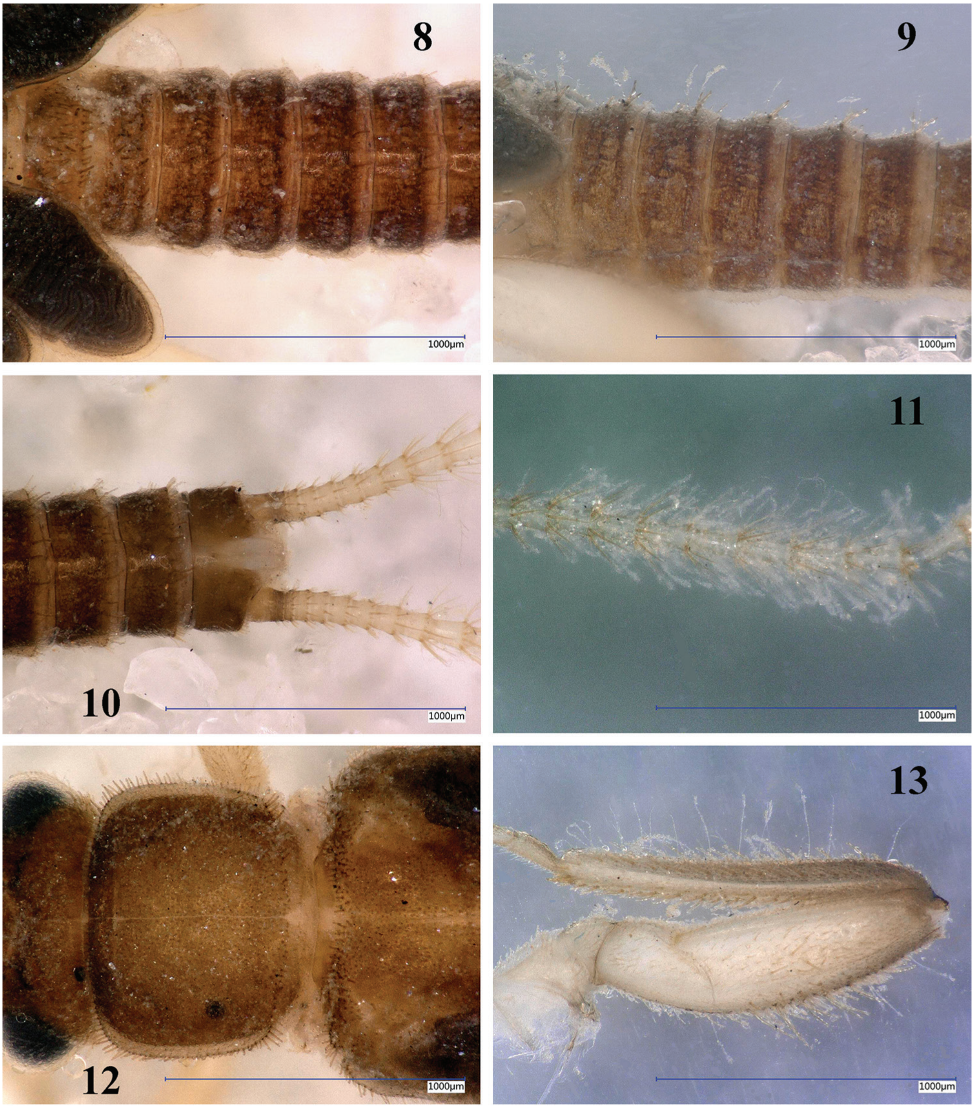


Figure 7. *Amphinemura albicauda* sp. n., habitus of matured female paratype larva. Scale bar: 0.5 mm.

Paragenital plate paired, forming quadrate brownish lobe connected with posterolateral corner of subgenital plate. Sternum IX trapezoidal, median half much protruded anteriorly, in ventral aspect with anterior indentation. Paraproct and cerci brownish.

Female inner genitalia (Fig. 6): Inner sclerite is medially separated into triangular sclerites located anteriorly to the subgenital plate; anterior portion of the sclerite halves with a



Figures 8–13. *Amphinemura albicauda* sp. n., matured larva paratypes. **8** Terga I–VI, dorsal view **9** Terga II–VII, lateral view **10** Terga VII–X and basal third of cerci, dorsal view **11** Cercomeres 9–18, dorsal view **12** Pronotum, dorsal view **13** Right hind leg, outer face. Scale bar: 1 mm.

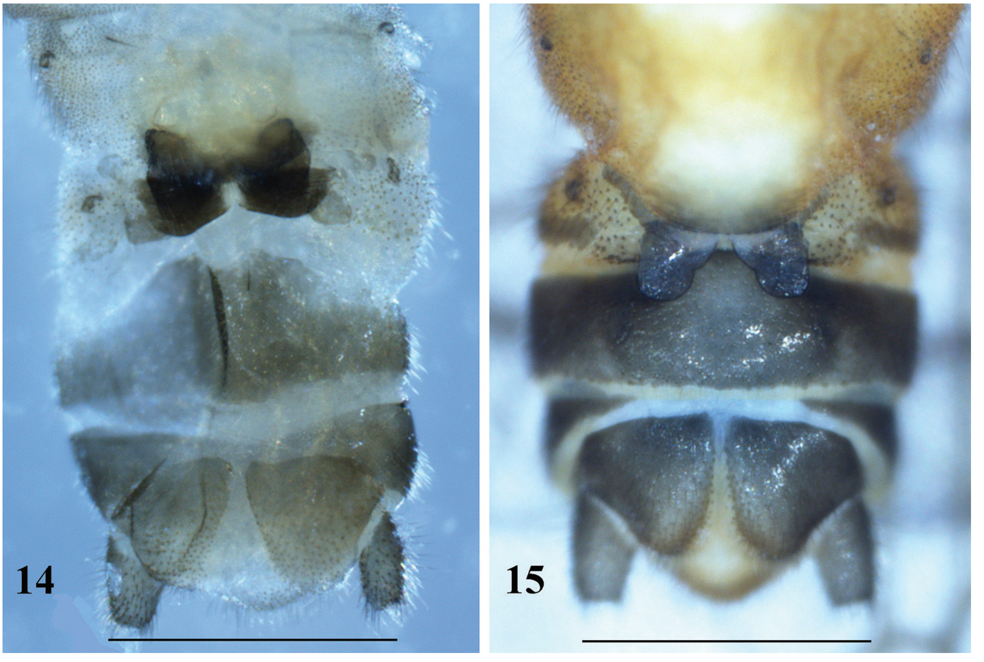
small, ear-shaped projection, easily observed in oblique or caudal view. Between the sclerites is a membranous tunnel with a tubular median sclerite leading to spermathecal ductus.

Mature larva (Fig. 7): Body relatively slender and small, body length without antennae and cerci 5.5–6.0 mm. General color brown, with some indistinct pattern on pronotum and abdomen, legs and antennae light brown but cerci contrasting white and hairy. Setation long and distinct. Legs moderately long, width of hind femora ca ¼ of their length. The pronotum is trapezoidal, wider than long, as wide as head. Cervical gills long, inner gills with 7, outer with 8 branches. Wing pads more than twice as long as the corresponding segments. Abdomen relatively slender and uniformly brown, integument light matt brown, first 2 abdominal segments divided by pleura. Posterior margin of sternum IX of the male larva short triangular, sternum VIII of female larva slightly incised; paraprocts blunt. Cerci long, with 24–26 cylindrical segments; length of the 15^{th} segment is about 3 times of its width.

Setation of the larva (Figs 8–13): Head, antennae and palpi with dense short setae. Pronotum covered with very short setae; marginal setae distinct and blunt, row interrupted in anteromedial and posteromedial third, corners have setae as long as one 15^{th} of pronotum width (Fig. 12). Setae on meso- and metanotum as long as longest marginal setae on pronotum; wing pads with long and acute, whitish setae. Legs with dense setation, all tibiae bear distinct swimming hairs as long as femur width (Fig. 13). Longest acute setae of all femora are longer than half of the corresponding femur width, long setae on fore femur arranged in an incomplete transversal line. Tarsi and claws relatively long. Tergal segments covered with thin setae, all segments bear a pair of thin, erect and irregularly curved hairs that are distinct in lateral view and reach nearly the segment length (Fig. 9); posterior margin with row of 14–16 acute setae, of various length, longest nearly reaches half of segment length (Figs 8, 10). Cercal segments with dense and long, white setation; setae sparser and shorter on basal and apical segments (Figs 10, 11). Cercomeres 14–16 with intercalary setae-like fine hairs, longer than the segment width, and an apical whorl of 14–17 acute setae that are as long as segment length (Fig. 11).

Type material. Holotype male (HIST): CHINA, Shaanxi Province, Hanzhong, Foping County, Changjiaoba Town, lower section of a large unnamed stream in Dizhuang valley, 895 m, 33°33.344'N, 107°59.018'E, 2018.IV.21, leg. W.H. Li, R.R. Mo and D. Murányi. Paratypes: same data as holotype: 1 male and 1 female, 1 female larva (HIST), 1 male larva (CAUC) 1 male and 1 female larva, with pharate adult terminalia dissected (HNHM).

Affinities. The new species belongs to a lineage of *Amphinemura* that is distributed in Oriental areas of China and Vietnam. This lineage can be characterized by the horn-shaped lateral processes of the dorsal sclerite, and pointed ventral sclerite of the male epiproct. It is defined here as the *A. sinensis* species group. The species belonging to this group are *A. ancistroidea* Li & Yang, 2007a, *A. caoae* Stark & Sivec, 2010, *A. cestroidea* Li & Yang, 2005, *A. chui* Wu, 1935, *A. divergens* Stark & Sivec, 2010, *A. elongata* Li, Yang & Sivec, 2005, *A. fleurdelia* (Wu, 1949), *A. furcostyla* (Wu, 1973), *A. giay* Stark & Sivec, 2010, *A. guangdongensis* Yang, Li & Zhu, 2004, *A. hamiornata* Li & Yang, 2008b, *A. leigong* Wang & Du in Wang et al. 2006, *A. licenti* (Wu, 1938), *A. malleicapitata* Li & Yang, 2006, *A. maoi* (Wu, 1938), *A. nanlingensis* Yang, Li & Sivec, 2005, *A. nigritubulata* Li & Yang, 2008d, *A. sinensis* (Wu, 1926), *A. tianmushana* Li & Yang, 2011, *A. viet* Stark & Sivec, 2010, and *A. yao* Mo, Yang, Wang & Li, 2017. Males of the new species can be distinguished from other members of the group by their armed outer lobe of the paraproct, which is unique within the group, and also by the distinctive shape of the processes of epiproct. The female outer genital sclerites are less distinctive, but the inner sclerite is unique because of the ear-shaped projections. The larva is distinctive by its conspicuous white and rather hairy cerci.



Figures 14, 15. Female terminalia in ventral view **14** *Amphinemura albicauda* sp. n., paratype **15** *Amphinemura dingoidea* sp. n., paratype. Scale bar: 0.5 mm.

Distribution and ecology. The new species was found at a single locality at the lower section of a large stream at moderate elevation (Figs 31, 35). The stream runs between forests, less-used agricultural areas, and ruderal bush. Its width varies between 3 and 8 m, and its maximum depth is less than 1 m. Rocky rapids are mixed with nearly stagnant pools and slow, stony sections. The substrate is mostly stony or sandy, with a moderate amount of debris (Fig. 32). Both last instar larvae and fully mature adults were present, suggesting April is the peak season of its emergence. Accompanying stoneflies were *A. sinensis,* a *Rhopalopsole* sp. collected as females, a few larvae of a *Neoperla* sp., and larvae of a *Kamimuria* sp. that occurred in high numbers.

Etymology. The specific name is composed of the Latin words *albus* (white) and *cauda* (tail), and refers to the distinctive white cerci of the larva.

Remarks. The adults and larvae were associated on the basis of pharate male and female adult terminalia dissected from matured larvae.

Amphinemura dingoidea **sp. n.**
http://zoobank.org/6C899234-61F5-4469-BAA6-2BED14A6EBAC
Figs 15–28, 33–35

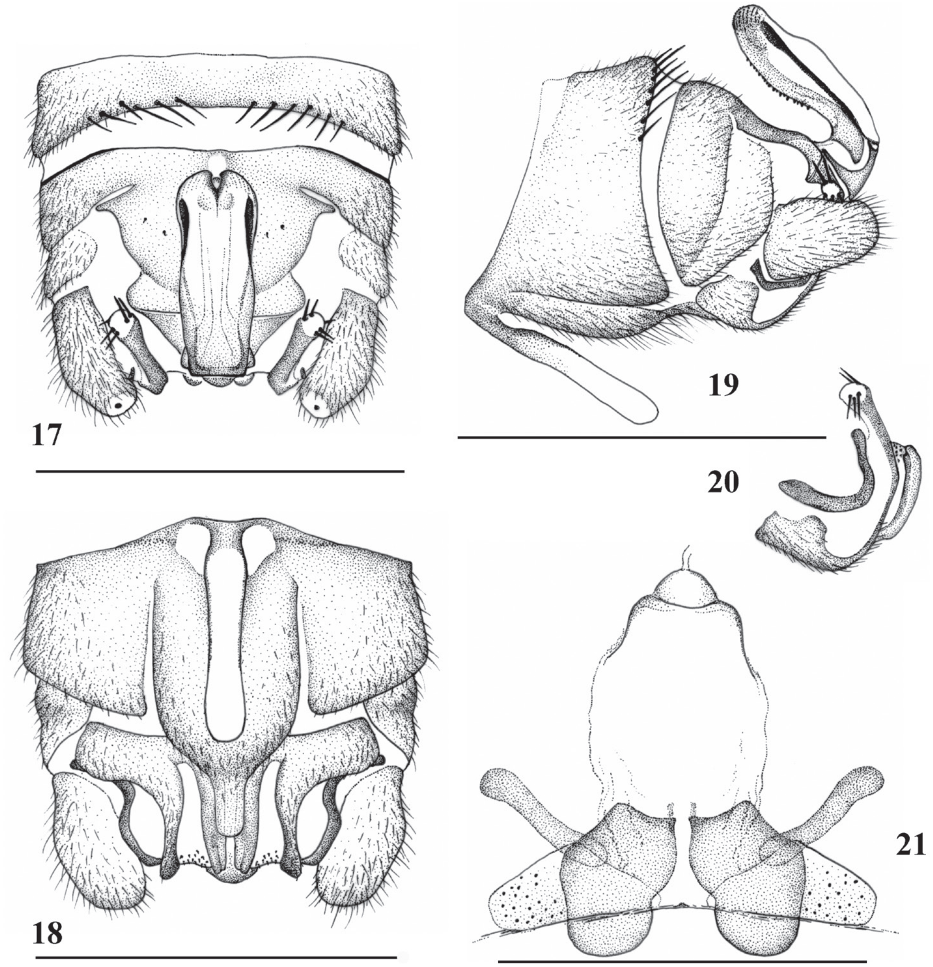
Diagnosis. Male: tergum IX with long setae, ventral vesicle very long, epiproct weakly modified but with apical notch, paraproctal inner lobe long and slightly bilobed, me-

Figure 16. *Amphinemura dingoidea* sp. n., habitus of male holotype adult. Scale bar: 1 mm.

dian lobe long, curved and with 5 or 6 apical spines, outer lobe short and lacks spine. Female: subgenital plate strongly bilobed with dome-like median notch, paragenital plate with two branches, inner genitalia simple. Larva: general color light brown with specific, distinct dark brown dorsal pattern, setation long.

Description. Adult habitus (Fig. 16): Head dark brown without pattern; compound eyes dark brown; antennae dark brown; palpi light brown. Pronotum lighter than head, arrangement of brown pattern similar to larvae. Legs generally brown, coxae and venter of femora lighter. Abdominal segments reddish brown, terminalia brown.

Male (Figs. 17–20): Forewing length 6.3–6.5 mm. Tergum IX lightly but evenly sclerotized, two paramedial groups of 6–8 long setae present along posterior margin (Fig. 17). Vesicle of sternum IX very long, constricted medially, about 5 times longer than wide (Figs 18, 19). Hypoproct basal half rectangular, medial portion rounded, apex short and up-curved (Fig. 18). Tergum X widely sclerotized, medial light area beneath epiproct very small and narrow, with 3 or 4 very small lateral spinules (Fig. 17). Cercus lightly sclerotized, stout and short. Epiproct (Figs 16, 17, 19) weakly modified, nearly rectangular with lightly sinuous margin, less than 3 times longer than wide, apically scaled and with a dark medioapical notch. Dorsal sclerite mostly membranous, basal sclerite narrow, lateral sclerite evenly thin and S-curved, basal portion hidden beneath the large membrane, appearing as weak stripe in dorsal aspect, apical portion black in dorsal and lateral aspects and ending subapically (Figs 17, 19); ventral sclerite



Figures 17–21. *Amphinemura dingoidea* sp. n., terminalia of the adult paratypes. **17** Male terminalia, dorsal view **18** Male terminalia, ventral view **19** Male terminalia, lateral view **20** Male paraproct, oblique lateral view **21** Female inner genitalia, dorsal view. Scale bar: 0.5 mm.

with weak ridge fringed by a row of short ventral teeth (Fig. 19). Paraproct trilobed (Figs 17–20): inner lobe slightly bilobed, relatively elongate and reach end of terminalia, basally connected to median lobe and membranous portion between the lobes bear minute apical setae; median lobe sclerotized, up-curved at apical half, apical portion in dorsal aspect with 5 or 6 medium-sized spines: 2 or 3 upper spines and 3 lateroapical spines (Figs 17, 19, 20); outer lobe sclerotized and medially curved along cerci, apex up-curved, without spines (Figs 18, 20).

Female (Fig. 15): Forewing length 7.5–7.8 mm. Sternum VII posteriorly greatly produced in a large semicircular pregenital plate, the plate mainly being pale but posterior margin brown. Sternum VIII forms bilobed subgenital plate with a deep medial indentation at inner margin of the lobes, the median notch between subgenital plate dome-like. Paragenital plate with two branches, lower plate large, rounded and lightly pigmented lobe with hairs in ventral surface, upper plate forming a dark brown sloping sclerite seemingly like the pod brim. Sternum IX with anterior margin slightly protruded medially. Paraproct and cerci brownish.

Female inner genitalia (Fig. 21): Inner structure under pregenital plate is simple and membranous, lightly sclerotized, ovum-shaped anterior shield attached to the spermathecal ductus; attached muscles linking with margin of paragenital plate easily observed.

Mature larva (Fig. 22): Body relatively stout and small, body length without antennae and cerci 5.5 mm. General color light brown with distinct and characteristic dark brown dorsal pattern: head mostly dark brown with well delimited light brown pattern on occiput; scape and pedicel dark brown, rest of the antennae and palpi light brown; pronotum mostly pale, dark brown pattern similar to adults; meso-, metanotum and wing pads mostly pale but with distinct, paired Z-shaped dark pattern; legs light brown, apex of femora and base of tibiae darker; abdominal terga I–II entirely pale, terga III–V entire dark, terga VI–X laterally dark with medial pale area gradually widened towards the apex; cerci pale brown. Ventral aspect of the body entirely pale. Setation long and distinct. Legs moderately short, width of hind femora about one-third of their length. The pronotum is rounded trapezoidal, wider than long, slightly wider than head. Cervical gills long, inner gills with 6, outer with 7 branches. Wing pads more than twice as long as the corresponding segments. Abdomen relatively stout, integument light matt brown, first 3 abdominal segments fully, further 3 partly divided by pleura. Posterior margin of sternum IX of the male larva short and blunt triangular. Cerci long, with 25 slightly clubbed segments; length of the 15^{th} segment is more than 3 times of its width.

Setation of the larva (Figs 23–28): Head, antennae and palpi with short setae. Pronotum covered with short setae; marginal setae distinct and blunt, row continuous but setae shorter in anteromedial and posteromedial third, corners have setae as long as $1/15^{th}$ of pronotum width (Fig. 27). Setae on meso- and metanotum with marginal setae as long as longest marginal setae on pronotum; wing pads with short setae besides marginal ones. Legs with relatively sparse but diverse setae, all tibiae bear sparse and indistinct swimming hairs as long as tibia width (Fig. 28). Longest acute setae of all femora are about as long as half of the corresponding femur width, not arranged in line but restricted to apical half. Tarsi and claws normal. Tergal segments covered with thin setae and a few short hairs; posterior margin with row of 14–16 acute and erect setae, of various length, longest reaches more than half of segment length (Figs 23, 24). Cercal segments with relatively sparse and moderately long setation, apical whorl of setae consist of both dark and whitish setae; setae sparser and shorter on the basal segments (Figs 25, 26). Cercomeres 14–16 with intercalary setae-like indistinct fine hairs, as long as the segment width, and an apical whorl of 8 or 9 acute setae that are much shorter than segment length (Fig. 26).

Type material. Holotype male (HIST): CHINA, Shaanxi Province, Hanzhong, Foping County, brook in Lover's Valley by Foping Old Town, 885 m, 33°31.838'N, 107°59.432'E, 2018.IV.21, leg. W.H. Li, R.R. Mo and D. Murányi. Paratypes:

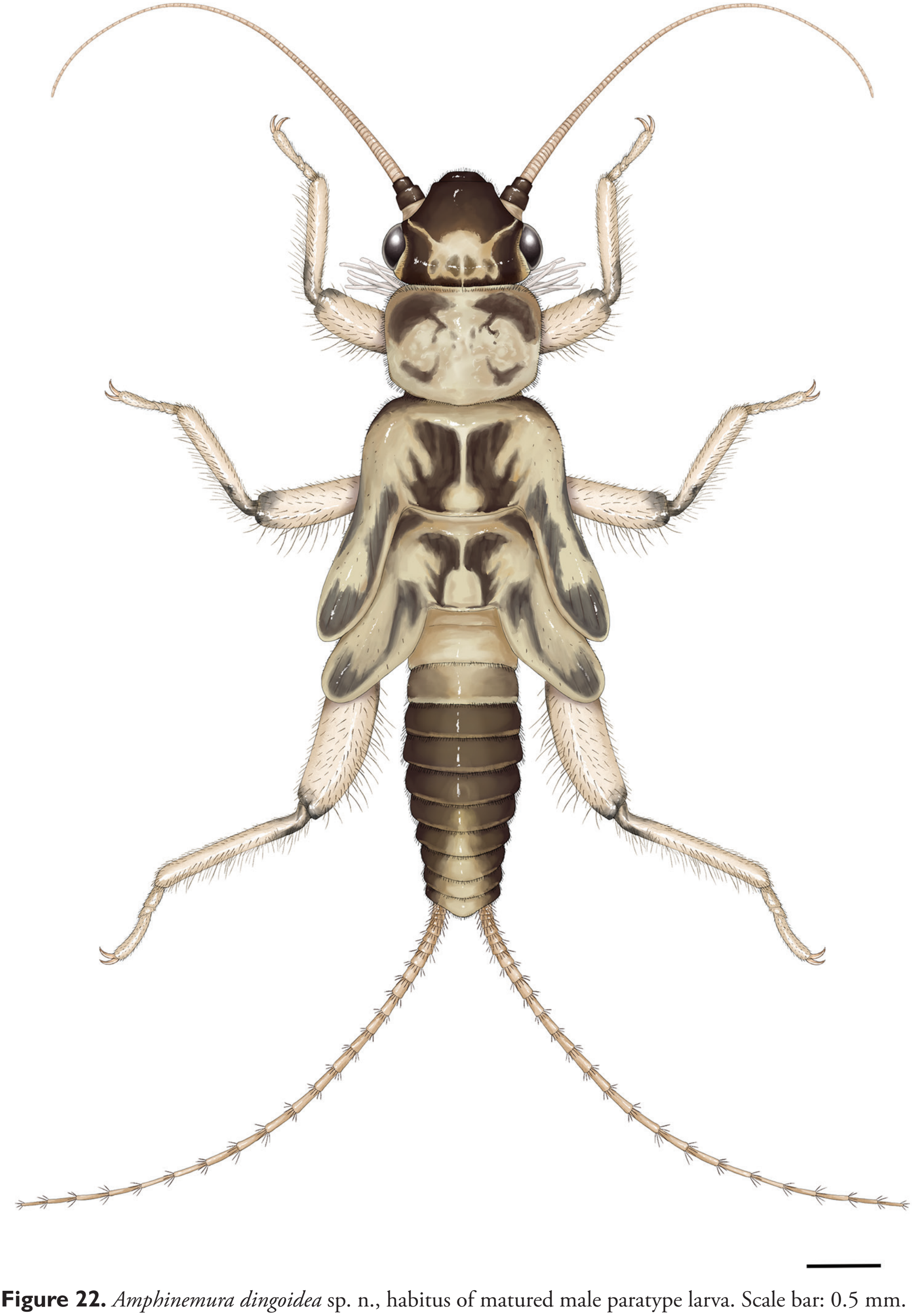
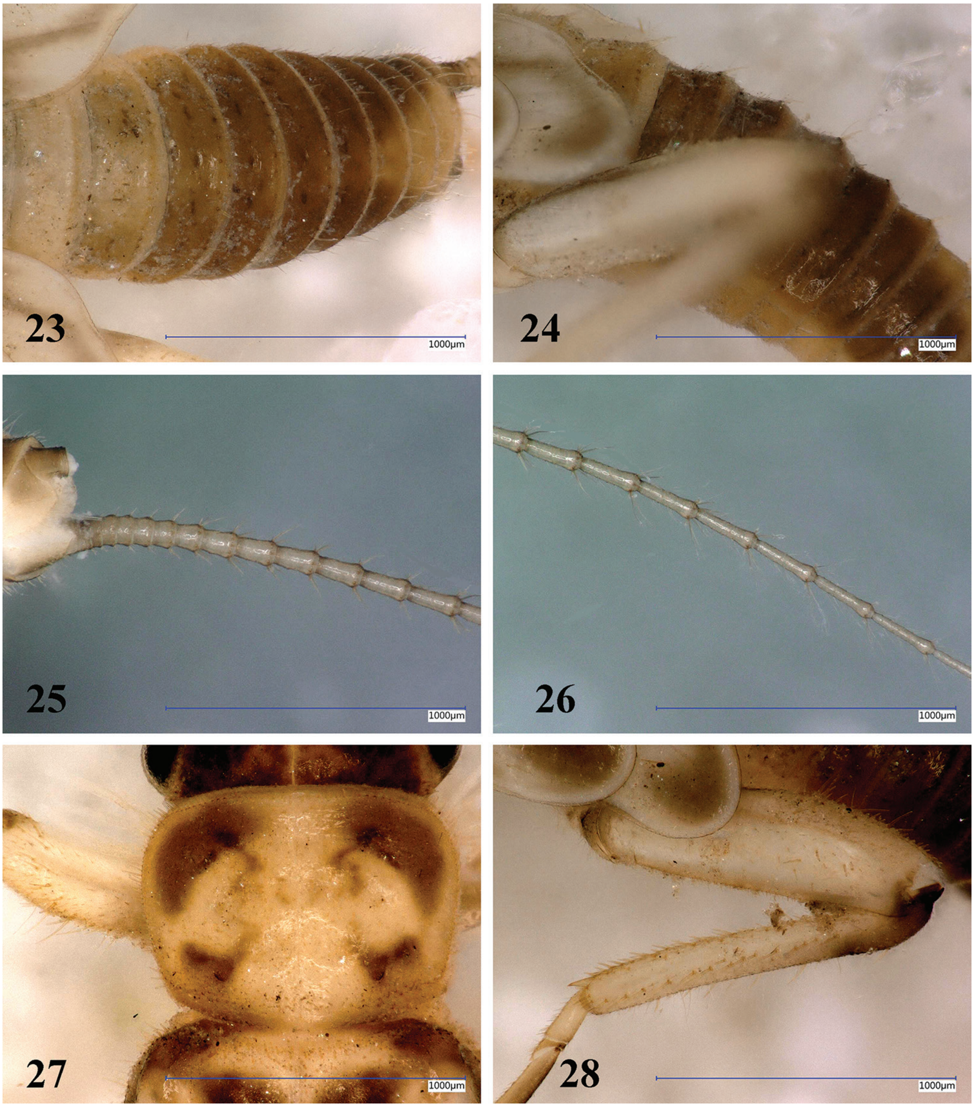


Figure 22. *Amphinemura dingoidea* sp. n., habitus of matured male paratype larva. Scale bar: 0.5 mm.



Figures 23–28. *Amphinemura dingoidea* sp. n., matured larva paratype. **23** Abdomen, dorsal view **24** Terga I–VII, lateral view **25** Paraproct and basal half of cerci, oblique ventral view **26** Cercomeres 14–21, oblique ventral view **27** Pronotum, dorsal view **28** Left hind leg, outer face. Scale bar: 1 mm.

same data as holotype: 2 females, 1 male larva (HIST), 1 male, 2 females (HNHM); Shaanxi Province, Hanzhong, Foping County, Changjiaoba Town, steep forest brook in Dizhuang valley, 980 m, 33°33.543'N, 107°58.263'E, 2018.IV.21, leg. W.H. Li, R.R. Mo and D. Murányi: 3 larvae (HNHM).

Affinities. On the basis of the simple epiproct and rather elongated median lobe of the paraproct, the male of *A. dingoidea* is similar to several other Chinese species, e.g. *A. curvispina* (Wu, 1973), *A. filarmia* Li & Yang, 2000, *A. microhamita* Li, Dong

& Yang, 2018, and *A. ovalis* Li & Yang, 2005. However, the combination of the long and slightly bilobed inner lobe and short, spine-less outer lobe of paraproct, together with simple but apically notched epiproct, distinguish the new species from all congeners. The female can be easily distinguished from females of the hitherto known Asian *Amphinemura* species on the basis of the distinctive shape of their subgenital plate combined with rather simple inner genitalia. The larva is distinctive by its rather conspicuous pale and dark brown color pattern.

Distribution and ecology. Most specimens were found along a small forest brook (Fig. 33), but a single male was collected by the upper, faster flowing section of the same large stream where *A. albicauda* was collected at the lower section (Fig. 35). Both localities are at moderate elevations, have fast current, and the littoral vegetation consists of deciduous forest, willow bush, and dense littoral grasses and sedges. The width of the brook at the type locality is less than 1 m and usually less than 10 cm in depth, but deeper, nearly stagnant pools also occur. The substrate is bedrock with small stones and variable debris (Fig. 34). Only a single mature larva was found among several other Nemouridae larvae, whereas most adults were fully mature and abundant among the adult stoneflies collected at this stream, suggesting late April is after the peak emergence. At the type locality, accompanying stoneflies were Nemouridae: *Amphinemura sinensis*, *A. unihamata* (Wu, 1973), *Sphaeronemoura grandicauda* (Wu, 1973), an unidentified *Indonemoura* sp. found only as premature larvae, and a yet undetermined *Nemoura* sp. of the *ovocercia* species group. In the Dizhuang valley, the paratype male was caught together with the recently described *Cryptoperla nangongshana* Huo & Du, 2018.

Etymology. The specific name refers to the shape of the pod-like subgenital plate and the pot-like anterolateral branches, which overall resembles the Chinese "ding", an ancient pot unique in Chinese culture.

Remarks. The adults were associated with the single mature larva on the basis of the distinct pronotal pattern and similar, contrasting body color.

Amphinemura sinensis (Wu, 1926)

Fig. 35

Material examined. CHINA, Shaanxi Province, Hanzhong, Foping County, brook in Lover's Valley by Foping Old Town, 870 m, 33°31.819'N, 107°59.335'E, 2018.IV.21, leg. W.H. Li, R.R. Mo and D. Murányi: 1 male (HNHM); Shaanxi Province, Hanzhong, Foping County, Changjiaoba Town, lower section of a large unnamed stream in Dizhuang valley, 895 m, 33°33.344'N, 107°59.018'E, 2018.IV.21, leg. W.H. Li, R.R. Mo and D. Murányi: 1 male (HIST).

Distribution and ecology. This species was described from Jiangsu Province, later reported also from Beijing and Henan (Yang and Li 2018), and is the only member of the *sinensis* group that occurs in Palaearctic areas of China. We collected this species at the type localities of the new species above (Figs 31–34). Our new records represent the first records from Shaanxi.

Remarks. From both streams where we found this species, we also collected mature larvae that are probably belong to this species. These are very similar to the larva of *A. albicercia* in color pattern and setation, but lacks the distinctive, dense white hairs on the cerci.

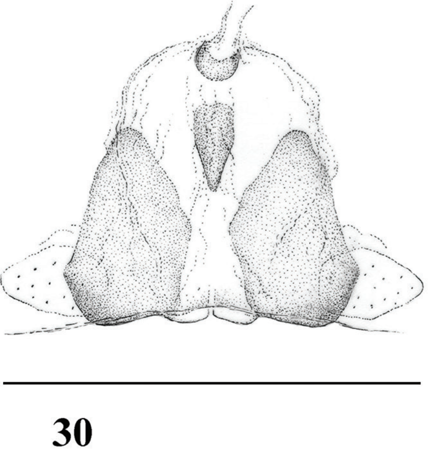
Amphinemura unihamata (Wu, 1973)

Figs 29, 30, 35

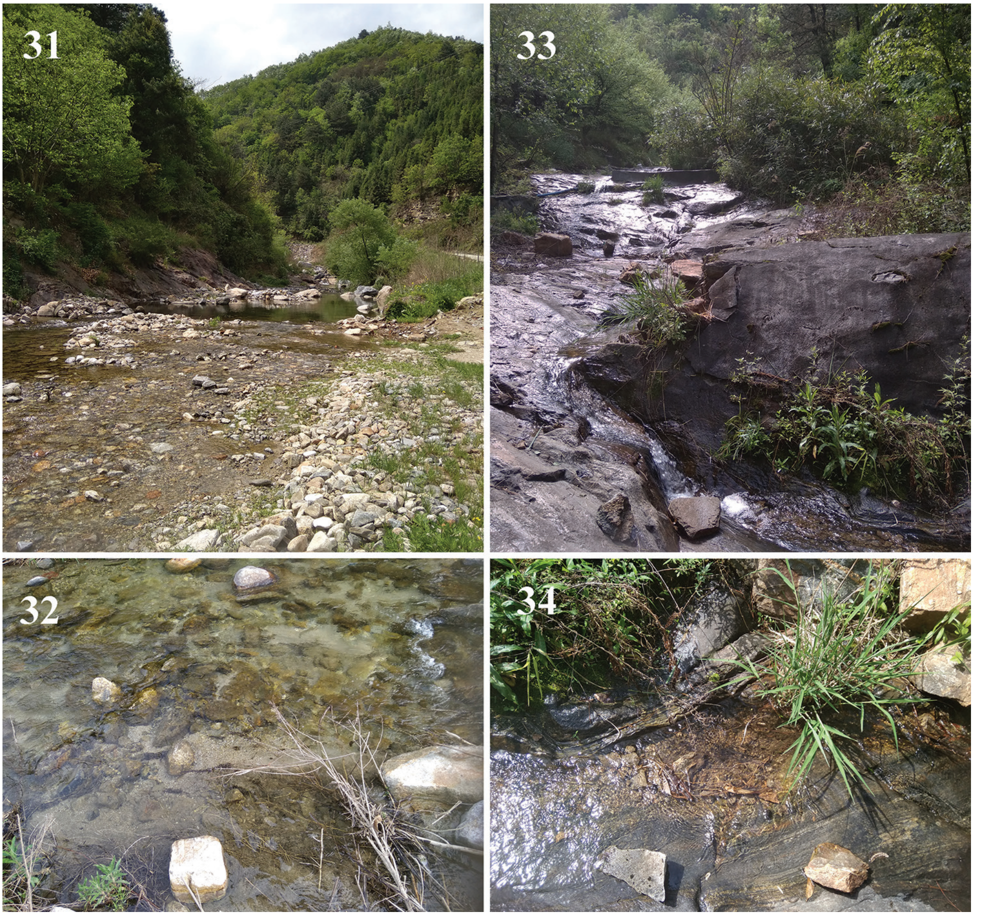
Description of the female. (Fig. 29) Forewing length 9.0–9.6 mm. Sternum VII posteriorly produced in a wide but short, semicircular pregenital plate, the plate pale. Sternum VIII forms trapezoid subgenital plate that is about half as wide as the segment, posterior lobes distinctly divided into rounded sublobes, nearly equal in size; posteromedial indentation narrow and triangular, with white medial area widened towards anterior edge. Paragenital plate large, rounded, but pale and rather indistinct. Sternum IX with anterior margin slightly protruded medially. Paraproct and cerci brown.

Female inner genitalia (Fig. 30): Inner structure consist of a beak-like, dark median sclerite positioned under pregenital plate at the anterior edge of subgenital plate, is simple and a smaller but thick, ring-shaped anterior shield attached to the spermathecal ductus; membranous portion wide and attached to the paragenital plates.

Material examined. CHINA, Shaanxi Province, Hanzhong, Foping County, steep forest brook in Panda valley, 1270 m, 33°40.322'N, 107°58.190'E, 2018.



Figures 29–30. *Amphinemura unihamata* (Wu, 1973), terminalia of adult female. **29** Terminalia in ventral view **30** Inner genitalia, dorsal view. Scale bar: 0.5 mm.



Figures 31–34. Type localities. **31, 32** *Amphinemura albicauda* sp. n., Dizhuang valley, 895 m, 33°33.344'N, 107°59.018'E **33, 34** *Amphinemura dingoidea* sp. n., Lover's Valley, 885 m, 33°31.838'N, 107°59.432'E.

IV.20, leg. W.H. Li, R.R. Mo and D. Murányi: 1 male (HNHM); Shaanxi Province, Hanzhong, Foping County, brook in Lover's Valley by Foping Old Town, 885 m, 33°31.838'N, 107°59.432'E, 2018.IV.21, leg. W.H. Li, R.R. Mo and D. Murányi: 2 females (HNHM); same brook and date, a few hundred meters downstream, 870 m, 33°31.819'N, 107°59.335'E: 1 male (HIST), 1 female (HNHM).

Distribution and ecology. Previously, this species was known only from the holotype, collected in Sichuan. It was recently redescribed by Yang et al. (2015). We found it at the type locality of *A. dingoidea* (Figs 31, 32) and a similar small brook at slightly higher elevation, representing its first records from Shaanxi.

Remarks. *Amphinemura annulata* Du & Ji in Ji et al., 2014 was described one year earlier before the specific identity of *A. unihamata* was resolved by Yang et al. (2015). The holotype of *A. annulata* was designated from Zhejiang Province but the paratype series included specimens also from Shanxi, Shaanxi, and Guizhou provinces. The two species are probably conspecific, but without examination of holotype of *A. annulata*, we do not propose a synonymy of *A. unihamata* at this time.

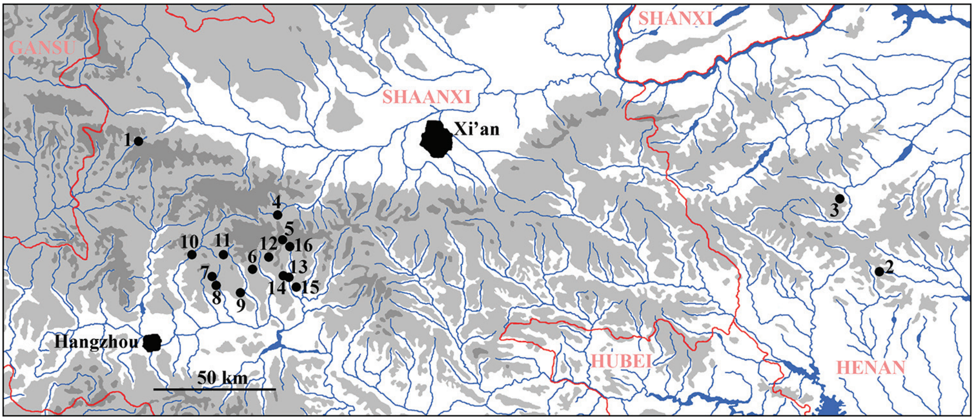


Figure 35. Known distribution of Amphinemurinae in the Qinling Mountains **1** *Amphinemura lingulata*, *Mesonemoura membranosa* **2** *A. didyma*, *A. mamillata*, *Indonemoura auriformis*, *M. tritaenia* **3** *Sphaeronemoura separata* **4** *A. annulata*, *A. lingulata* **5** *A. annulata* **6** *A. dicroidea* **7** *A. longihamita*, *S. grandicauda* **8** *A. microhamita* **9** *A. microhamita* **10** *A. microhamita*, *A. multispina* **11** *S. grandicauda* **12** *S. grandicauda* **13** *A. albicauda*, *A. sinensis* **14** *A. dingoidea* **15** *A. dingoidea*, *A. sinensis*, *A. unihamata*, *Indonemoura* sp., *S. grandicauda* **16** *A. unihamata*.

Concluding remarks: the Amphinemurinae known from the Qinling Mountains

Sloping from the Qinghai-Tibet Plateau to the North China Plain, the Qinling Mountains form the boundary between the Palaearctic and Oriental realms and are an important biodiversity hotspot (Chen et al. 2008, Zhang et al. 2014). Even though one of the earliest Chinese publications on stoneflies was published on species from the Qinling (Klapálek 1908), the Amphinemurinae remained unknown until the last ten years.

The first known Amphinemurinae species, *Mesonemoura membranosa* Du & Zhou in Du et al., 2007a was described from the central part of the mountain range in Shaanxi. Subsequently, four additional species were reported from the eastern extremities of the range in Henan Province: *M. tritaenia* Li & Yang, 2007b, *A. mamillata* Li & Yang, 2008e, and *Indonemoura auriformis* Li & Yang, 2008e were described as new, whereas *A. didyma* was reported from Baotianman as new for Henan (Li and Yang 2008e). Later, *Sphaeronemoura separata* Li, Murányi & Yang, 2014 was described from the ranges in Henan, while Ji et al. (2014) described *A. annulata* Du & Ji in Ji et al., 2014 with paratypes from the Shaanxi ranges, and *A. lingulata* Du & Wang in Ji et al., 2014 from the holotype and some paratypes from the central ranges in Shaanxi. More recently, Li et al. (2018) described three species, *A. dicroidea* Li, Dong & Yang, 2018, *A. longihamita* Li, Dong & Yang, 2018, and *A. microhamita* Li, Dong & Yang, 2018, from the Shaanxi areas of the Qinling, and reported *A. multispina* (Wu, 1973) and *S. grandicauda* (Wu, 1973) from the same ranges.

Including the newly described or reported species in the present paper, 16 species are now known from the Qinling Mountains. However, this number must be just a fraction of the possible diversity. Seven of the species described from the range are known only from their Qinling type localities, whereas *A. mamillata* was later found also in Ningxia (Yang and Li 2018), and the type series of *A. annulata* included specimens from Zhejiang, Shanxi, and Guizhou. The paratypes of *A. lingulata* were from Sichuan (Ji et al. 2014). Among species subsequently reported from the Qinling, *A. didyma* is also known from Ningxia and Inner Mongolia, *A. multispina, A. unihamata*, and *S. grandicauda* from Sichuan, and *A. sinensis* from Jiangsu, Beijing, and Henan (Yang and Li 2018). These distributions support well the idea that the Qinling Mountains, as a border zone between realms, include species distributed mainly in the Palaearctic as well as other species that are members of the Oriental fauna. However, none of the known species have dispersed far into the both realms, e.g. no species is known to occur in northeastern or southern China.

Amphinemura is a widespread Holarctic and Oriental genus, whereas *Indonemoura* Baumann, 1975, *Mesonemoura* Baumann, 1975, and *Sphaeronemoura* Shimizu & Sivec, 2001 are distributed primarily in the Oriental Region. Among these three genera, *Indonemoura* is not known to occur on the mainland north to Qinling, further supporting the uniqueness of this Palaearctic and Oriental border region.

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Morphological characters of immature stages of Palaearctic species of *Cleopomiarus* and *Miarus* and their systematic value in Mecinini (Coleoptera, Curculionidae, Curculioninae)

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Abstract

The relationship between the genera *Cleopomiarus* and *Miarus* of Mecinini (Curculionidae, Curculioninae) was tested on the basis of morphological characters from the immature stages. The mature larvae of five *Cleopomiarus* species (*C. distinctus* (Boheman, 1845), *C. graminis* (Gyllenhal, 1813), *C. longirostris* (Gyllenhal, 1838), *C. medius* (Desbrochers des Loges, 1893), and *C. meridionalis* (H. Brisout de Barneville, 1863)), three *Miarus* species (*M. abnormis* Solari, 1947, *M. ajugae* (Herbst, 1795), and *M. campanulae* (Linnaeus, 1767)), and the pupae of four *Cleopomiarus* species (*C. distinctus*, *C. graminis*, *C. longirostris*, and *C. medius*) and two *Miarus* species (*M. abnormis* and *M. ajugae*) are described in detail for the first time. To confirm the taxonomic identification of some larvae, DNA COI barcode was obtained and compared with those of adults. The immature stages of the species herein studied were compared with those known from other genera in tribe Mecinini. It is suggested that *Miarus* and *Cleopomiarus* may be monophyletic based on several shared distinctive characters. Larvae of *Miarus* have a characteristic maxillary mala with six finger-like *dms* of two sizes (one or two *dms* very long and the rest of medium length), this feature being apparently unique among weevils. Other genus-specific character states are observed in the pupae, such as the length of setae on the head, rostrum and pronotum, including the number of *rs* on the rostrum, *ds* on pronotum, and finally the shape of the urogomphi. A key to the described larvae and pupae were respectively presented. New biological and distributional data on some species are reported.

Keywords
biology, *Cleopomiarus*, distribution, mature larva, Mecinini, *Miarus*, morphology, pupa

Introduction

The Mecinini is a tribe of the subfamily Curculioninae (Curculionidae) and comprises six genera: *Cleopomiarus* Pierce, 1919, *Gymnetron* Schoenherr, 1825, *Mecinus* Germar, 1821, *Miarus* Schoenherr, 1826, *Rhinumiarus* Caldara, 2001 and *Rhinusa* Stephens, 1829 (Caldara 2001; Caldara et al. 2014; Alonso-Zarazaga et al. 2017). Whereas *Rhinumiarus* is only found in South America, the other five genera are largely distributed in the Palaearctic region. Moreover, *Cleopomiarus* and *Gymnetron* are well known in the Afrotropical region, with many species, especially in South Africa (Caldara 2003, 2005). In the last twenty years, these genera were subjected to a careful taxonomic revision (not yet completed for *Rhinusa*) and a phylogenetic analysis based on adult morphology (Caldara 2001, 2003, 2005, 2007; Caldara et al. 2010, 2013; Caldara and Fogato 2013).

With regard to the biology, the larvae of Mecinini develop in roots, shoots, leaves and flowers, many of them causing the organs of the host plants to swell or develop into galls; moreover, some species of *Rhinusa* are inquilines in galls produced by other species of the same genus (Hoffmann 1958; Arzanov 2000; Caldara 2001, 2003, 2005, 2007; Korotyaev et al. 2005). The larvae are predominantly associated with the families Scrophulariaceae, Plantaginaceae, and Campanulaceae (sensu APG 2016). *Mecinus* species live on Plantaginaceae, while *Gymnetron* and *Rhinusa* species live on both Scrophulariaceae and Plantaginaceae, which are two closely related families placed together in the Order Lamiales (Olmstead et al. 2001; Albach et al. 2005; APG 2016). The Palaearctic species of *Gymnetron* live on *Veronica* (Caldara 2008), currently included in Plantaginaceae (Olmstead et al. 2001; Albach et al. 2005), while those in the Afrotropical region, where Plantaginaceae are poorly represented, appear to live on various genera of Scrophulariaceae distributed mainly in the southern hemisphere (Caldara 2003; Caldara et al. 2010). In contrast, the Palaearctic species of *Miarus* and *Cleopomiarus* are associated with the genera of Campanulaceae in the subfamily Campanuloideae (*Campanula*, *Jasione*, *Phyteuma*), whereas the *Cleopomiarus* species in South Africa and in the southern part of North America live on the genera of the subfamilies Campanuloideae (*Roella*, *Wahlenbergia*) and Lobelioideae (*Lobelia*) (Caldara 2005, 2007; Caldara and Legalov 2016; Prena and O'Brien 2017). However, it is noteworthy that the systematics of Campanuloideae, especially of *Campanula* s.l. and close genera is still highly unstable (see APG 2016). This plant family is less phylogenetically close to

Scrophulariaceae and Plantaginaceae and placed in Order Asterales (APG 2016). *Cleopomiarus* and *Miarus* are very closely related each other and morphologically somewhat far from the other mecinine genera, as recent taxonomic revisions have shown (Caldara 2001, 2005, 2007; Caldara and Legalov 2016; Jiang et al. 2018).

The general habitus of the imagoes of all *Cleopomiarus* and *Miarus* species is very uniform, and there are few external characters allowing differentiation of many species. Species recognition is often possible only by the careful examination of male or female genitalia. The presence of a deep prosternal canal and free claws are two easily observed external characters that immediately allow the separation of *Cleopomiarus* and *Miarus* from other Mecinini. The shape of the penis and the sclerites of the endophallus, the slightly more pronounced convexity of the male pygidium, and the more globose femora distinguish *Cleopomiarus* from *Miarus*. Moreover, in many species of *Cleopomiarus*, meso- and metafemora are dentate, and the uncus of the male metatibiae is enlarged, whereas the fifth ventrite of *Miarus* often shows a median fovea and two teeth placed posterolaterally. Finally, both genera feed on Campanulaceae, a family of plants apparently not parasitized by any other weevil. Preliminary molecular studies appear to confirm the systematic separation of these two genera, whereas several species of *Miarus*, well identified on the basis of morphological characteristics, tend to have very similar DNA fragments on mitochondrial COI gene (Vahtera and Muona 2006; Hendrich et al. 2015; Horecka et al. 2017; I Toševski, unpublished data). It is clear that more characters are required to separate these two genera from each other and from other Mecinini genera.

A detailed study of immatures might reveal more defining characters. To date, larvae of only 19 Mecinini species have been described (Gardner 1934; Emden 1938; Scherf 1964; Anderson 1973; Lee and Morimoto 1988; May 1993; Gosik 2010; Jiang and Zhang 2015), while descriptions of pupae are known for ten Mecinini species (Scherf 1964; Anderson 1973; Gosik 2010; Jiang and Zhang 2015). However, there are only four detailed descriptions of larvae and pupae that can be used for an adequate taxonomic comparison; these include immatures of three species of *Gymnetron* (Jiang and Zhang 2015) and one species of *Rhinusa* (Gosik 2010). In fact, the comparison with other previously described immatures, e.g., *Gymnetron anagallis* Marshall, 1933 (Gardner 1934; Emden 1938); *Mecinus heydenii* Wencker, 1866 (Emden 1938); *M. janthinus* Germar, 1821 (Scherf 1964); *Gymnetron beccabungae* (Linnaeus, 1760), *G. villosulum* Gyllenhal, 1838; *Rhinusa collina* (Gyllenhal, 1813); *R. linariae* (Panzer, 1795); *Cleopomiarus graminis*, *C. hispidulus* (LeConte, 1876), and *Miarus campanulae* (Emden 1938; Scherf 1964; Anderson 1973) is somewhat problematic due to the missing details of the chaetotaxy and/or the absence of quality drawings.

Therefore, the purpose of this study was the following: 1) to describe larvae and pupae of *Miarus* and *Cleopomiarus* in detail for the first time, confirming when necessary the identity of the immatures by the study of the DNA COI barcode; 2) to find characters distinctive between these two genera and between the species; and 3) to investigate the relationships of these two genera with other genera of the same tribe and other tribes within Curculioninae.

Materials and methods

Insect collection

Immature specimens examined in this study came from material preserved at the British Museum of Natural History (London), the Department of Zoology University collection of Maria Curie-Skłodowska (Lublin) and from personal collections of the two authors (RC and IT) which are deposited in the collection of the Group Function of Invertebrate and Plant Biodiversity in Agro-Ecosystems of the Crop Research Institute (Prague, Czech Republic). In the last case, the specimens were collected and placed in tubes with 95% ethyl alcohol generally with a few adults. Since it is well known that more than one species of the complex *Miarus* + *Cleopomiarus* can be found on the same plant (Caldara 2007; Caldara and Legalov 2016), to be completely sure of the identification of some immatures, the DNA COI barcode of some specimens was also studied and compared with adults found in the same plant or with data already deposited in GenBank. The collectors identified the plants.

Morphological descriptions

Part of the larval and pupal material was preserved in Pampel fixation liquid (see Trnka et al. 2015) and used for the morphological descriptions. To prepare the slides, we followed May (1994): a larva was decapitated, and the head was cleared in a 10% potassium hydroxide (KOH) solution and then rinsed in distilled water. After clearing, the mouthparts were separated from the head capsule, and the head capsule and all mouthparts were mounted on permanent microscope slides in Euparal. All other body parts were mounted on temporary microscope slides in 10% glycerine.

The observations and measurements were conducted using a light microscope with calibrated oculars (Olympus BX 40 and Nikon Eclipse 80i). The following characters were measured for each larva: head width, body length (larvae fixed in a C-shape were measured in segments), and body width in the widest place (i.e., metathorax or abdominal segments I–IV). For the pupae, the length and width at the widest place were measured. The lengths of all setae are visible on Figures.

Drawings were created with a drawing tube on a light microscope and processed by a computer (Adobe Photoshop, Corel Photo-Paint 11, GIMP 2). The numbers of setae of the bilateral structures are given for one side.

We used the terms and abbreviations for the setae of the mature larvae and pupae found in Scherf (1964), May (1977, 1994), and Marvaldi (1998, 1999).

Molecular analysis

For molecular analysis, DNA was extracted from larvae and adults collected from seed capsules or flowers of plants belonging to the Campanulaceae. The barcoding region of the mitochondrial cytochrome c oxidase subunit I gene (mtCOI) was used to confirm the identity of the sampled larvae and the corresponding adults previously determined by using morphological characteristics (Caldara 2007; Caldara and Legalov 2016). Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. The barcoding region of the mtCOI gene was amplified using the *de novo* designed primer pair for *Miarus* and *Cleopomiarus* species, MiaF (5' CATGATCAGGAATACTMGGAACATC 3') and MiaR (5' GCTCGTGTATCAACATCTATTCC 3'). The MiaF/MiaR primers amplified a mtCOI product of 838 bp, which consisted of 635 bp of the barcoding region (Hebert et al. 2003).

Each PCR reaction was carried out in a volume of 20 µl [1 µl of DNA, 11.8 µl of H2O, 2 µl of High Yield Reaction Buffer A (1 × 1.5 mM MgCl2), 1.8 µl of MgCl2 (2.25 mM), 1.2 µl of dNTP (0.6 mM), 1 µl of each primer of the pair MiaF/MiaR (0.5 µM) and 0.2 µl of KAPATaq DNA polymerase (0.0375 U/µl) (Kapa Biosystems Inc. USA)]. The PCR protocol consisted of an initial denaturation at 95 °C for 5 min; 35 cycles consisting of three steps, i.e., 1 min at 94 °C, 1 min at 54 °C and 1.5 min at 72 °C; and a final extension step at 72 °C for 7 min. After PCR amplification, the products were separated on a 1% agarose gel, stained with ethidium bromide, and visualized under a UV transilluminator. The amplified products were sequenced by Macrogen Inc. (Seoul, Korea). The sequence data were deposited in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) under accession number MH558545–MH558548.

Results

Morphology of immature stages
Description of the mature larva (L3)

Genus *Cleopomiarus* Pierce, 1919

Description. *Measurements* (in mm). Body length: 2.20–8.70. Body width (metathorax or abdominal segments I–II) 0.73–2.44. Head width: 0.35–1.16.

General. Body elongated, slender, rounded in cross section.

Colouration. From yellow to pale brown head. All thoracic and abdominal segments from distinctly white to slightly yellow.

Vestiture. Setae on body thin, in different colouration, distinctly different in length; piliform, often with some asperities.

Head capsule. Head oval or suboval, slightly or more flattened laterally, endocarinal line present and very distinct, more than half the length of frons. Frontal sutures on the head in different sizes, and ever extended to antennae. One or two stemmata (st), anterior stemma in the form of a pigmented spot with convex cornea behind the antenna. Dorsum of the epicranium with five setae; des_3 located anteriorly on epicranium close border with frontal suture. Frons with four setae; fs_2 absent; fs_4; and fs_5 subequal. Head also with two *les* and two *ves*. Epicranial area with three *pes* and 2–3 sensilla.

Antennae located at the end of the frontal suture on each side, membranous and distinctly convex basal article bearing 3–4 sensilla and one conical sensorium, the later elongated, narrow.

Clypeus transverse-shaped, approximately 2.5–3 times as wide as long with two *cls*, and one sensillum (clss) between setae; all very close to margin with frons.

Mouthparts. Labrum with three piliform *lms*; anterior margin bisinuate. Epipharynx with three finger-like *als*; with 2–3 *ams*; and 0–1 *mes*; labral rods (lr) distinct, elongated. Mandibles distinctly broad, bifid, teeth of unequal height; slightly truncate; both setae piliform. Maxilla stipes with one *stps*, two *pfs* and one *mbs* and one sensillum; mala with six finger-like *dms*; five *vms*; all *vms* distinctly shorter than *dms*. Maxillary palpi with two palpomeres; basal palpomere with one short *mxps* and two sensilla; distal palpomere with one sensillum and a group of micro cuticular apical processes. Prelabium oval-shaped, with one *prms*; ligula with sinuate margin and 1–2 *ligs*; premental sclerite well sclerotized but without anterior and posterior extensions, U-shaped. Labial palpi with two palpomeres (partially appears as one palpomere); each of the palpomeres with one sensillum, distal palpomere with cuticular apical processes. Postlabium with three *pms*, all located laterally.

Thorax. Prothorax slightly smaller than meso- and metathorax. Spiracle bicameral, placed between the pro- and mesothorax (see, e.g., Skuhrovec et al. 2015). Prothorax with 9–10 *prns*; two *ps*; and one *eus*. Mesothorax with one *prs*, three *pds*; one *as*; two long and one short *ss*; one *eps*; one *ps*; and one *eus*. Chaetotaxy of metathorax almost identical to that of mesothorax. Each pedal area of thoracic segments well separated, with 5–6 *pda*.

Abdomen. Abdominal segments I–III of almost equal length, next abdominal segments decreasing gradually to the terminal parts of the body. Abdominal segment X reduced to four anal lobes of unequal size, the lateral lobes being distinctly the largest, the dorsal and the ventral lobes being very small. Anus located terminally. Eight spiracles, bicameral, all spiracles functional, close to the anterior margin. Abdominal segments I–VII with one *prs*; three *pds*, pds_2 the longest one; one long and one minute *ss*; two long *eps*; one *ps*; one *lsts*; and two *eus*. Abdominal segment VIII with one *prs*; 2–3 *pds*, if there are three setae, then pds_2 the longest one; one long and one minute *ss*; two long *eps*; one *ps*; one *lsts*; and two *eus*. Abdominal segment IX with four *ds*; 1–2 *ps*; and 1–2 *sts*. Abdominal segment X with one minute seta present or absent.

Cleopomiarus distinctus **(Boheman, 1845)**

Figures 1–10

Material examined. 17 L3 larvae: 7 exx., 29.07.2010, Gródek ad Hrubieszów, CE Poland, leg. E. Szwaj, det. J. Łętowski; 10 exx., ex seed capsules of *Campanula cervicaria* L., 05.07.2017, Stara Planina, Babin Zub, east Serbia, leg. I. Toševski, all collected in association with adults det. R. Caldara. Accession numbers of sequenced specimens: MH558546.

Figure 1. *Cleopomiarus distinctus* mature larva habitus.

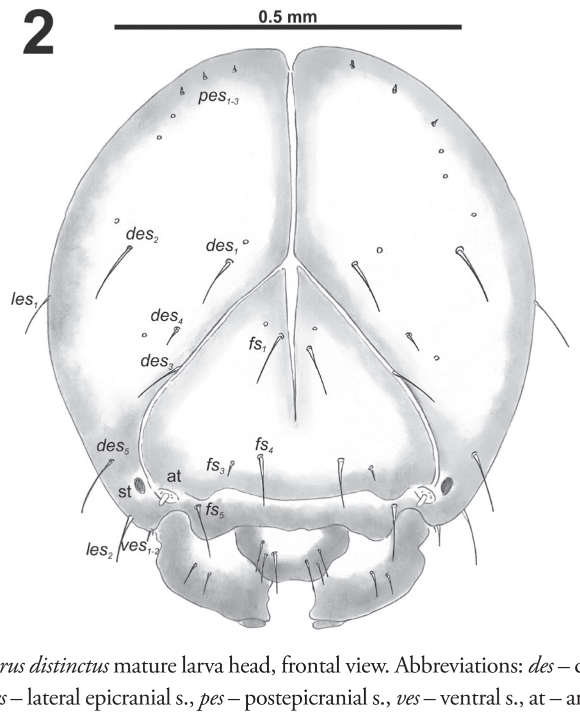
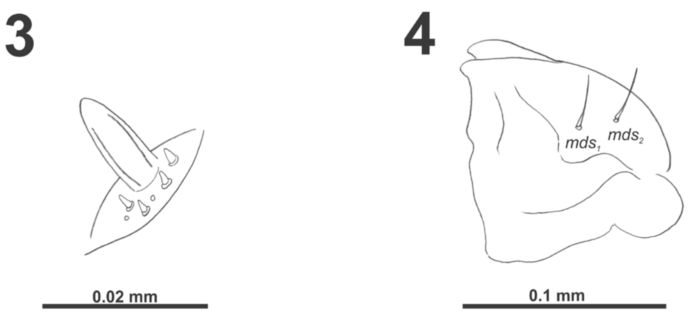
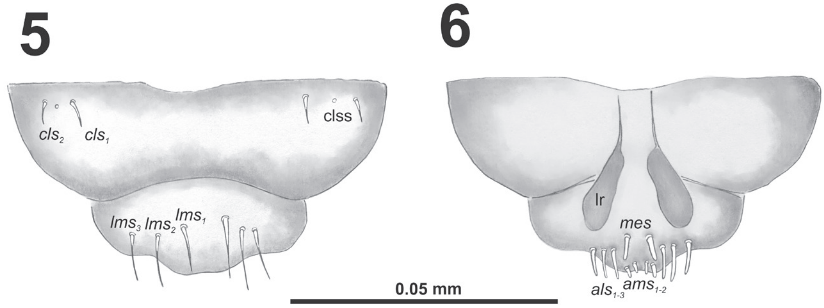


Figure 2. *Cleopomiarus distinctus* mature larva head, frontal view. Abbreviations: *des* – dorsal epicranial s., *fs* – frontal epicranial s., *les* – lateral epicranial s., *pes* – postepicranial s., *ves* – ventral s., at – antenna, st – stemmata.



Figures 3–4. *Cleopomiarus distinctus* mature larva. **3** Antenna **4** Right mandible. Abbreviation: *mds* – mandible dorsal s.



Figures 5–6. *Cleopomiarus distinctus* mature larva, mouthparts. **5** Labrum and clypeus **6** Epipharynx. Abbreviations: *als* – anteriolateral s., *ams* – anteromedial s., *cls* – clypeal s., *lms* – labral s., *mes* – median s., clss – clypeal sensillum, lr – labral rods.

Description. *Measurements* (in mm). Body length: 4.43–5.57 (mean 4.90). Body width (metathorax or abdominal segments I–II) up to 1.37. Head width: 0.70–0.84 (mean 0.71).

General. Body elongated, slender, curved, rounded in cross section (Fig. 1).

Colouration. Black head (Fig. 1). All thoracic and abdominal segments from distinctly white to slightly yellow (Fig. 1).

Vestiture. Setae on body thin, light yellow to greyish, distinctly different in length (minute to very long).

Head capsule (Fig. 2). Head oval, slightly flattened laterally. Frontal sutures distinct, seem as pallid stripes. Anterior stemma (st), in the form of a small pigmented spot. Des_{1-3} and des_5 long; des_4 short (Fig. 2). Fs_1 long; fs_2 absent; fs_3 very short; fs_4 long; and long fs_5 (Fig. 2). Les_1 and les_2 as long as des_5; both *ves* medium to very short. Epicranial area with three *pes* and two sensilla in line with des_2.

Antennae bearing one relatively elongated conical sensorium; and basal membranous article with four sensilla equal in length, and two pores (Fig. 3).

Clypeus (Fig. 5) approximately three times as wide as long with two *cls* of medium size, equal in length, and one sensillum; anterior margin sinuate.

Mouthparts. Labrum (Fig. 5) almost two times as wide as long, with three piliform *lms*, almost equal in the length; all located more or less anteromedially, lms_2 and lms_3

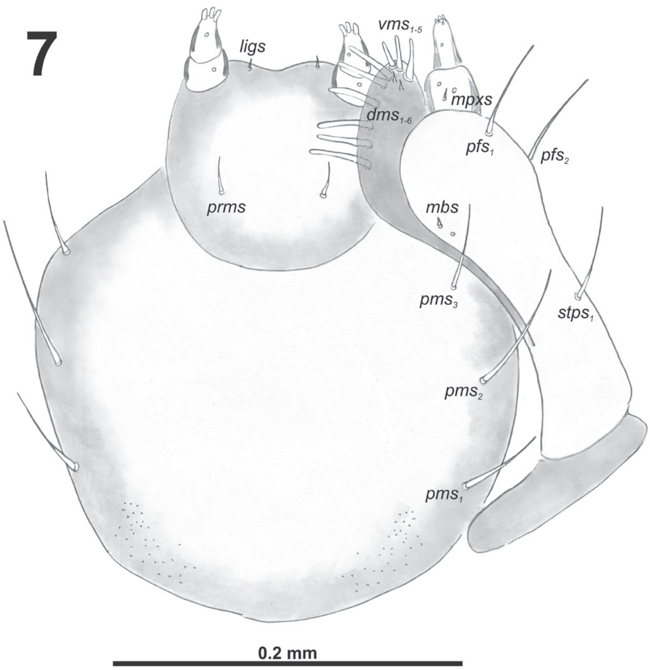
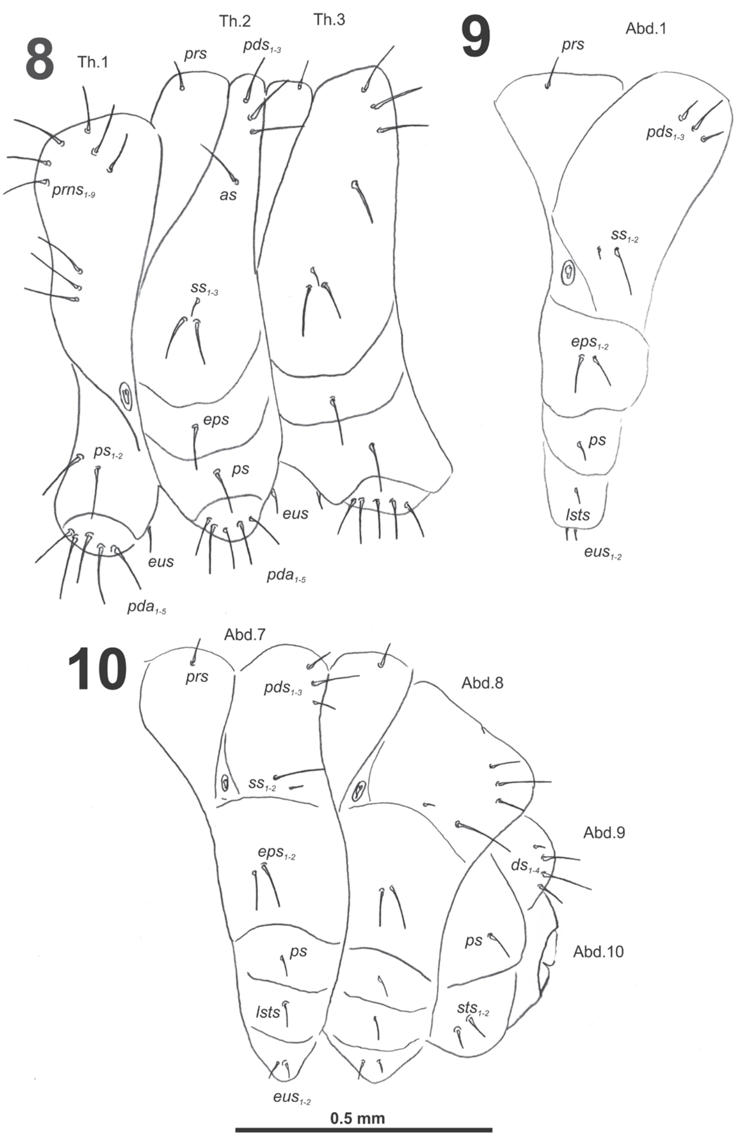


Figure 7. *Cleopomiarus distinctus* larval mouthparts, maxillolabial complex, ventral view right maxilla. Abbreviations: *dms* – dorsal malar s., *vms* – ventral malar s., *mpxs* – maxillary palps s., *mbs* – basioventral s., *pfs* – palpiferal s., *stps* – stipital s., *prms* – premental s., *pms* – postmental s., *ligs* – ligular s.

distinctly reach labral margin. Epipharynx (Fig. 6) with three medium sized finger-like *als*, all similar in length; with two rather short, equal in length *ams*; and one medium size, finger-like *mes*; labral rods (lr) distinct, elongated, slightly convex. Mandibles (Fig. 4) bifid; cutting edge with a blunt tooth; bearing with two setae in medium size, piliform, and aligned longitudinally. Maxilla (Fig. 7) stipes with long *stps* and both *pfs*, minute *mbs*, and one sensillum close to *mbs*; mala with six medium sized finger-like *dms*; five *vms*, three medium size, two very short. Maxillary palpi: basal palpomere with one short *mxps* and two sensilla; distal palpomeres with medium, cuticular apical processes; length ratio of basal and distal palpomeres 1:1. Prelabium (Fig. 7) with one short *prms*; ligula with one minute *ligs*; premental sclerite narrow, ring-shaped. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres 1:1.2; each of the palpomeres with one sensillum, distal palpomeres with medium, cuticular apical processes. Postlabium (Fig. 7) with long *pms_1* located basally, very long *pms_2* located medially and long *pms_3* located apically; membranous area basolaterally sparsely and finely asperate.

Thorax. Prothorax (Fig. 8) with nine very long *prns*, weakly pigmented dorsal sclerite present with six long *prns*, this sclerite subdivided in two triangular plates medially; two long *ps*; and one short *eus*. Meso- and metathorax (Fig. 8) with one medium *prs*, three long *pds*; one long *as*; two very long and one minute *ss*; one long



Figures 8–10. *Cleopomiarus distinctus* mature larva, habitus. **8** Lateral view of thoracic segments **9** Lateral view of abdominal segment I **10** Lateral view of abdominal segments VII–X. Abbreviations: *as* – alar s., *ds* – dorsal s., *eps* – epipleural s., *eus* – eusternal s., *lsts* – laterosternal s., *pda* – pedal s., *pds* – postdorsal s., *prns* – pronotal s., *prs* – prodorsal s., *ss* – spiracular s., *ps* – pleural s., *sts* – sternal s., Th1–3 – number of thoracic segments, Ab1–10 – number of abdominal seg.

eps; one long *ps*; and one very short *eus*. Each pedal area of the thoracic segments with five very long *pda*.

Abdomen. Abdominal segments I–VII (Figs 9–10) with one medium *prs*; one long and two medium size *pds* (order: medium, long, medium); one very long and one minute *ss*; two very long *eps*; one medium *ps*; one medium *lsts*; and two very short *eus*.

Abdominal segment VIII (Fig. 10) with one very short to minute *prs*; one short and two long to relatively long *pds* (order: short, long, relatively long); one long and one minute *ss*; two very long *eps*; one medium *ps*; one medium *lsts*; and two very short *eus*. Abdominal segment IX (Fig. 10) with four short *ds*; one medium *ps*; and two short *sts*. Abdominal segment X (Fig. 10) without seta.

Biology. This species lives on various species of *Campanula* (*C. glomerata* L., *C. incurva* Auch., *C. latifolia* L., *C. persicifolia* L., *C. rapunculus* L., *C. rhomboidalis* L., *C. thyrsoides* L., *C. trachelium* L.) in central Europe (Hoffmann 1958; Smreczyński 1976; Caldara and Legalov 2016). It was never reported to feed on *C. cervicaria* L., a species widely distributed in Europe. Larvae are seed feeders developing inside seed capsules.

Remarks. This is one of the most variable species and with the widest Palaearctic distribution in the genus (Europe and central and northern Asia to the Russian Far East) (Caldara and Legalov 2016; Jiang et al. 2018). The three most variable characters in adults are the colour of the dorsal vestiture, which varies from whitish grey to light brown, the density of the elytral scales, which sometimes completely cover the integument, and the length of the rostrum, especially in the female and Anatolian populations. It is clear that it would be very interesting to perform a detailed molecular study of these populations. Apart from the characters of the shape of the rostra, the uncus of the male metatibiae and that of the penis, this species differs from *C. graminis* and related species also by the more angulate shape of the elytral base. Also the immatures of *C. distinctus* can easily be separated from those of *C. graminis* by several characters in larvae: postlabium with medium size pms_1 and pms_3, a very long pms_2 (Fig. 7) and a membranous area of postlabium basolaterally finely asperate as well as in pupae: *Vs* and *sos* absent (or as microsetae) (Fig. 82), pronotum with one *sls* (Fig. 83), and abdominal segments I–VII without ventral setae (Fig. 82). Finally, we could confirm that these two species are well separated molecularly as previously reported (Vahtera and Muona 2006; Hendrich et al. 2015; Horecka et al. 2017).

Cleopomiarus graminis (Gyllenhal, 1813)

Figures 11–20

Material examined. 11 L3 larvae: 6 exx., 18.07.2010, Wólka ad Lublin, CE Poland, leg. E. Szwaj, det. J. Łętowski; 5 exx., ex seed capsules of *Campanula macrostachya* Waldst. et Kit. ex Willd., Dobra, Iron Gate, east Serbia. 13.07.2015, leg. I. Toševski, all collected in association with adults, det. R. Caldara. Accession numbers of sequenced specimens: MH558545.

Description. *Measurements* (in mm). Body length: 3.75–6.27 (mean 4.80). Body width (metathorax or abdominal segments I–II) up to 1.63. Head width: 0.65–0.78 (mean 0.71).

General. Body elongated, slender, curved, rounded in cross section (Fig. 11).

Figure 11. *Cleopomiarus graminis* mature larva habitus.

Colouration. Pale brown head (Fig. 11). All thoracic and abdominal segments from distinctly white to slightly yellow (Fig. 11).

Vestiture. Setae on body thin, slightly from orange to pale brown, distinctly different in length (minute to very short or long to very long). Cuticle distinctly asperate.

Head capsule (Fig. 12). Head oval, slightly flattened laterally. Frontal sutures narrow, but distinct. Anterior stemma (st), in the form of a large pigmented spot. *Des*$_{1-3}$ and *des*$_5$ long; *des*$_4$ short to very short (Fig. 12). *Fs*$_1$ long; *fs*$_2$ absent; *fs*$_3$ very short; *fs*$_4$ long; and long *fs*$_5$ (Fig. 12). *Les*$_1$ and *les*$_2$ as long as *des*$_5$; both *ves* very short. Epicranial area with two sensilla and three minute *pes* in line with *des*$_2$.

Antennae bearing one medium size conical sensorium, and basal membranous article with three sensilla different in length, two behind conical sensorium, and one ahead of it (Fig. 13).

Clypeus (Fig. 15) approximately 2.5–3 times as wide as long with two short *cls*, *cls*$_1$ slightly shorter than *cls*$_2$, and one sensillum; anterior margin sinuate.

Mouthparts. Labrum (Fig. 15) less than two times as wide as long, with three piliform *lms*, different in the length; *lms*$_1$ located anteromedially, very close to margin of clypeus, *lms*$_2$ located in the middle, and *lms*$_3$ located anterolaterally; *lms*$_1$ and *lms*$_2$ of medium size, and *lms*$_3$ distinctly shorter than the previous two; only *lms*$_2$ distinctly reaches labral margin. Epipharynx (Fig. 16) with three long finger-like *als*, all of identical in length; with three *ams* in different length, *ams*$_1$ and *ams*$_2$ piliform of medium size, finger-like short *ams*$_3$ and enlarged in middle, and also located more close to lr; without *mes*; labral rods (lr) distinct, elongated, oval. Mandibles (Fig. 14)

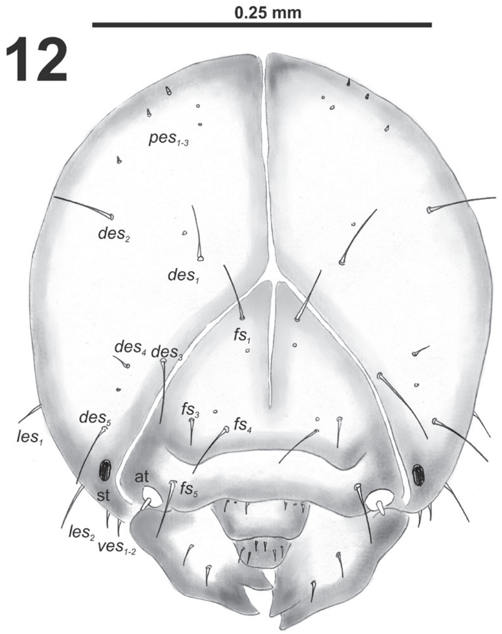
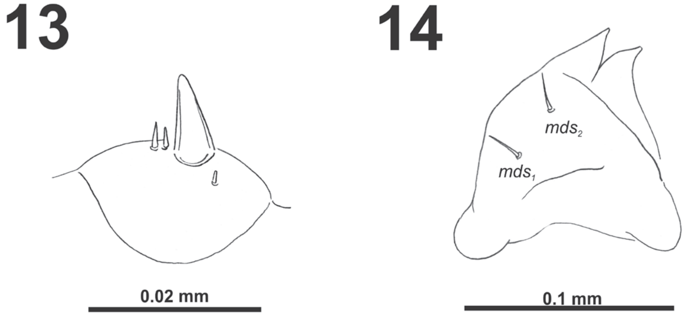
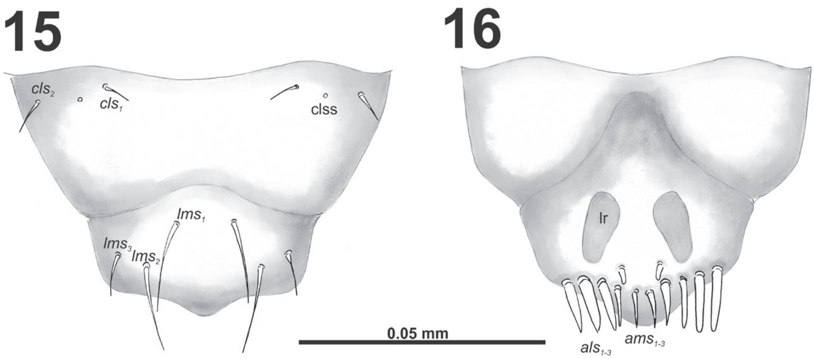


Figure 12. *Cleopomiarus graminis* mature larva head, frontal view. Abbreviations: *des* – dorsal epicranial s., *fs* – frontal epicranial s., *les* – lateral epicranial s., *pes* – postepicranial s., *ves* – ventral s., at – antenna, st – stemmata.



Figures 13–14. *Cleopomiarus graminis* mature larva. **13** Antenna **14** Right mandible. Abbreviation: *mds* – mandible dorsal s.

bifid; bearing with two setae in medium size, piliform, and aligned longitudinally, mds_1 located basally; mds_2, located distinctly apically. Maxilla (Fig. 17) stipes with very long *stps* and pfs_2, medium pfs_1, very short to minute *mbs*, and sensillum close to *mbs*; mala with six medium sized finger-like *dms*; five *vms*, different in length, three setae medium size, and two setae very short. Maxillary palpi: basal palpomere with one short *mxps* and two sensilla; distal palpomere with some cuticular apical processes; length ratio of basal and distal palpomeres 1:0.8. Prelabium (Fig. 17) with one short *prms*; ligula with two very short to minute *ligs*; premental sclerite broad, ring-shaped. Labial palpi with two palpomeres; length ratio of basal and distal pal-



Figures 15–16. *Cleopomiarus graminis* mature larva, mouthparts. **15** Labrum and clypeus **16** Epipharynx. Abbreviations: *als* – anteriolateral s., *ams* – anteromedial s., *cls* – clypeal s., *lms* – labral s., *mes* – median s., clss – clypeal sensillum, lr – labral rods.

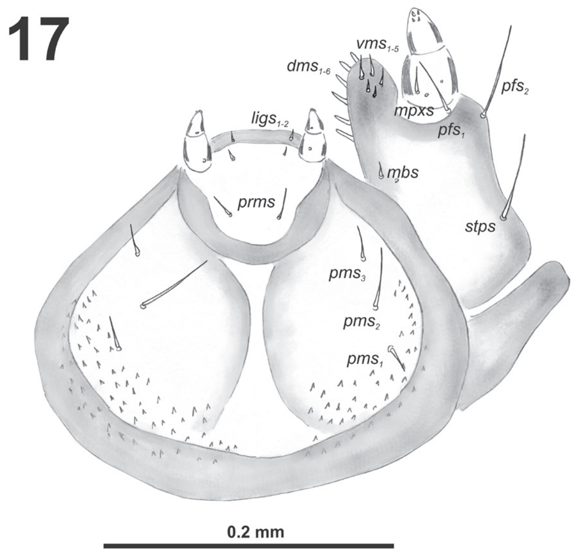
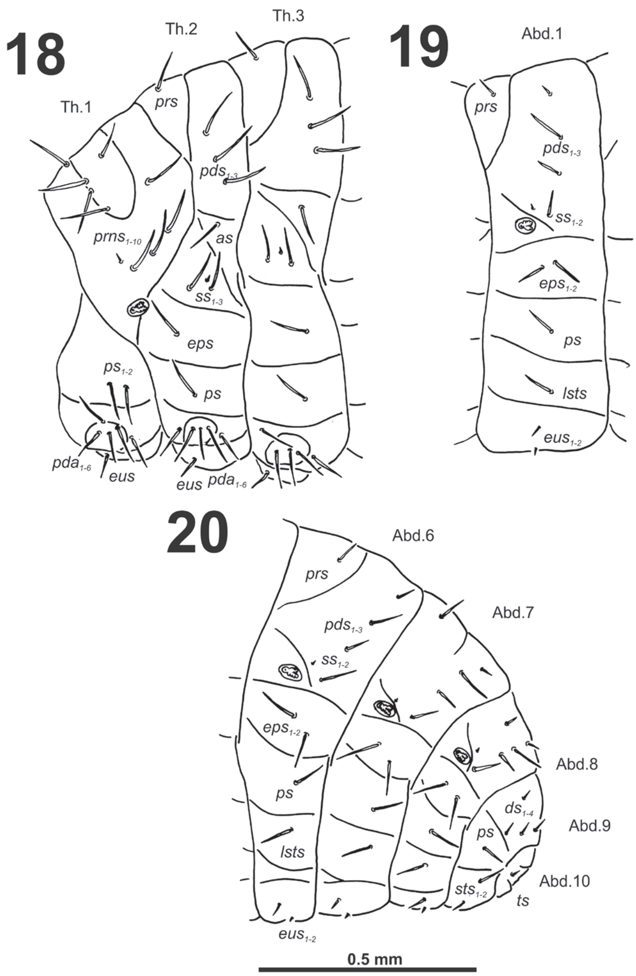


Figure 17. *Cleopomiarus graminis* larval mouthparts, maxillolabial complex, ventral view right maxilla. Abbreviations: *dms* – dorsal malar s., *vms* – ventral malar s., *mpxs* – maxillary palps s., *mbs* – basioventral s., *pfs* – palpiferal s., *stps* – stipital s., *prms* – premental s., *pms* – postmental s., *ligs* – ligular s.

pomeres 1:0.8; each of the palpomeres with one sensillum, distal palpomere with cuticular apical processes. Postlabium (Fig. 17) with short pms_1 located basally, very long pms_2 located medially and short pms_3 located apically; membranous area basolaterally distinctly asperate.

Thorax. Prothorax (Fig. 18) with nine very long and one very short to minute *prns*, small pigmented dorsal sclerite present with five long *prns*, this sclerite subdivided in two triangular plates medially; two very long to long *ps*; and one short *eus*. Meso- and metathorax (Fig. 18) with one long *prs*, three very long *pds*; one very long *as*; two very



Figures 18–20. *Cleopomiarus graminis* mature larva, habitus. **18** Lateral view of thoracic segments **19** Lateral view of abdominal segment I **20** Lateral view of abdominal segments VI–X. Abbreviations: *as* – alar s., *ds* – dorsal s., *eps* – epipleural s., *eus* – eusternal s., *lsts* – laterosternal s., *pda* – pedal s., *pds* – postdorsal s., *prns* – pronotal s., *prs* – prodorsal s., *ss* – spiracular s., *ps* – pleural s., *sts* – sternal s., *ts* – terminal s., Th1–3 – number of thoracic segments, Ab1–10 – number of abdominal seg.

long and one very short to minute *ss*; one very long *eps*; one very long *ps*; and one short to very short *eus*. Each pedal area of the thoracic segments with 5–6 very long *pda*.

Abdomen. Abdominal segments I–VII (Figs 19–20) with one medium *prs*; one short and two very long to long *pds* (order: short, very long, long); one very long and one minute *ss*; two long *eps*; one very long *ps*; one long *lsts*; and two short to very short *eus*. Abdominal segment VIII (Fig. 20) with one very short to minute *prs*; one short and two long to relatively long *pds* (order: short, long, relatively long); one long and one minute *ss*; two very long *eps*; one long *ps*; one long *lsts*; and two short to very short *eus*. Abdominal segment IX (Fig. 20) with three relatively long and one short to very short *ds*; one relatively long and sometimes one minute *ps*; and one relatively long to short and one short to very short *sts*. Abdominal segment X (Fig. 20) with one very short seta (*ts*).

Biology. Larvae were collected while feeding on the seeds of several species of *Campanula*, mainly *C. glomerata*, *C. persicifolia*, and *C. rotundifolia* L. (Hustache 1932; Hoffmann 1958; Smreczyński 1976; Lohse and Tischler 1983; Caldara and Legalov 2016) without producing galls. The species was not previously reported on *Campanula macrostachya* Waldst. and Kit. ex Willd., a taxon distributed from Ukraine along the Balkans until Anatolia. Pupae, as well as immatures of *M. ajugae*, were also collected on *Adenophora liliifolia* (L.) A. DC, although in another Serbian locality (see below). This genus, however, is very closely related to *Campanula* (Cano-Maqueda and Talavera 2011).

Remarks. This is a very common and variable species with a wide European and Asian distribution from the Iberian Peninsula to eastern China (Caldara and Legalov 2016; Jiang et al. 2018). The two most variable characters in adults are the colour of the dorsal vestiture, which varies from whitish grey to light brown, and the density of the elytral scales, sometimes completely covering the integument. The rostrum varies somewhat in length and curvature, especially in the female. *Cleopomiarus graminis* is very closely related to *C. longirostris* as demonstrated by our data on the molecular fragment COI (I Toševski, unpublished data). Therefore, the differences between these two taxa found in the study of the immature stages, especially in the larvae – antennae with a very long conical sensorium and three sensilla (Figs 13, 33), dorsal setae (except des_4) long (Figs 12, 32), prothorax with nine very long and one very short to minute *prns* (Figs 18, 38) – are very important in order to confirm the specific rank of both taxa. On the other hand, the larva of *C. longirostris* is distinctly longer than the larva of *C. graminis*. With regard to the differences from *C. distinctus*, another widespread sympatric species sometimes confused with *C. graminis*, see the Remarks for the former taxon.

Cleopomiarus longirostris (Gyllenhal, 1838)

Figures 21–30

Material examined. 11 L3 larvae: south-eastern France, Menton, July 2007, ex capsules of *Campanula trachelium* L., leg. and det. R. Caldara, all determined by association with reared adults.

Description. *Measurements* (in mm). Body length: 6.60–8.70 (mean 8.3). Body width (abdominal segments I–III) up to 2.44. Head width: 1.05–1.16 (mean 1.10).

General. Body elongated, slender, curved, rounded in cross section (Fig. 21).

Colouration. Pale brown head with indistinct pattern around frontal sutures (Fig. 21). All thoracic and abdominal segments from distinctly white to slightly yellow (Fig. 21).

Vestiture. Setae on body thin, orange, distinctly different in length (minute to very short or long to very long). Cuticle slightly asperate.

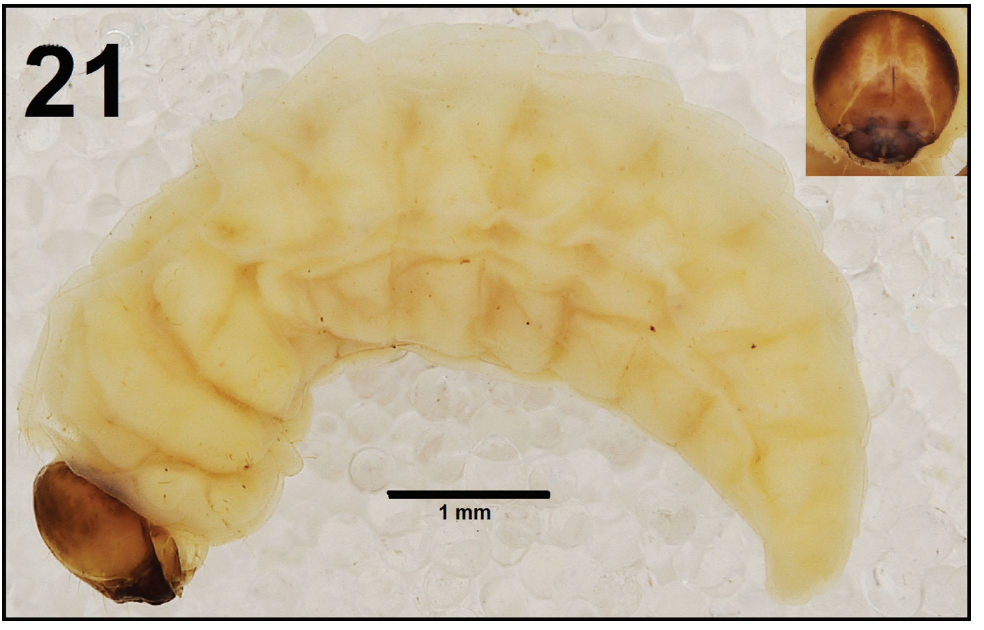


Figure 21. *Cleopomiarus longirostris* mature larva habitus.

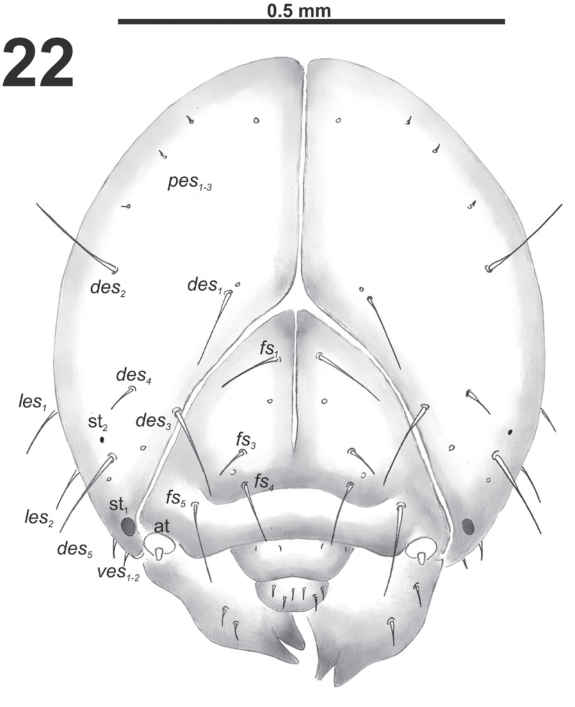
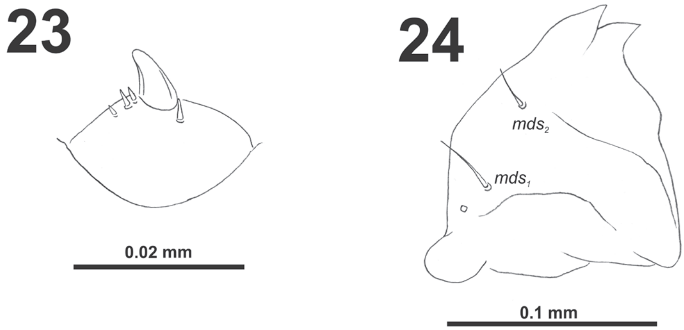
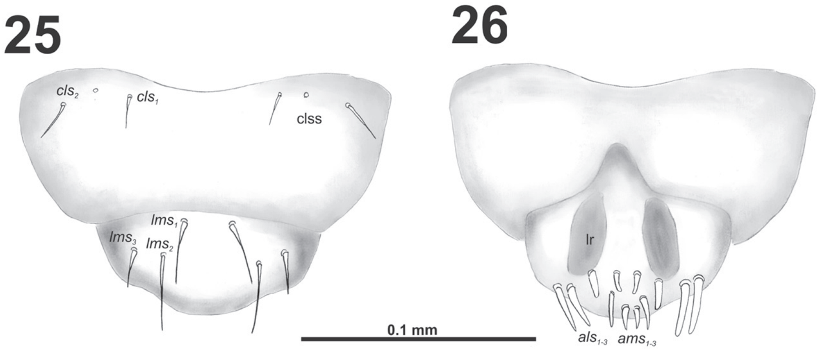


Figure 22. *Cleopomiarus longirostris* mature larva head, frontal view. Abbreviations: *des* – dorsal epicranial s., *fs* – frontal epicranial s., *les* – lateral epicranial s., *pes* – postepicranial s., *ves* – ventral s., at – antenna, st – stemmata.



Figures 23–24. *Cleopomiarus longirostris* mature larva. **23** Antenna **24** Right mandible. Abbreviation: *mds* – mandible dorsal s.



Figures 25–26. *Cleopomiarus longirostris* mature larva, mouthparts. **25** Labrum and clypeus **26** Epipharynx. Abbreviations: *als* – anteriolateral s., *ams* – anteromedial s., *cls* – clypeal s., *lms* – labral s., *mes* – median s., clss – clypeal sensillium, lr – labral rods.

Head capsule (Fig. 22). Head oval, slightly flattened laterally. Frontal sutures medium width, distinct. Two pairs of stemmata (st), anterior one in the form of a large pigmented spot; and posterior one in form of a very small pigmented spot, located on each side close des_5. $Des_{1–3}$ long; des_4 short and des_5 long to very long (Fig. 22). Fs_1 long; fs_2 absent; fs_3 short; fs_4 long; and long fs_5 (Fig. 22). Les_1 and les_2 as long as des_5; both *ves* very short. Epicranial area with three *pes* and two sensilla in line with des_2.

Antennae bearing one medium size conical sensorium, and basal membranous article with four sensilla different in length, three behind conical sensorium, and one ahead of it (Fig. 23).

Clypeus (Fig. 25) approximately 2.5 times as wide as long with two short *cls*, cls_2 distinctly longer than cls_1, and one sensillum.

Mouthparts. Labrum (Fig. 25) less than 2.5 times as wide as long, with three piliform *lms*, different in the length; lms_1 located anteromedially, close to margin, lms_2 located in the middle, and lms_3 located posterolaterally; lms_1 and lms_2 of medium size, and lms_3 distinctly shorter than the previous two; only lms_2 distinctly reaches labral margin. Epipharynx (Fig. 26) with three long finger-like *als*, two *als* of identical in

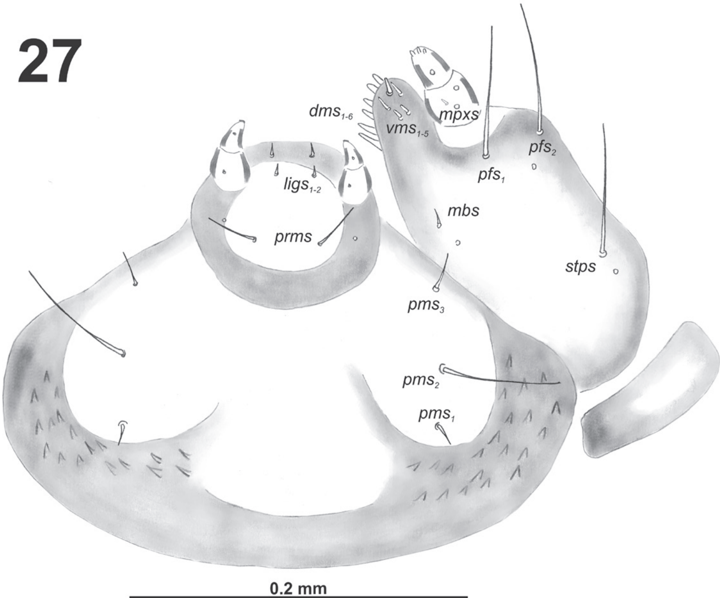
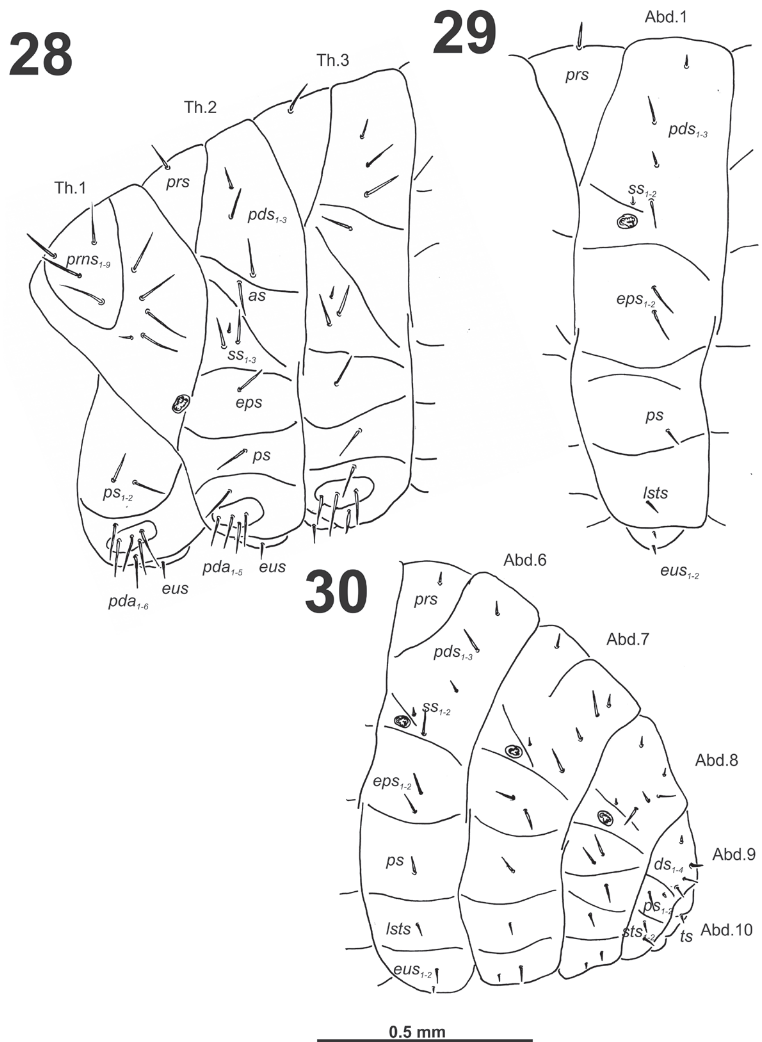


Figure 27. *Cleopomiarus longirostris* larval mouthparts, maxillolabial complex, ventral view right maxilla. Abbreviations: *dms* – dorsal malar s., *vms* – ventral malar s., *mpxs* – maxillary palps s., *mbs* – basioventral s., *pfs* – palpiferal s., *stps* – stipital s., *prms* – premental s., *pms* – postmental s., *ligs* – ligular s.

length, and the third one distinctly shorter and also located more close to labral rods (lr); with three *ams* in different length, ams_1 and ams_2 piliform and short, finger-like ams_3 and enlarged in middle, and also located more close to lr; without *mes*; labral rods (lr) distinct, elongated, oval. Mandibles (Fig. 24) bifid; mds_1 relatively long, piliform, located basally; mds_2 medium size, piliform, located distinctly apically and laterally. Maxilla (Fig. 27) stipes with very long *stps* and both *pfs*; very short to minute *mbs*, and sensillum close to *mbs*; mala with six medium sized finger-like *dms*; five *vms*, different in length, three setae medium size, and two setae very short. Maxillary palpi: basal palpomere with one short *mxps* and two sensilla; distal palpomere with, cuticular apical processes; length ratio of basal and distal palpomeres 1:0.8. Prelabium (Fig. 27) with one relatively long *prms*; ligula with two very short to minute *ligs*; premental sclerite broad, ring-shaped. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres 1:0.8; each of the palpomeres with one sensillum, distal palpomere with short, cuticular apical processes. Postlabium (Fig. 27) with very short pms_1 located basally, very long pms_2 located medially and short to medium size pms_3 located apically; membranous area basolaterally distinctly asperate.

Thorax. Prothorax (Fig. 28) with eight very long to long and one very short to minute *prns*, small pigmented dorsal sclerite present with four long *prns*, this sclerite subdivided in two triangular plates medially; two very long to long *ps*; and one short to very short *eus*. Meso- and metathorax (Fig. 28) with one long *prs*, three medium to long *pds*; one very long to long *as*; two very long and one very short to minute *ss*; one



Figures 28–30. *Cleopomiarus longirostris* mature larva, habitus. **28** Lateral view of thoracic segments **29** Lateral view of abdominal segment I **30** Lateral view of abdominal segments VI–X. Abbreviations: *as* – alar s., *ds* – dorsal s., *eps* – epipleural s., *eus* – eusternal s., *lsts* – laterosternal s., *pda* – pedal s., *pds* – postdorsal s., *prns* – pronotal s., *prs* – prodorsal s., *ss* – spiracular s., *ps* – pleural s., *sts* – sternal s., *ts* – terminal s., Th1–3 – number of thoracic segments, Ab1–10 – number of abdominal seg.

long *eps*; one long *ps*; and one short to very short *eus*. Each pedal area of the thoracic segments with 5–6 very long to long *pda*.

Abdomen. Abdominal segments I–VII (Figs 29–30) with one short *prs*; one long and two short to very short *pds* (order: short, long, short); one long and one minute *ss*; two very long to long *eps*; one relatively long *ps*; one short *lsts*; and two very short *eus*. Abdominal segment VIII (Fig. 30) with one very short to minute *prs*; one relatively long and two very short *pds* (order: very short, long, very short); one relatively long and one minute *ss*; two relatively long *eps*; one short *ps*; one short *lsts*; and two very short *eus*. Abdominal segment IX (Fig. 30) with three relatively long and one very short to minute *ds*; one relatively long and sometimes one minute *ps*; and two short to very short *sts*. Abdominal segment X (Fig. 30) with one very short seta (*ts*).

Biology. We can confirm that larvae of this species feed on seed capsules of *Campanula trachelium* L., where they pupate without producing galls. It is noteworthy that adults did not exit by making a hole in the capsules but remained inside with the rostrum folded in the ventral canal until these opened spontaneously and forcefully, blowing up the seeds. On the other hand, it would be impossible, especially for the female, to straighten up the very long rostrum inside the capsule due to the limited available space. This is a more advantageous behaviour and apparently opposite to that of *Rhopalapion longirostre* (Olivier, 1807), another species where the female rostrum is more than twice as long as the stout male rostrum. In this species, Wilhelm et al. (2011) argued that the long rostrum is presumably an advantage for this weevil because its larvae can feed on plant parts with high energy density into buds (i.e., pollen grains) and that natural selection favours rostrum elongation. However, these authors reported that the elongated rostrum of females also bears a high risk when metamorphosed weevils attempt to leave their site of pupal development, which is the dry seed chambers, and therefore mortality during escaping may counteract selection for rostrum elongation, thus placing a limit on rostrum exaggeration. It is noteworthy that *R. longirostre* does not possess a ventral canal, which allows it to retain the folded rostrum.

Remarks. This species is only known from France, Italy, and Switzerland, where it is quite common. The adult is very closely related to *C. graminis,* as also supported by preliminary molecular studies (I Toševski, unpublished data), from which it differs only by the very long rostrum especially in the female and usually by the larger size (Caldara and Legalov 2016). Therefore, the larval differences between these two taxa, in *C. longirostris* antennae bearing one medium size conical sensorium and four sensilla (Fig. 23), dorsal setae (except des_4) extremely long (Fig. 22), prothorax with eight very long and one very short to minute *prns* (Fig. 28), are very important since they allow easy separation of these two species.

Cleopomiarus medius **(Desbrochers des Loges, 1893)**

Figures 31–40

Material examined. 13 L3 larvae, ex seed capsules of *Campanula lingulata* Waldst. and Kit., 26.06.2017, Staničenje, Pirot, east Serbia, leg. I. Toševski, all collected in association with adults, det R. Caldara. Accession numbers of sequenced specimens: MH558547.

Description. *Measurements* (in mm). Body length: 5.10–7.30 (mean 5.67). Body width (metathorax or abdominal segments I–II) up to 2.02. Head width: 0.83–0.96 (mean 0.91).

General. Body elongated, slender, weakly curved, rounded in cross section (Fig. 31).

Figure 31. *Cleopomiarus medius* mature larva habitus.

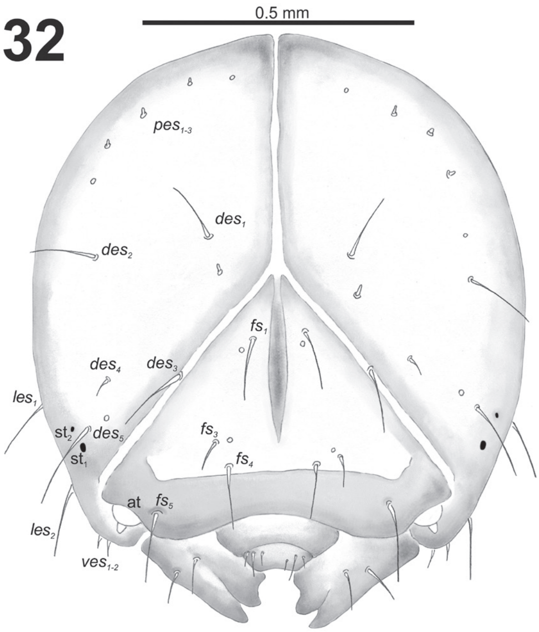
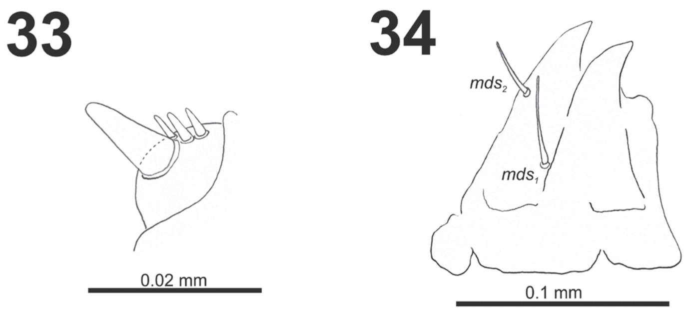
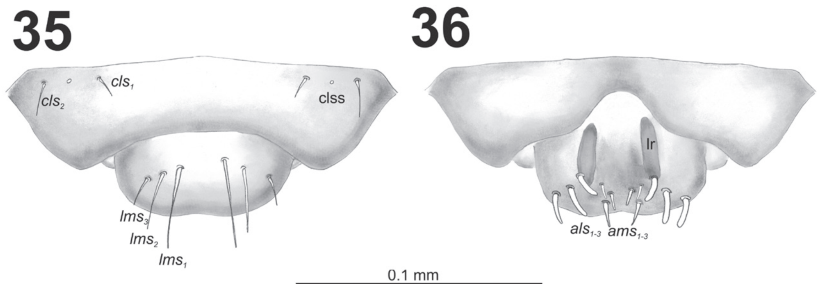


Figure 32. *Cleopomiarus medius* mature larva head, frontal view. Abbreviations: *des* – dorsal epicranial s., *fs* – frontal epicranial s., *les* – lateral epicranial s., *pes* – postepicranial s., *ves* – ventral s., at – antenna, st – stemmata.



Figures 33–34. *Cleopomiarus medius* mature larva. **33** Antenna **34** Right mandible. Abbreviation: *mds* – mandible dorsal s.



Figures 35–36. *Cleopomiarus medius* mature larva, mouthparts. **35** Labrum and clypeus **36** Epipharynx. Abbreviations: *als* – anteriolateral s., *ams* – anteromedial s., *cls* – clypeal s., *lms* – labral s., *mes* – median s., clss – clypeal sensillum, lr – labral rods.

Colouration. Pale brown or almost yellow head (Fig. 31). All thoracic and abdominal segments from white to slightly yellow (Fig. 31).

Vestiture. Setae on body thin, slightly from orange to brown, distinctly different in length (minute to very short or long). Cuticle distinctly asperate.

Head capsule (Fig. 32). Head suboval. Frontal sutures distinct. Endocarina distinctly widened in the middle of the length. Two small stemmata (st), located close to des_5. $Des_{1–2}$ and des_5 very long; des_3 medium size; des_4 short (Fig. 32). Fs_1 long; fs_2 absent; fs_3 medium; fs_4 long; and fs_5 very long (Fig. 32). Les_1 and les_2 as long as des_5; both *ves* very short. Epicranial area with two sensilla and three *pes* in line with des_2.

Antennae bearing one very long conical sensorium, and basal membranous article with three sensilla almost equal in length (Fig. 33).

Clypeus (Fig. 35) approximately 4.25 times as wide as long with two almost equal in length *cls*: cls_2 some longer than cls_1, and one sensillum; anterior margin sinuate.

Mouthparts. Labrum (Fig. 35) two times as wide as long, with three piliform *lms*, different in the length; lms_1 and lms_2 located medially, and lms_3 located anterolaterally; lms_1 very long and reaches distinctly the labrum margin, lms_2 long, and lms_3 short, three times as shorter than lms_1. Epipharynx (Fig. 36) with three medium sized finger-like *als*, and three medium to short *ams* in different shape; labral rods (lr) distinct,

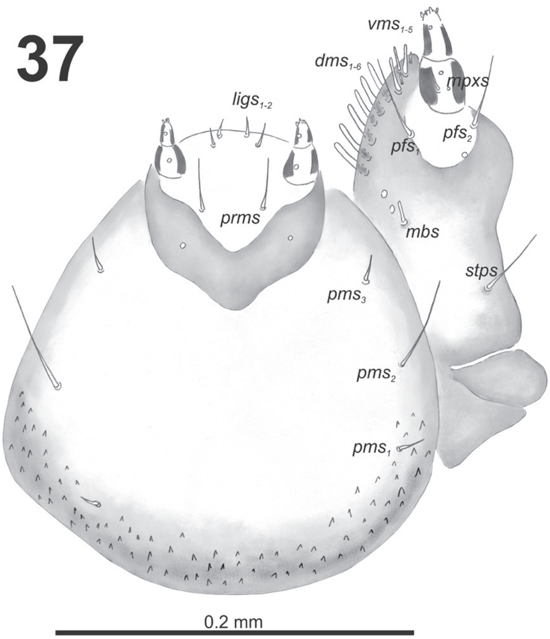
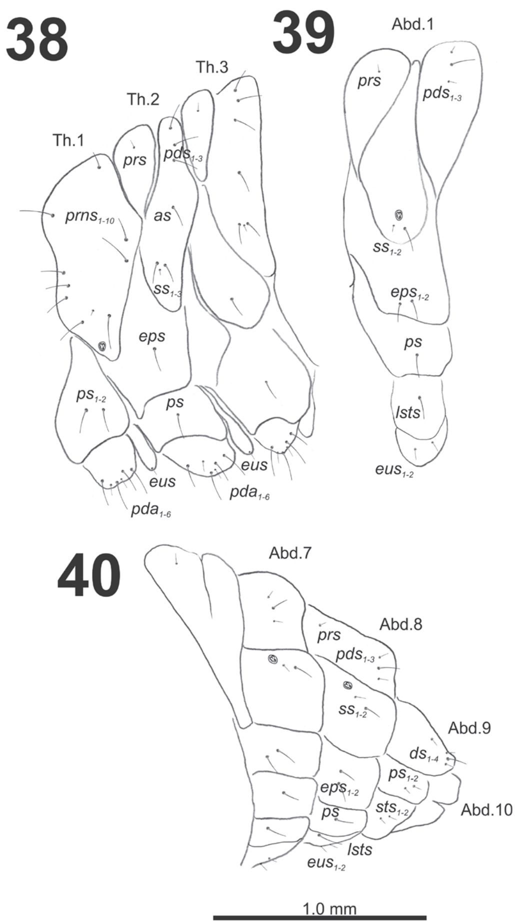


Figure 37. *Cleopomiarus medius* larval mouthparts, maxillolabial complex, ventral view right maxilla. Abbreviations: *dms* – dorsal malar s., *vms* – ventral malar s., *mpxs* – maxillary palps s., *mbs* – basioventral s., *pfs* – palpiferal s., *stps* – stipital s., *prms* – premental s., *pms* – postmental s., *ligs* – ligular s.

elongated. Mandibles (Fig. 36) bifid; bearing with two setae in short to medium size, piliform, and aligned longitudinally, mds_1 located basally; mds_2, located distinctly apically. Maxilla (Fig. 34) stipes with long *stps* and equal in length pfs_1 and pfs_2, very short to minute *mbs*, and two sensilla close to *mbs*; mala with six medium sized finger-like *dms*; five *vms*, different in length, three setae medium size, and two setae very short. Maxillary palpi: basal palpomere with one short *mxps* and two sensilla; distal palpomere with cuticular apical processes; length ratio of basal and distal palpomeres 1:0.8. Prelabium (Fig. 37) with one short *prms*; ligula with two short *ligs*; premental sclerite broad, ring-shaped. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres 1:0.9; each of the palpomeres with one sensillum, distal palpomere with short, cuticular apical processes. Postlabium (Fig. 37) with short pms_1 located basally, very long pms_2 located medially and short pms_3 located apically; membranous area basolaterally distinctly asperate.

Thorax. Prothorax (Fig. 38) with nine long and one very short *prns*; two long *ps*; and one short *eus*. Meso- and metathorax (Fig. 38) with one short *prs*, three long *pds*; one long *as*; two long and one very short to minute *ss*; one long *eps*; one long *ps*; and one short *eus*. Each pedal area of the thoracic segments with six different in length *pda*.

Abdomen. Abdominal segments I–VII (Figs 39–40) with one very short *prs*; two short and one long *pds* (order: short, long, short); one long and one minute *ss*; two long *eps*; one long *ps*; one long *lsts*; and two short *eus*. Abdominal segment VIII (Fig. 40)



Figures 38–40. *Cleopomiarus medius* mature larva, habitus. **38** Lateral view of thoracic segments **39** Lateral view of abdominal segment I **40** Lateral view of abdominal segments VI–X. Abbreviations: *as* – alar s., *ds* – dorsal s., *eps* – epipleural s., *eus* – eusternal s., *lsts* – laterosternal s., *pda* – pedal s., *pds* – postdorsal s., *prns* – pronotal s., *prs* – prodorsal s., *ss* – spiracular s., *ps* – pleural s., *sts* – sternal s., Th1–3 – number of thoracic segments, Ab1–10 – number of abdominal seg.

with one very short *prs*; three *pds* (order: short, long, short); one long and one minute *ss*; two long *eps*; one long *ps*; one long *lsts*; and two short *eus*. Abdominal segment IX (Fig. 40) with one medium long and three very short *ds*; two short *ps*; and two short *sts*. Abdominal segment X (Fig. 40) without seta.

Biology. Previously, the unique biological datum on this species was reported by Weill et al. (2011), who collected adults in Syria on *Michauxia campanuloides* L'Hér., a small genus of Campanulaceae distributed in the Middle East, possibly a synonym of *Campanula* (Crowl et al. 2014). Therefore, the observation that this species feeds on *Campanula lingulata* Waldst. and Kit. is unpublished. Moreover, adults were recently observed feeding on flowers of *Campanula sibirica* L. in eastern Serbia (I Toševski, pers. obs.), but larval development on this plant species is not confirmed. Like *C. distinctus* and *C. graminis*, larvae are seed feeders inside capsules of the host plant without producing galls.

Remarks. This species was previously known from Anatolia, Syria and many countries of the Balkans but not from Serbia. The adults of this species are characterized by a very long rostrum in the females. This character, however, is not uncommon in the Palaearctic *Cleopomiarus*. For example, this character is shared with *C. longirostris* and *C. distinctus*, two other taxa presented in this paper. It is distinguishable from these species by the less globose and moderately elongate elytra, and moreover by the shape of the male and female genitalia (Caldara and Legalov 2016). Other characters of the immatures allow easy separation of *C. medius* from these two species as well as from *C. graminis* (see keys to larvae and pupae). There are also substantial molecular differences between *C. medius* and other species (I Toševski, unpublished data).

Cleopomiarus meridionalis (H. Brisout, 1863)

Figures 41–50

Material examined. 10 L3 larvae: south-eastern France, Castellar (Menton), Juin 2005, ex seed capsules of *Campanula rapunculus* L., leg. and det. R. Caldara all collected in association with adults.

Description. *Measurements* (in mm). Body length: 2.20–3.15 (mean 2.8). Body width (metathorax or abdominal segments I–II) up to 0.73. Head width: 0.35–0.51 (mean 0.45).

General. Body elongated, slender, curved, rounded in cross section (Fig. 41).

Colouration. Pale brown or almost yellow head (Fig. 41). All thoracic and abdominal segments from distinctly white to slightly yellow (Fig. 41).

Vestiture. Setae on body thin, slightly from orange to pale brown, distinctly different in length (minute to very short or long to very long). Cuticle distinctly asperate.

Head capsule (Fig. 42). Head suboval, distinctly flattened laterally. Frontal sutures narrow, but distinct. Two stemmata (st), anterior one in the form of a small pigmented spot; and posterior one in form of a very small pigmented spot, located on each side close des_5. Des_{1-3} and des_5 very long; des_4 relatively long (Fig. 42). Fs_1 long to very long; fs_2 absent; fs_3 long medium, laterally to fs_4; fs_4 very long; and fs_5 very long (Fig. 42). Les_1 and les_2 as long as des_5; both *ves* medium size. Epicranial area with two sensilla and three *pes* in line with des_2.

Antennae bearing one very long conical sensorium, and basal membranous article with three sensilla different in length, two behind conical sensorium, and one ahead of it (Fig. 43).

Clypeus (Fig. 45) approximately three times as wide as long with two medium size *cls*, cls_1 distinctly longer than cls_2, and one sensillum; anterior margin sinuate.

Mouthparts. Labrum (Fig. 45) less than 2.5 times as wide as long, with three piliform *lms*, different in the length; lms_1 located posteromedially, very close to margin

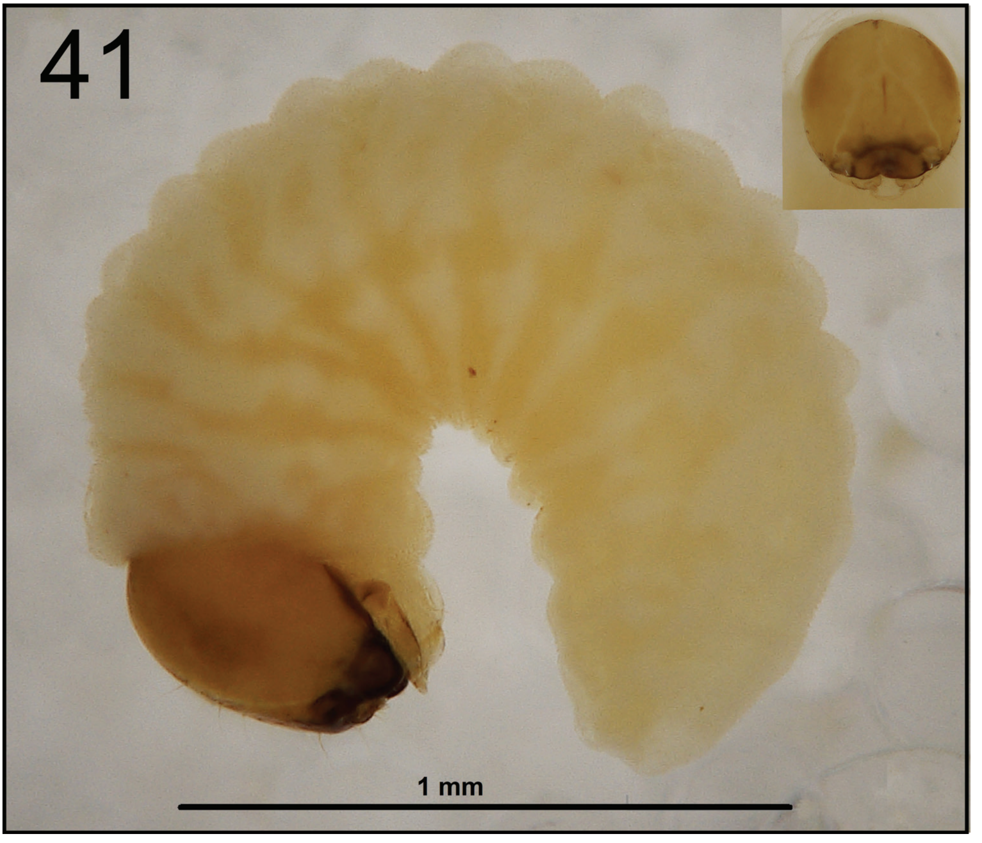


Figure 41. *Cleopomiarus meridionalis* mature larva habitus.

of clypeus, *lms*$_2$ located in the middle, and *lms*$_3$ located laterally; *lms*$_1$ very long and reaches distinctly the labrum margin, *lms*$_2$ long, and *lms*$_3$ medium size, more than twice times as short as *lms*$_1$. Epipharynx (Fig. 46) with three medium sized finger-like *als*, two *als* of identical in length, and the third one distinctly shorter and also located close to labral rods (lr); with three short *ams* in different shape, *ams*$_1$ and *ams*$_2$ piliform, finger-like *ams*$_3$ and enlarged in middle, and also located more close to lr; without *mes*; labral rods (lr) distinct, elongated, oval. Mandibles (Fig. 44) bifid; bearing with two setae in short to medium size, piliform, and aligned longitudinally., *mds*$_1$ located basally; *mds*$_2$, located distinctly apically. Maxilla (Fig. 47) stipes with very long *stps* and *pfs*$_2$, medium size *pfs*$_1$, very short to minute *mbs*, and sensillum close to *mbs*; mala with six medium sized finger-like *dms*; five *vms*, different in length, four setae medium size, and one seta very short. Maxillary palpi: basal palpomere with one short *mxps* and two sensilla; distal palpomere with short, cuticular apical processes; length ratio of basal and distal palpomeres 1:0.8. Prelabium (Fig. 47) with one very short *prms*; ligula with two very short to minute *ligs*; premental sclerite broad, ring-shaped. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres 1:0.9;

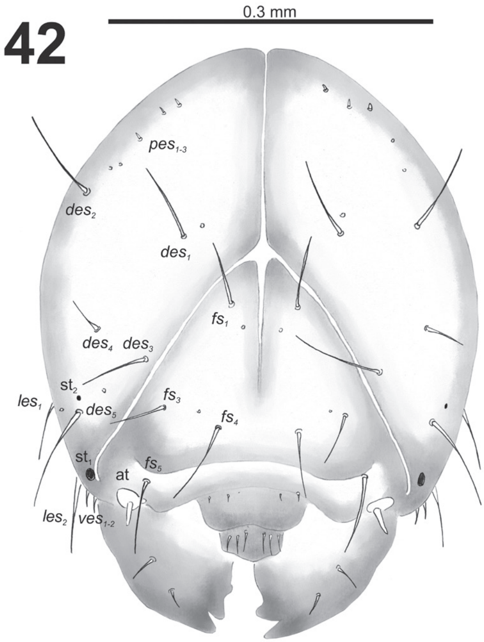
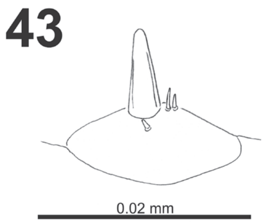
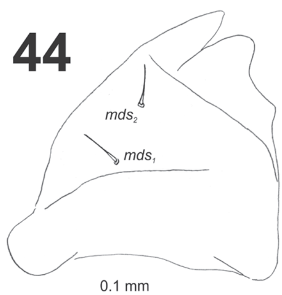


Figure 42. *Cleopomiarus meridionalis* mature larva head, frontal view. Abbreviations: *des* – dorsal epicranial s., *fs* – frontal epicranial s., *les* – lateral epicranial s., *pes* – postepicranial s., *ves* – ventral s., at – antenna, st – stemmata.

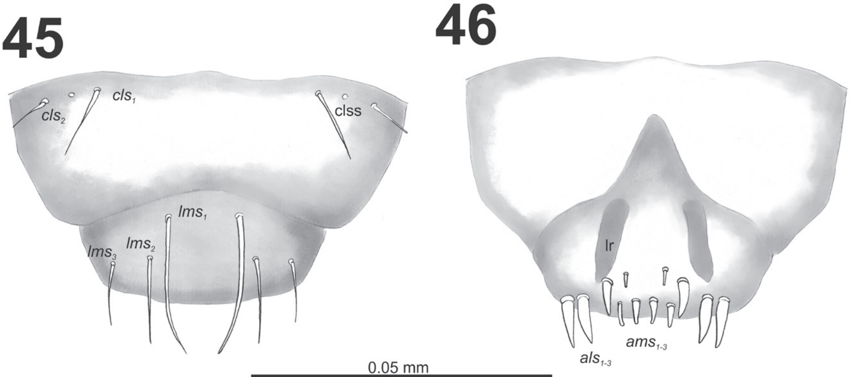




Figures 43–44. *Cleopomiarus meridionalis* mature larva. **43** Antenna **44** Right mandible. Abbreviation: *mds* – mandible dorsal s.

each of the palpomeres with one sensillum, distal palpomere with short, cuticular apical processes. Postlabium (Fig. 47) with short pms_1 located basally, long pms_2 located medially and short pms_3 located apically; membranous area basolaterally only a partly and finely asperate.

Thorax. Prothorax (Fig. 48) with nine very long and one very short to minute *prns*, small pigmented dorsal sclerite present with four long *prns*, this sclerite subdivided in two triangular plates medially; two very long to long *ps*; and one short *eus*. Meso- and metathorax (Fig. 48) with one long *prs*, three very long to long *pds*; one



Figures 45–46. *Cleopomiarus meridionalis* mature larva, mouthparts. **45** Labrum and clypeus **46** Epipharynx. Abbreviations: *als* – anteriolateral s., *ams* – anteromedial s., *cls* – clypeal s., *lms* – labral s., *mes* – median s., clss – clypeal sensillum, lr – labral rods.

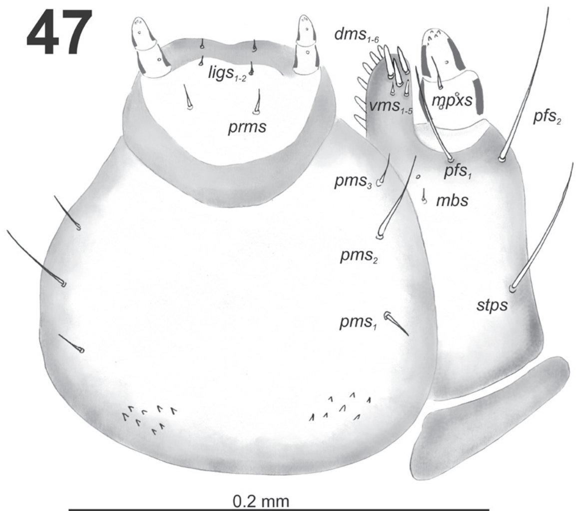
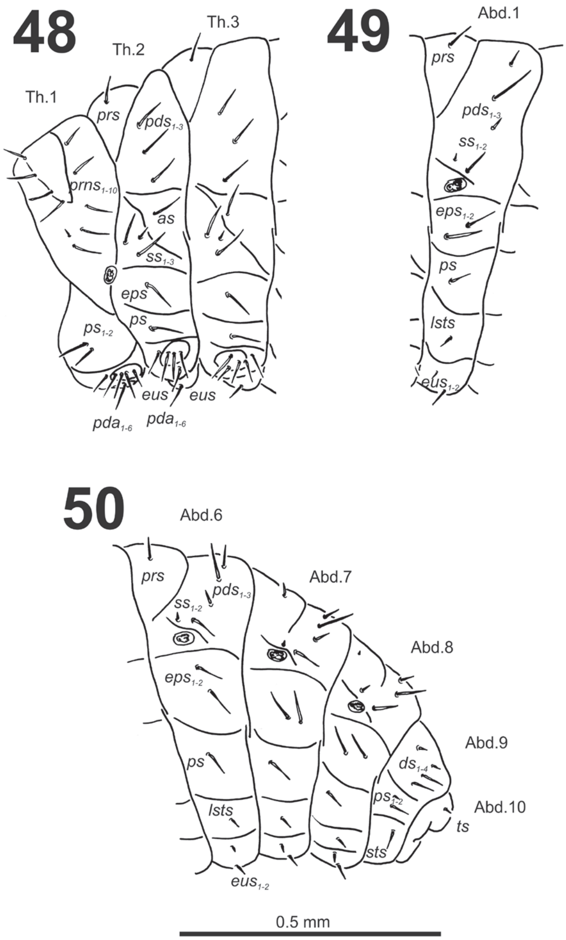


Figure 47. *Cleopomiarus meridionalis* larval mouthparts, maxillolabial complex, ventral view right maxilla. Abbreviations: *dms* – dorsal malar s., *vms* – ventral malar s., *mpxs* – maxillary palps s., *mbs* – basioventral s., *pfs* – palpiferal s., *stps* – stipital s., *prms* – premental s., *pms* – postmental s., *ligs* – ligular s.

long *as*; two very long and one very short to minute *ss*; one long *eps*; one very long to long *ps*; and one short to very short *eus*. Each pedal area of the thoracic segments with 5–6 very long *pda*.

Abdomen. Abdominal segments I–VII (Figs 49–50) with one long *prs*; two relatively long to short and one very long to long *pds* (order: relatively long, very long, short); one very long to long and one minute *ss*; two very long *eps*; one very long to long *ps*; one relatively long to short *lsts*; and one short to very short and one relatively long *eus*. Abdominal segment VIII (Fig. 50) with sometimes one very short to minute



Figures 48–50. *Cleopomiarus meridionalis* mature larva, habitus. **48** Lateral view of thoracic segments **49** Lateral view of abdominal segment I **50** Lateral view of abdominal segments VI–X. Abbreviations: *as* – alar s., *ds* – dorsal s., *eps* – epipleural s., *eus* – eusternal s., *lsts* – laterosternal s., *pda* – pedal s., *pds* – postdorsal s., *prns* – pronotal s., *prs* – prodorsal s., *ss* – spiracular s., *ps* – pleural s., *sts* – sternal s., *ts* – terminal s., Th1–3 – number of thoracic segments, Ab1–10 – number of abdominal seg.

prs; one short and one long to relatively long *pds* (order: short, long); one long and one minute *ss*; two very long *eps*; one very long to long *ps*; one relatively long to short *lsts*; and one short to very short and one relatively long *eus*. Abdominal segment IX (Fig. 50) with two relatively long and two short to very short *ds*; one relatively long and one minute *ps*; and one relatively long *sts*. Abdominal segment X (Fig. 50) with one very short seta (*ts*).

Biology. Adults of this species are usually collected on the flowers of *Campanula rapunculus* L., and we can confirm that larvae feed on seeds of this plant as previously reported by Hoffmann (1958).

Remarks and comparative notes. This species is widely distributed and common in southern Europe, whereas it appears rare in North Africa and the Middle East. Adults can be confused with some related species such as *C. plantarum* (Germar, 1823), *C. micros* (Germar, 1821) and *C. reitteri* (Caldara & Legalov, 2016), from which they differ by some external characters and the shape of their genitalia (Caldara and Legalov 2016). In contrast, this species is poorly related morphologically to the other species of *Cleopomiarus* studied here. This difference is confirmed also by the larval morphology, which differs from all of the other species mainly by a longer fs_3 that is almost as long as $fs_{4.}$

Genus *Miarus* Schoenherr, 1826

Description. *Measurements* (in mm). Body length: 3.80–8.39. Body width (metathorax or abdominal segments I–II) 1.55–2.04. Head width: 0.57–0.83.

General. Body slender, C-curved, rounded in cross section.

Colouration. From black to dark brown head. All thoracic and abdominal segments yellowish, with some asperities.

Vestiture. Setae on body thin, in different colouration, distinctly different in length; piliform.

Head capsule. Head almost rounded, sometimes slightly flattened laterally, endocarinal line present and distinct, more than half the length of frons. Frontal sutures on the head narrow and loosened, but distinct, and ever extended to the antennae. One stemma (st), in the form of a pigmented spot with convex cornea. Dorsum of the epicranium with four or five setae; des_3 located anteriorly on epicranium close border with frontal suture. Frons with three or four setae; fs_2 absent. Head also with two *les* and two *ves*. Epicranial area with two or three *pes* and more or without sensilla.

Antennae located at the end of the frontal suture on each side, membranous and distinctly convex basal article bearing one very long conical sensorium; basal membranous article with 1–4 sensilla.

Clypeus transverse-shaped, approximately 2.5–3.5 times as wide as long with two *cls*, and one sensillum (clss) between setae; all very close to margin with frons.

Mouthparts. Labrum with three piliform *lms*; anterior margin bisinuate. Epipharynx with three finger-like *als*; with two *ams*; and 0–2 *mes*; labral rods (lr) elongated. Mandibles distinctly broad, bifid, teeth of unequal height; slightly truncate; both setae piliform and located apically. Maxilla stipes with one *stps*, two *pfs* and one *mbs* and one sensillum; mala with six finger-like *dms*, in two sizes, first or first and second *dms* very long as *pfs*, next medium length; five *vms*; all *vms* distinctly shorter than *dms*. Maxillary palpi with two palpomeres; basal palpomere with one short *mxps* and two sensilla; distal palpomere with one sensillum and a group of micro cuticular apical processes. Prelabium oval-shaped, with one *prms*; ligula with sinuate margin and 2–3 *ligs*; premental sclerite feebly visible. Labial palpi with two palpomeres; each of the palpomeres with one sensillum, distal palpomere with cuticular apical processes. Postlabium with three *pms*, all located laterally.

Thorax. Prothorax slightly smaller than meso- and metathorax. Spiracle bicameral, placed between the pro- and mesothorax (see, e.g., Skuhrovec et al. 2015). Prothorax with ten *prns*; two *ps*; and one *eus*. Mesothorax with one *prs*, three *pds*; one *as*; two long and one short *ss*; one *eps*; one *ps*; and one *eus*. Chaetotaxy of metathorax almost identical to that of mesothorax. Each pedal area of the thoracic segments well separated, with 5–6 *pda*.

Abdomen. Abdominal segments I–III of almost equal length, next abdominal segments decreasing gradually to the terminal parts of the body. Abdominal segment X reduced to four anal lobes of unequal size, the lateral lobes being distinctly the largest, the dorsal and the ventral lobe very small. Anus located terminally. Eight spiracles, bicameral, all spiracles placed medially or anteromedially and functional. Abdominal segments I–VIII with one *prs* (sometimes abdominal segment VIII without); three *pds*, pds_2 the longest one; one long and one minute *ss*; two long *eps*; one *ps*; one *lsts*; and two *eus*. Abdominal segment IX with 3–4 *ds*; 1–3 *ps*; and two *sts*. Abdominal segment X with one minute seta present or absent.

Miarus abnormis Solari, 1947

Figures 51–60

Material examined. 5 L3 larvae: north-eastern Italy, Venezia Giulia, Duino (Trieste), Rilke path, August 2017, ex galls on capsules of *Campanula pyramidalis* L., leg. E. Tomasi, all collected in association with adults, det. R. Caldara.

Description. *Measurements* (in mm). Body length: 3.50–4.75 (mean 3.9). Body width (abdominal segment II) up to 1.65. Head width: 0.57–0.65 (mean 0.60).

General. Body moderately elongated, rather stout, curved, rounded in cross section (Fig. 51).

Colouration. Almost black head (Fig. 51). All thoracic and abdominal segments from greyish-white to yellowish; prodorsum with brownish dorsal sclerite; all abdominal segments covered with fine spiculation (Fig. 51).

Vestiture. Setae on body thin, brown, rather short or minute, piliform.

Head capsule (Fig. 52). Head oval, slightly flattened laterally. Endocarinal line present and very distinct. Stemma placed below des_5. Des_{1-3} and des_5 long; des_4 medium size (Fig. 52). Fs_1 long; fs_2 absent; fs_3 and fs_4 medium size; and fs_5 long (Fig. 52). Les_1 and les_2 as long as des_5; one *ves* very short. Epicranial area with three *pes* (in line with des_2), and also two sensilla.

Antennae located at the end of the frontal suture on each side, membranous and distinctly convex basal article bearing one conical sensorium, relatively elongated; basal membranous article with four sensilla (styloconica) equal in length, and one (ampullacae) (Fig. 53).

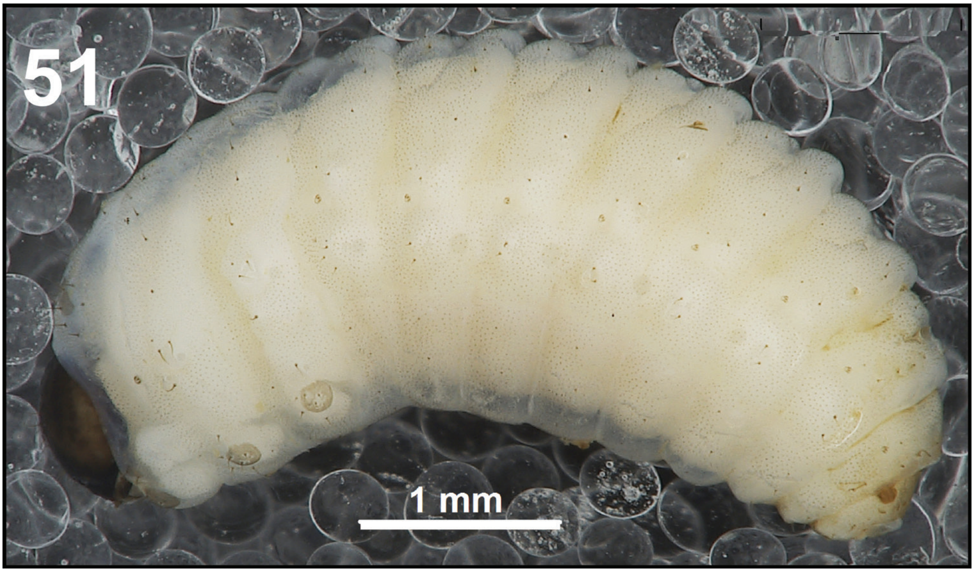


Figure 51. *Miarus abnormis* mature larva habitus.

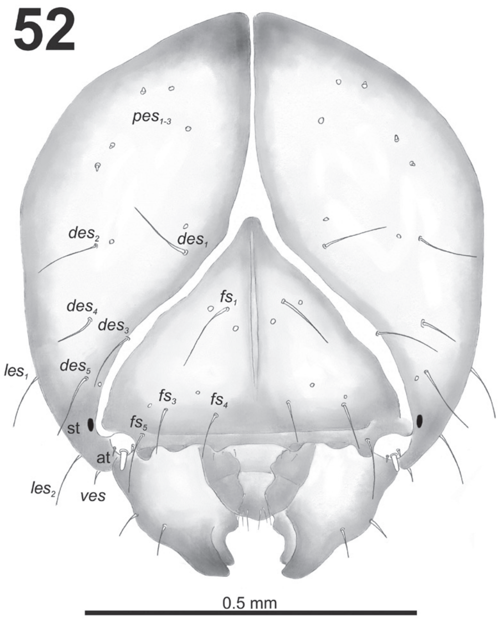
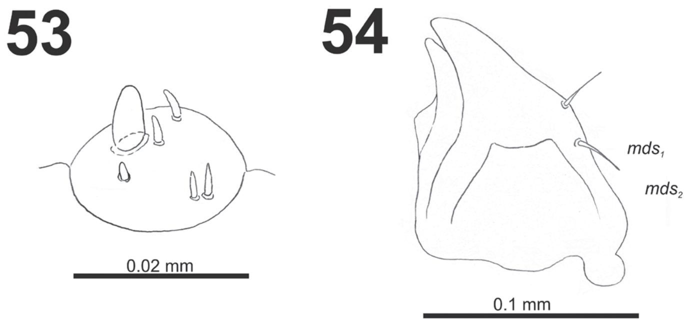
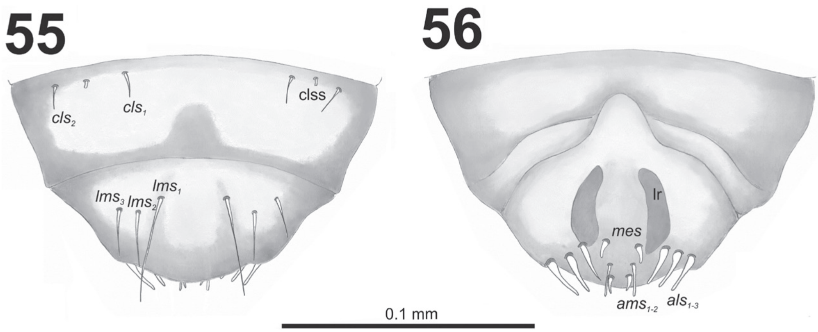


Figure 52. *Miarus abnormis* mature larva head, frontal view. Abbreviations: *des* – dorsal epicranial s., *fs* – frontal epicranial s., *les* – lateral epicranial s., *pes* – postepicranial s., *ves* – ventral s., at – antenna, st – stemmata.

Clypeus (Fig. 55) trapezium-shaped, approximately 3.3 times as wide as long with two medium size, equal in length *cls*, and one sensillum (clss) between setae; all very close to margin with frons; anterior margin of clypeus rounded to inside.



Figures 53–54. *Miarus abnormis* mature larva. **53** Antenna **54** Right mandible. Abbreviation: *mds* – mandible dorsal s.



Figures 55–56. *Miarus abnormis* mature larva, mouthparts. **55** Labrum and clypeus **56** Epipharynx. Abbreviations: *als* – anteriolateral s., *ams* – anteromedial s., *cls* – clypeal s., *lms* – labral s., *mes* – median s., clss – clypeal sensillum, lr – labral rods.

Mouthparts. Labrum (Fig. 55) 2.5 times as wide as long, with three piliform *lms*, lms_1 twice longer than (equal in the length) lms_2 and lms_3; all located more or less anteromedially, all reach labral margin; anterior margin double sinuate. Epipharynx (Fig. 56) with three medium sized finger-like *als*, all similar in length; with two rather short, different in length *ams*; and one medium size, finger-like *mes*; labral rods (lr) distinct, kidney-shaped. Mandibles (Fig. 54) distinctly broad, bifid, teeth of unequal height; slightly truncate; cutting edge with a blunt tooth; bearing with two setae in short size, piliform, and aligned longitudinally. Maxilla (Fig. 57): stipes with long *stps*, two long *pfs*, one minute *mbs* and two sensillae close to *mbs*; mala with six finger-like *dms* (first and second elongated, forth to sixth medium size); five *vms* (two medium size and three very short); all *vms* shorter than *dms*. Maxillary palpi with two palpomeres; basal palpomere with one short *mxps* and two sensilla; length ratio of basal and distal palpomeres almost 1:1; distal palpomere with one sensillum and a group of microcuticular processes apically. Prelabium (Fig. 57) oval-shaped, with one medium *prms*; ligula with sinuate margin and three minute *ligs*; premental sclerite narrow, ring-shaped, well visible. Labial palpi with one palpomere (partially seems as two palpomere); palpomere with one sensillum and medium, cuticular apical processes. Postlabium (Fig. 57) with

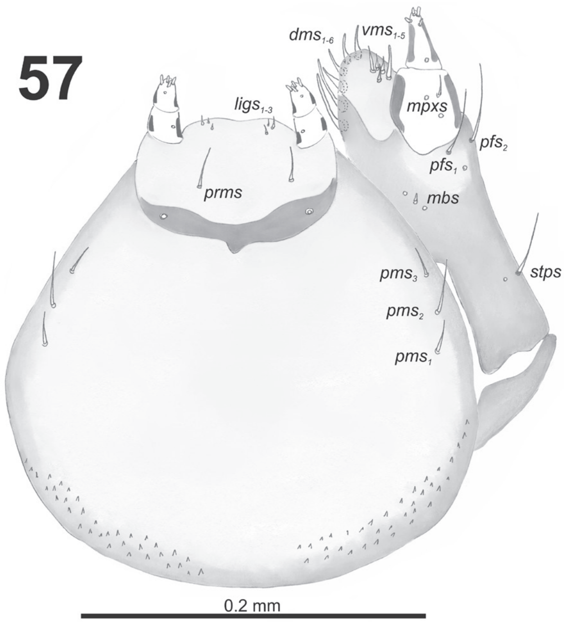


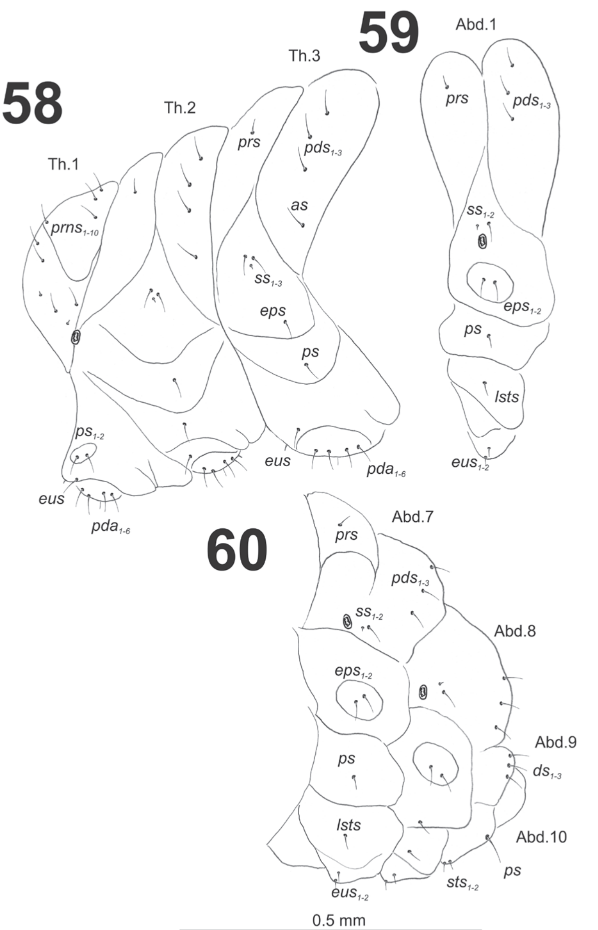
Figure 57. *Miarus abnormis* larval mouthparts, maxillolabial complex, ventral view right maxilla. Abbreviations: *dms* – dorsal malar s., *vms* – ventral malar s., *mpxs* – maxillary palps s., *mbs* – basioventral s., *pfs* – palpiferal s., *stps* – stipital s., *prms* – premental s., *pms* – postmental s., *ligs* – ligular s.

three *pms*, all located laterally; pms_1 and pms_3 short, pms_2 medium size; membranous area basolaterally finely asperate.

Thorax. Prothorax smaller than meso- and metathorax. Spiracle bicameral, placed between the pro- and mesothorax. Prothorax (Fig. 58) with ten *prns* (two minute and eight long), well pigmented dorsal sclerite with four long *prns*); two medium *ps*; and one short *eus*. Meso- and metathorax (Fig. 58) with one short *prs*, three medium *pds*; one medium *as*; two medium and one minute *ss*; one medium *eps*; one medium *ps*; and one very short *eus*. Chaetotaxy of metathorax (Fig. 58) almost identical to that of mesothorax. Each pedal area of the thoracic segments with six medium length *pda* (four of them placed on well-separated pedal areas, next two setae outside).

Abdomen. Abdominal segments I–VII (Figs 59, 60) with one short *prs*; three medium size *pds* (equal in length); one medium and one minute *ss*; two medium *eps*; one medium *ps*; one medium *lsts*; and two very short *eus*. Abdominal segment VIII (Fig. 60) without *prs*; three medium *pds*; one medium and one minute *ss* (sometimes absent); two medium *eps*; one medium *ps*; one medium *lsts*; and two very short *eus*. Abdominal segment IX (Fig. 60) with three medium *ds*; one relatively long *ps*; and two short *sts*. Abdominal segment X (Fig. 60) without seta.

Biology. The only detailed biological data are reported by Tomasi (2002), who observed that this species lives in Friuli-Venezia Giulia (Italy) on *Campanula pyramidalis* L., where larvae cause a distinct swelling of the calix of the flowers, which remain closed.



Figures 58–60. *Miarus abnormis* mature larva, habitus. **58** Lateral view of thoracic segments **59** Lateral view of abdominal segment I **60** Lateral view of abdominal segments VII–X. Abbreviations: *as* – alar s., *ds* – dorsal s., *eps* – epipleural s., *eus* – eusternal s., *lsts* – laterosternal s., *pda* – pedal s., *pds* – postdorsal s., *prns* – pronotal s., *prs* – prodorsal s., *ss* – spiracular s., *ps* – pleural s., *sts* – sternal s., *ts* – terminal s., Th1–3 – number of thoracic segments, Ab1–10 – number of abdominal seg.

Remarks. This species has a well-delimited distribution (south-eastern Poland, Austria, north-eastern Italy, Slovenia, Croatia, Serbia, Montenegro, Macedonia). It is easily distinguishable from all other species of *Miarus* by the shape of the body of the penis, which is characterized by the presence of two lateral flanges at its apex. However, for the external morphology, the *M. abnormis* adults are very similar to several other species, such as *M. ajugae* and *M. campanulae*, from which they can be distinguished only by the characters of the male ventrite five (fovea less deep, teeth less robust). Unfortunately, the females of these three species appear not to be distinguishable (Caldara 2007), and the molecular fragment COI poorly differentiates these species. Therefore, the differences between the immatures of these species are much important for the separation of these three species. According to the larval morphology, *M. abnormis* appears more closely related to *M. ajugae* than to *M. campanulae* due to several features (mala with six finger-like *dms*, different in length: two setae elongated, and four setae of medium length; epipharynx with 1–2 *mes*, and finally des_4 and fs_3 present), confirming what was suggested by the adult morphology (Caldara 2007). Moreover, larvae of *M. abnormis* differ from other *Miarus* species here studied mainly by an epipharynx with one finger-like *mes*.

Miarus ajugae **(Herbst, 1795)**

Figures 61–70

Material examined. 26 L3 larvae: 9 exx., 12.07.2009, Bychawa ad Lublin, CE Poland, leg. E. Szwaj, det. J. Łętowski; 12 exx, ex galls on capsules of *Adenophora liliifolia,* 30.06.2017, Kaludjerske Bare, Mt. Tara, Central Serbia, leg. I. Toševski, det. R. Caldara; 5 exx, ex galls on capsules of *Campanula bononiensis* L.*,* 14.07.2017, Zavojsko jezero, Pirot, east Serbia, leg. I. Toševski, all collected in association with adults, det. R. Caldara. Accession numbers of sequenced specimens: MH558548.

Description. *Measurements* (in mm). Body length: 4.50–8.39 (mean 5.70). Body width (metathorax or abdominal segments I–II) up to 2.04. Head width: 0.68–0.83 (mean 0.70).

General. Body slender, C-curved, rounded in cross section (Fig. 61).

Colouration. Head dark brown to black (Fig. 61). All thoracic and abdominal segments yellowish with fine speculation, with clearly separated dark pigmented pedal areas (Fig. 61).

Vestiture. Setae on body very thin, piliform, distinctly different in length (minute to very short or long to very long).

Head capsule (Fig. 62). Head almost rounded. Frontal sutures narrow and loosened, but distinct. One stemma (st), in the form of a large pigmented spot. Des_{1-3} and des_5 long; des_4 very short (Fig. 62). Fs_1 long; fs_2 absent; fs_3 short; fs_4 and fs_5 long (Fig. 62). Les_1 and les_2 as long as des_5; both *ves* very short. Epicranial area with three very short *pes* and also three sensilla.

Antennae bearing one very long conical sensorium, and basal membranous article with three sensilla and one pore (Fig. 63).

Clypeus (Fig. 65) approximately 3.5 times as wide as long with two short, almost equal in length *cls*, and one sensillum between them; anterior margin sinuate.

Mouthparts. Labrum (Fig. 65) 1.6 times as wide as long, with three piliform *lms*, rather equal in length; lms_1 located medially, lms_2 located anteromedially, and lms_3 located anterolaterally; all of them reaches labral margin. Epipharynx (Fig. 66) with three long finger-like *als*, all of identical in length; with two medium size *ams*; and two *mes,* first finger-like, second sharp and more slender; labral rods (lr) elongated, broad, slightly convergent posteriorly. Mandibles (Fig. 64) bifid; cutting edge with small blunt tooth; bearing with two setae in medium to long size, piliform, located apically and aligned longitudinally. Maxilla (Fig. 67) stipes with long *stps* and both *pfs*,

Figure 61. *Miarus ajugae* mature larva habitus.

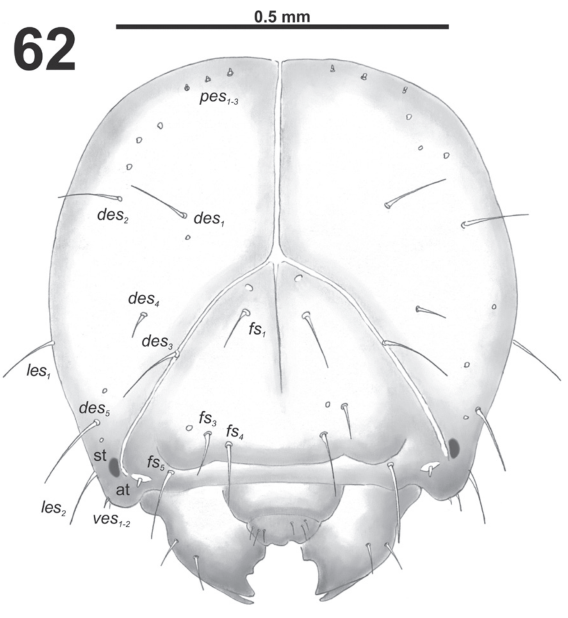
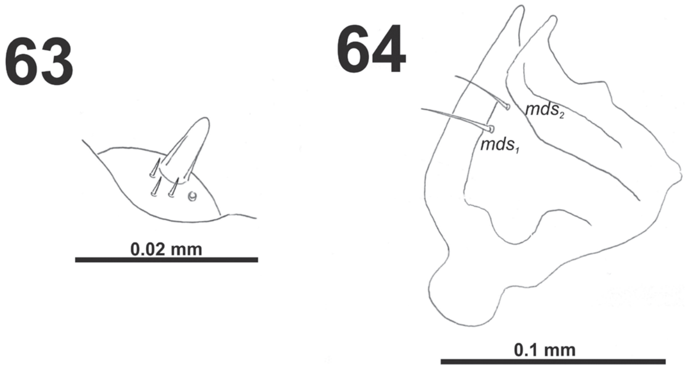
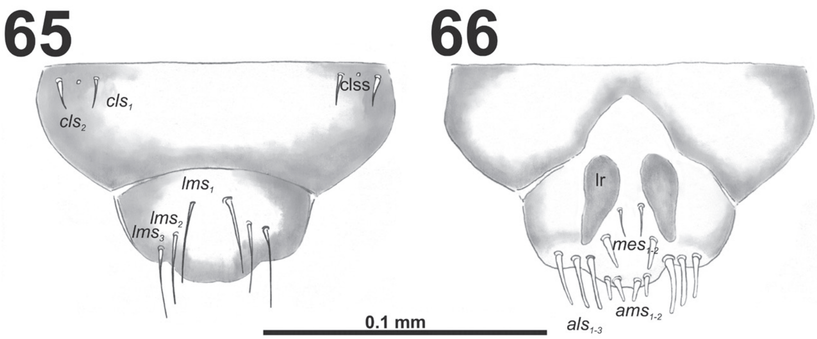


Figure 62. *Miarus ajugae* mature larva head, frontal view. Abbreviations: *des* – dorsal epicranial s., *fs* – frontal epicranial s., *les* – lateral epicranial s., *pes* – postepicranial s., *ves* – ventral s., at – antenna, st – stemmata.

very short to minute *mbs*, and one sensillum close to *mbs*; mala with six finger-like *dms*, different in length: first and second very long, forth to sixth medium size; five *vms*, different in length, three setae medium size, and two setae very short. Maxillary palpi:



Figures 63–64. *Miarus ajugae* mature larva. **63** Antenna **64** Right mandible. Abbreviation: *mds* – mandible dorsal s.



Figures 65–66. *Miarus ajugae* mature larva, mouthparts. **65** Labrum and clypeus **66** Epipharynx. Abbreviations: *als* – anteriolateral s., *ams* – anteromedial s., *cls* – clypeal s., *lms* – labral s., *mes* – median s., clss – clypeal sensillum, lr – labral rods.

basal palpomere with one short *mxps* and two sensilla; distal palpomere with cuticular apical processes; length ratio of basal and distal palpomeres 1:0.9. Prelabium (Fig. 67) with one medium *prms*; ligula with two minute *ligs*; premental sclerite narrow, ring-shaped. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres 1:0.7; each of the palpomeres with one sensillum, distal palpomere with cuticular apical processes. Postlabium (Fig. 67) with short pms_1 located basally, long pms_2 located medially and short pms_3 located apically; membranous area without any asperities.

Thorax. Prothorax (Fig. 68) with ten *prns* (nine very long and one minute), small pigmented dorsal sclerite present with five long *prns*, this sclerite subdivided in two triangular plates medially; two long *ps*; and one short *eus*. Meso- and metathorax (Fig. 68) with one medium *prs*, three long *pds*; one very long *as*; two long and one minute *ss*; one long *eps*; one long *ps*; and one short *eus*. Chaetotaxy of metathorax (Fig. 68) almost identical to that of mesothorax. Each pedal area of the thoracic segments with six very long *pda*.

Abdomen. Abdominal segments I–VII (Figs 69–70) with one medium *prs*; two medium and one long to very long *pds* (order: medium, very long, medium); one very long and one minute *ss*; two long *eps*; one medium *ps*; one medium *lsts*; and two short

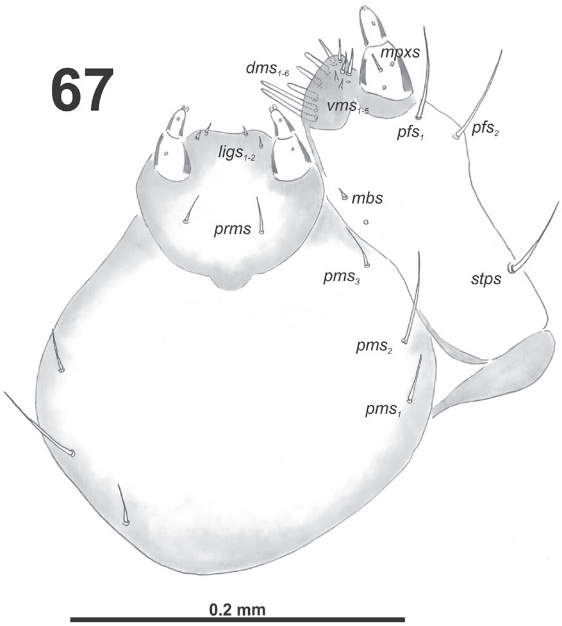
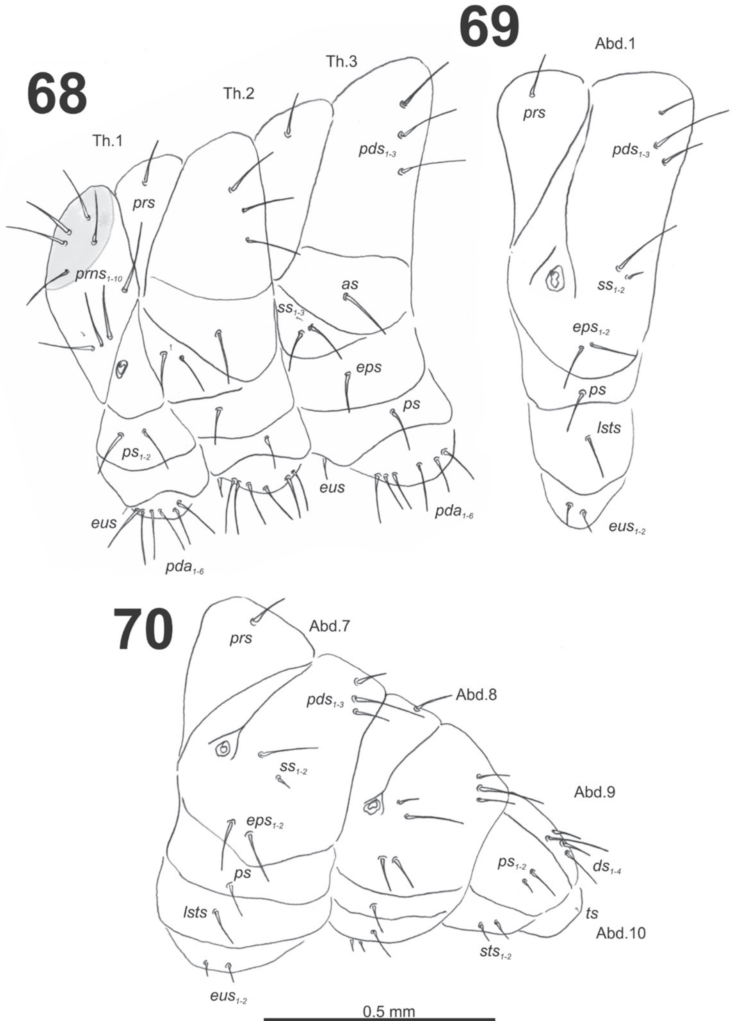


Figure 67. *Miarus ajugae* larval mouthparts, maxillolabial complex, ventral view right maxilla. Abbreviations: *dms* – dorsal malar s., *vms* – ventral malar s., *mpxs* – maxillary palps s., *mbs* – basioventral s., *pfs* – palpiferal s., *stps* – stipital s., *prms* – premental s., *pms* – postmental s., *ligs* – ligular s.

eus. Abdominal segment VIII (Fig. 70) with one short *prs*; two short and one long *pds* (order: short, long, short); one long and one minute *ss*; two medium *eps*; one medium *ps*; one medium *lsts*; and two short *eus*. Abdominal segment IX (Fig. 70) with two relatively long and two short *ds*; two different in length *ps*; and two short *sts*. Abdominal segment X (Fig. 70) with one very short seta (*ts*).

Biology. *Miarus ajugae* was collected on various species of the genus *Campanula* (*C. carpathica* Jacq., *C. glomerata* L., *C. latifolia* L., *C. macrorrhiza* Gay ex DC, *C. media* L., *C. patula* L., *C. persicifolia* L., *C. rapunculoides* L., *C. rapunculus* L., *C. rhomboidalis* L., *C. rotundifolia* L., *C. trachelium* L.) and *Phyteuma* (*P. orbiculare* L., *P. spicatum* L.) (Caldara 2007). However, it was never reported previously to feed on *Campanula bononiensis* L. and *Adenophora liliifolia* (L.) A. DC. (see also Biology of *C. graminis*).

Remarks. This species with large Palaearctic distribution (from France and northwestern Africa along all Europe to the Far East) is very closely related to *M. campanulae*, from which it differs mainly by the shape of the apex of the body of the penis (Caldara 2007). Unfortunately, molecular studies on the fragment COI revealed poor differences between these two species (Vahtera and Muona 2006; Hendrich et al. 2015, I. Toševski, unpublished data). Therefore, the consistent differences which we found in larval morphology between *C. ajugae* and *C. campanulae* are very important in order to confirm the validity of these two taxa at species level. Larvae of *M. ajugae* differ from all other species mainly by an epipharynx with two *mes,* first finger-like, second sharp and slender.



Figures 68–70. *Miarus ajugae* mature larva, habitus. **68** Lateral view of thoracic segments **69** Lateral view of abdominal segment I **70** Lateral view of abdominal segments VII–X. Abbreviations: *as* – alar s., *ds* – dorsal s., *eps* – epipleural s., *eus* – eusternal s., *lsts* – laterosternal s., *pda* – pedal s., *pds* – postdorsal s., *prns* – pronotal s., *prs* – prodorsal s., *ss* – spiracular s., *ps* – pleural s., *sts* – sternal s., *ts* – terminal s., Th1–3 – number of thoracic segments, Ab1–10 – number of abdominal seg.

Miarus campanulae (Linnaeus, 1767)

Figures 71–80

Material examined. 9 L3 larvae: 30.07.1939, ex *Campanula*, Store Dyrehave, Denmark, leg. J.P. Kryger, collected in association with adults, det. Van Emden, coll. British Museum of Natural History (London).

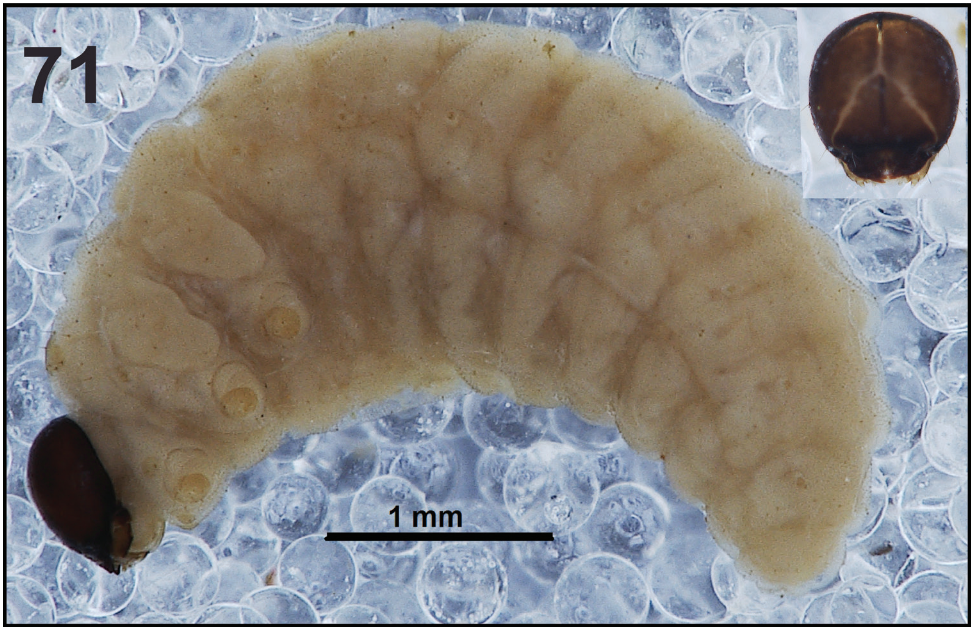


Figure 71. *Miarus campanulae* mature larva habitus.

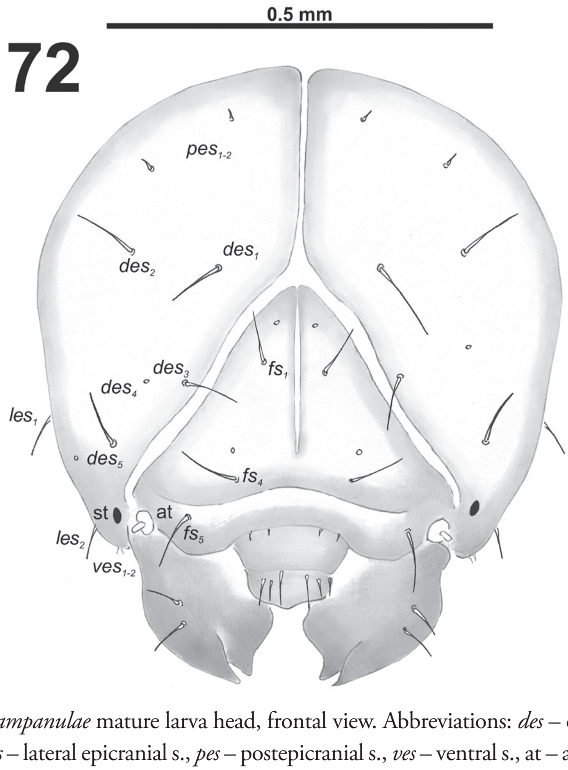
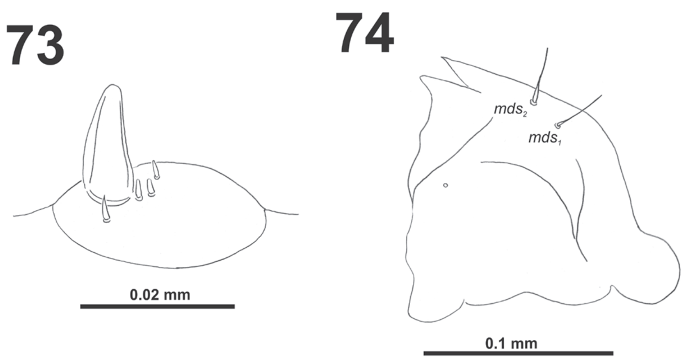
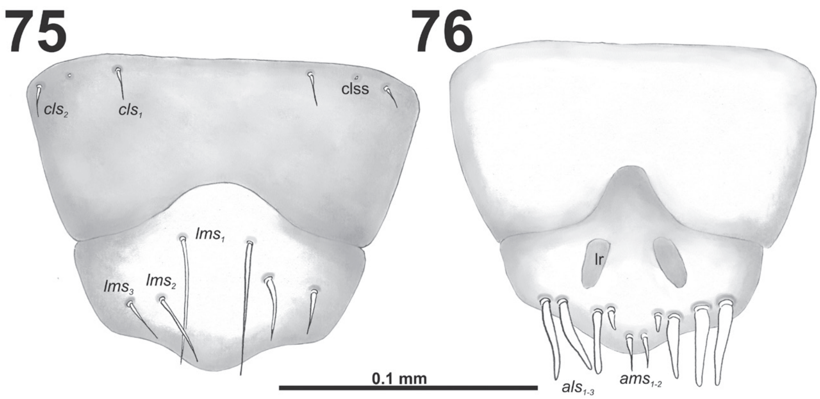


Figure 72. *Miarus campanulae* mature larva head, frontal view. Abbreviations: *des* – dorsal epicranial s., *fs* – frontal epicranial s., *les* – lateral epicranial s., *pes* – postepicranial s., *ves* – ventral s., at – antenna, st – stemmata.



Figures 73–74. *Miarus campanulae* mature larva. **73** Antenna **74** Right mandible. Abbreviation: *mds* – mandible dorsal s.



Figures 75–76. *Miarus campanulae* mature larva, mouthparts. **75** Labrum and clypeus **76** Epipharynx. Abbreviations: *als* – anteriolateral s., *ams* – anteromedial s., *cls* – clypeal s., *lms* – labral s., *mes* – median s., clss – clypeal sensillum, lr – labral rods.

Description. *Measurements* (in mm). Body length: 3.80–5.96 (mean 5.20). Body width (metathorax or abdominal segments I–II) up to 1.61. Head width: 0.58–0.64 (mean 0.61).

General. Body slender, C-curved, rounded in cross section (Fig. 71).

Colouration. Brown to dark brown head (Fig. 71). All thoracic and abdominal segments yellowish with distinct asperation (Fig. 71).

Vestiture. Setae on body thin, slightly from dark orange to brown, distinctly different in length (minute to very short or long to very long) distinct asperate.

Head capsule (Fig. 72). Head almost round, very slightly flattened laterally. Frontal sutures narrow and loosened, but distinct. One stemma (st), in the form of a large pigmented spot. *$Des_{1–3}$* and *des_5* long; *des_4* absent (Fig. 72). *Fs_1* long; *fs_2* and *fs_3* absent; *fs_4* and *fs_5* long (Fig. 72). *Les_1* and *les_2* as long as *des_5*; both *ves* short. Epicranial area with two *pes*.

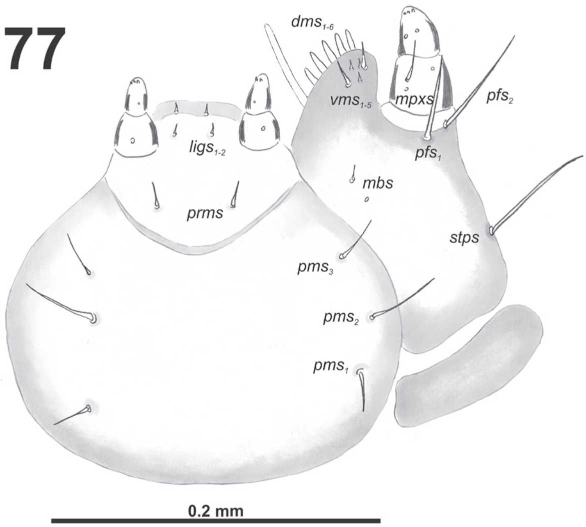
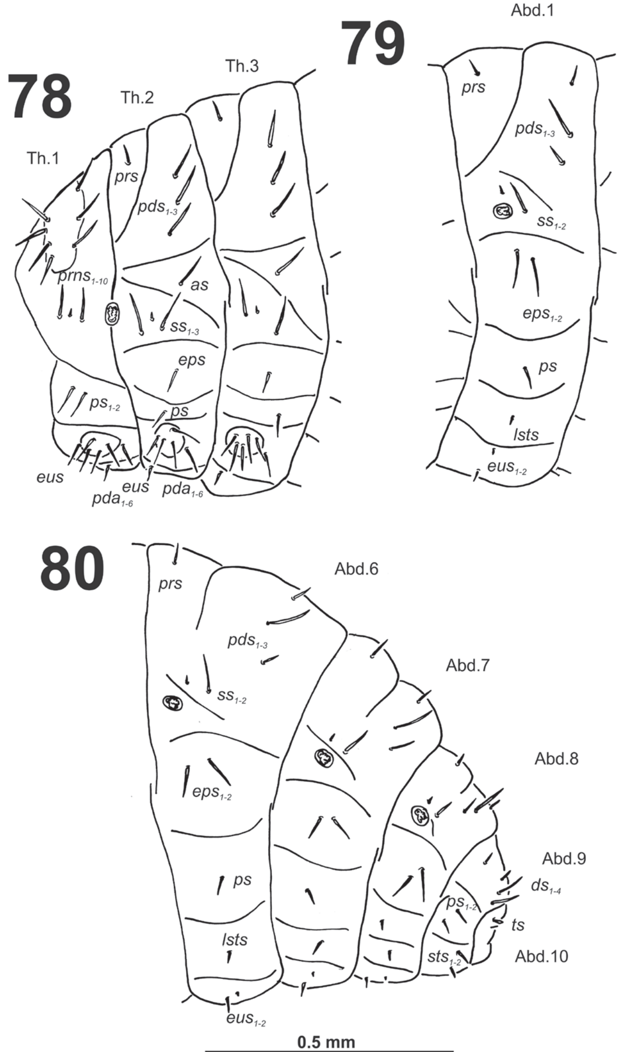


Figure 77. *Miarus campanulae* larval mouthparts, maxillolabial complex, ventral view right maxilla. Abbreviations: *dms* – dorsal malar s., *vms* – ventral malar s., *mpxs* – maxillary palps s., *mbs* – basioventral s., *pfs* – palpiferal s., *stps* – stipital s., *prms* – premental s., *pms* – postmental s., *ligs* – ligular s.

Antennae bearing one very long conical sensorium, and basal membranous article with four sensilla different in length, three behind conical sensorium, and one ahead of it (Fig. 73).

Clypeus (Fig. 75) approximately 2.5–3 times as wide as long with two short to very short *cls*, localized posterolaterally, cls_1 slightly longer than cls_2, and one sensillum between them; anterior margin sinuate.

Mouthparts. Labrum (Fig. 75) less than two times as wide as long, with three piliform *lms*, different in the length; lms_1 located medially, lms_2 located anteromedially, and lms_3 located anterolaterally; lms_1 long, lms_2 medium size, and lms_3 distinctly shorter than the previous two; no one distinctly reaches labral margin. Epipharynx (Fig. 76) with three long finger-like *als*, all of identical in length; with two *ams* in different length, ams_1 piliform of medium size, and finger-like short ams_2; without *mes*; labral rods (lr) indistinct, slightly elongated, oval. Mandibles (Fig. 74) bifid; bearing with two setae of medium to long size, piliform, both located apically. Maxilla (Fig. 77) stipes with very long *stps* and pfs_1, long pfs_2, very short to minute *mbs*, and one sensillum close to *mbs*; mala with six finger-like *dms*, different in lengths: first very long (as long as pfs_1), next medium size; five *vms*, different in length, two setae medium size, and three setae very short. Maxillary palpi: basal palpomere with one short *mxps* and two sensilla; distal palpomere with short, cuticular apical processes; length ratio of basal and distal palpomeres 1:0.9. Prelabium (Fig. 77) with one short *prms*; ligula with two very short to minute *ligs*; premental sclerite narrow, ring-shaped. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres 1:0.8; each of the palpomeres with one sensillum, distal palpomere with short, cuticular apical processes. Postlabium



Figures 78–80. *Miarus campanulae* mature larva, habitus. **78** Lateral view of thoracic segments **79** Lateral view of abdominal segment I **80** Lateral view of abdominal segments VI–X. Abbreviations: *as* – alar s., *ds* – dorsal s., *eps* – epipleural s., *eus* – eusternal s., *lsts* – laterosternal s., *pda* – pedal s., *pds* – postdorsal s., *prns* – pronotal s., *prs* – prodorsal s., *ss* – spiracular s., *ps* – pleural s., *sts* – sternal s., *ts* – terminal s., Th1–3 – number of thoracic segments, Ab1–10 – number of abdominal seg.

(Fig. 77) with short pms_1 located basally, long pms_2 located medially and short pms_3 located apically; membranous area without any asperities.

Thorax. Prothorax (Fig. 78) with nine very long and one very short to minute *prns*, small pigmented dorsal sclerite present with seven long *prns*, this sclerite subdivided in two triangular plates medially; two very long to long *ps*; and one medium *eus*. Meso- and metathorax (Fig. 78) with one medium *prs*, three very long *pds*; one very long *as*; two very long and one very short to minute *ss*; one long *eps*; one long *ps*; and one medium to long *eus*. Each pedal area of the thoracic segments with 5–6 very long *pda*.

Abdomen. Abdominal segments I–VII (Figs 79–80) with one medium to long *prs*; two medium to short and one very long to long *pds* (order: medium/short, very long, medium); one very long and one minute *ss*; two long *eps*; one medium to short *ps*; one medium to short *lsts*; and one medium to short and one very short to minute *eus*. Abdominal segment VIII (Fig. 80) with one very short *prs*; two short and one long to relatively long *pds* (order: short, long, short); one long and one minute *ss*; two very long *eps*; one medium to short *ps*; one medium to short *lsts*; and one medium to short and one very short to minute *eus*. Abdominal segment IX (Fig. 80) with two relatively long and two short to very short *ds*; two relatively long and sometimes one minute *ps*; and one relatively long to short and one short to very short *sts*. Abdominal segment X (Fig. 80) with one very short seta (*ts*).

Biology. Larvae live and pupate in the capsules of several species of *Campanula* (*C. cochleariifolia* Lam., *C. patula* L., *C. persicifolia* L., *C. rapunculoides* L., *C. rapunculus* L., *C. rotundifolia* L., *C. scheuchzeri* Vill. *C. trachelium* L.), and *Phyteuma spicata* L. where they cause distinct swelling (Hoffmann 1958; Buhr 1964)

Remarks. This species has a wide European distribution and is very similar to *M. ajugae*. Only the examination of the penis allows easy separation of these two taxa. Therefore, as reported in the Remarks of *C. ajugae*, the discovery of clearly distinctive characters between the larvae of these two species is extremely important. Moreover, larvae of *M. campanulae* differ from the two other species studied mainly by an epipharynx without *mes*, and des_4 and fs_3 absent.

Key to larvae (mature larva, L3)

The following key is based on the larvae of five *Cleopomiarus* and three *Miarus* species described in this paper. Unfortunately, the previous description of *Cleopomiarus hispidulus* (Anderson 1973) cannot be included due to missing details about the chaetotaxy used in our key.

1	Mala with 6 finger-like *dms*, all more or less in equal length (Figs 7, 17, 27, 37, 47)	***Cleopomiarus* 2**
–	Mala with 6 finger-like *dms*, in two sizes; 1 or 2 *dms* very long and rest of them in medium length (Figs 57, 67, 77)	***Miarus* 6**
2	Fs_3 long, almost as long as fs_4 (Fig. 42)	***C. meridionalis***
–	Fs_3 short or very short, always distinctly shorter than fs_4 (Figs 2, 12, 22, 32)	**3**
3	Postlabium with medium size pms_1 and pms_3, and very long pms_2 (Fig. 7). Membranous area of postlabium basolaterally finely asperate (Fig. 7)	***C. distinctus***
–	Postlabium with short pms_1 and pms_3, and very long pms_2 (Figs 17, 27, 37). Membranous area of postlabium basolaterally sparsely and distinctly asperate (Figs 17, 27, 37)	**4**

4 Antennae bearing one medium size conical sensorium, and 4 sensilla (Fig. 23). Dorsal setae (except *des*$_4$) extremely long (Fig. 22). Head width over 1.05 mm. Prothorax with 8 very long and 1 very short to minute *prns* (Fig. 28). Body length over 6.60 mm .... ***C. longirostris***

– Antennae with very long conical sensorium, and 3 sensilla (Figs 13, 33). Dorsal setae (except *des*$_4$) long (Figs 12, 32). Head width under 0.80 mm. Prothorax with 9 very long and 1 very short to minute *prns* (Figs 18, 38). Body length under 6.30 mm **5**

5 Clypeus (Fig. 35) broad, approximately 4.25 times as wide as long; labrum 2.0 times as wide as long .... ***C. medius***

– Clypeus (Fig. 15) narrow, 2.5–3 times as wide as long, labrum less than 2 times as wide as long .... ***C. graminis***

6 Mala with 6 finger-like *dms*, different in length: 1 seta very long as *pfs*$_1$, and 5 setae in medium length (Fig. 77). Epipharynx (Fig. 76) without *mes*. *Des*$_4$ and *fs*$_3$ absent (Fig. 72) .... ***M. campanulae***

– Mala with 6 finger-like *dms*, different in length: 2 setae elongated, and 4 setae in medium length (Figs 57, 67). Epipharynx (Figs 56, 66) with 1–2 *mes*. *Des*$_4$ and *fs*$_3$ present (Figs 52, 62) **7**

7 Epipharynx (Fig. 66) with 2 *mes,* first finger-like, second sharp and slender. Antennae with very long conical sensorium (Fig. 63) .... ***M. ajugae***

– Epipharynx (Fig. 56) with 1 finger-like *mes*. Antennae bearing medium size conical sensorium (Fig. 53) .... ***M. abnormis***

Description of pupae

Genus *Cleopomiarus* Pierce, 1919

Description. *Measurements* (in mm). Body length: 3.00–6.50. Body width: 1.50–3.80. Head width: 0.65–1.10.

Body moderately slender or stout. Smooth, dark brown or black spotted cuticle. Rostrum long or very long, from four up to five times as long as wide, reached up to meso- or metacoxae. Antennae elongated. Pronotum from 2.2 up to 2.9 times as wide as long. Mesonotum distinctly shorter than metanotum. Abdominal segments I–V of equal length; abdominal segments VI and VII diminish gradually; abdominal segment VIII almost semicircle; abdomen segment IX distinctly reduced. Spiracles on abdominal segments placed dorsolaterally: on abdominal segments I–V functional; and on segment VI atrophic on next ones invisible. Urogomphi (ur) stout and short, conical, each of them with sclerotized apex.

*Chaetotaxy***:** setae piliform, in a different size. Head capsule with 0–1 *vs*, 0–1 *sos*, and two *os.* Rostrum with 0–1 *pas* and one *rs* (placed medially and apically). Pronotum with: two *as*, one *ds*, 1–2 *sls*, 0–1 *ls* and three *pls*. Setae on head, rostrum and pronotum very thin, light and relatively short. Dorsal parts of meso- and metathorax with 2–3 setae placed medially. Apex of each femora with one *fes*. Abdominal segments I–VIII with two setae laterally and sometimes 2–3 short setae ventrally. Dorsal parts of abdominal segments I–VII with 4–5 setae, and abdominal segment VIII with 3–4 setae dorsally. Abdominal segment IX with 2–4 micro-setae ventrally.

Cleopomiarus distinctus (Boheman, 1845)

Figures 81–84

Material examined. 12 specimens: 2 ♂; 2 ♀, 03.08.2010, Gródek ad Hrubieszów, CE Poland, leg. E. Szwaj, det. J. Łętowski; 2 ♂; 6 ♀, ex seed capsules of *Campanula cervicaria* L., 05.07.2017, Stara Planina, east Serbia, leg. I. Toševski, det. R. Caldara.

Description. *Measurements* (in mm). Body length: 3.00–4.10 (mean 3.20). Body width: 1.50–2.60 (mean 1.80). Head width: 0.65–0.77 (mean 0.67).

Body moderately slender (Figs 81–84). Rostrum long, approximately 4.0 times as long as wide, reached up to mesocoxae. Antennae moderately elongated. Pronotum 2.2 times as wide as long. Urogomphi (ur) stout (Figs 81–84).

*Chaetotaxy***:** setae very thin, greyish, piliform, medium size to short. Head capsule with only two *os* of different in length (second pair placed on eye spots). Rostrum with one *pas* and one *rs* (Figs 82, 83). Pronotum with: two *as*, one *ds*, one *sls*, and three *pls* (Figs 83, 84). All setae of prothorax almost equal in length (Fig. 83). Dorsal parts of meso- and metathorax with three setae placed medially. Apex of each femora with one *fes* (Figs 82–84). Abdominal segments I–VIII with two setae laterally. Dorsal parts of abdominal segments I–VII with four setae: $d_{1–3}$ placed postero-medially, d_4 postero-laterally, abdominal segment VIII with only three setae dorsally. Abdominal segment IX with two micro-setae ventrally.

Cleopomiarus graminis (Gyllenhal, 1813)

Figures 85–88

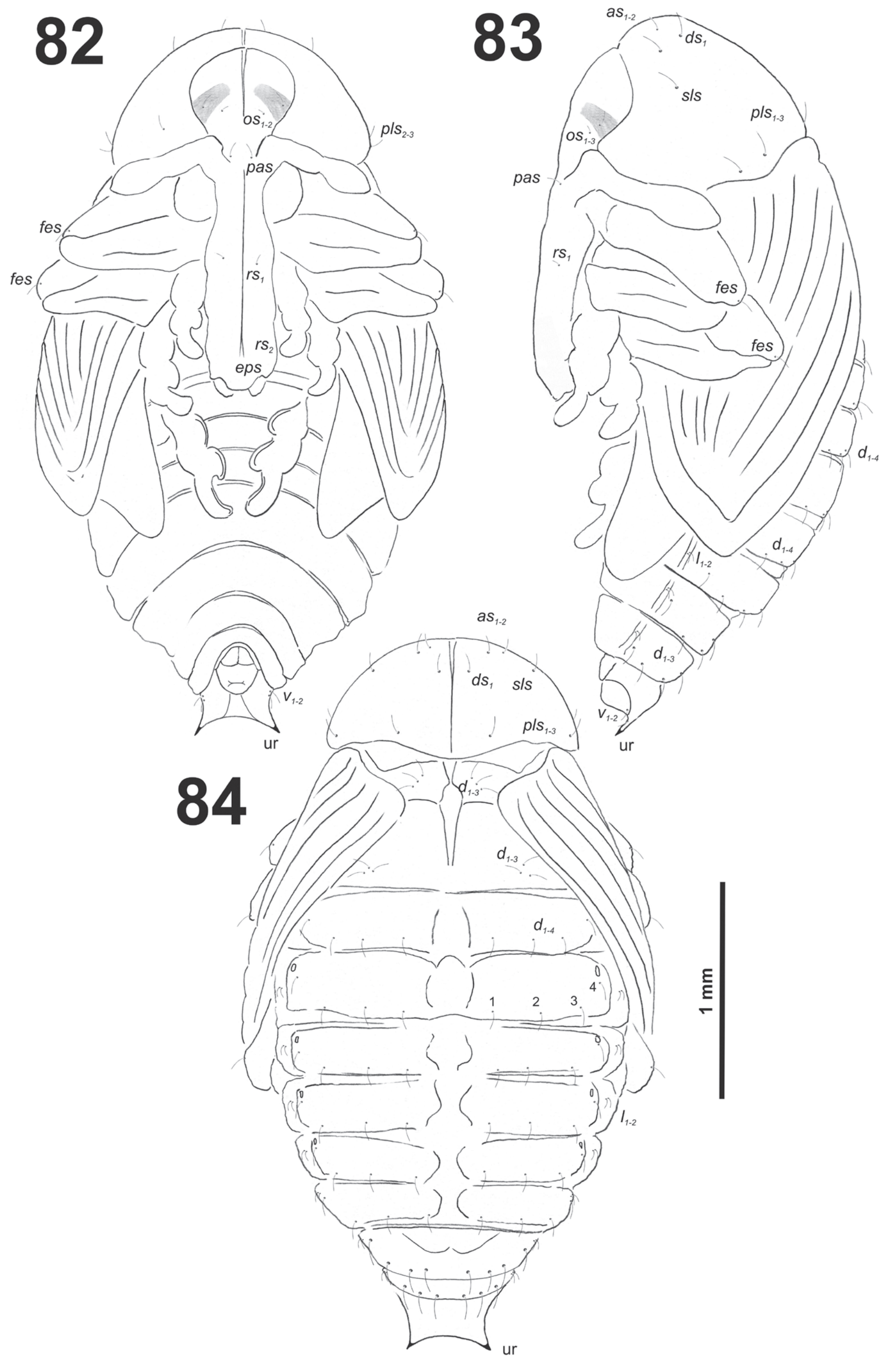
Material examined. 15 specimens: 2 ♂, 3 ♀, 27.07.2010, Wólka ad Lublin, CE Poland, leg. E. Szwaj, det. J. Łętowski; 4 ♂, 2 ♀, ex seed pods of *Adenophora liliifolia*, Dobra, Iron Gate, east Serbia, leg. I. Toševski, det. R. Caldara; 4 ♀, ex seed pods of *Campanula macrostachya,* 13.07.2015, Dobra, Iron Gate, east Serbia, leg. I. Toševski, det. R. Caldara.

Description. *Measurements* (in mm). Body length: 3.60–4.10 (mean 3.70). Body width: 2.10–2.25 (mean 2.15). Head width: 0.65–0.70 (mean 0.67).

Body moderately slender (Figs 85–88). Rostrum long, approximately four times as long as wide, reached up to mesocoxae. Antennae slender and elongated. Pronotum 2.9 times as wide as long. Urogomphi (ur) moderately stout (Figs 85–88).

Figure 81. *Cleopomiarus distinctus* pupa habitus, ventral view.

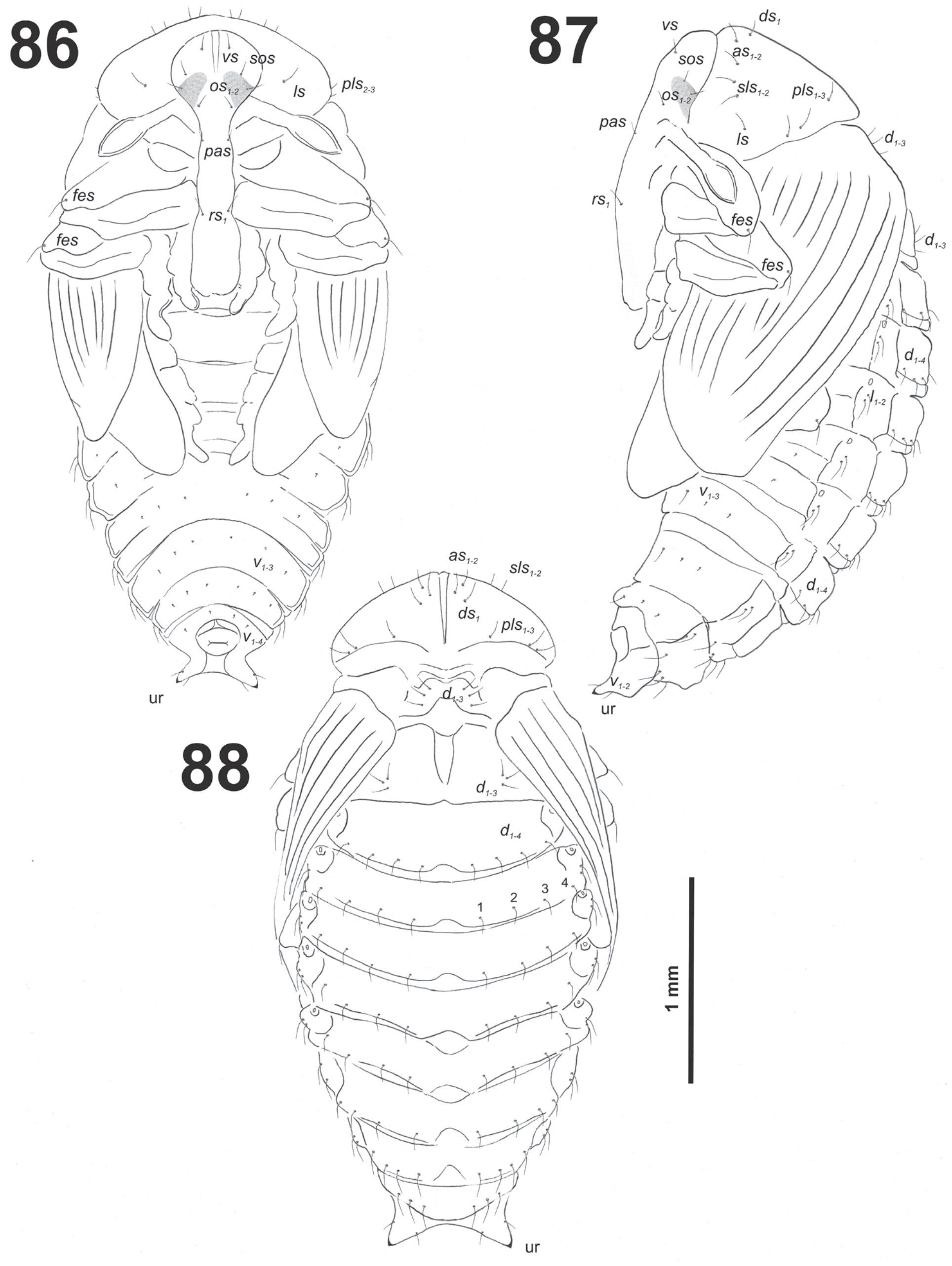
*Chaetotaxy***:** setae very thin, greyish, piliform, medium size to short. Head capsule with one *vs*, one *sos*, and two *os* of different length (second pair placed on eye spots). Rostrum with one *pas* and one *rs* (Figs 86, 87). Pronotum with: two *as*, one *ds*, two *sls*, one *ls*, and three *pls* (Figs 87, 88). All setae of prothorax equal in length (Fig. 88). Dorsal parts of meso- and metathorax with three setae placed medially. Apex of each femora with one *fes* (Figs 86, 87). Abdominal segments I–VIII with



Figures 82–84. *Cleopomiarus distinctus* pupa habitus and chaetotaxy. **82** Ventral view **83** Lateral view **84** Dorsal view. Abbreviations: *d* – dorsal s., *ds* – discal s., *fes* – femoral s., *l*, – lateral s., *os* – orbital s., *pas* – postantennal s., *pls* – posterolateral s., *rs* – rostral s., *sls* – super lateral s., *v* – ventral s., ur – urogomphi.

Figure 85. *Cleopomiarus graminis* pupa habitus, ventral view.

two setae laterally and three micro setae ventrally. Dorsal parts of abdominal segments I–VII with four setae: d_{1-3} postero-medially, and d_4 postero-laterally. Abdominal segment VIII with four setae dorsally. Abdominal segment IX with four micro-setae ventrally.



Figures 86–88. *Cleopomiarus graminis* pupa habitus. **86** Ventral view **87** Lateral view **88** Dorsal view. Abbreviations: *d* – dorsal s., *ds* – discal s., *fes* – femoral s., *l*, *ls* – lateral s., *os* – orbital s., *pas* – postantennal s., *pls* – posterolateral s., *rs* – rostral s., *sls* – super lateral s., *sos* – super orbital s., *v* – ventral s., *vs* – vertical s., ur – urogomphi.

***Cleopomiarus longirostris* (Gyllenhal, 1838)**
Figures 89–92

Material examined. 18 specimens: 8 ♂, 10 ♀, south eastern France, Menton, July 2007, ex seed capsules of *Campanula trachelium* L., leg. and det. R. Caldara

Description. *Measurements* (in mm). Body length: 5.20–6.50 (mean 5.60). Body width: 3.00–3.80 (mean 3.30). Head width: 0.90–1.10 (mean 1.00).

Body stout (Figs 89–92). Rostrum very long, almost five times as long as wide, reached almost up to metacoxae. Antennae slender and moderately elongated. Pronotum 2.5 times as wide as long. Urogomphi (ur) short (Figs 90–92).

89

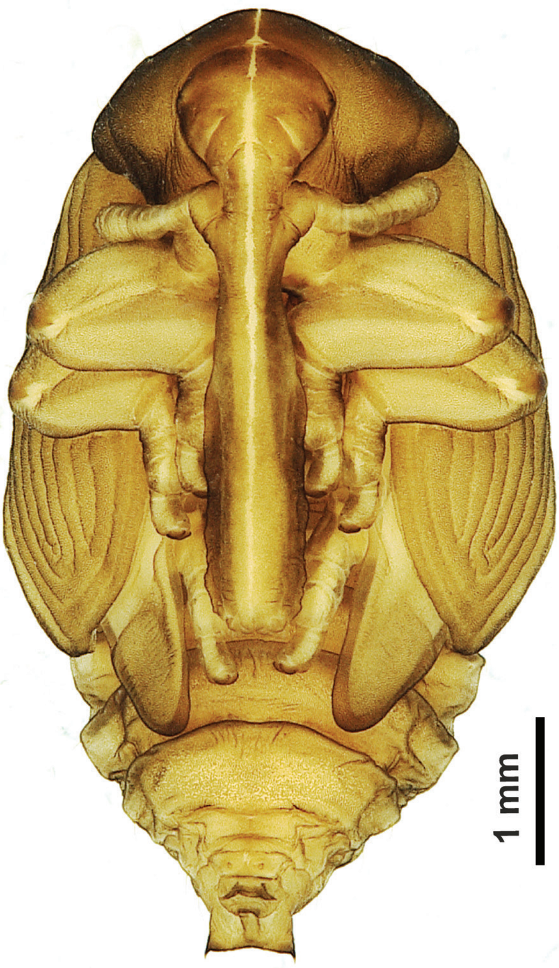
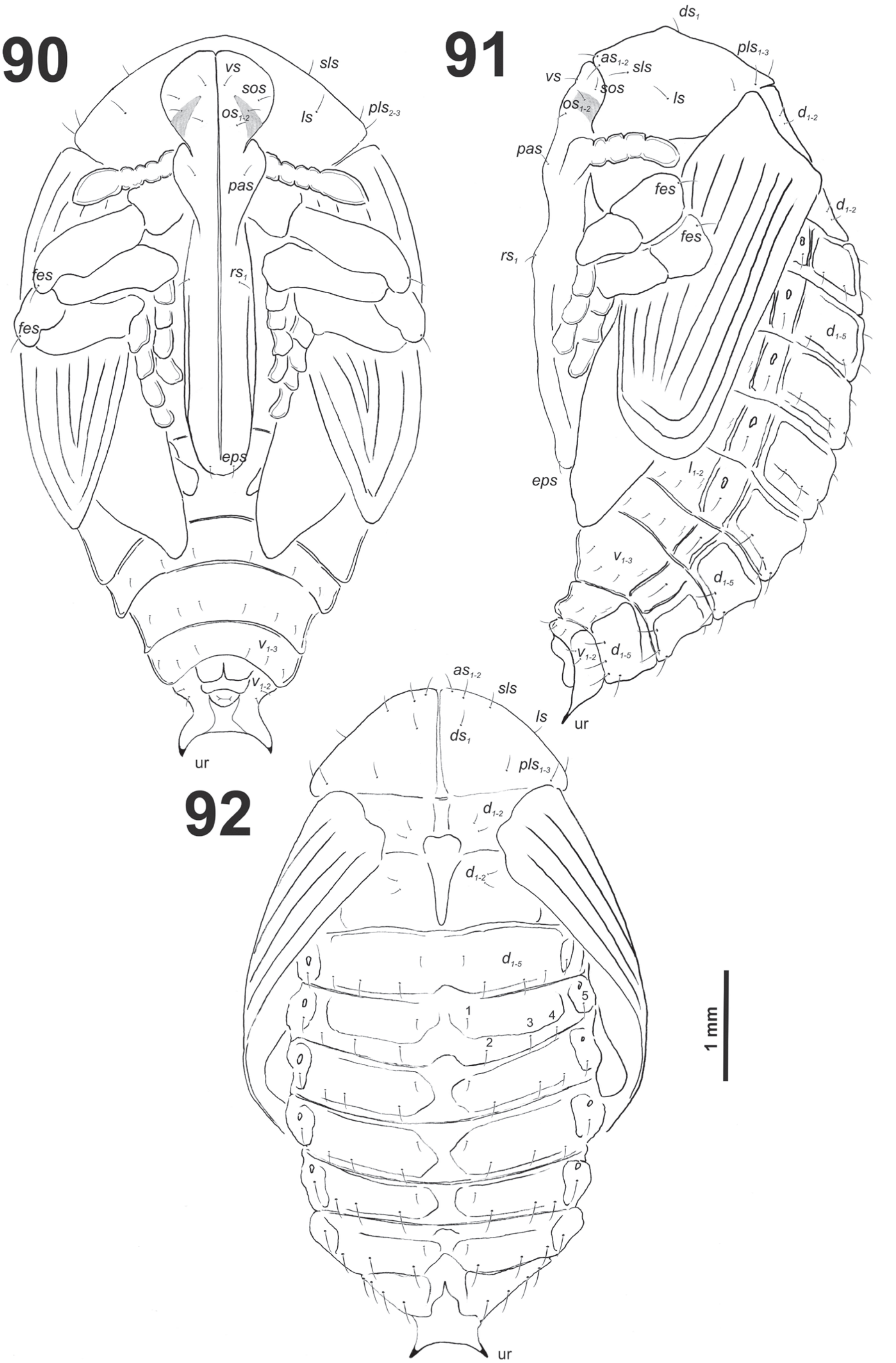


Figure 89. *Cleopomiarus longirostris* pupa habitus, ventral view.



Figures 90–92. *Cleopomiarus longirostris* pupa habitus. **90** Ventral view **91** Lateral view **92** Dorsal view. Abbreviations: *d* – dorsal s., *ds* – discal s., *es* –epistomal s., *fes* – femoral s., *l*, *ls* – lateral s., *os* – orbital s., *pas* – postantennal s., *pls* – posterolateral s., *rs* – rostral s., *sls* – super lateral s., *sos* – super orbital s., *v* – ventral s., *vs* – vertical s., ur – urogomphi.

*Chaetotaxy***:** sparse, setae very thin, short, piliform. Head capsule with one *vs*, one *sos*, and two *os* of different length (second pair placed on eye spots). Rostrum with one *rs* and one *es* (Figs 90, 91). Pronotum with: two *as*, one *ds*, one *ls*, one *sls*, and three *pls* (Figs 91, 92). All setae of prothorax equal in length (Fig. 92). Dorsal parts of meso- and metathorax with two setae placed medially. Apex of each femora with one *fes* (Figs 90–92). Abdominal segments I–VIII with two setae laterally and three medium long setae ventrally. Dorsal parts of abdominal segments I–VII with five setae: d_1 placed antero-medially, $d_{2–4}$ postero-medially, d_5 postero-laterally. Abdominal segment VIII with only three setae dorsally. Abdominal segment IX with two micro-setae ventrally.

Cleopomiarus medius (Desbrochers des Loges, 1893)

Figures 93–96

Material examined. 4 specimens: 2 ♂, 2 ♀, ex seed capsules of *Campanula lingulata* Waldst. and Kit., 26.06.2017, Staničenje, Pirot, east Serbia, leg. I. Toševski, det R. Caldara.

Description. *Measurements* (in mm). Body length: 4.40–4.60. Body width: 2.40–2.50. Head width: 0.70–0.80.

Body moderately stout (Figs 93–96). Rostrum very long, approximately 4.5 times as long as wide, reached beyond mesocoxae. Antennae slender and moderately elongated. Pronotum 2.4 times as wide as long. Urogomphi (ur) stout (Figs 94–96).

*Chaetotaxy***:** sparse, setae short to very short, light, piliform. Head capsule with one very short *vs*, and two *os* of different length, both placed between eye spots. Rostrum with one *rs* (Figs 94, 95). Pronotum with: two *as*, one *ds*, two *sls*, one *ls* and three *pls* (Figs 95, 96). All setae of prothorax equal in length (Fig. 96). Dorsal parts of meso- and metathorax with three setae placed medially. Apex of each femora with one *fes* (Figs 94, 95). Abdominal segments I–VIII with two setae laterally and three medium long setae ventrally. Dorsal parts of abdominal segments I–VII with four setae: $d_{1–3}$ postero-medially, d_4 postero-laterally. Abdominal segment VIII with only three setae dorsally. Abdominal segment IX with four micro-setae ventrally.

Genus *Miarus* Schoenherr, 1826

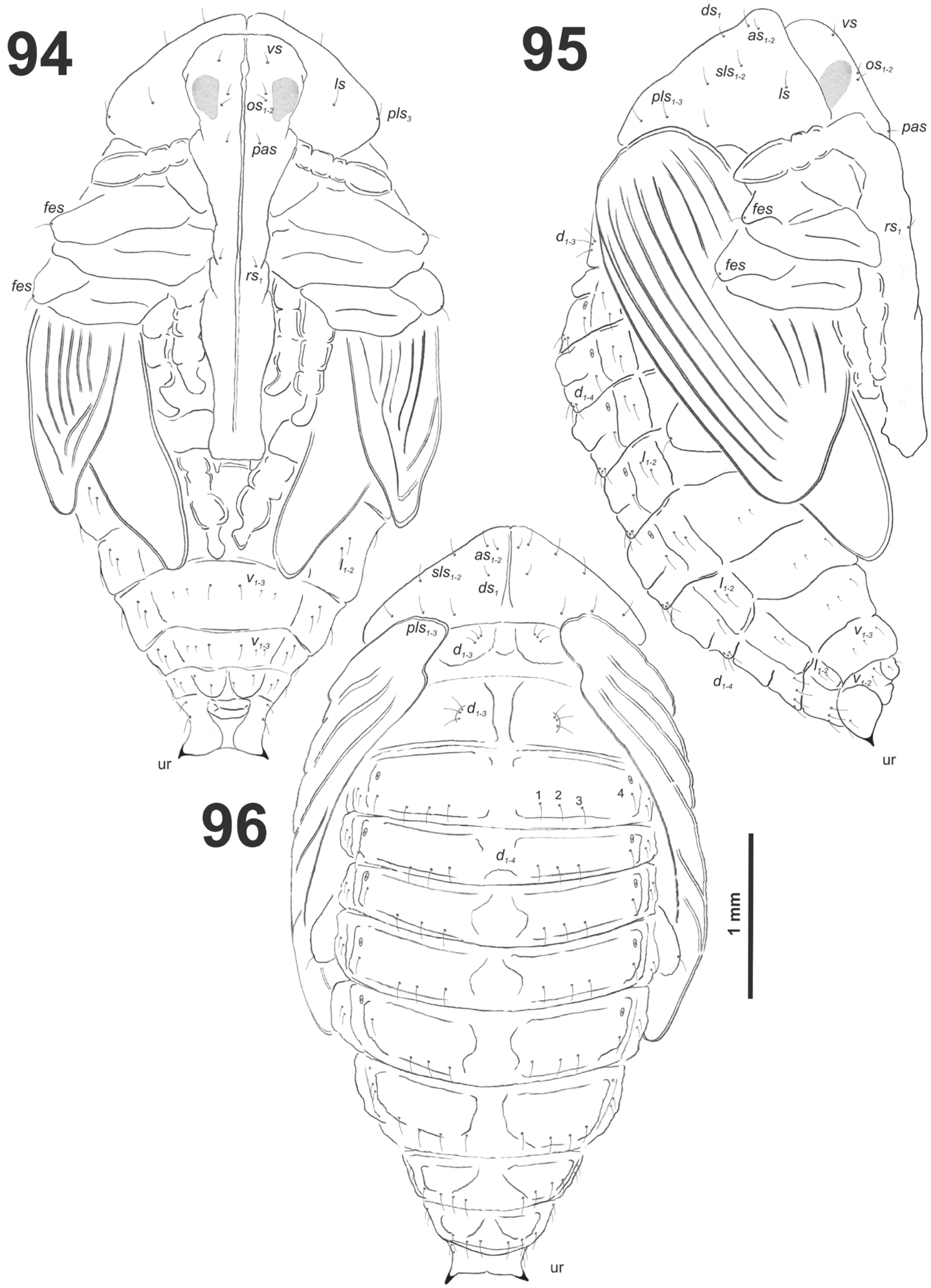
Description. *Measurements* (in mm). Body length: 3.20–4.00. Body width: 1.90–2.70. Head width: 0.55–0.70.

Body moderately slender or stout. Smooth, dark brown or black spotted cuticle. Rostrum long or very long, from 2.3 up to 3.5 times as long as wide, reached up to meso- or metacoxae. Antennae elongated. Pronotum from 2.1 up to 2.5 times as wide as long. Mesonotum distinctly shorter than metanotum. Abdominal segments I–V of equal length; abdominal segments VI and VII diminish gradually; abdominal segment VIII almost semicircle; abdomen segment IX distinctly reduced. Spiracles on abdomi-

Figure 93. *Cleopomiarus medius* pupa habitus, ventral view.

nal segments placed dorsolaterally: on abdominal segments I–V functional; and on segment VI atrophic on next ones invisible. Urogomphi (ur) slender and elongated, conical, each of them with sclerotized apex.

*Chaetotaxy***:** setae piliform, in a different size. Head capsule with one *vs*, one *sos*, and 3–4 *os.* Rostrum with one *pas* and three *rs* (placed medially and apically). Prono-



Figures 94–96. *Cleopomiarus medius* pupa habitus. **94** Ventral view **95** Lateral view **96** Dorsal view. Abbreviations: *d* – dorsal s., *ds* – discal s., *fes* – femoral s., *l*, *ls* – lateral s., *os* – orbital s., *pas* – postantennal s., *pls* – posterolateral s., *rs* – rostral s., *sls* – super lateral s., *v* – ventral s., *vs* – vertical s., ur – urogomphi.

tum with: two *as*, two *ds*, two *sls*, one *ls* and three *pls*. All setae of prothorax almost equal in length. Dorsal parts of meso- and metathorax with three setae placed medially. Apex of each femora with one *fes*. Abdominal segments I–VIII with two setae laterally and sometimes 3–4 short setae ventrally. Dorsal parts of abdominal segments I–VII with 4–5 setae, and abdominal segment VIII with 3–4 setae dorsally. Abdominal segment IX with 4–6 micro-setae ventrally.

Miarus abnormis Solari, 1947

Figures 97–100

Material examined. 3 specimens: 1 ♂, 2 ♀, north-eastern Italy, Venezia Giulia, Duino (Trieste), Rilke path, August 2017, ex galls on capsules of *Campanula pyramidalis* L., leg. E. Tomasi, det. R. Caldara.

Description. *Measurements* (in mm). Body length: 3.55–3.60. Body width: 1.90–2.00. Head width: 0.55–0.60.

Body moderately slender (Figs 97–100). The cuticle densely covered with asperities. Rostrum medium length, approximately 2.3 times as long as wide, reached up to mesocoxae. Antennae moderately elongated. Pronotum 2.1 times as wide as long. Urogomphi (ur) slender and elongated, conical, each of them with sclerotized apex (Figs 98–100).

*Chaetotaxy***:** setae brownish to dark brown, piliform, short to medium size. Head capsule with one *vs*, one *sos*, and three *os* of equal length; rostrum with three *rs* (rs_1 and rs_2 placed medially, rs_3 more apically) and one *es* (Figs 98, 99). Pronotum with: two *as*, two *ds*, two *sls*, one *ls* and three *pls* (Figs 99. 100). Setae on head and rostrum slightly shorter than those on prothorax. All setae of prothorax almost equal in length (Fig. 100). Mesothorax with three setae placed medially; metathorax with three setae placed laterally. Apex of each femora with one *fes* (Figs 98–100). Abdominal segments I–VIII with two short, thin setae laterally and three short setae ventrally. Dorsal parts of each abdominal segments I–VIII with four setae: d_{1-3} placed postero-medially, d_4 postero-laterally. Abdominal segment IX with six microsetae ventrally.

Miarus ajugae (Herbst, 1795)

Figures 101–104

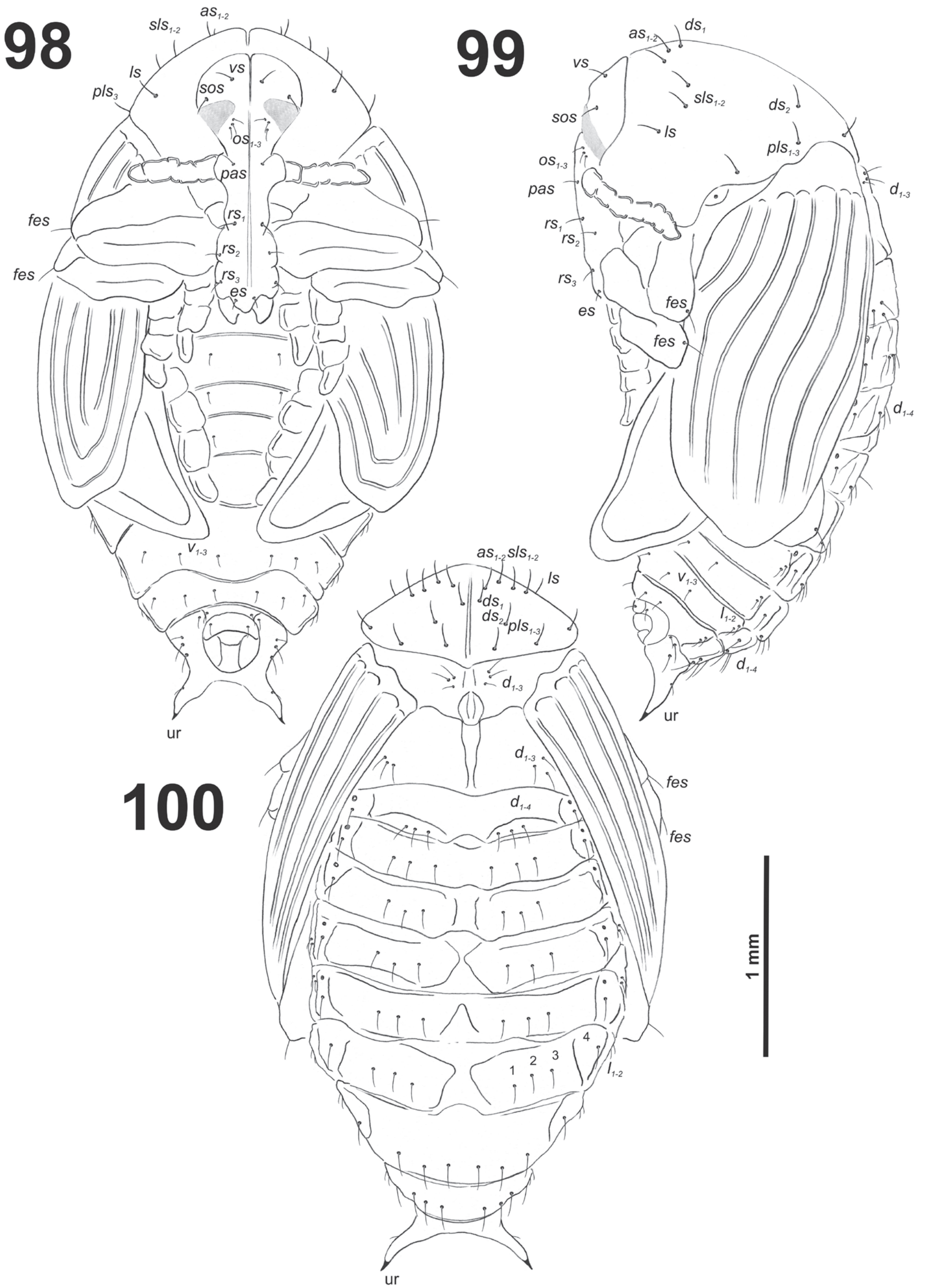
Material examined. 12 specimens: 2 ♂, 1 ♀, 24.08.2009, Ciechanki, CE Poland, leg. E. Szwaj, det. J. Łętowski; 3 ♂, 2 ♀, 30.06.2017, ex galls on capsules of *Adenophora liliifolia,* 30.06.2017, Kaludjerske Bare, Mt. Tara, Central Serbia, leg. I. Toševski, det. R. Caldara; 3 ♂, 1 ♀, ex galls on capsules of *Campanula bononiensis,* 14.07.2017, Zavojsko jezero, Pirot, east Serbia, leg. I. Toševski, det. R. Caldara.

Description. *Measurements* (in mm). Body length: 3.20–4.00 (mean 3.40). Body width: 1.90–2.70 (mean 2.10). Head width: 0.65–0.70 (mean 0.65).

Figure 97. *Miarus abnormis* pupa habitus, ventral view.

Body rather stout (Figs 101–104). Rostrum moderately elongated, approximately 3.5 times as long as wide, reached almost up to metacoxae. Antennae rather stout and moderately elongated. Pronotum 2.5 times as wide as long. Urogomphi (ur) slender and elongated (Figs 102–104).

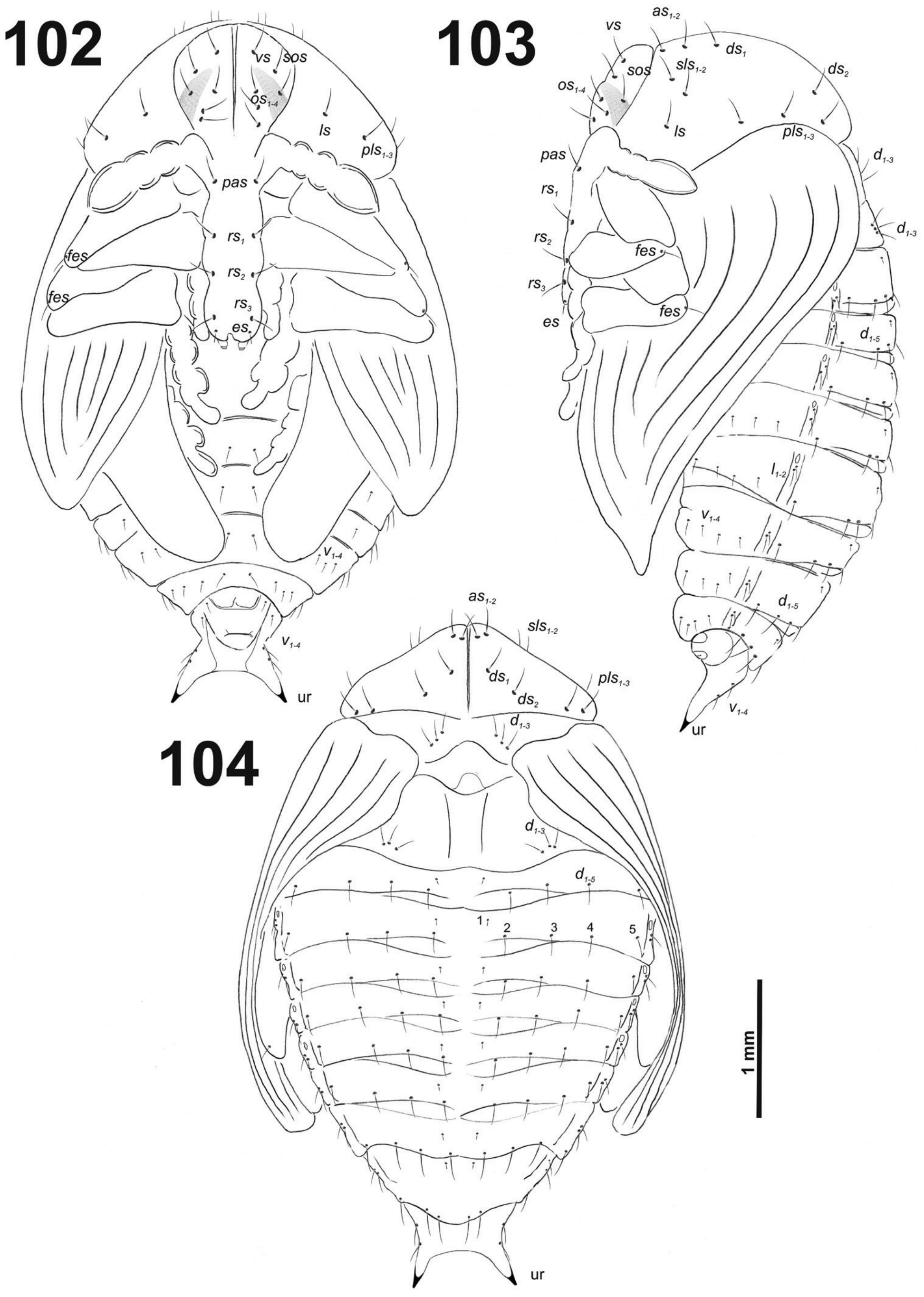
Chaetotaxy: setae piliform, greyish to dark brown, different in length, short to medium size. Head capsule with one *vs*, one *sos*, and four *os* of different length (fourth placed on eye spots). Rostrum with one *pas*, and three *rs* (rs_1 and rs_2 placed medially, rs_3 more apically) and one *es* (Figs 102, 103). Pronotum with: two *as*, two *ds*, one *ls*, two *sls*, and three *pls* (Figs 103, 104). All setae of prothorax equal in length (Fig. 104). Dorsal parts of meso- and metathorax with three setae placed medially. Apex of each femora with one *fes* (Figs 102, 103). Abdominal segments I–VIII with two setae laterally and four medium



Figures 98–100. *Miarus abnormis* pupa habitus. **98** Ventral view **99** Lateral view **100** Dorsal view. Abbreviations: *d* – dorsal s., *ds* – discal s., *es* – epistomal s., *fes* – femoral s., *l*, *ls* – lateral s., *os* – orbital s., *pas* – postantennal s., *pls* – posterolateral s., *rs* – rostral s., *sls* – super lateral s., *sos* – super orbital s., *v* – ventral s., *vs* – vertical s., ur – urogomphi.

Figure 101. *Miarus ajugae* pupa habitus, ventral view.

long setae ventrally. Dorsal parts of abdominal segments I–VII with five setae: d_1 placed antero-medially, $d_{2–4}$ postero-medially, d_5 postero-laterally, and abdominal segment VIII with only three setae dorsally. Abdominal segment IX with four micro-setae ventrally.



Figures 102–104. *Miarus ajugae* pupa habitus. **102** Ventral view **103** Lateral view **104** Dorsal view. Abbreviations: *d* – dorsal s., *ds* – discal s., *es* – epistomal s., *fes* – femoral s., *l*, *ls* – lateral s., *os* – orbital s., *pas* – postantennal s., *pls* – posterolateral s., *rs* – rostral s., *sls* – super lateral s., *sos* – super orbital s., *v* – ventral s., *vs* – vertical s., ur – urogomphi.

Key to pupae

The following key is based on pupae of four *Cleopomiarus* and two *Miarus* species described in this paper. Unfortunately, the description of *Cleopomiarus hispidulus* published previously (Anderson 1973) could not be included due to missing details about the chaetotaxy used in our key.

1 Rostrum with 1 *rs* (Figs 82, 86, 90, 94). Setae on head, rostrum and pronotum very thin, light and relatively short (Figs 83, 87, 91, 95). Pronotum with 1 *ds* (Figs 84, 88, 92, 96). Urogomphi stout and short (Figs 82, 86, 90, 94) .... **2 *Cleopomiarus***

– Rostrum with 3 *rs* (Figs 98, 102). Setae on head, rostrum and pronotum brown, prominent and relatively long (Figs 99, 103). Pronotum with 2 *ds* (Figs 100, 104). Urogomphi relatively slender and elongated (Figs 100, 104) .... **5 *Miarus***

2 Rostrum extremely elongated (at least 4.5 times as long as wide) (Figs 89, 93). Head with 2 *os* (Figs 90, 94) **3**

– Rostrum elongated (ca. 4.0 times as long as wide) (Figs 81, 85). Head with 3 *os* (Figs 82, 86) **4**

3 Body length over 5.20 mm; head width over 0.90 mm. Pronotum with 1 *sls*; *es* present (Fig. 91) .... ***Cleopomiarus longirostris***

– Body length under 4.60 mm; head width under 0.80 mm. Pronotum with 2 *sls*; *es* absent (Fig. 95) .... ***C. medius***

4 *Vs* and *sos* present (as long as other setae on head) (Fig. 86). Pronotum with 2 *sls* (Fig. 87). Abdominal segments I–VII with 3 ventral setae (Fig. 87) .... ***C. graminis***

– *Vs* and *sos* absent (or as microsetae) (Fig. 82). Pronotum with 1 *sls* (Fig. 83). Abdominal segments I–VII without ventral setae (Fig. 82) .... ***C. distinctus***

5 Body length usually over 3.60 mm (Fig. 101). Head width over 0.65 mm. Head with 4 *os* (Fig. 102).. ***Miarus ajugae***

– Body length usually under 3.50 mm (Fig. 97). Head width under 0.60 mm. Head with 3 *os* (Fig. 98).. ***M. abnormis***

Discussion

Comparison with immature stages of known Mecinini

Presently, it is somewhat difficult to compare the immatures of *Cleopomiarus* and *Miarus*, which we have just described, with those of other genera of the Mecinini. As above reported, the description of most of the 19 species previously described is somewhat problematic because of missing details about the chaetotaxy and/or absence of quality drawings. Only the recent descriptions of three species of *Gymnetron* (Jiang and

Zhang 2015) and one species of *Rhinusa* (Gosik 2010) are useful for a comparison with our data, although some categorizations of setae in the former paper are very disputable (e.g., thoracic and abdominal dorsal setae) and create an unfounded differential diagnosis, precluding the construction of a key to the tribe. Having said this, we think that there are important but still disputable character states within the tribe Mecinini.

The most important morphological character of larvae in this tribe is probably the count of palpomeres on the labial palpi. The basal state in weevils is the presence of two palpomeres on labial palpi (Marvaldi 1997), but it is known that *Gymnetron* species has only one palpomere (May 1993; Jiang and Zhang 2015). The count of these palpomeres in some descriptions in this paper, mainly in *Cleopomiarus*, is disputable. There is not a distinct separation of basal palpomere from the labium, which can appear to be only one palpomere. This state in *Cleopomiarus* and partially in *Miarus* could be an intermediate stage in the reduction in the *Gymnetron* species; however, this should be compared with other Mecinini genera such as *Rhinusa* and *Mecinus* and discussed only within the whole tribe.

Another crucial genus-specific character in Mecinini larvae is the state of the thoracic and abdominal spiracles. All known larvae of *Cleopomiarus* and *Miarus* species have bicameral spiracles on the thorax and abdomen, but other groups within tribe have only unicameral spiracles (*Gymnetron* species; Jiang and Zhang 2015) or thoracic spiracle bicameral and abdominal spiracles unicameral (some *Rhinusa* and *Mecinus*; Anderson 1973; May 1993).

The count of some setae on the epipharynx (especially *ams* and *mes*) in Curculionidae has not been completely resolved, but this has been discussed in previous papers (e.g., Tychiini: Skuhrovec et al. 2014, 2015; Gosik et al. 2017). According to Marvaldi (1998, 1999), the standard status of the epipharyngeal setae in weevils is two *ams* and three *mes*, but when the position of the distal *mes* is very close to the anterior margin, it appears as *ams*. There are also situations with some out of line positions of the *als* where the position is very close to the tormae (then it appears to be a *mes*) or close to the *ams*. The final decision about the count of each seta is important, but not crucial, and the comparison between groups/genera should be done together for all three of these epipharyngeal setae in order to make fewer mistakes in the creation of a differential diagnosis of genera in the tribe.

The last observed important characteristic within the Mecinini tribe is the vestiture of the body with distinct asperities; however, this feature can be variable within each genus due to specific environmental conditions within plant tissues. This feature will possibly be discussed after other detailed descriptions within the Mecinini tribe.

Comparison of the immature stages of *Cleopomiarus* and *Miarus*

Before this study, larvae of only two *Cleopomiarus* species (*C. graminis* and *C. hispidulus*) and one *Miarus* species (*M. campanulae*) had been described (Emden 1938; Scherf 1964; Anderson 1973), while a description of the pupae was available for *C. hispidulus* (Anderson 1973). Therefore, a detailed redescription of the two species, *Cleopomiarus graminis* and *Miarus campanulae*, has been necessary for their incorporation into the key. The previous descriptions were almost without the chaetotaxy with a few exceptions (e.g., number of *ams* and *als* in *C. graminis*) and included only basal morphological descriptions such as the number of teeth on the mandible or colouration of the head and body. The present detailed descriptions of the immature stages of five *Cleopomiarus* and three *Miarus* species allow a comparison of both genera.

The comparative study of these immatures supports the theory that these genera may be monophyletic based on several unique characteristics (see below), as already suggested in a phylogenetic study by Caldara (2001) on the basis of the adult morphological characters. Larvae of *Miarus* have mala with six finger-like *dms* in two sizes: one or two very long *dms* with the rest being medium length. This appears to be a unique characteristic in weevils. More genus-specific characters are in the pupae, which are more conservative in chaetotaxy. The main differential characters in pupae among known species include the following: (1) setae on the head, rostrum and pronotum very thin, light and relatively short (*Cleopomiarus*) vs brown, prominent and relatively long (*Miarus*); (2) rostrum with one *rs* (*Cleopomiarus*) vs rostrum with three *rs* (*Miarus*); (3) pronotum with one *ds* (*Cleopomiarus*) vs pronotum with two *ds* (*Miarus*); and finally (4) urogomphi (ur) stout and short (*Cleopomiarus*) vs relatively slender and elongated (*Miarus*). Less genus-specific character states in the larvae than in pupae were also shown in another tribe of the Curculioninae (Tychiini) with regard to genera *Tychius* Germar, 1817 and *Sibinia* Germar, 1817 (see Skuhrovec et al. 2014, 2015).

Differences between immatures at the species level

Our study shows that all the species considered can be identified by the examination of larvae and pupae based on at least one character state. It is noteworthy that several taxa examined only by the study of imagoes were difficult to separate. Therefore, finding distinctive supplementary characters is welcome. This is true for *C. longirostris* vs *C. graminis* and especially *M. ajugae* vs *M. campanulae*. The latter case is particularly emblematic. In these two taxa the adults may be separated by the shape of the apical part of the penis. On the contrary they seem indistinguishable on the basis of barcoding (Vahtera and Muona 2006; Hendrich et al. 2015).

Importance of molecular data

We have confirmed that a molecular study of immatures is very important in cases where it is necessary to be sure on the identity of a species as already demonstrated by Horecka et al. (2017) just for *Miarus* and *Cleopomiarus*. This is true especially when it is known that more than one related species can live on the same plant or when imagoes are not available or finally when one has to study specimens not personally collected and preserved in a museum. It is noteworthy that the data filed on GenBank or BOLD are becoming larger and larger and therefore more and more useful for such an adequate comparison. Therefore, it is also important to continue to implement these data. In this regard, it is noteworthy that we reported the barcode of *C. medius* for the first time.

Biological considerations

It is obvious that only a careful search of immature and a careful study of their biological cycle can distinguish true host plants from those used only as a refuge or adult food when the host plants are not yet or no longer available. Our observations confirm that the species of both genera *Cleopomiarus* and *Miarus* are monophagous, although never strictly monophagous, to oligophagous (Bernays and Chapman 1994). Moreover, our data show that the larvae of *Miarus* are gall-inducers as previously reported (Buhr 1964; Tomasi 2002) but that the *Cleopomiarus* species do not form galls. However, this apparently different biological behaviour requires confirmation, since it is well known that several closely related species of Mecinini, especially in the genera *Gymnetron* and *Rhinusa*, do not have the same behaviour with regard to induction of galls (Caldara 2008; Caldara et al. 2010).

Conclusions

Our data show that detailed descriptions of the immature stages of the Mecinini species are important for further studies of generic taxonomic relationships within the Mecinini group. The detailed descriptions of larvae and pupae of five *Cleopomiarus* and three *Miarus* species are reported in this study. Although the number remains low in comparison with the total number of species of *Cleopomiarus* and *Miarus*, these results demonstrate the possibility of identifying the immature stages in these species, as was done in other groups of weevils (see Skuhrovec et al. 2014, 2015). This is our first paper about the Mecinini group. Detailed descriptions of the genera *Gymnetron*, *Rhinusa,* and *Mecinus* and a tribe overview will be published soon.

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Comparative larval ultramorphology of some myrmecophilous Aleocharinae (Coleoptera, Staphylinidae), with a first description of the larvae of *Amidobia talpa* (Heer O, 1841) and *Oxypoda haemorrhoa* (Mannerheim C.G., 1830), associated with the *Formica rufa* species group

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Abstract
The paper describes the external structures of the late larval stages of two Palearctic myrmecophilous staphylinids: *Amidobia talpa* and *Oxypoda haemorrhoa* associated with the *Formica rufa* species group. This is the first-ever description of the larva of *Amidobia*, and the only complete, detailed account of the morphology of this developmental stage in the genus *Oxypoda* currently available. For the first time in these two genera, 13 and 10 larval diagnostic features, respectively, are proposed. Morphological differences have been established between known and the newly described larvae of five species (genera) of myrmecophilous and one non-myrmecophilous Aleocharinae, belonging to three tribes. *Amidobia talpa* and *O. haemorrhoa* are probably typical, tiny predators, like most other Aleocharinae, including non-myrmecophilous ones. Being very small and highly mobile, they are ignored by worker ants. Not surprisingly, no particular larval morphological modifications were found to enable them to survive among ants. Such features have, however, evolved in the larvae of larger aleocharines, that is, those that are perceived by ants and are wholly integrated with their hosts in the ant nest (e.g. *Lomechusa*). This comparative analysis of the functional morphology of the larvae of known myrmecophilous Aleocharinae is a springboard to further such studies of these interesting insects.

Keywords
Aleocharines, Red wood ants, Coleoptera, developmental stages, ecological preferences, external structure, late larval instars, larva, morphology, myrmecophile, rove beetles, symbionts

Introduction

Red wood ants from the *Formica rufa* Linnaeus, 1761 species group are regarded as key insect species in European woodlands because of their vast numbers and the invaluable biocoenotic contribution they make to the ecosystems they inhabit. Being polyphagous predators, they have a major, multidimensional effect on the invertebrate fauna in that they limit the numbers of many harmful woodland phytophages. On the other hand, the presence of ants has a very positive effect on a range of tiny woodland creatures, such as aphids, which ants protect and defend, obtaining honeydew in return (Szujecki 1998, Laakso and Setälä 2000). Moreover, these ants build complex nests with an extensive inner space, which provides a distinctive microclimate with stable levels of temperature and humidity, as well as constant and diverse food resources. It is therefore an optimal but at the same time highly specific microhabitat for a great number of myrmecophilous invertebrates, predominantly insects (Hölldobler and Wilson 1990, Päivinen et al. 2002, Staniec and Zagaja 2008, Parmentier et al. 2014, Parker 2016).

Among the insects associated with ants, beetles (Coleoptera) are the richest and the most diverse in form. According to Hölldobler and Wilson (1990), there are many thousands of myrmecophilous coleopteran species in 35 families. By way of example, as many as 166 species of beetle have been found in the nests of *Formica rufa* (Päivinen 2002). By far the most numerous group of myrmecophilous beetles are from the family Staphylinidae (rove beetles), the majority of which, in turn, belong to the subfamily Aleocharinae (Wilson 1979, Kistner 1979, Kronauer and Pierce 2011, Parker 2016) represented, among others, by *Amidobia talpa* Heer O, 1841 and *Oxypoda haemorrhoa* Mannerheim C.G., 1830.

The genus *Amidobia* Thomson C.G., 1858 (Athetini) contains eight Palearctic species, of which only *A. talpa* (Heer O., 1841) occurs in Europe (Löbl and Löbl 2015). This is a very small myrmecophile (body length ca 1.5 mm), inhabiting mainly the nests of *Formica rufa* and related species (Lohse 1974, Burakowski et al. 1981, Koch 1989). To date, this rove beetle has been reported from the nests of *Formica rufa*, *F. pratensis* Retzius, 1783, *F. aquilonia* Yarrow, 1955, *F. polyctena* Foerster, 1850, *F. lugubris* Zetterstedt, 1838, *F. truncorum* Fabricius, 1804, *F. execta* Nylander, 1846 and *Lasius fuliginosus* Latreille, 1798 (Päivinen et al. 2002, 2003, Staniec and Zagaja 2008, Parmentier et al. 2014). Its distribution range lies in central Europe and Fennoscandia, extending beyond the Arctic Circle in the north, and reaching the Caucasus, Siberia and the north of the Korean peninsula in the east. It is probably present throughout Poland, but there are still no records of it from many regions (Burakowski et al. 1981, Löbl and Löbl 2015).

The genus *Oxypoda* Mannerheim, 1830, one of the most species rich aleocharine genera, has a worldwide distribution (Newton et al. 2000, Löbl and Löbl 2015). *Oxypoda haemorrhoa* (Mannerheim C.G., 1830) (Oxypodini) belongs to the subgenus *Bessopo-*

ra Thomson, 1859, which has 100 species in the Palearctic, 13 of which occur in Poland (Melke 2014, Löbl and Löbl 2015). This rove beetle is widely distributed from North Africa across continental Europe as far as Iceland and northern Fennoscandia; it has also been recorded in Siberia. In Poland, it probably inhabits all parts of the country except the higher mountain areas, although some regions currently lack records (Burakowski et al. 1981). This myrmecophile is one of the smaller European representatives of the genus (body length: 2.0–2.7 mm). It lives mainly in ant nests from the *Formica rufa* group: *F. rufa*, *F. pratensis*, *F. polyctena*, *F. aquilonia* and *F. lugubris*, but one also comes across it in the nests of *F. truncorum*, *F. execta, F. sanguinea* Latreille, 1798*, F. suecica* Adlerz, 1902*, F. nigricans* Emery, 1909 and *Lasius fuliginosus* (Päivinen et al. 2002, 2003, Staniec and Zagaja 2008, Lapeva-Gjonova 2013, Parmentier et al. 2014). It has sometimes been found in the neighbourhood of anthills, under plant debris (Burakowski et al. 1981).

Myrmecophilous Aleocharinae, like other beetles associated with ants, are fascinating organisms for research because of their highly interesting morphological, ecological and behavioural adaptations to the distinctive conditions found in ant nests (Kolbe 1971, Hölldobler and Wilson 1990). In this context, however, very few data are available concerning the structure of their larval stages. Contemporary data on this subject relate to just three species: *Pella laticollis* Märkel F., 1844, *Thiasophila angulata* (Erichson W.F., 1837) and *Lomechusa pubicollis* Brisout de Barneville Ch.N.F., 1860. The first-mentioned is associated primarily with *Lasius fuliginosus*, the other two with ants from the *Formica rufa* group (Staniec et al. 2009, Zagaja et al. 2014, Staniec et al. 2017).

Presumably, the larval structure of these myrmecophiles, among other characteristics, which actively live and forage in the anthill throughout their development, should well reflect the extent and nature of their integration with their hosts. Therefore, detailed morphological data of the larval forms should prove useful for discovering the distinctive adaptations of myrmecophilous species to life in ant nests and also the relations between them and their hosts.

The links of numerous Staphylinidae with such a characteristic habitat like ant nests are reflected in various degrees of specialization. In the context of host-guest interactions, Wasmann (1894) classified arthropods inhabiting anthills into ectoparasites, endoparasites, trophobionts, synechtry, synoics and symphiles. Myrmecophilous aleocharines can be placed in these last three categories. *Lomechusa pubicollis* is a representative of the symphilous Aleocharinae. Like all symphiles, this rove beetle is the most highly integrated with its host; evidence for this can be found *inter alia* in a number of adaptive morphological features in its larval stage, recently described by Staniec et al. (2017). In turn, both *Amidobia talpa* and *Oxypoda haemorrhoa* are probably synoics, that is, myrmecophiles feeding on debris or other organisms inhabiting the anthill; being small and highly mobile, they are probably overlooked by their hosts. On the other hand, both these beetles will readily feed on ant larvae, thus exhibiting typical features of synechtry (Parmentier et al. 2015, own observations). The myrmecophilous *Thiasophila angulata* (Erichson W.F., 1837) has a similar lifestyle (Zagaja et al. 2017), but the morphology of its larva is no different from that of the larvae of other, related but non-myrmecophilous aleocharine beetles (Zagaja et al. 2014).

It is apposite, therefore, to pose the following questions: 1) Does the myrmecophily of *A. talpa* and *O. haemorrhoa* have any effect on the external structure of their larvae? 2) Are their ecological preferences of no great importance in this respect, as in the case of *T. angulata*? 3) How does the extent of guest-host integration affect the morphology of aleocharine larvae so far examined?

The chief aim of this paper is therefore to describe in detail the morphology, including the chaetotaxies and external ultrastructure, of the larval stages of *Amidobia talpa* and *Oxypoda haemorrhoa* and to compare them with the external larval structures of other, well-known myrmecophilous aleocharines.

Materials and methods

Material examined

Larval stages were obtained by rearing 34 adults of *Amidobia talpa* and 9 adults of *Oxypoda haemorrhoa*. Specimens of both species were collected on May 5, 2017, at Lake Moszne (51°26'57.4"N, 23°07'34.0"E) and Lake Długie (51°27'04.0"N 23°09'39.9"E), situated in the Polesie National Park near Lublin (SE Poland). The insects were sifted from the nest material of *Formica polyctena*. Live beetles of *A. talpa* and *O. haemorrhoa* were placed in transparent plastic containers (diameter 10 cm, height 4 cm) filled with nest substrate and observed in the laboratory from May 9 to June 24, and from May 11 to June 21, respectively, at room temperature (22–25 °C). Adults and larvae of various species of ants, including *F. rufa* and small springtails, were supplied as a source of adult food.

Study techniques

Larvae of both species were killed in boiling water and preserved in ethanol (75%).

To prepare temporary microscope slides, some larvae were macerated in cold 10% KOH for two to three hours, immersed in lactic acid for subsequent preparation and mounting of antennae, mouthparts, sensory structures, chaetotaxy of the body, legs and urogomphi. They were then traced from photos taken with an Olympus DP72 or Olympus DP21 digital camera mounted on a binocular Olympus SZX16 or Olympus BX63 compound microscope (Figs 9, 13, 14, 17, 18, 22–46, 48, 49, 52, 53, 53a, 54, 54a, 55, 56). The final image adjustments were made using CorelDraw Graphics Suite 2018.

Habitus illustrations of larvae, structure of setae, chaetotaxy of head, functional position of mouthparts, structural details of antennae, microstructure, spiracles and various details of their external structure were recorded using SEM, type VEGA3 TESCAN (Figs 1–8, 10–12, 15, 16, 19–21, 42a, b, 43a, 44a, 47, 47a, 50, 51, 53b, 54b). For the SEM work, larval specimens taken from alcohol were briefly dried and placed directly in the SEM chamber for observation.

Table 1. Measurements of larval instars of *Amidobia talpa* and *Oxypoda haemorrhoa*. Symbols and abbreviations: larval instars, A – average, N – number of specimens examined, M – measurement, R – range, SV – standard variation.

Species (larval instars/N)	M	R	A	SV
Amidobia talpa (all larval instars/28)	Body length	1.00–2.55	1.99	0.45
	Thorax length	0.38–0.69	0.55	0.09
	Head width	0.16–0.23	0.20	0.02
	Prothorax length	0.15–0.30	0.21	0.40
	Prothorax width	0.18–0.27	0.23	0.03
Oxypoda haemorrhoa (all larval instars/17)	Body length	2.46–3.32	2.79	0.37
	Thorax length	0.40–0.80	0.68	0.09
	Head width	0.22–0.27	0.25	0.02
	Prothorax length	0.19–0.33	0.27	0.03
	Prothorax width	0.23–0.32	0.28	0.02

Measurements and their abbreviations

Measurements of the larvae of both species, made using an Olympus BX63 compound microscope in cellSens Dimension v1.9 software, are given in millimetres, as explained in detail in Pietrykowska-Tudruj and Staniec (2012). Measurements (Table 1) were made on freshly killed specimens. The terms of morphological structures, chaetotaxy (selected aspects only) and their abbreviations generally follow Ashe and Watrous (1984) and Staniec et al. (2018), with modifications in some of the figures. The material examined for the measurements is listed in Table 1. The material examined for morphological descriptions includes four or five specimens of the late-instar larva of each species. The voucher specimens are deposited in the collections of the Department of Zoology, Maria Curie Skłodowska University, Lublin.

Results

Generic diagnosis of the late larval instar of *Amidobia* and *Oxypoda*

The combination of characteristics distinguishing mature larvae of *Amidobia* and *Oxypoda* from known larvae of other genera within the subfamily Aleocharinae are as follows (Paulian 1941, Pototskaya 1967, Topp 1975, Ashe 1981, Ashe and Watrous 1984, Ashe 1985, Ahn 1997, Jeon and Ahn 2009, Staniec et al. 2009, 2010, 2016, 2018, Zagaja et al. 2014, the present study): *Amidobia* – (1) body extremely slim; (2) head wider (1.1 ×) than pronotum; (3) sensory appendage of antennal segment II longer (1.1 ×) than antennal article III; (4) labrum rectangular; (5) anterior margin of labrum shallowly excised in the centre; (6) seta Ld2 of labium spiniform; (7) epipharynx with about 20 long cuticular processes and 2 pores centrally; (8) mandible stocky with 2 preapical teeth; (9) mala wide, distinctly dilated anteriorly; (10) adoral margin of mala with 10–12 teeth

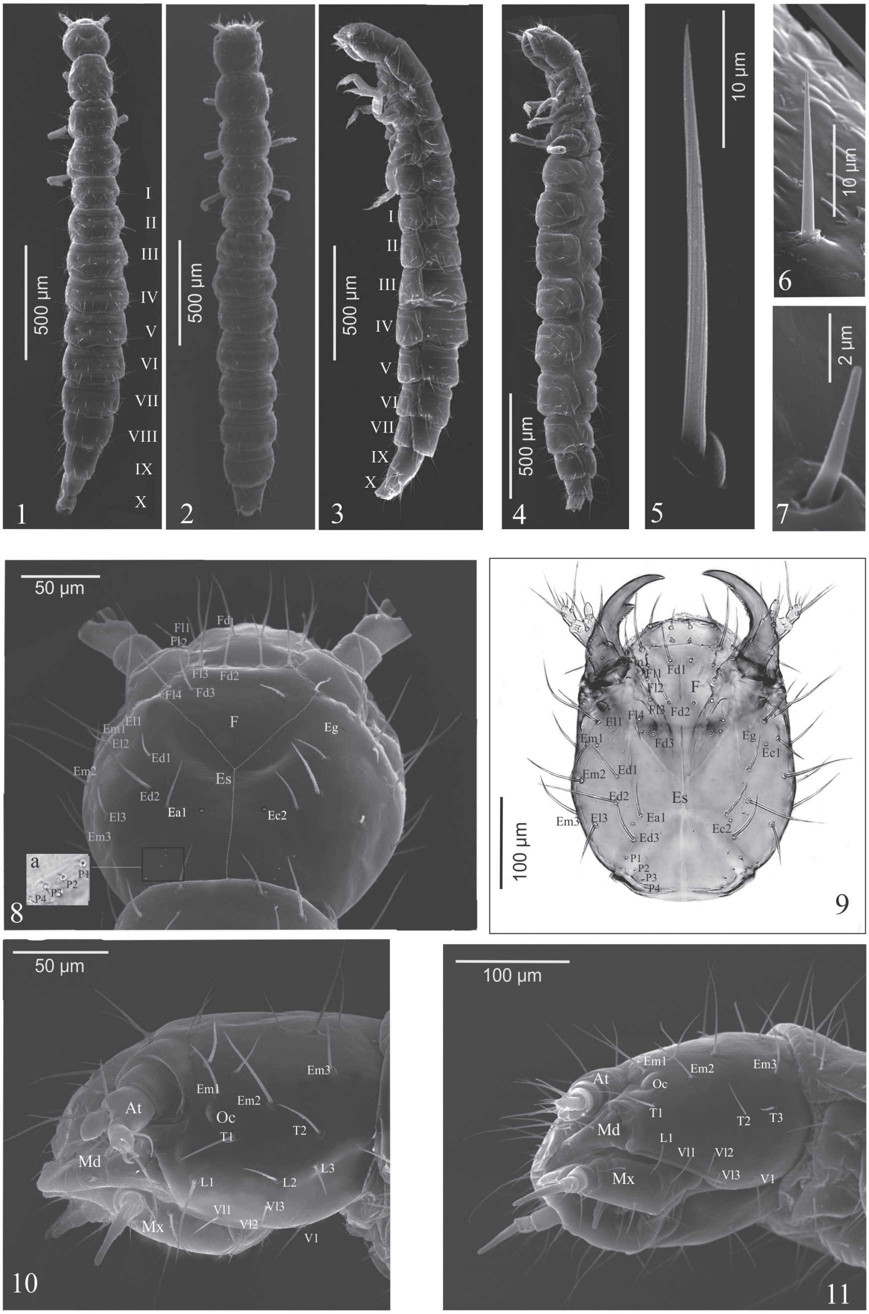
(4–5 small distally); (11) dorsal side of mala with about 25 cuticular processes; (12) ligula dome-like, as long as wide; (13) spiracle with unique perforation; *Oxypoda* – (1) head sides weakly rounded; (2) labrum semi-circular; (3) seta Ld2 of labium very short, verrucous; (4) epipharynx with about 100–150 short cuticular processes and 10 centrally; (5) mandible slender with 1 preapical tooth; (6) interior edge of apical and preapical tooth serrate; (7) adoral margin of mala with 11–15 teeth including 1 biggest proximally and a few small distally; (8) mala slender, narrowed anteriorly; (9) dorsal side of mala with about 15 cuticular processes; (10) ligula finger-like, almost twice as long as wide.

Comparative morphological description of the late-instar larvae of *Amidobia talpa* (*A.p.*) and *Oxypoda haemorrhoa* (*O.h.*)

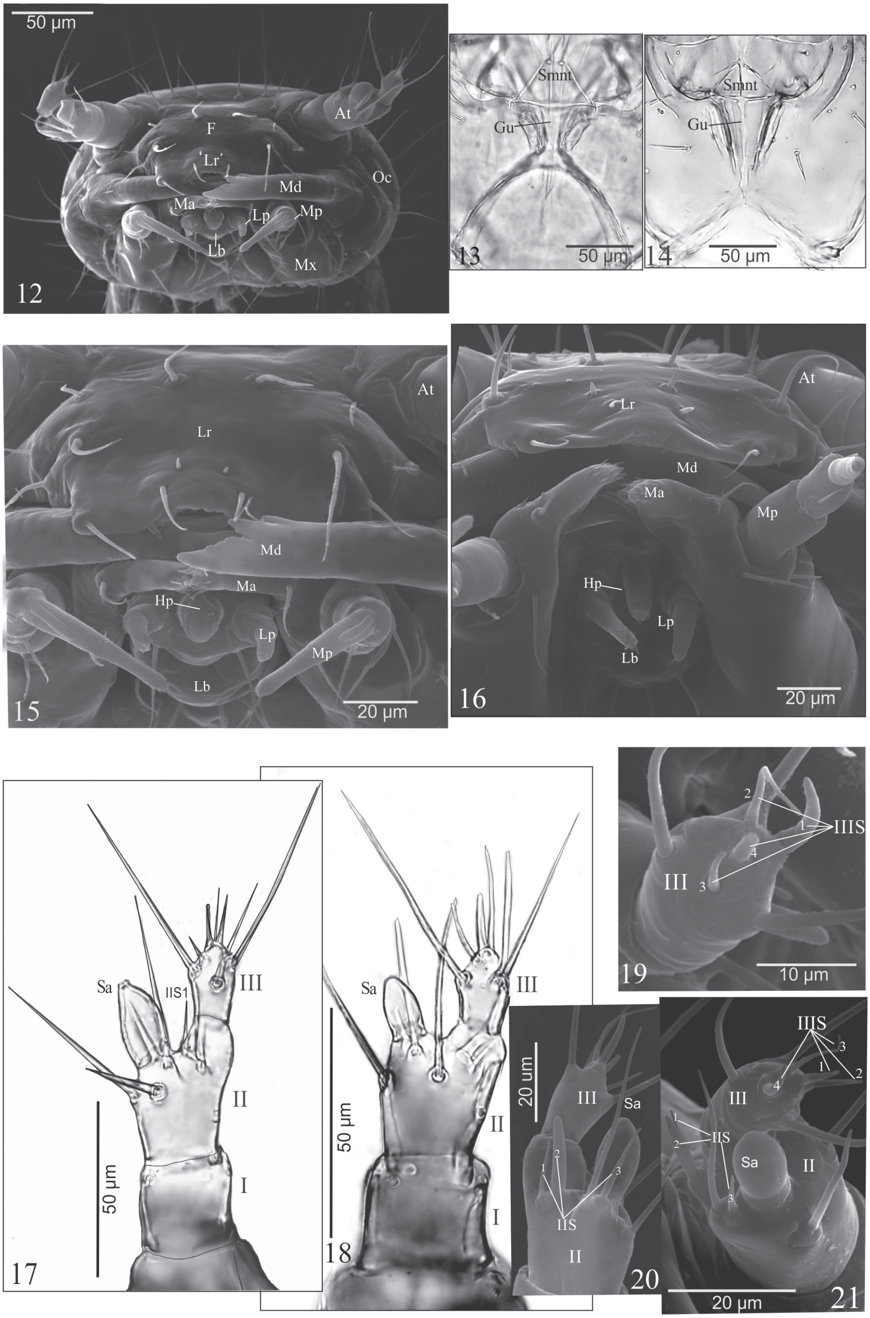
Body narrow, elongate, semi-cylindrical, segments IX and X distinctly narrower than the others; *A.p.* (Figs 1, 3) – head distinctly wider than prothorax and distinctly narrower than metathorax, abdomen gradually widening to segments V or VI, then tapering to terminal segment of body; *O.h.* (Figs 2, 4) – head distinctly narrower than pro- and metathorax, abdominal segments III-VII of more or less equal width. Colour: *A.p.* and *O.h.* – head poorly sclerotized, stemmata and mandible distinctly darker; thorax, legs and abdomen whitish, tergites somewhat darker, slightly and gradually darkening to terminal segments. Setae of different length, light brown, setose with longitudinal grooves (Figs 5–7).

Head. Shape: *A.p.* (Fig. 8) – distinctly (1.2 ×) wider than long, widest at level of setae El3, lateral margins distinctly rounded; *O.h.* (Fig. 9) – slightly (1.1 ×) longer than wide, widest at level of setae Em2, lateral margins moderately rounded. Chaetotaxy of dorsal side with 40 setae in *A.p.* – 14 frontal [2(Fd1–3, Fl1–4)], 18 epicranial [2(Ea1, Ed1–2, Ell-3, Em1–3)], 8 posterior micro setae (2P1–4), a pair of epicranial campaniform sensillae (Ec2) and epicranial glands (Eg) (Fig. 8); 42 in *O.h.* – 16 frontal [2(Fd1–3, Fm1, Fl1–4)], 18 epicranial [2(Ea1, Ed1–3, Ell, Fl3, Em1–3)], 8 posterior micro setae (P1–4), 2 pairs of epicranial campaniform sensillae (Ec1–2) and epicranial glands (Eg) (Fig. 9). Lateral margin with 10 setae [2(T1–2, L1–3)] in *A.p.* (Fig. 10); 8 setae [2(T1–3, L1)] in *O.h.* (Fig. 11). *A.p.* and *O.h.* – dorsal ecdysial lines (Es) bifurcate at about half the head length (Figs 8, 9); each side of head with one small, oval, weakly convex ocellus (Oc) (Figs 10, 11). Functional position of antennae (At), labrum (Lr), mandibles (Md), maxillae (Mx) with maxillary palps (Mp), mala (Ma), hypopharynx (Hp) and labium (Lb) with labial palps (Lp) as in Figs 12, 15 – *A.p.* and Fig. 16 – *O.h.* Gula (Gu) triangular: short, length to width ratio = 1 : 1.4 – *A.p.* (Fig. 13); elongate, length to width ratio = 1.5 : 1 – *O.h.* (Fig. 14).

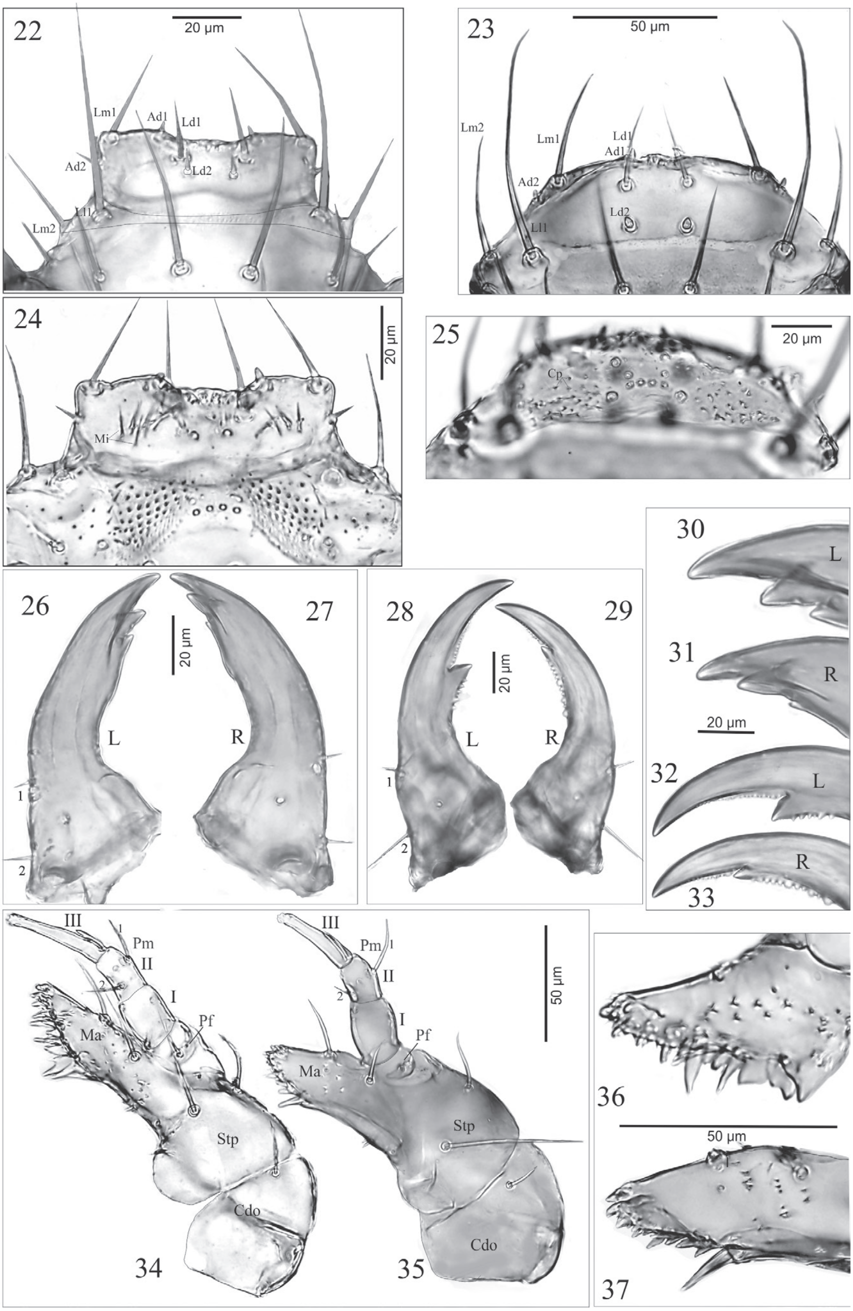
Antenna (Figs 17–21): three-articled, length ratio of articles I–III: 1.5 : 1.0 : 2.0 – *A.p.* or 1.2 : 1.0 : 1.6 – *O.h.* Article I: as long as wide – *A.p.* or 1.2 × wider than long – *O.h.* with 4 pores; article II 1.5 × as long as wide – *A.p.*, *O.h.*, with 3 macro setae, one acorn-shaped – *A.p.* or semi-spherical – *O.h.*, sensory appendage (Sa), 1.6 – *A.p.* or 1.9 – *O.h.* × as long as wide (Figs 17, 18), and 3 solenidia ventrally of different size (IIS1–3) – *A.p*, *O.h.* (Figs 20, 21); Sa 1.2 × longer – *A.p.* or 1.3 × shorter – *O.h.* than



Figures 1–11. *A. talpa* (**1, 3, 5, 8, 8a, 10**), *O. haemorrhoa* (**2, 4, 6, 7, 9, 11**), mature larva. **1–4** habitus in dorsal (**1, 2**) and lateral (**3, 4**) aspect **5–7** abdominal setae **8–11** head in dorsal (**8, 9**) and lateral (**10, 11**) aspect. Abbreviations: I-X, abdominal segments; At, antenna; Ec, epicranial campaniform sensilla; Ed, epicranial dorsal setae; Eg, epicranial gland; El, epicranial lateral setae; Em, epicranial marginal setae; Es, epicranial suture; F, frons; Fd, frontal dorsal setae; Fl, frontal lateral setae; L, lateral setae; P, posterior setae; T, temporal setae; V, ventral setae; Vl, ventral lateral setae.



Figures 12–21. *A. talpa* (**12, 13, 15, 17, 19, 20**), *O. haemorrhoa* (**14, 16, 18, 21**), mature larva. **12** head in fronatl aspect **13, 14** gular region **15, 16** functional position of mouthparts in frontal aspect **17–21** right antenna in dorsal aspect (**17, 18**), entire article III in anterior (**19**) and in ventral (**20**) aspect, entire article II and III in anterior aspect (**21**). Abbreviations: I-III, antennal articles; IIS, IIIS, solenidia of antennal article II or III; At, antenna; F, frons; Gu, gula; Hp, hypopharynx; Lp, labial palp; Lr, labrum; Ma, mala; Md, mandible; Mx, maxilla; Pm, maxillary palp; Oc, ocellus; Sa, sensory appendage; Smnt, submentum.



Figures 22–37. *A. talpa* (**22, 24, 26, 27, 30, 31, 34, 36**), *O. haemorrhoa* (**23, 25, 28, 29, 32, 33, 35, 37**), mature larva **22, 23** labrum **24, 25** epipharynx **26–29** left (L) and right (R) mandible, in dorsal aspect; **30–33** anterior region of left (L) and right (R) mandible, in dorsal aspect **34, 35** right maxilla in ventral aspect **36, 37** right mala in ventral aspect. Abbreviations: I-III, articles of maxillary palp; 1, 2, mandibular setae; Ad, additional setae; Cp, cuticular processes; Cdo, cardo; La, labral anterior setae; Ld, labral dorsal setae; Lm, labral marginal setae; Mi, microtrichia; Ma, mala; Pf, palpifer; Pm, maxillary palp; Stp, stipes.

article III; article III 1.5 – *A.p.* or 1.6 – *O.h.* × as long as wide, with 3 macro setae and 4 solenidia apically (IIIS1–4) of different length and diameter (Figs 19, 21).

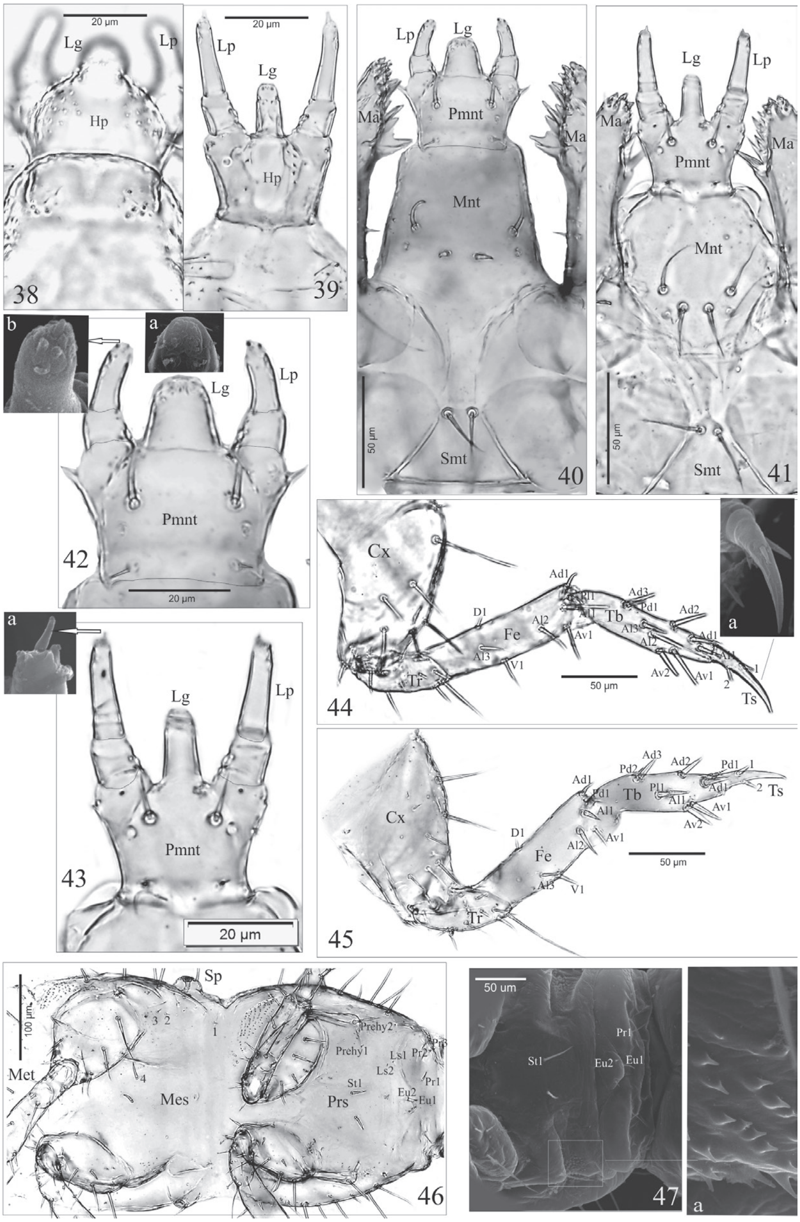
Labrum (Figs 22, 23): posterior portion rectangular – *A.p.* (Fig. 22) or almost semi-circular – *O.h.* (Fig. 23) in outline, central region of anterior margin slightly excised – *A.p.* or rounded – *O.h.*; in *A.p.* length ratio of excised region and whole anterior margin 1:2.6; with 8 macro setae [2(Ld1, Lm1, Ll1, Lm2)], 2 micro setae (Ld2), spine-shaped centrally – *A.p.* or verrucous posteriorly – *O.h.*, and 4 additional short setae on anterior (Ad1) and lateral (Ad2) margin; separated from clypeal region by membranous area – *A.p*, *O.h*.

Epipharynx (adoral surface of labrum) membranous (Figs 24, 25), with two groups of a few microtrichia (Mi) each pointing towards anterior margin of labrum and a pair of pores centrally – *A.p.* (Fig. 24) or with sharp cuticular processes (Cp) directed towards central portion and 10 pores – *O.h.* (Fig. 25).

Mandibles (Figs 26–33): stocky, moderately bent, strongly widened basally – *A.p.* or slender, strongly bent, moderately widened basally – *O.h.*, with 2 micro setae (coded: 1 – upper, 2 – lower) on outer margin almost of equal length – *A.p.* (Figs 26, 27) or seta 2 distinctly longer than 1 – *O.h.* (Figs 28, 29), and 2 pores; incisor lobe with one large subapical tooth and two such teeth of different size, the upper one larger than the lower one – *A.p* (Figs 26, 27) or incisor lobe with 1 large and 1 smaller subapical tooth – *O.h.* (Figs 28, 29); differences between left (L) and right (R) mandibles in *A.p* and *O.h.* as in Figs 30–33.

Maxilla (Mx) (Figs 34–37): consisting of triangular – *A.p.* (Fig. 34) or tetragonal – *O.h.* (Fig. 35) cardo (Cd) divided by sclerotized ridge into two unequal parts, wide stipes (Stp), obliquely truncate mala (Ma) distinctly widened – *A.p.* (Fig. 36) or slightly narrowed – *O.h.* (Fig. 37) at adoral margin, palpifer (Pf) and three-articled maxillary palp (Pm); cardo with one ventral seta; stipes with two setae; palpifer with one seta; mala fused with stipes, with two setae, one pore and approx. 25 – *A.p.* or about 15 – *O.h.* triangular cuticular processes ventrally; adoral margin of mala (functional positions in Figs 15, 16) with sparse group of a few micro teeth apically and ctenidium of 6 macro teeth, different sizes and shape proximally – *A.p.* (Fig. 36) or about 10 small teeth of equal length and 1 long tooth spinose proximally – *O.h.* (Fig. 37). Maxillary palp (Pm) (Figs 34, 35): length ratio of articles I–III: 1.1 : 1.0 : 2.0 – *A.p.* or 1.5 : 1.0 : 2 – *O.h.*; article I slightly wider than second, 1.3 – *A.p.* or 1.4 –*O.h.*× as long as wide with two pores; article II 1.5 – *A.p.* or 1.3 – *O.h.*× as long as wide with two setae (coded: 1, 2) equal in length – *A.p.* (Fig. 34) or unequal in length – *O.h.* (Fig. 35); article III narrower than I and II, tapering slightly to apex, 5.2 × – *A.p.* or 3.8 × – *O.h.* as long as wide, with one digitiform sensory appendage basally 0.4 × as long as article, one pore and a few tiny sensory apically.

Hypopharynx (Hp) (Figs 38, 39): membranous, surface (except central area) with approx. 25 – *A.p.* (Fig. 38) or a few tiny microtrichiae – *O.h.* (Fig. 39). Ligula (Lg): dome-like, gradually tapering to the top, as long as wide at base, separated from prementum by transverse line – *A.p.* (Figs 40, 42) or finger-like, tapering slightly to top, 1.9 × as long as wide, fused with prementum (Figs 41, 43); surface of apex with microsculpture resembling dermato-glyphics – *A.p.*, *O.h.* (Fig. 42a). Prementum (Pmnt) trapeziform, 1.3 × – *A.p.* or 1.1 × – *O.h.* as wide at base as long, with 4 setae (2 long,



Figures 38–47. *A. talpa* (**38, 40, 42, 42a, b, 44, 44a**), *O. haemorrhoa* (**39, 41, 43, 43a, 45, 46, 47, 47a**), mature larva. **38, 39** hypopharynx **40, 41** labium **42, 43** prementum with labial palp, apex of ligula (**42a**) and labial palp (**42b, 43a**), **44, 45** legs and tarsungulus (**44a**), **46** pre- and mesosternum **47** anterior portion of presternum and microstructure (**47a**). Abbreviations: 1, 2, 3, 4, setae of mesosternum; 1, 2, setae of tarsungulus; Ad, anterodorsal setae; Al, anterolateral setae; Av, anteroventral setae; Cx, coxa; D, dorsal setae; Eu, eusternum; Fe, femur; Hp, hypopharynx; Lg, ligula; Lp, labial palp; Ls, laterosternum; Ma, mala; Mes, mesosternum; Mnt, mentum; Pd, posterodorsal setae; Pl, posterolateral; Pmnt, prementum; Pr, prosternum; Prehy, prehypopleuron; Prs, presternum; Smt, submentum; Sp, spiracle; St, sternellum; Tb, tibia; Tr, trochanter; Ts, tarsungulus; V, ventral setae.

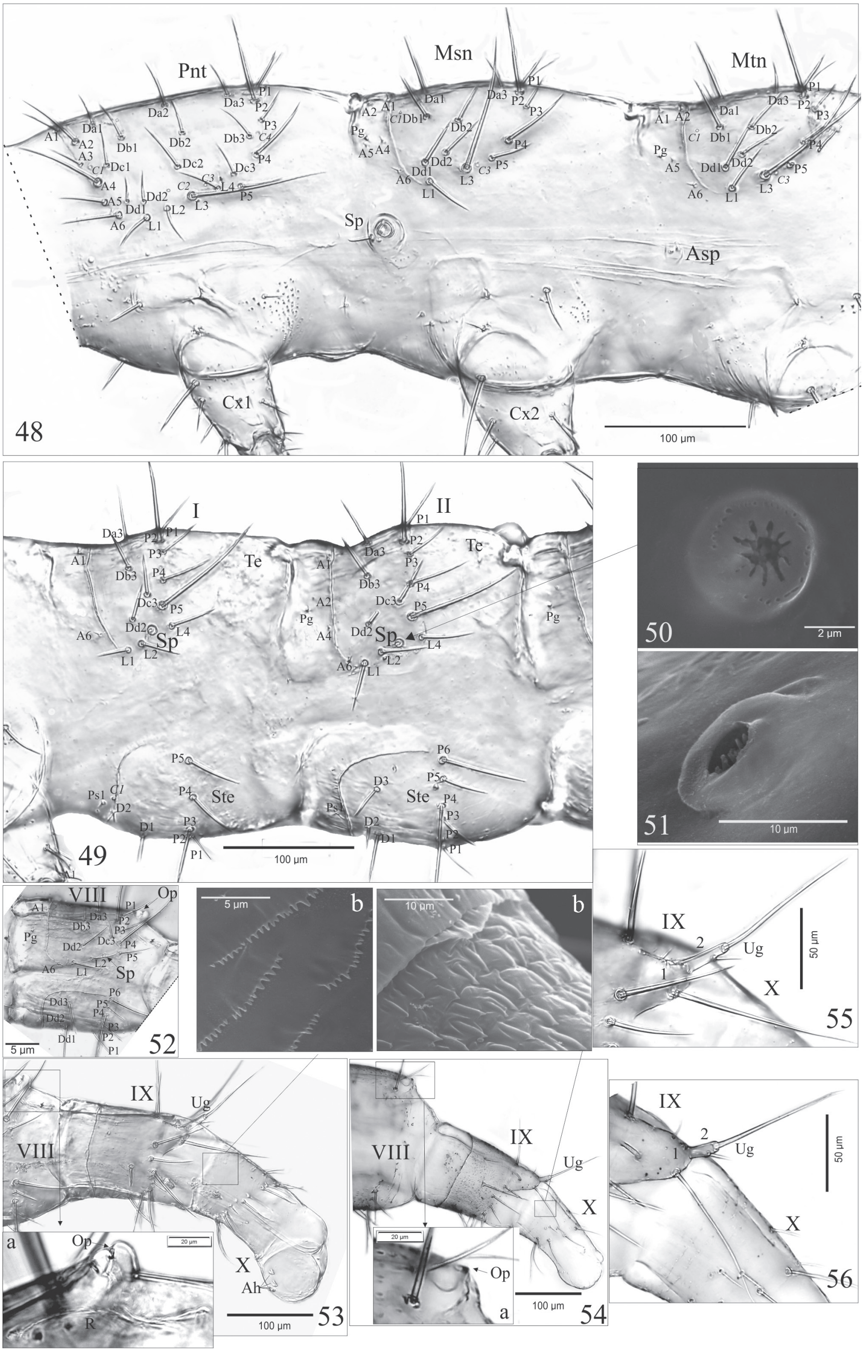
2 short) and a pair of pores (Figs 42, 43). Labial palp (Pl) two-articled, length ratio of articles I and II: 1 : 2.4 – *A.p.* or 1 : 2.1 – *O.h.,* article I 1.4 × as wide as long – *A.p.* or as long as wide – *O.h.*, article II 2.9 × – *A.p.* or 3.1 × – *O.h.* as long as wide, with a few sensory appendages apically, almost equal in length – *A.p.* (Fig. 42b) or one sensory appendage distinctly longer than the others – *O.h.* (Fig. 43a).

Thorax. Foreleg (Figs 44, 45): consists of stocky coxa (Cx), short trochanter (Tr), elongate – *A.p.* (Fig. 44) or moderately elongate – *O.h.* (Fig. 45) femur (Fe), slim – *A.p.* or quite stocky – *O.h.* tibia (Tb) and tarsungulus (Ts) slightly curving inwards (Fig. 44a); Cx, Tr, Fe, Tb and Ts: 1.8, 2,3, 3.6, 4.3 and 5.6 × – *A.p.* or 1.1, 2.1, 2.9, 3.0 and 3.5 × – *O.h.* as long as wide respectively; Fe, Tb and Ts with 8 (Ad1, Al1–3, Av1, D1, V1, Pl1 – *A.p.*, *O.h.*), 9 (Ad1–3, Al1–3, Av1–2, Pd1 – *A.p.* or Ad1–3, Al1, Av1–2, Pd1–2, Pl1 – *O.h.*) and 2 (1, 2) setae respectively. Length ratio of Fe, Tb and Ts: 1.8 : 1.7 : 1.0 – *A.p.* or 2.4 : 2.0 : 1.0 – *O.h.*

Pro- (Prs), meso- (Mes) and metasternum (Met) membranous (Figs 46, 47): Prs with 20 setae [2(Eu1–2, Ls1–2, Pr1–3, Prehy1–2, St1)] and microstructure on sides (Fig. 47a), Mes and Met each with 8 setae (coded: 1–4). Prothorax (Pnt) 1.4–1.5 × as long as mesothorax (Msn), mesothorax and metathorax (Mtn) almost equal in length (Fig. 48): Pnt with 50 setae [2(A1–6, Da1–3, Db1–3, Dc1–3, Dd1–2, L1–4, P1–4)] and 8 pores (2[C1–4]); Msn with 36 setae [2(A1–2, A4–6, Da1, Da3, Db1–2, Dd1–2, L1, L3, P1–5)], 4 pores and 1 pair of paratergal glands (Pg), [(2C1, C3)]; chaetotaxy of metanotum identical with that of mesonotum; lateral area between pro- and mesothorax with a pair of large, functional spiracles (Sp), and between meso- and metathorax with a pair of atrophied spiracles (Asp) and one micro seta (Fig. 48).

Abdomen. Chaetotaxy of tergites: I with 28 setae [2(A1, A6, Da3, Db3, Dc3, Dd2, L1–2, L4, P1–5)], II-VII with 32 setae [2(A1, A2, A4, A6, Da3, Db3, Dc3, Dd2, L1–2, L4, P1–5)] and 1 pair of paratergal glands (Pg) (Fig. 49); VIII with 30 setae [2(A1, A6, Da2–3, Db2, Dc2–3, L1, L3–4, P1–5)], 2 pores (C5) and a pair of glands (Pg) (Fig. 52). Tergal gland reservoir (R) clearly developed with split opening (Op) at the posterior margin of abdominal tergite VIII; Op with unique structure for *A.p.* (Figs 53, 53a) and for *O.h.* (Figs 54, 54a). Chaetotaxy of sternites: I (Fig. 49) with 16 setae (2[D1–2, Ps1, P1–5]); II–VIII (Figs 49, 52) with 20 setae (2[D1–3, Ps1, P1–6]). Abdominal tergites I-VIII each with a pair of functional spiracles laterally (Sp) (Figs 49, 52) of unique structure for *A.p.* (Fig. 50) and for *O.h.* (Fig. 51). Segments IX and X distinctly narrower than the others with tergites and sternites fused in uniform ring (Fig. 53); segment IX with 24 setae (6 micro) (Fig. 53). Segment X with 14 setae and 4 anal hooks terminally (Ah) (Fig. 53) and unique microstructure for *A.p.* (Fig. 53b) and for *O.h.* (Fig. 54b). Urogomphi (Ug) of segment IX (Figs 53–56): two-articled, article I fused to tergum IX; article I (coded: 1) wide and turgid with 4 setae (3 macro); article II (coded: 2) slender, finger-shaped, moderately elongate and tapering apically, 2.8 × as long as wide at the base – *A.p.* (Figs 53, 55) or truncated, with sides almost parallel, 2.5 × as long as wide at the base – *O.h.* (Figs 54, 56), with 1 short seta subapically, 1 macro seta apically and a pore basally; length ratio of Ug and apical seta: 1 : 1.4 – *A.p.* or 1 : 1.3 – *O.h.*; length ratio of urogomphus (without apical seta) and segment X (pygopod): 1 : 1.5 – *A.p.* or 1 : 1.4 – *O.h.*

Table 1 lists some differences in larval measurements (particular instars are not distinguished) between *A. talpa* and *O. haemorrhoa*.



Figures 48–56. *A. talpa* (**48, 49, 50, 52, 53, 53a, b, 55**), *O. haemorrhoa* (**51, 54, 54a, b, 56**), mature larva. Thoracal (**48**) and abdominal segment I–II (**49**), VIII (**52**), VIII–X (**53, 54**) and urogomphus (**55, 56**) in lateral aspect. Abbreviations: 1, 2, article of urogomphi; VII – X, abdominal segments; A, anterior setae; Ah, anal hooks; Asp, atrophied spiracles; C, campaniform sensilla; Cx, coxa; D, Da-c, discal setae; L, lateral setae; Msn, mesonotum; Mtn, metanotum; Op, opening of gland reservoir; P, posterior setae; Pg, pretergal gland; Pnt, pronotum; Ps, presternal sensilla; R, gland reservoir; Sp, spiracle; Ste, sternite; Te, tergite; Ug, urogomphus.

Table 2. Similarities and differences in external morphology between known late larval instars of some myrmecophilous and non-myrmecophilous Aleocharinae species. Abbreviations: A–article; a–anterior; arrg.–arrangement; As–apical seta of urogomphus; *ang.–angulata;* ap.–apical; c–central; cent. reg.–central region; cut. proc.–cuticular processes; Fe–femur; *haem.–haemorrhoa*; L–left mandible; l–long; Lr–length ratio; LWr–length to width ratio; Ma–mala; mod.–moderately; mic.–micro; mod.–moderately; Msn–mesonotum; Ns–number of setae; p–posterior; Pm–maxillary palp; Pnt–pronotum; preap.–preapical; poor.–poorly; *pub.–pubicollis* R–right mandible; S–segment; s–short; Sa–sensory appendage of antennal article II; Sas–subapical setae of urogomphus; St.–sternite; strong.–strongly; Tb–tibia; Ts–tarsungulus; Ug–urogomphus; w.–without; weak.–weakly; Wr–width ratio; ?–lack of data; *–body measurements of all known larval instars. Data based on Zagaja et al. (2014, 2017), Staniec et al. (2009, 2017, 2018), and the present study.

Ecological preferences	**Myrmecophilous species**					**Non-myrmecophilous species**
Host	*Lasius fuliginosus*	Formica rufa group				–
Tribe of Aleocharinae	Lomechusini		Oxypodini		Athetini	
Species	*Pella laticollis*	*Lomechusa pub.*	*Thiasophila ang.*	*Oxypoda haem.*	*Amidobia talpa*	*Dinaraea aequata*
level of integration with host	preadaptation to integration	peak of integration (symphile)	preadaptation to integration	non-integrated? (synoics)		–
Character						
Body length	4.30–4.80	4.99–6.70	2.72–4.40	2.46–3.32*	1.00–2.55*	3.01–3.78
Body shape	moderately elongate	dumpy	elongate	elongate	elongate	elongate
Cuticle	mod. sclerotized	membranous	mod. sclerotized	poor. sclerotized	poor. sclerotized	mod. sclerotized
Setae: structure	setose	blunt, jagged distally	setose	setose	setose	setose
Head						
Width	0.57–0.63	0.87–0.97	0.41–0.48	0.22–0.27*	0.16–0.23*	0.42–0.45
LWr	1:1	1:1.4	1:1	1.1:1	1:1.2	1:1
Ocelli	present	absent	present	present	present	present
Ns: dorsal side	40	70	42	40	40	40
Sides	distinctly rounded	distinctly rounded	distinctly rounded	weakly rounded	distinctly rounded	distinctly rounded
Wr of head and Pnt	1:1.3	1:1.5	1:1.3	1:1.1	1.1:1	1:1.1
Antenna						
Lr of AI-III	1.4:2.3:1	1.2:1.7:1	1.5:1.9:1	1.3 : 1.6 : 1	1.3:1.9:1	1:1.7:1
LWr of AI, AII, AIII	1.2:1/2.4:1/2.2:1	1:2.8/1:1.1/1:1.6	1.2:1/1.4:1/2:1	1:1.2/1.5:1/1.6:1	1:1/1.5:1/1.5:1	1:1/1.8:1/1.4:1
LWr of Sa	1.5:1	1.4:1	2.1:1	1.9:1	1.6:1	1.8:1
Lr Sa and AIII	1:1.6	1.1:1	1:1.1	1:1.3	1.1:1	1:1

Ecological preferences	**Myrmecophilous species**					**Non-myrmecophilous species**
Host	*Lasius fuliginosus*	Formica rufa group				–
Tribe of Aleocharinae	Lomechusini		Oxypodini		Athetini	
Species	*Pella laticollis*	*Lomechusa pub.*	*Thiasophila ang.*	*Oxypoda haem.*	*Amidobia talpa*	*Dinaraea aequata*
level of integration with host	preadaptation to integration	peak of integration (symphile)	preadaptation to integration	non-integrated? (synoics)		–
Labrum						
Shape	trapeziform	trapeziform	semi-circular	semi-circular	rectangular	trapeziform
Anterior margin	almost straight	slightly rounded	distinctly rounded	distinctly rounded	excised in the centre	cent. reg. protruding, crenate
Seta Ld2: structure	mic. setose	macro, setose	macro, peg shaped	mic. verrucous	mic. spiniform	mic. spiniform
Labium and clypeus	separated	fused	separated	separated	separated	separated
Epipharynx						
Cuticular processes: No.	well above 200	well above 200	150–200	100–150	about 20	50–80
Cut. proc.: length	short	short	short	short	long	short
Cut. proc. in central area	absent	present	absent	absent	absent	present
No. of pores of a/c/p	2/8 (in 1 row)/ 4	?	1–2/9/4	2/6 (in 2 rows)/2	0/2/0	2/4/4
Mandible						
Shape	slender, bent weakly	slender, strong, bent	slender, weak, bent	slender, strong, bent	stocky, mod., bent	slender, weak, bent
Interior edge ap. tooth	slightly undulating	smooth	smooth	serrate	smooth	smooth
Edge below preap. tooth	slightly undulating	smooth	smooth	serrate	smooth	serrate
No. of preap. teeth	1-R and 1-L	0	1-R and1-L	R-1 and L-1	R-2 and L-2	4-R, 5-L
Number/length of setae	2/almost equal	3/almost equal	2/almost equal	2/different	2/almost equal	2/equal
Maxilla						
Mala: ant. marg. with:	27–31 equal teeth	8 equal setae	20 teeth (11 small)	11 teeth (1 big)	12 teeth (4 small)	23 teeth (15 small)
Mala: shape	wide, equilateral, sclerotized	lobar, membranous	slender, slightly dilated anteriorly	slender, slightly narrowed anteriorly	wide, distinctly dilated anteriorly	slender, distinctly dilated anteriorly
Ma: No. cut. proc./arrg.	about 80/singly	numerous/in rows	55–60/singly	15/singly	25/ singly	40/singly
Stipes and mala	fused	separated	separated	fused	fused	separated
Pm: Lr A I-III	1.7:1:2.3	1.4:1:2.0	1.6:1:2	1.5:1.0:2.0	1.1:1.0:2.0	1.6:1:2.2
Pm: LWr of AI-III	2.2:1/1.4:1/5.2:1	1:1.8/1:1.9/2.4:1	1.5:1/1.3:1/4.7:1	1.4:1/1.3:1/3.8:1	1.3:1/1.5:1/5.2:1	1.8:1/1.5:1/6.8:1
Lr: Pm and Ma	1.5:1	1:1.2	1.1:1	1.1:1	1.2:1	1.2:1

Ecological preferences	**Myrmecophilous species**					**Non-myrmecophilous species**
Host	*Lasius fuliginosus*	Formica rufa group				–
Tribe of Aleocharinae	Lomechusini		Oxypodini		Athetini	
Species	*Pella laticollis*	*Lomechusa pub.*	*Thiasophila ang.*	*Oxypoda haem.*	*Amidobia talpa*	*Dinaraea aequata*
level of integration with host	preadaptation to integration	peak of integration (symphile)	preadaptation to integration	non-integrated? (synoics)		–
Labium						
Lg: shape	transverse, short,	transverse, very short	finger-like, 1.8 × as long as wide	finger-like, 1.9 × as long as wide	domelike, as long as wide	finger-like, 2.5 × as long as wide
Lg/anterior margin	sinuate	rounded	truncated	truncated	truncated	truncated
Lr: Lg and Lp	1:2.2	1:3.9	1:1.9	1:2	1:1.5	1:1
Lg and Pmnt	fused	fused	separated	fused	separated	separated
Pl: Lr A I and II	1:1.3	1:1	1:1.6	1:2.1	1:2.4	1:2.1
Pl: LWr of AI/AII	1.1:1/2.4:1	1:1/1.6:1	2:1/3.2:1	1:1/3.1:1	1:1.4/2.9:1	1.1:1/3.5:1
Thorax						
Ns: Pnt, Msn	52, 40	110, 80	52, 38	50. 36	50, 36	50, 38
Lr: Fe, Tb, Ts	2.3:2.2:1	2.2:1.9:1	2.1:2.2:1	2.4:2.0:1.0	1.8:1.7:1.0	1.9:2.2:1
LWr: Fe, Tb, Ts	4.4:1/4.5:1/5.2:1	2.2:1/2.3:1/3.0:1	3.6:1/5.2:1/5.2:1	2.9:1/ 3.0:1/3.5:1	2..6:1/4.3:1/5.6:1	3.0:1/5.3:1/7.1:1
Ns: Fe, Tb, Ts	8, 9, 2	30–34, 22–25, 2	8, 9, 2	8, 9, 2	8, 9, 2	7, 9,2
Abdomen						
Ns: Tergite I, II-VII	30, 30 each	70, 80 each	30, 32 each	28, 32	28, 32	32 each
Ns: St. I, each II-VIII	16, 20	100, 110	14, 20 each	16, 20	16, 20	16, 20 each
Urogomphi	present	absent	present	present	present	present
Ug: Lr AI, AII, Sap	1:1.1:1.2	–	1:2.2:2.6	2.4:1.0:4.8	1.4:1.0:3.1	1:1.5:1.7
LWr AII	2.7:1	–	3.7:1	2.5:1	2.8:1	4.1:1
Lr Ug (w. As) to S X	1:1.7	–	1:1	1:1.4	1:1.5	1:1.5

Discussion and summary

This paper gives a detailed description of the external structure of the hitherto unknown larval stage of *Amidobia talpa* and *Oxypoda haemorrhoa* – Palearctic, myrmecophilous staphylinids belonging to the subfamily Aleocharinae – which are associated with the *Formica rufa* species group. It also gives the first description of the larva of *Amidobia*, and at present, the only complete, detailed account of the larval morphology of *Oxypoda*. The existing fragmentary descriptions of *Oxypoda* larvae, with only a few schematic drawings relating to just two species – *O. spectabilis* and *O. longipes* – were written 40–50 years ago (Pototskaya 1967, Topp 1978); in the context of contemporary comparative studies they are therefore practically useless. The diagnostic features presented above – 13 for *Amidobia* and 10 for *Oxypoda* – are based mainly on this description of the late larval instars of *A. talpa* and *O. haemorrhoa*. They mainly involve the structural details of the mouthparts, and in the case of the former taxon, of the antennae and spiracles as well. These features were established with reference to well-known larvae of other aleocharine species of diverse ecological preferences (Ashe and Watrous 1984, Staniec et al. 2009, 2010, 2017, 2018, Zagaja et al. 2014), among which anthill symbionts, represented by the two titular staphylinids, are deserving of particular scrutiny.

Relationships between myrmecophiles and their hosts exhibit varying degrees of advancement (Wasmann 1894, Hölldobler 1971, Hölldobler and Wilson 1990, Stoeffler et al. 2011, Staniec et al. 2017, Zagaja et al. 2017), which may be correlated with certain morphological adaptations in actively living developmental forms, including larvae. The data listed in Table 2 summarize the current state of our studies, which focus on the external structure of the larval stages of European myrmecophilous aleocharine species, especially in relation to the differing degrees of integration with their hosts (Stoeffler et al. 2011, Staniec et al. 2009, 2017, 2018, Zagaja et al. 2014). Five of the six species (genera) are known ant symbionts, associated with two species of these hymenopterans. Only *Dinaraea aequata* Erichson W.F. 1837 is a typical saproxylic (non-myrmecophilous) beetle, totally unconnected with these social insects. In this configuration, therefore, it functions as a control species.

By far the largest number of characteristic features of the external structure, compared with other myrmecophilous and non-myrmecophilous aleocharines, were found in the larva of the symphilous genus *Lomechusa* Brisout de Barneville Ch.N.F., 1860 (Table 2). These beetles are the most highly integrated with their hosts, both behaviourally and morphologically (Hölldobler 1967, 1970, Parmentier et al. 2014, Parker 2016), and this also applies to the larval stages (Staniec et al. 2017). The morphological adaptations of their larvae are associated with the specific conditions prevailing within the anthill: absence of ocelli, a white body, and the close and continuous interaction in the host-guest system (e.g. absence of urogomphi; dense, asymmetrical chaetotaxy; membranous cuticle; short legs; some elements of mouthparts shortened). In addition, this distinctive structure of the larval stage of *Lomechusa* is accompanied by a passive lifestyle, possible trophallaxis and chemical mimicry.

The classification of the degree of integration of the other four myrmecophilous species of Aleocharinae is not so obvious. Nonetheless, there do seem to be certain differences between them in this respect. Stoeffler et al. (2011), referring to Hölldobler (1981), suggests that in *Pella laticollis* there is behavioural pre-adaptation towards a closer relationship with the host. Evidence for this could be the presence in adult beetles, in contrast to the other representatives of this genus, of glands modifying the behaviour of the ants, which enables the beetles to live unmolested in the near neighbourhood of the anthill. Again, on the basis of existing classifications (Wasmann 1894, Kistner 1979) and observations of different behavioural aspects of *Thisophila angulata*, Zagaja et al. (2017) place this species among host-integrated myrmecophiles. However, Stoeffler et al. (2011) demonstrated that the relations of *T. angulata* with ants resemble a pre-adaptation (initial phase) to a closer relationship with them, rather than complete integration, as in the case of *P. laticollis*. The relationships with hosts of the smallest of this group of beetles – *Amidobia talpa* and *Oxypoda haemorrhoa* – were not examined. It seems, however, that according to Wasmann's (1894) classification, they are probably synoics, that is, myrmecophiles feeding on detritus or other organisms inhabiting the anthill. Opportunistically, they may also consume eggs and small larvae of ants. Because they are small and highly mobile, these beetles are probably completely ignored by the host (Parmentier et al. 2015, own observations).

In view of the above it cannot be surprising that, with the exception of *Lomechusa*, discussed earlier, the other myrmecophilous larvae analysed here do not possess any outstanding features distinguishing them from non-myrmecophilous species (Table 2). Therefore, the morphological differences between the aleocharine larvae examined here are probably a reflection of the biotic and abiotic conditions specific to the particular microhabitat they occupy rather than of more general habitat preferences (myrmecophily or non-myrmecophily). The crucial aspect of this situation thus appears to be the trophic specialization of these tiny predators, a question as yet incompletely understood. That is why the greatest number of differences between them concerns the structural details of the mouthparts (e.g. shape of labrum and ligula, structure of epipharynx, mandibles, mala, length of articles of maxillary and labial palps), this differentiation being strictly linked with the food resources these larvae consume (Table 2). Other characteristic features of these larvae include the detailed structure of the antennae, the urogomphi, less often the structure of the spiracles (*A. talpa*) and head shape (*O. haemorrhoa*). The present morphological analysis has not revealed any features characteristic of the several tribes (Table 2). This might indicate, on the one hand, the need to reassess the systematics of the higher taxonomic units in Aleocharinae, but on the other, that the larval characteristics of these rove beetles are of minimal usefulness in phylogenetic studies.

Therefore, as studies to date have shown, the characteristic morphology of the aleocharine larvae examined to date is not due to their myrmecophily alone. Likewise, the larval stages of myrmecophiles, which exhibit behavioural pre-adaptations to integration with host ants (*P. laticollis*, *T. angulata*), do not possess any visible external structural features pointing to associations with ants (Staniec et al. 2009, Zagaja et al. 2014). By contrast, symphiles, i.e. aleocharine species wholly integrated with their hosts and obligatorily dependent on them (e.g. *Lomechusa*), do exhibit a far-reaching restructuring of the body, particularly that of the larva. The unique morphological features of larvae (Table 2) are the result of advanced adaptations to life in an anthill and to constant interactions with their inhabitants (Hölldobler 1967, Parker 2016, Staniec 2017). In this context, the structure of the newly-described larvae of *Amidobia* and *Oxypoda* is typical of tiny, predacious Aleocharinae, not associated with ants (Table 2). In all probability, because they are highly mobile and very small (max. lengths up to 2.6 and 3.3 mm respectively), they are, like the adult forms, entirely ignored by the worker ants. They can thus live unmolested among ants without the need to possess the morphological adaptations that have evolved in the larger and slower symphiles. Similar, co-existential strategies in other small insect species associated with ants were described by Parker (2016) and Parmentier et al. (2015).

This analysis of the comparative morphology of known myrmecophilous aleocharine larvae in the context of the type of interaction with hosts is merely a preamble to far more extensive research on this subject. Unfortunately, as knowledge of the larval stage, not only of myrmecophilous but of other members of this very numerous staphylinid subfamily, remains fragmentary, the formulation of more comprehensive generalizations is as yet not possible. Moreover, there is still no information whatsoever on the detailed external larval structure of a number of other interesting, symbiotic European aleocharines. This situation can be illustrated by the genus *Dinarda* Leach W.E., 1819. Its members exhibit behaviour testifying to quite an advanced degree of integration with hosts, including the possibility of their being fed by ants on the principle of regurgitation (Hölldobler and Wilson 1990). It may well be that the mouthparts of *Dinarda* larvae are adapted to this form of feeding in the same way as in *Lomechusa* (Staniec et al. 2017) and that they possess other features emerging from their close relationships with ants. Where the degree of advancement of integration with hosts is concerned, such features would help to place *Dinarda* right after members of *Lomechusa*, and certainly in front of the other myrmecophiles listed in Table 2. But further studies are needed in order to find a definitive answer to this question.

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RESEARCH ARTICLE

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A new species of *Sphaerius* Waltl from China (Coleoptera, Myxophaga, Sphaeriusidae)

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Abstract
A new species, *Sphaerius minutus* **sp. n.**, is described and illustrated from Jinggangshan Mts., Jiangxi Province, China. It is the first species of this family described from East Asia. This species lives under wet stones at the edge of rivers.

Keywords
China, Coleoptera, new species, Oriental Region, *Sphaerius*, Sphaeriusidae

Introduction

Sphaeriusidae are a group of tiny beetles, usually 0.5–1.3 mm in length, most of which are semi-aquatic, living in moist substrates by stream or river banks, but some species are strictly terrestrial (Löbl 1995, Endrödy-Younga 1997, Beutel and Arce-Pérez 2005). The family contains a single genus, *Sphaerius* Waltl, 1938 with 22 valid species, distributed in all continents expect Antarctica (Reichardt 1973, Löbl 1995, Lawrence and Ślipiński 2013). Among all faunal regions, the Oriental Region has the largest number of species. Reichardt (1973) recorded 18 known species of Sphaeriusidae, eight of which were from Southeast Asia. Subsequently four more terrestrial species were reported from South Asia (Löbl 1995). However, none of the species have ever been described from East Asia although this family has been reported from China once by Hall (2003) but without any detailed information. This family has also been reported from several places in Japan but no specimen was identified to species level (Kamezawa and Matsubara 2012). Other than that, there are no other records of this family from China or other East Asian countries. Based on the specimens collected from Jiangxi, China, a new species of *Sphaerius* is described. It is the first species of *Sphaerius* described from East Asia.

Materials and methods

Specimens of the new species were dissected, and the genitalia were placed in a drop of glycerol on glass slides. After photography, the genitalia were transferred to a plastic plate attached to the respective specimen. SEM photographs were taken with a Phenom Prox scanning electronic microscope, and photographs of habitus and male genitalia were taken with a Zeiss SteREO Discovery V20 Microscope and a Zeiss Axioskop 40 Microscope respectively. Illustrations of elytron and male genitalia were drawn with Adobe Illustrator CS6 based on the material and photographs.

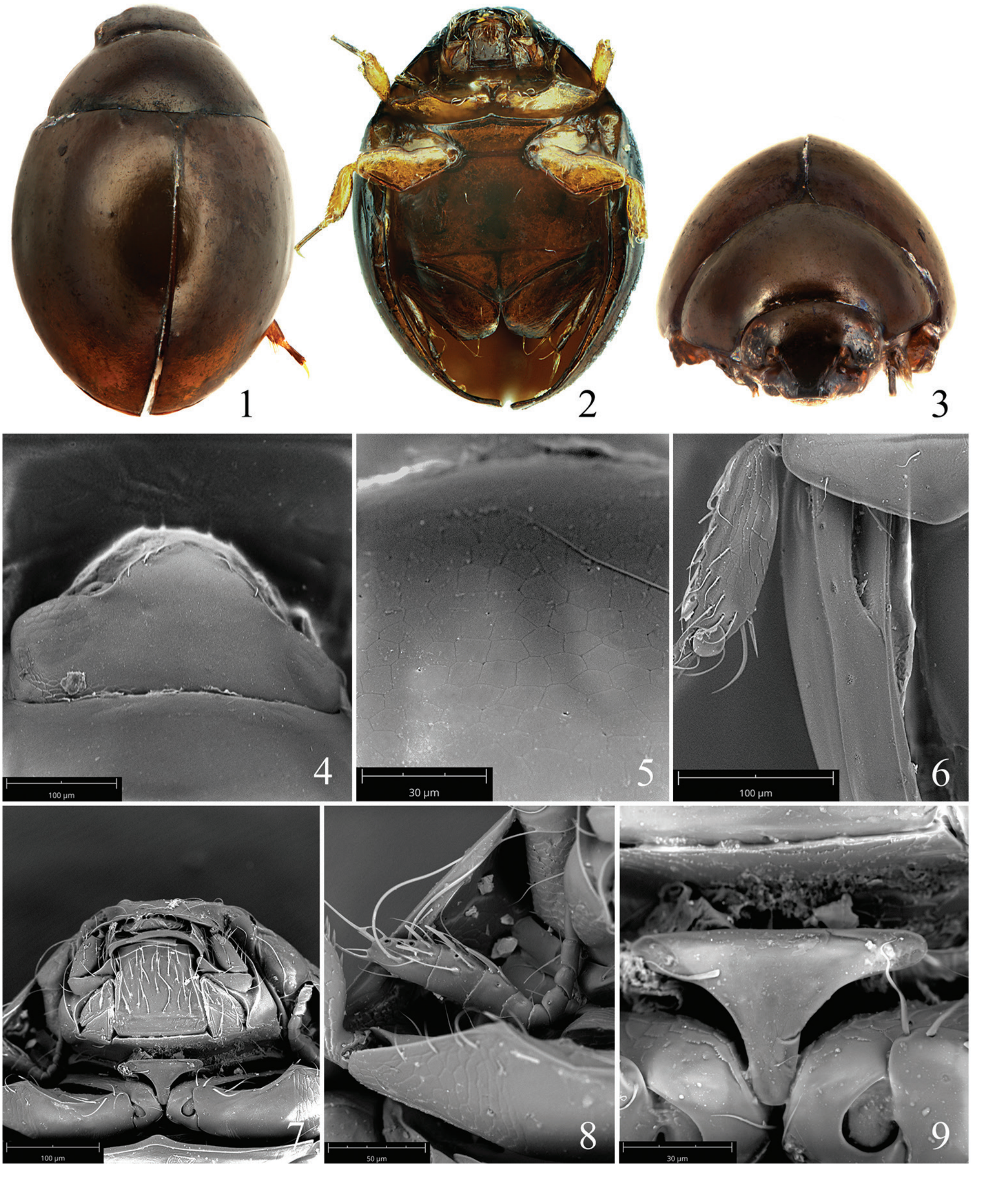
One paratype was deposited in National Museum, Prague, Czech Republic (**NM**), and the remaining material examined is at the Entomological Collection of Sun Yat-sen University, Guangzhou, China (**SYSU**).

Results

***Sphaerius minutus* sp. n.**
http://zoobank.org/1D941BED-6051-427B-8080-B1D415975C64
Figs 1–18

Type material. Holotype: male: China, Jiangxi Province, Jinggangshan Mountain, 1.3 km southwest of Xiping County, 26°33'4"N, 114°12'2"E, 850 m, at the edge of a stream, beneath a stone, caught, 24 June 2011, Fenglong Jia leg. **Paratype**: five specimens: same data as holotype.

Diagnosis. Length 0.9–1.0 mm, broadly oval, strongly convex in form. Dorsal surface smooth and shiny, without any punctation or striae. Each elytron with 8–12 conical and rough asperities. Antennae with last four antennomeres slightly clubbed, but not as strongly dilated as in other species of the genus. Prosternal process T-shaped, strongly elevated, with anterior margin straight and lateral margin smoothly concave. Pro- and mesotibiae each bear a ventral disc at the apex. Median lobe of the aedeagus long and laterally compressed, apex distinctly dilated, apical angle rounded, never prominent; left paramere digitus-shaped, half the length of the median lobe; right paramere petaliform.



Figures 1–9. *Sphaerius minutus* sp. n. **1–3** habitus (**1** dorsal view **2** ventral view **3** frontal view) **4** dorsal view of head **5** micro-reticulation on pronotum **6** epipleuron **7** ventral view of head **8** antenna **9** prosternal process.

Description. Body widely oval, strongly convex in form. 0.9–1.0 mm in length, ca. 1.5× as long as wide, widest in the middle. Dorsal surface uniformly brown to dark brown, smooth and shining, without any punctation (Fig. 1). Ventral surface reddish brown, with front legs and mid legs yellow (Fig. 2). Micro-reticulation distinct on head and pronotum at magnification of more than × 1000, but fainter on elytra. The meshes of reticulation pentagonal or hexagonal (Fig. 5).

Head. Short and broad. Frons attenuated anteriorly, with several setae along lateral margin. Clypeus short and broad, with anterior margin straight, fronto-clypeal suture indistinct. Compound eyes developed, strongly protruding (Figs 3, 4). Mentum flat, shovel-like, slightly attenuated anteriorly, anterior margin slightly rounded; lateral margin with two longitudinal ridges on each side (Fig. 7). Antennae inserted near eyes, extending well beyond the posterior margin of prosternum, 11–segmented; antennomere 3 long, about as long as the following 4 antennomeres combined; last 4 antennomeres slightly clubbed, but not dilated as strongly as in other species, at most twice as wide as previous antennomeres, with rough and long setae at apex (Fig. 8).

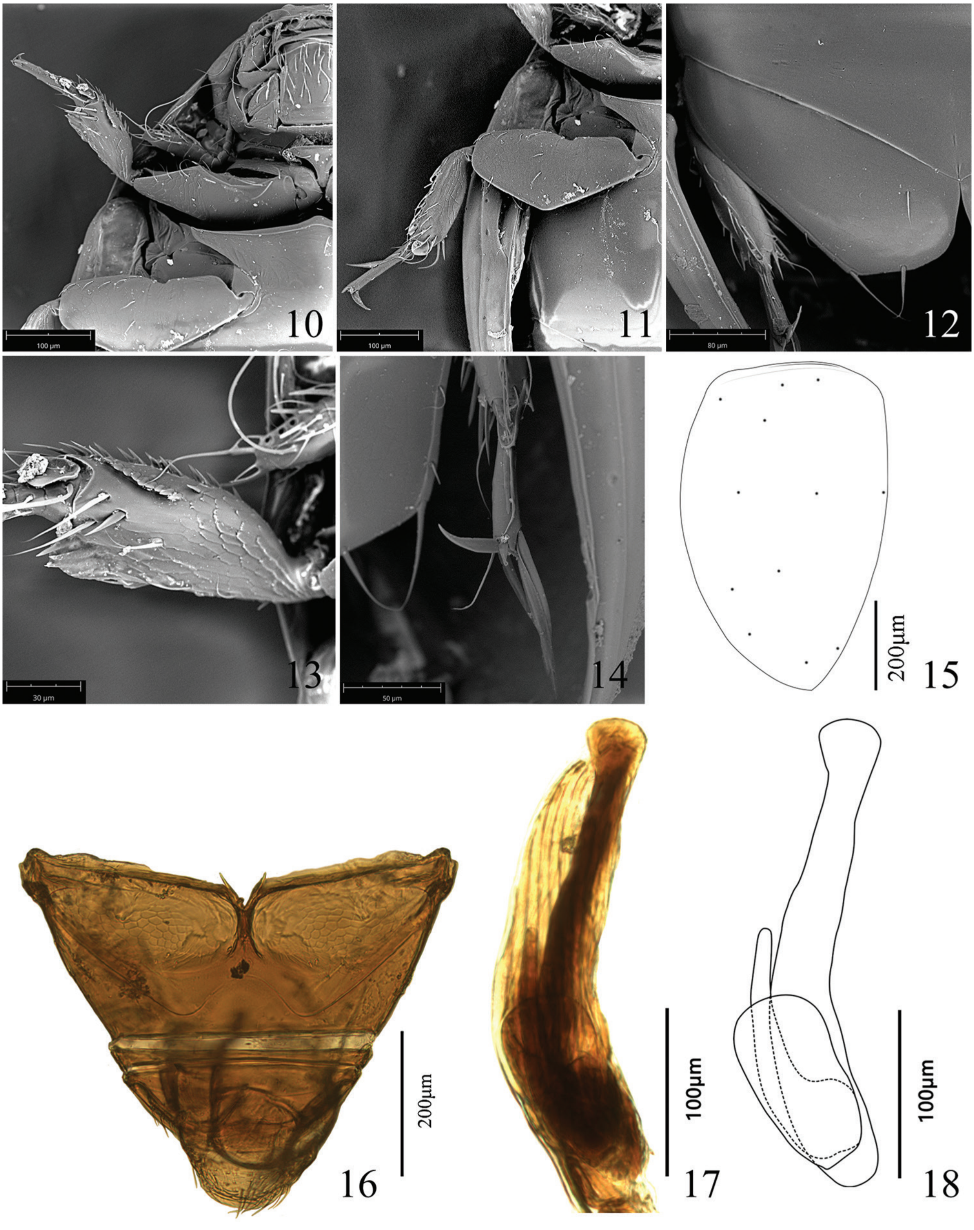
Pronotum. Attenuated anteriorly, ca. 0.35× as long as wide, lateral margin smoothly rounded; anterior angle produced and acute, posterior angle rectangular, slightly rounded; posterior margin slightly narrower than base of elytra. Scutellum almost equilaterally triangular, small and flat, with length a bit narrower than wide (Fig. 1).

Elytra. Smooth and shining, without any punctation or striae, lateral margin smoothly rounded (Fig. 5). Elytron with 8–12 conical and rough asperities arranged as in Fig. 15, some of which are obsolete or absent in some specimens. Epipleuron developed, constricted posteriorly, with a deep and broad furrow along basal third; outer edge with several round depressions composed of a cluster of micro-holes (Figs 6, 11).

Ventral surface. Prosternal process T-shaped, strongly elevated, ca. 0.6× as long as wide, extending to about half of procoxae; anterior margin straight, while lateral margin smoothly concave (Fig. 9). Mesoventrite short and broad, largely elevated at middle, constricted posteriorly, reaching the middle of mesocoxae, fused with the much larger metaventrite; metaventrite ca. 2.5× as long as mesoventrite; meso- and metaventrite smooth and flat, bearing very sparse bristles (Fig. 2).

Legs. Coxae transverse, procoxae close to each other, mesocoxae widely separated by meso-metaventrite, while metacoxae contiguous, forming a large transverse coxal plate with two setae at outer apical angle and middle of the posterior margin (Fig. 12). Profemur robust and broad, basal half of anterior margin rather concave, forming a cavity for head; mesofemur strongly dilated, short and broad, posterior side margin straight, forming obtuse angle; metafemur hidden under coxal plate. Protibia short and broad, bearing a row of short setae at inner edge and a row of denticles near the apex (Fig. 13); pro- and mesotibiae both bearing some long setae and a ventral disc at the apex (Figs 10, 11). Tarsus 3-segmented, first and second tarsomeres very short, shorter than wide, third tarsomere much longer, more than 3/4 as long as the entire tarsus; some setae on tarsus flattened and broadened, transforming into banding-shaped (Fig. 14). Claws quite well developed, strongly unequal, the smaller one ca. 0.5× as long as the other one in front legs, and much smaller in mid and hind legs (Figs 10, 11, 14).

Abdomen. Abdomen with three visible ventrites; first ventrite large and broad with two large coxal cavities occupying almost the entire ventrite, the basal half was divided into two parts by a longitudinal furrow in the middle; third ventrite shorter than first ventrite; second ventrite extremely narrow, ca. 0.15× as long as the third ventrite, anterior and posterior margins straight and parallel (Fig. 16).



Figures 10–18. *Sphaerius minutus* sp. n. **10** front leg **11** mid leg **12** hind leg **13** protibia **14** metatarsus **15** asperities on left elytron **16** abdomen **17** lateral view of male genitalia **18** illustration of male genitalia (lateral view).

Male genitalia. Median lobe long and laterally compressed, gradually narrowed toward apex, apex distinctly dilated, with apical angle rounded; left paramere digitus-shaped, half the length of the median lobe; right paramere petaliform (Figs 17, 18).

Etymology. The species name comes from the Latin adjective *minutus* (= tiny), referring to the minute body size of this species.

Distribution. Only known from type locality in Jiangxi Province, China.

Biology. This species was found under a stone on the muddy shore of a running stream. They were tightly attached to the lower surface of the stone when the stone was removed. Biology of larvae unknown.

Differential diagnosis. The new species closely resembles *S. papulosus* Lense, 1940 described from Myanmar. These two species share several character states. Besides the similar body shape and size, their antennal clubs are composed of four antennomeres, they both have smooth and shiny dorsal surface and with similar reticulation on pronotum, and they both have some conical and rough asperities on the elytra. However, the new species has a different pattern in the arrangement of the asperities and the metatarsus is 3-segmented instead of 2-segmented.

The new species differs from other species of *Sphaerius* by its less dilated antennal club. Additionally, the smooth, uniformly brown dorsal surface, the T–shaped prosternal process, the serial denticles on protibiae, and ventral disc on pro- and mesotibiae are also important characters to identify the new species. As for the median lobe, the Himalayan species have a more or less prominent apex, while the new species has a round and more dilated apex.

Discussion

Based on their size and small wings and the fact that there is no record of Sphaeriusidae having been collected with traps, we infer that their ability to migrate is weak. All known species of *Sphaerius* in the Oriental Region occur in Nepal, Vietnam, and other areas of Southeast Asia (Lense 1936, 1940, Löbl 1995). The locality of the new species is rather distant from those of known species which suggests, besides the morphology, that we are dealing with an undescribed species.

Sphaeriusidae are frequently overlooked by collectors because of their minute size and special habitat environment. So, we believe that with proper collecting methods in suitable areas, more species would be discovered. The Oriental Region is obviously the hot spot of Sphaeriusidae, and all the Oriental species were described from countries bordering China. Therefore, we strongly believe there are more species distributed in south China.

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RESEARCH ARTICLE

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A taxonomic revision and molecular phylogeny of the eastern Palearctic species of the genera *Schizomyia* Kieffer and *Asteralobia* Kovalev (Diptera, Cecidomyiidae, Asphondyliini), with descriptions of five new species of *Schizomyia* from Japan

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Abstract

The genus *Asteralobia* (Diptera, Cecidomyiidae, Asphondyliini, Schizomyiina) was erected by Kovalev (1964) based on the presence of constrictions on the cylindrical male flagellomeres. In the present study, we examine the morphological features of *Asteralobia* and *Schizomyia* and found that the male flagellomeres are constricted also in *Schizomyia galiorum*, the type species of *Schizomyia*. Because no further characters clearly separating *Asteralobia* from *Schizomyia* were observed, we synonymize *Asteralobia* under *Schizomyia*. Molecular phylogenetic analysis strongly supports our taxonomic treatment. We describe five new species of *Schizomyia* from Japan, *S. achyranthesae* Elsayed & Tokuda, **sp. n.**, *S. diplocyclosae* Elsayed & Tokuda, **sp. n.**, *S. castanopsisae* Elsayed & Tokuda, **sp. n.**, *S. usubai* Elsayed & Tokuda, **sp. n.**, and *S. paederiae* Elsayed & Tokuda, **sp. n.**, and redescribe three species, *S. galiorum* Kieffer, *S. patriniae* Shinji, and *S. asteris* Kovalev. A taxonomic key to the Japanese *Schizomyia* species is provided.

Keywords

Cecidomyiinae, gall midges, Schizomyiina, taxonomic key

Introduction

Schizomyia Kieffer is a cosmopolitan genus of the subtribe Schizomyiina (Diptera, Cecidomyiidae, Cecidomyiinae, Asphondyliini) with 53 species associated with diverse plant families (Gagné and Jaschhof 2017, Elsayed et al. in press). The genus includes species with needle-like ovipositors, four-segmented palpi, and larval terminal segments with four or fewer pairs of terminal papillae (Gagné and Menjivar 2008). Most of these species induce bud galls, but some induce leaf galls (Gagné 1989). Some *Schizomyia* species are agricultural pests, e.g. *S. loroco* Gagné, which induces flower galls on loroco, *Fernaldia pandurata* (A. DC.) Woodson (Apocynaceae), in El Salvador (Gagné and Menjivar 2008). A few species have been used as potential biological control agents, e.g., *S. macrofila* (Felt), which induces flower galls on *Amsinckia* spp. weeds in California (Pantone and Brown 1985).

Kovalev (1964), in his revision of the Russian Far East gall midges of the tribe Asphondyliini, erected the genus *Asteralobia* based on the presence of shallow or deep constrictions on the cylindrical male flagellomeres. Although *Asteralobia* has been treated as an independent genus of Schizomyiina and presently contains 12 species (Gagné and Jaschhof 2017), some studies have indicated that *Asteralobia* could be subsumed under *Schizomyia* because of the lack of known synapomorphic differences between them (e.g. Tokuda et al. 2003; Gagné and Jaschhof 2017). In the present study, we re-examined morphological features of *Asteralobia* and *Schizomyia* and analyzed molecular phylogenetic relationships between them, which have led us to the conclusion that *Asteralobia* should be synonymized under *Schizomyia*. In addition, we describe five new species of *Schizomyia* from Japan and provide a taxonomic key to the *Schizomyia* species from Japan.

Materials and methods

Collecting and rearing methods

Galls induced by six gall midge species were collected from various localities in Japan (Table 1). Some galls were dissected to obtain larval specimens, while the remaining galls were kept in plastic bags until the departure of mature larvae from the galls. Thereafter, larvae were transferred to plastic cups (120 mm in diameter, 110 mm in depth) containing a mixture of wet peat moss and sand (1:1), covered with fine mesh, and fixed with a rubber band. Cups containing larvae obtained from fruit galls on *Achyranthes bidentata* Blume (Amaranthaceae) and *Trachelospermum asiaticum* (Sieb. et Zucc.) Nakai (Apocynaceae), and flower bud galls on *Paederia foetida* L. (Rubiaceae) and *Patrinia villosa* (Thunb.) (Valerianaceae) were transferred to the field on the Saga University campus, Saga Prefecture (about 5.5 m a.s.l.), and half-buried in the soil to allow the mature larvae to overwinter under natural conditions. These cups were brought back to the laboratory in April to rear adults and were kept at room temperature. In cases of larvae obtained from flower bud galls on *Diplocyclos palmatus* (L.) Jeffrey (Cucurbitaceae) and *Castanopsis sieboldii* (Makino) Hatus. (Fagaceae), the cups containing larvae were maintained at room temperature in the laboratory until adult emergence.

Table 1. Collection data of the newly described and redescribed Japanese *Schizomyia* species.

Gall midge	Host Plant and galls	Collection site	Collecting date	Collector
Schizomyia achyranthesae sp. n.	Fruit bud galls on *Achyranthes bidentata* Blume (Amaranthaceae)	Tokushima City, Tokushima Prefecture	6 October 2001	M. Yukinari
		Kyushu University, Ito Campus, Fukuoka Prefecture	30 October 2012	J. Yukawa et al.
		Mount Hinokuma, Saga Prefecture	16 October 2014	A.K. Elsayed & M. Tokuda
		Mount Tara, Saga Prefecture	22 October 2014	A.K. Elsayed & M. Tokuda
		Mount Tenzan, Saga Prefecture	29 October 2001	A.K. Elsayed & M. Tokuda
		Mount Tara, Saga Prefecture	9 October 2015	M. Tokuda
		Takeo City, Saga Prefecture	10 October 2015	A. Kita
Schizomyia diplocyclosae sp. n.	Flower bud galls on *Diplocyclos palmatus* (L.) Jeffrey (Cucurbitaceae)	Kinjo town, Naha City, Okinawa Prefecture	13 January 1977	S. Yamauchi
		Shuri, Naha City, Okinawa Prefecture	January 1977	S. Yamauchi
		Gogayama, Nakijin village, Okinawa Prefecture	4 March 2002	M. Tokuda
		Hantagawa, Naha City, Okinawa Prefecture	10 February 2016	T. Ganah-Kikumura
Schizomyia paederiae sp. n.	Flower bud galls on *Paederia foetida* L. (Rubiaceae)	Nishino-omote, Nishino-omote City, Nokubi, Kagoshima Prefecture	24 September 2014	K. Ogata
		Ogorori City, Misawa, Aomori Prefecture	August 2016	K. Matsunaga
Schizomyia patriniae (Shinji 1938)	Flower bud galls on *Patrinia villosa* (Thunb.) (Valerianaceae)	Kyuragi, Karatsu City, Saga Prefecture	12 October 2015	M. Tokuda
Schizomyia castanopsisae sp. n.	Flower bud galls on *Castanopsis sieboldii* (Fagaceae)	Hachijojima Island	6 December 2014	T. Kikuchi
		Shikinejima Island	10 December 2014	M. Tokuda
		Niijima Island	11 December 2014	M. Tokuda
		The Izu Islands	9 December 2016	M. Tokuda
Schizomyia usubai sp. n.	Fruit galls on *Trachelospermum asiaticum* (Sieb. et Zucc.) Nakai (Apocynaceae)	Mount Takakuma, Kagoshima Pref.	November 1969	J. Yukawa
		Imuta Lake-side, Kedouin Town, Kagoshima Prefecture	2 November 1978	S. Sako
		Torinosu, Tanabe City, Wakayama Prefecture	2 November 2016	I. Matoba
		Mount Onigasawa, Nishino-omote, Nishino-omote City, Kagoshima Prefecture	4 November 2016	K. Ogata

The pupal exuviae protruding from the surface of the soil in the plastic cups were collected at the same time carefully. Reared specimens were preserved in 75% ethanol for morphological study and 99.5% ethanol for the molecular phylogenetic study.

Taxonomy

Gall midge specimens were mounted on slides in Canada balsam using the technique outlined in Gagné (1994), except for the clearing step following Elsayed et al. (2018). The slide-mounted specimens were examined under a bright-field and phase-contrast microscope (H550L, Nikon, Tokyo) and illustrations were made with the aid of a drawing tube. A semi-motorized fluorescence microscope (BX53, Olympus, Tokyo) was used to photograph some of the characters from mounted specimens with the aid of a microscope-attached camera (DP22, Olympus, Tokyo).

Morphological terminology basically follows McAlpine et al. (1981) for adults, but the thoracic sclerite "mesanepisternum" follows Gagné (1968) and the wing venation follows Yukawa (1971). Larval and pupal terminology follows Gagné (1994). All the types of the newly described species are deposited in the collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Japan (KUEC).

Adult and immature specimens of *Asteralobia humuli* Shinji, *A. patriniae* Shinji, *A. sasakii* Monzen and *A. soyogo* Kikuti, and larvae of *A. doellingeriae* Kovalev were examined in KUEC. Adults of *A. asteris* Kovalev, *A. calathidiphaga* Kovalev, *A. doellingeriae*, and *A. solidaginis* Kovalev, as well as four females and two males of *S. galiorum* were examined in the B. M. Mamaev Collection in the Museum of Nature and Human Activities, Hyogo, Japan. A female and a pupa of *Schizomyia galiorum* Kieffer were examined in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

Molecular phylogenetic study

Total DNA was extracted from larval or adult specimens and fragments of the mitochondrial genes, cytochrome oxidase subunit I (COI) and 12S small ribosomal subunit, were sequenced and aligned following Elsayed et al. (2017). The following sets of primers were used for the cytochrome oxidase subunit I (COI) gene: J–1718 (5'–GGA GGA TTT GGA AAT TGA TTA GTT CC–3') (Simon et al. 1994) and COIA (5'–CCC GGT AAA ATT AAA ATA TAA ACT TC–3') (Funk et al. 1995); COIS (5–GGA TCA CCT GAT ATA GCA TTC CCA TAT TGG–3') and COIA (5'–CCC GGT AAA ATT AAA ATA TAA ACT TC–3') (Funk et al., 1995); or LCO1490 (5'–GGT CAA CAA ATC ATA AAG ATA TTG G–3') and HCO2198 (5'–TAA ACT TCA GGG TGA CCA AA AAT CA–3') (Folmer et al. 1994). The following primer set was used for the 12S small ribosomal subunit gene: SR–J–14199 (5'–TAC TAT GTT ACG ACT TAT–3') and SR–N–14594 (5'–AAA CTA GGA TTA GAT ACC C–3') (Kambhampati and Smith 1995). The obtained sequences were deposited in the DNA Data Bank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank under the accession numbers given in Table 2.

The sequence data were analyzed with the maximum likelihood (ML) method using MEGA (ver. 6.0) (Tamura et al. 2013). Sequences of *Asphondylia gennadii* Marchal

Table 2. Gall midge species used for genetic analysis.

Gall midge	Host Plant	Collection site	Collector	*COI* accession no.	*12S* accession no.
Schizomyia achyranthesae sp. n.	*Achyranthes bidentata* Blume (Amaranthaceae)	Mount Tara, Saga Pref., Japan	M. Tokuda	LC426387–LC426389	LC426410-LC426412
S. (=*Asteralobia*) *asteris* (Kovalev)	*Aster tataricus* L. fil. (Asteraceae)	Smolyaninovo, Primorsky Territory, Russia	M. Tokuda et al.	LC426390	LC426413
S. buboniae (Frauenfeld)	*Deverra tortuosa* (Desf.) DC. (Apiaceae)	Borg Al-Arab District, Alexandria, Egypt	A.K. Elsayed	LC426391-LC426393	LC426414-LC426416
S. (=*Asteralobia*) *doellingeriae* (Kovalev)	*Aster scaber* Thunb. (Asteraceae)	Shkotovo, Primorsky Territory, Russia	M. Tokuda	LC422074¶	LC422101¶
S. diplocyclosae sp. n.	*Diplocyclos palmatus* (L.) Jeffrey (Cucurbitaceae)	Hantagawa, Naha City, Okinawa Pref., Japan	T. Ganaha-Kikumura	LC426394-LC426396	LC426417-LC426419
S. (=*Asteralobia*) *kovalevi* (Skuhravá)	*Patrinia scabiosifolia* Fisch. (Valerianaceae)	Dukhovskoye, Primorsky Territory, Russia	M. Tokuda et al.	LC422068¶	LC422095¶
S. (=*Asteralobia*) *patriniae* (Shinji)	*Patrinia villosa* (Thunb.) (Velerianaceae)	Kyuragi, Saga City, Saga Pref., Japan	M. Tokuda	AB176718§	LC422105¶
S. galiorum (Kieffer)	*Galium mollugo* L. (Rubiaceae)	Wescot Downs, Surrey, UK	K.M. Harris	AB213410#	LC422108¶
S. (=*Asteralobia*) *humuli* (Shinji)	*Humulus japonicus* Siebold & Zucc. (Cannabaceae)	Ogi City, Saga Pref., Japan	A.K. Elsayed	LC426397-LC426399	LC426420-LC426422
S. paederiae sp. n.	*Paederia foetida* L. (Rubiaceae)	Nokubi, Nishinoomote City, Kagoshima Pref., Japan	K. Ogata	LC426400-LC426402	LC426423-LC426425
S. (=*Asteralobia*) *sasakii* (Monzen)	*Ilex crenata* Thunberg (Aquifoliaceae)	Mount Daimonji, Kyoto Pref., Japan	N. Uechi	LC422071¶	LC422098¶
S. (=*Asteralobia*) *solidaginis* (Kovalev)	*Solidago pacifica* Juz. (Asteraceae)	Mount Litovka, Primorsky Territory, Russia	M. Tokuda et al.	LC426403	LC426426
S. (=*Asteralobia*) *soyogo* (Kikuti)	*Ilex pedunculosa* Miq. (Aquifoliaceae)	Mount Daimonji, Kyoto Pref., Japan	N. Uechi	LC422075¶	LC422102¶
S. castanopsisae sp. n.	*Castanopsis sieboldii* (Makino) Hatus. (Fagaceae)	Hachijojima Island, Tokyo, Japan	T. Kikuchi	LC426404-LC426406	LC426427-LC426429
S. usubai sp. n.	*Trachelospermum asiaticum* (Sieb. et Zucc.) Nakai (Apocynaceae)	Mount Onigasawa, Nishino-omote, Nishino-omote City, Kagoshima Pref., Japan	K. Ogata	LC426407-LC426409	LC426430-LC426432

Sequences obtained from the Genbank: §Tokuda et al. (2004); #Tokuda et al. (2005); ¶Elsayed et al. in press.

(AB198012 and AB115580) and *Pseudasphondylia neolitseae* Yukawa (AB334237 and LC422092) were used as outgroup species. The best model was identified with the jModelTest 2 software (Darriba et al. 2012, Guindon and Gascuel 2003) on the basis of hierarchical likelihood ratio tests (hLRT) was GTR+G+I.

Results

Taxonomy

Genus *Schizomyia* Kieffer, 1889

Schizomyia Kieffer, 1889: 183. Type species: *S. galiorum* Kieffer, 1889.
Parasphondylia Kieffer, 1913: 93. Type species: *P. variicornis* Kieffer, 1913.
Asteralobia Kovalev, 1964: 419. Type species: *A. doellingeriae* Kovalev, 1964. Syn. n.
Euasteralobia Kovalev, 1964: 430, as subg. of *Asteralobia*. Type species, *Asteralobia calathidiphaga* Kovalev (mon.).

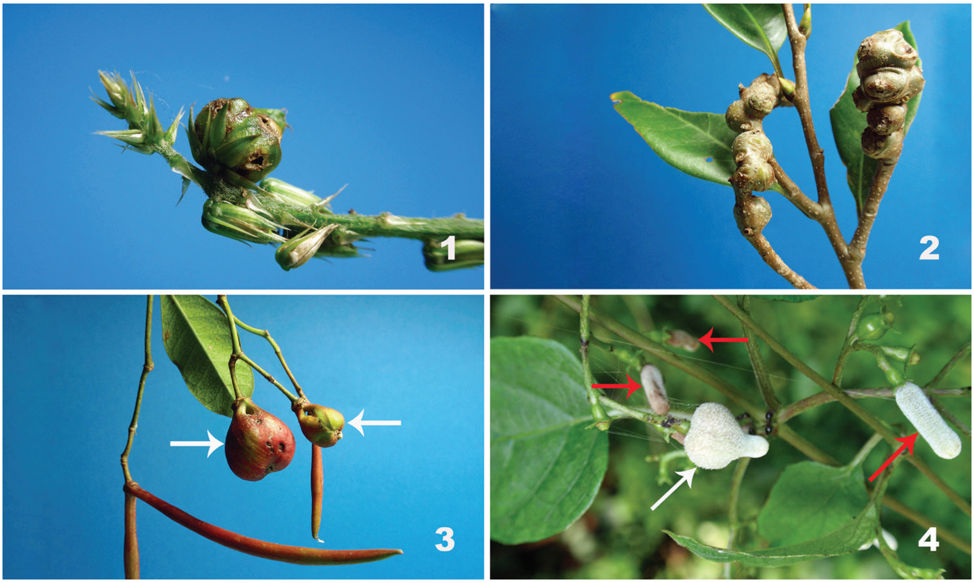
Kovalev (1964) erected the genus *Asteralobia*, all occurring in Russian Far East and Japan, based on the presence of constrictions on male flagellomeres. However, this character is observed in the type species of *Schizomyia*, *S. galiorum* (Fig. 69), and no other characters were found to differentiate between these genera. Therefore, the 12 species of *Asteralobia*, namely *A. asteris* Kovalev, *A. calathidiphaga* Kovalev, *A. clematidis* Fedotova, *A. doellingeriae* Kovalev, *A. humuli* Shinji, *A. kovalevi* Skuhravá, *A. patriniae* Shinji, *A. sasakii* Monzen, *A. soyogo* Kikuti, *A. solidaginis* Kovalev, *A. spiraeae* Fedotova and *A. veronicastrum* Fedotova, are combined here under *Schizomyia*.

Schizomyia is a cosmopolitan genus of 53 species, which are associated with over 30 host plants (Gagné and Jaschhof 2017; Elsayed et al. in press). With such a broad host range, *Schizomyia* is considered a catch-all genus defined only by plesiotypic characters and lack of synapomorphies (Gagné and Marohasy 1997; Gagné 1994). However, *Schizomyia* can be distinguished from the other genera of Schizomyiina by the following combination of characters: palpi four-segmented; tarsomeres I without ventroapical extension (Elsayed et al. in press), except *S. maricaensis* Sousa & Maia (Sousa and Maia 2007) and *S. novoguineensis* Kolesik (Kolesik and Butterill 2015); ovipositor protrusible, with needlelike protrusible portion; and larva usually with bilobed spatula and eight or fewer terminal papillae.

Schizomyia achyranthesae Elsayed & Tokuda, sp. n.

http://zoobank.org/AC909591-4D08-489E-AC19-9221F770F0D6
Figs 5–17; Table S1

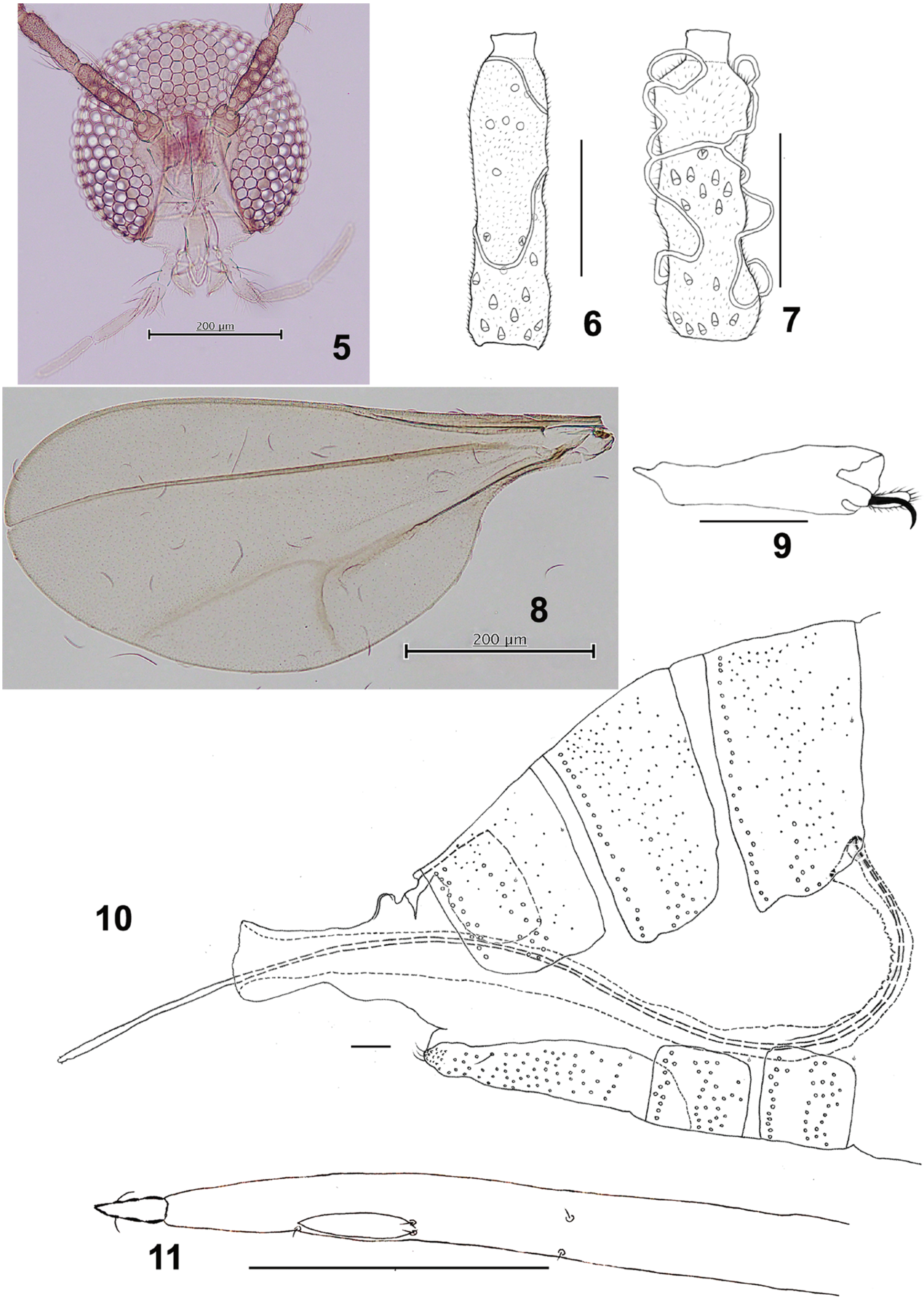
Etymology. The species name, *achyranthesae*, is based on the generic name the host plant, *Achyranthes bidentata* (Amaranthaceae).



Figures 1–4. Galls of *Schizomyia* spp. **1** Fruit gall induced by *S. achyranthesae* on *Achyranthes bidentata* (Amaranthaceae) **2** Inflorescence galls induced by *S. castanopsisae* on *Castanopsis sieboldii* (Fagaceae) **3** Fruit galls (arrows) induced by *S. usubai* on *Trachelospermum asiaticum* (Apocynaceae) **4** A flower bud gall (white arrow) induced by *S. paederiae* on *Paederia foetida* (Rubiaceae) [red arrows indicate normal flower buds].

Type material. *Holotype*: 1♂ (KUEC): reared by A. K. Elsayed from a larva obtained from a fruit gall on *A. bidentata*, collected from Mount Tara, Saga Prefecture, Japan, on 7.x.2015, M. Tokuda leg., the larva departed from gall between 10–19.x.2015 and the adult male emerged on 3.ix.2016. *Paratypes:* All paratypes were reared from fruit galls on *A. bidentata* in Japan. 3 larvae: collected from Tokushima City, Tokushima Prefecture on 6.x.2001, M. Yukinari leg., larvae departed from galls on 12.x.2001; 5 larvae: collected from Tokushima City, Tokushima Prefecture on 30.x.2012, J. Yukawa et al. leg., larvae departed from galls on 30.x.2012; 8♀, 4 Pupal exuviae: collected from Takeo City, Saga Prefecture on 10.x.2015, A. Kita leg., larvae departed from galls between 13–19.x.2015, adults emerged on 1.ix.2016; 3♀: collected from Mount Hinokuma, Saga Prefecture on 16.x.2014, A. K. Elsayed & M. Tokuda leg., larvae departed from galls on 22.x.2014, adults emerged in summer 2015; 4 pupal exuviae: collected from Takeo City, Saga Prefecture on 10.x.2015, larvae departed from galls between 13–19.x.2015, adults emerged on 29.viii.2016; 10♂, 6♀: same data as holotype.

Description. *Head* (Fig. 5): Compound eyes separated on vertex by a diameter of 0.0–0.75 facets, eye bridge 7–8 facets long, facets hexagonal. Fronto-clypeus with 15–21 setae (n = 6). Labrum and labella setose. Palpus 4-segmented: first segment ca 34 µm, second 1.3 times as long as the first, third 1.5 as long as the second, fourth 1.3 as long as the third. Antenna: scape slightly wider than long, pedicel rounded, flagellomeres I and II partially fused; female flagellomeres I–IX cylindrical with 2 connected rings of circumfila (Fig. 6); distal flagellomeres successively shorter; flagellomere X about as long as wide; flagellomere XI rounded, slightly wider than long;



Figures 5–11. *Schizomyia achyranthesae*. **5** Head **6** Ventral view of female antennal flagellomere V **7** Ventral view of male flagellomere V **8** Wing **9** Tarsomere V and acromere **10** Terminal part of female abdomen **11** Ovipositor apex. Scale bars: 50 µm (**6, 7, 9–11**), 200 µm (**5, 8**).

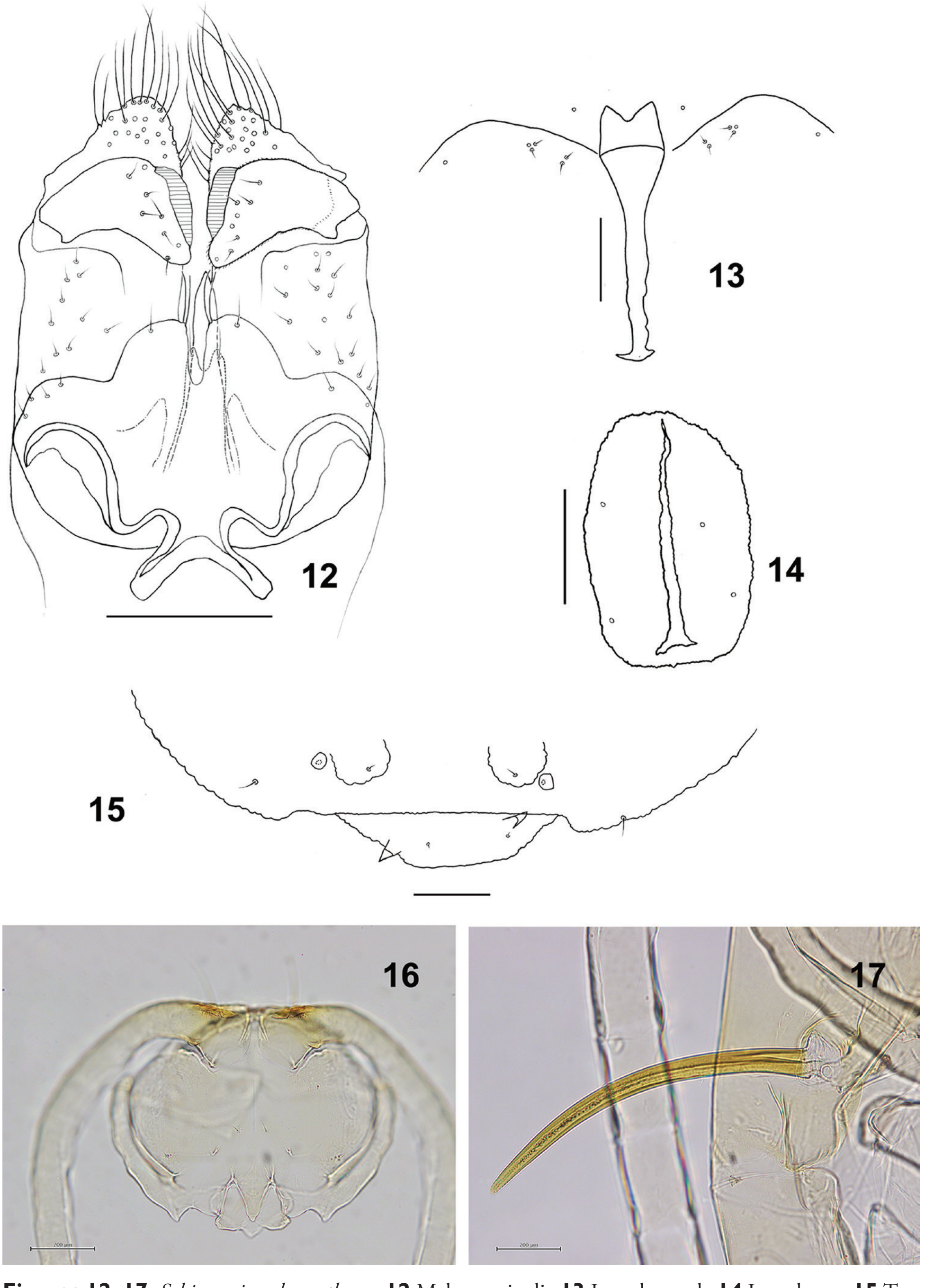
flagellomere XII rudimentary, partially fused with XI; male flagellomeres cylindrical with sinuous circumfila (Fig. 7).

Thorax: Wing (Fig. 8) length 1.60–2.02 mm (n = 6) in female, 1.43–1.73 mm (n = 5) in male: R_1 join C before wing midlength, R_5 join C just after wing apex, C broken after the conjunction with R_5; wing fold present; M_{3+4} forked with Cu. Tarsal claws untoothed, bent after midlength; empodia shorter than claws, with long setulae apically; pulvilli not discernable (Fig. 9). Anepimeral setae 19–23 (n = 8); mesanepisternum scales 22–38 (n = 6); lateral scutal setae 21–28 (n = 8). Lengths of leg segments as in Suppl. material 1: Table S1.

Female abdomen (Figs 10, 11): Tergites with anterior pair of trichoid sensilla; tergites I–VII rectangular and evenly covered with scales, tergites I–VI with a posterior row of setae and some scattered setae laterally at midlength; tergite VII with 1–2 posterior rows of setae and some scattered setae laterally at midlength; tergite VIII bare, notched laterally, posterior margin with a pair of well-developed dorsal lobes. Sternites II–VI rectangular, bare and less pigmented medially, with the lateral pair of trichoid sensilla situated anterior to the sclerotized sternite, several scattered setae on the anterior half, and 1–2 rows of setae posteriorly; sternite VII about 2.6 times as long as preceding sternites, with anterior pair of trichoid sensilla laterally situated on the sternite and setae covering posterior two-thirds. Ovipositor: protrusible needlelike portion about 4 times as long as sternite VII (Fig. 10); cerci fused, each lobe with few setae (Fig. 11).

Male abdomen: Tergites I–VII as in female; tergite VIII weakly sclerotized medially, with anterior pair of trichoid sensilla. Sternites II–VI as in female; sternite VII with lateral pair of trichoid sensilla situated anterior to the sclerotized sternite, several setae scattered anteriorly, median membranous bare area and 1–2 posterior row of setae; sternite VIII setose, with lateral pair of trichoid sensilla situated intersegmentally between sterna VII and VIII. Terminalia (Fig. 12): gonocoxite extending ventrally as convex lobe beyond of gonostylus; gonostylus stout, with unfused and compressed denticles, dorsally with setae on distal third, ventrally with a cluster of setae on the basal half. Cerci notched, each cercus with 4 strong setae and a few fine setae. Hypoproct shorter than cerci and aedeagus, bilobed, with a seta on each lobe. Parameres about half length of hypoproct. Aedeagus gradually tapering, acute apex, longer than cerci.

Mature larva (Figs 13–15): Cylindrical, yellow. Number and position of thoracic and abdominal spiracles as in other Asphondyliini (see Möhn 1961); 6 dorsal papillae present on all thoracic segments and abdominal segments I–VII, each with a seta; abdominal segment VIII with 2 dorsal lobes, each with a setose dorsal papilla. Sternal spatula bilobed (Fig. 13) with posterior portion about 3.6 times as wide as the base of the anterior free portion. Two groups of lateral papillae on all thoracic segments, the inner group of 2 setose papillae and the outer group of 2 setose and 1 asetose papillae. Two sternal papillae on each thoracic segment and 4 sternal papillae on abdominal segments I–VIII, all without setae and situated on slight swellings. Ventral papillae each with a seta on meso- and metathoracic segments and on abdominal segments I–VII. Anus situated ventrally, with simple opening and 4 asetose anal papillae (Fig. 14). Two



Figures 12–17. *Schizomyia achyranthesae*. **12** Male terminalia **13** Larval spatula **14** Larval anus **15** Terminal larval segment dorsally **16** Ventral view of pupal head **17** Pupal prothoracic spiracle. Scale bars: 50 µm (**12–15**), 100 µm (**16, 17**).

pairs of pleural papillae present on all thoracic segments and abdominal segment VIII, and 3 pairs on abdominal segments I–VII. Terminal segment (Fig. 15) with 2 setose and 2 corniform terminal papillae.

Pupa (Figs 16, 17): Exuviae not pigmented except prothoracic spiracles and dorsal spines of abdomen. Antennal horns short; 2 pairs of cephalic papillae present, a pair with short seta; 2 pairs of lower facial papillae, a pair with seta; 3 lateral facial papillae present on each side, 1 with short seta, 2 without setae. Prothoracic spiracle, slightly curved, 23–29 µm long (n = 6), connected with trachea to the tip. Spiracles present on abdominal segments II–VI. Abdominal terga I–VIII each with anterior pair of trichoid sensilla and 2 pleural papillae; terga I–VII each with 3 pairs of dorsal papillae, only outermost pair with a seta; tergum VIII with a pair of dorsal papillae, each with a seta. Abdominal terga II–VIII each with 3–4 rows of spines on median third.

Distribution. Japan: Honshu, Shikoku, Kyushu (Yukawa and Masuda 1996) and Tanegashima Island (Yukawa et al. 2013).

Gall and life history. *Schizomyia achyranthesae* induces subglobular fruit galls on *A. bidentata*, 5.07–5.17 mm in diameter (n = 5) (Fig. 1) [Gall No. C-246 in Yukawa and Masuda (1996)]. Based on Yukawa and Masuda (1996) and present observations, each gall contains 1–13 chambers and each chamber contains a single larva. The galls appear in September. The mature larvae leave the galls between October and November and overwinter in the soil. The adults of *S. achyranthesae* emerge during the flowering season of the host plant in August and September.

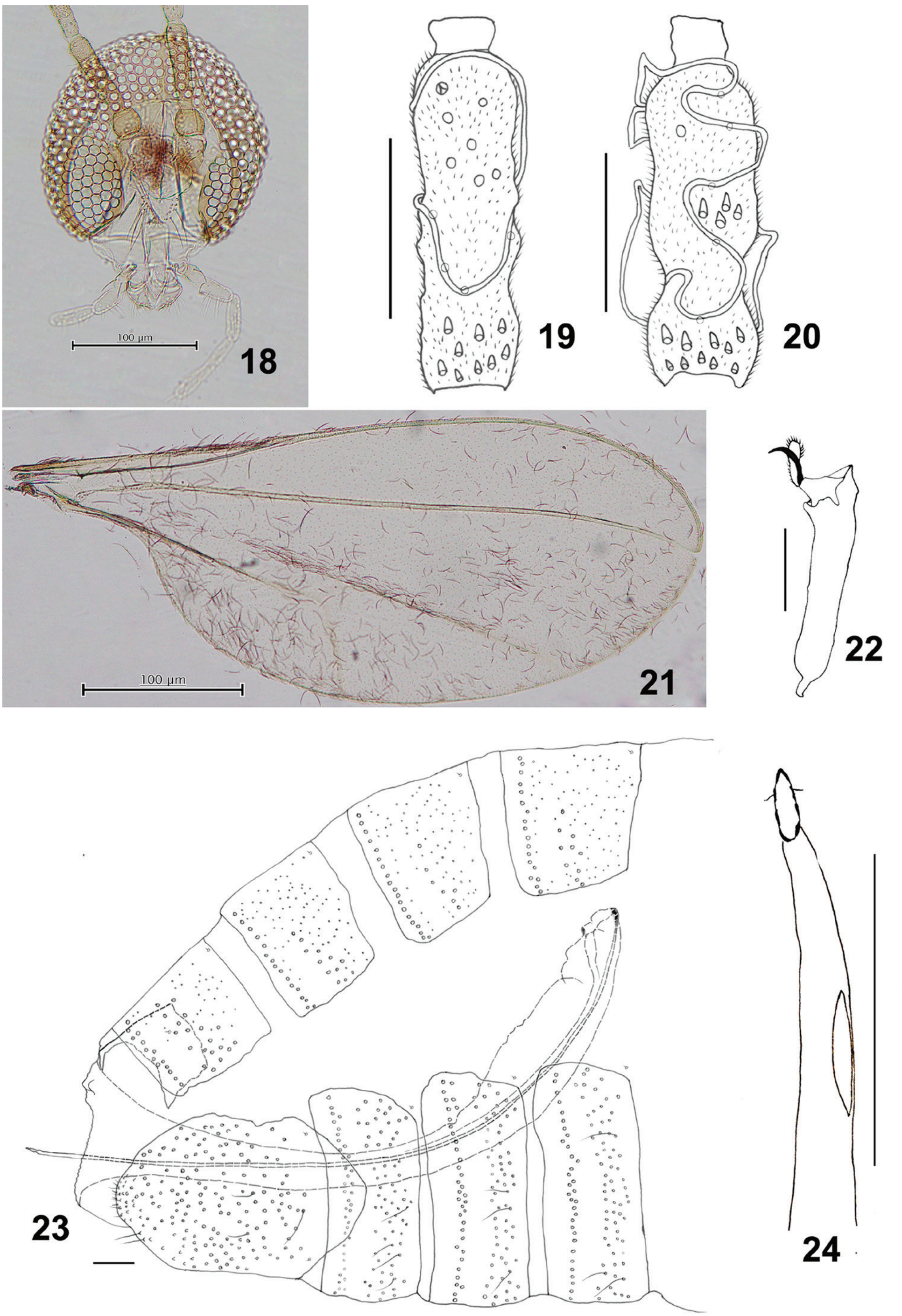
Remarks. *Schizomyia achyranthesae* is distinguishable from the known *Schizomyia* species, except *S. asteris* and *S. solidaginis,* by its shallowly constricted male flagellomeres, lateral position of anterior pair of trichoid sensilla and presence of four larval terminal papillae, as well as two setose papillae in inner group of lateral papillae. *S. achyranthesae* can be separated from *S. solidaginis* based on the larval characters as follows: *S. achyranthesae* possesses a more elongated sternal spatula than *S. solidaginis*; the inner group of lateral papillae consists of two setose papillae in *S. achyranthesae*, but one setose and one asetose papillae in *S. solidaginis*; the anal opening is simple in *S. achyranthesae*, while branched in *S. solidaginis.* Then, *S. achyranthesae* can be separated from *S. asteris* by the following features: female cerci is less divided in *S. achyranthesae*; dorsal setae are present on the gonostylus in *S. achyranthesae*, but absent in *A. asteris*; and the gonocoxite is only slightly extends ventrally beyond the gonostylus in *S. achyranthesae,* and the larval anal opening is simple in *S. achyranthesae* while branched in *S. asteris*.

Schizomyia diplocyclosae Elsayed & Tokuda, sp. n.

http://zoobank.org/C884D6A9-466D-45A8-9C63-A772B85E2539
Figs 18–29; Table S1

Characters given in *S. achyranthesae* except for the following:

Etymology. The species name, *diplocyclosae*, is based on the generic name of the host plant, *Diplocyclos palmatus* (Cucurbitaceae).



Figures 18–24. *Schizomyia diplocyclosae.* **18** Head **19** Ventral view of female flagellomere V **20** Dorsal view of male flagellomere V **21** Wing **22** Tarsomere V and acromere **23** Terminal part of female abdomen **24** Ovipositor apex Scale bars: 100 µm (**18, 19**), 50 µm (**20–24**).

Type material. *Holotype*: 1♂ (KUEC): reared from a larva obtained from a flower bud gall on *D. palmatus*, collected from Hantagawa, Naha City, Okinawa Prefecture, Japan on 10.ii.2016, T. Ganaha-Kikumura leg., emerged on 14.iii.2016. *Paratypes*: All paratypes were reared from flower bud galls on *D. palmatus* in Japan. 4 larvae: collected from Gogayama, Nakijin Village, Okinawa Prefecture on 4.iii.2002, M. Tokuda leg., departed from galls on 9.iii.2002; 2 larvae: collected from Kinjo cho, Naha City, Okinawa Prefecture on 13.i.1977, S. Yamauchi leg.; 4 pupal exuviae: collected from Shuri, Naha City, Okinawa Prefecture, emerged in February 1977, S. Yamauchi leg.; 2 pupal exuviae, 3♂, 5♀: same data as holotype; 5 pupal exuviae, 3♂, 2♀: collected from Hantagawa, Naha City, Okinawa Prefecture on 10.ii.2016, T. Ganaha-Kikumura leg., emerged on 15.iii.2016; 1 pupal exuviae: collected from Hantagawa, Naha City, Okinawa Prefecture on 10.ii.2016, T. Ganaha-Kikumura leg., emerged on 16.iii.2016; 1 pupal exuviae: collected from Hantagawa, Naha City, Okinawa Prefecture on 10.ii.2016, T. Ganaha-Kikumura leg., emerged on 21.iii.2016.

Description. *Head* (Fig. 18): Fronto-clypeus with 17–24 setae (n = 6). Palpus: first segment ca 38.3 μm, second 1.4 times as long as the first, third 1.3 as long as the second, fourth 1.5 as long as the third.

Thorax: Wing (Fig. 21) length 2.15–2.26 mm (n = 5) in female, 1.70–2.12 (n = 5) in male. Empodia slightly longer than tarsal claws (Fig. 22). Anepimeral setae 11–18 (n = 8); mesanepisternum scales 20–38 (n = 8); lateral scutum setae 26–48 (n = 8). Lengths of leg segments as in Suppl. material 1: Table S1.

Female abdomen (Figs 23, 24): Posterior margin of tergite VIII with a pair of slightly developed dorsal lobes. Sternite VII about 2.5 times as long as preceding sternites. Ovipositor: distal protrusible needle-like portion about 3 times as long as sternite VII.

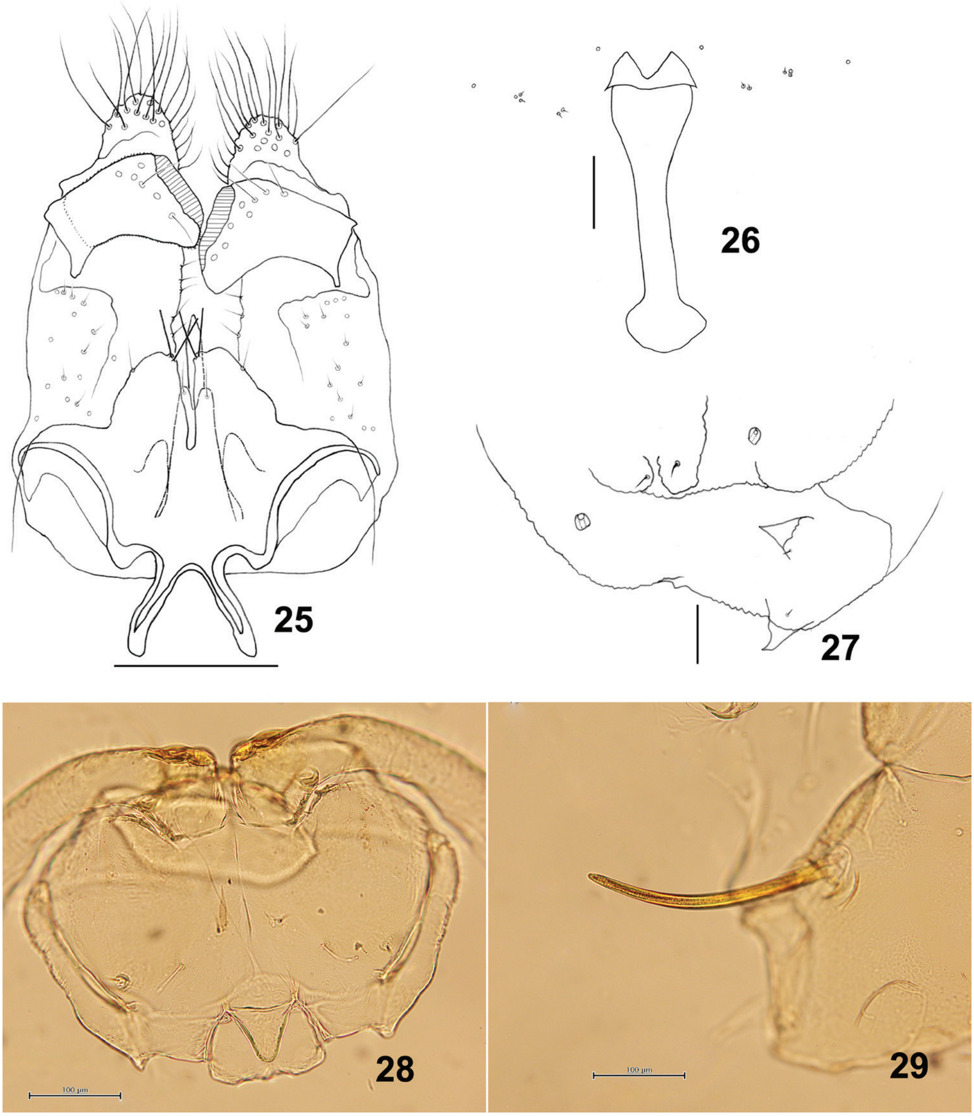
Male abdomen: Terminalia (Fig. 25): Gonostylus dorsally with several setae on distal half.

Mature larva: Sternal spatula with posterior portion about 2.8 times as wide as the base of the anterior free portion (Fig. 26). Larval anus with 2 asetose anal papillae.

Pupa (Figs 28, 29): Prothoracic spiracle 280–310 μm long (n = 6).

Distribution. Japan: Kikaijima Island and Okinawa-honto Island (Yukawa and Masuda 1996).

Gall and life history. *Schizomyia diplocyclosae* induces subglobular and pale green flower bud galls on *D. palmatus*, about 6–10 mm in diameter. Each gall consists of 10–45 chambers and each chamber contains a single larva [Gall No. C-409 in Yukawa and Masuda (1996)]. Galls become mature between December and March and larvae depart from galls to drop to the ground. The adults of *S. diplocyclosae* emerge in February and March when the larvae were reared under laboratory temperature (Yukawa and Masuda 1996, present data). Similar flower bud galls were found on *Melothria liukiuensis* Nakai (Cucurbitaceae) and considered to be induced by this species or a closely related one (Yamauchi et al. 1982, Yukawa and Masuda 1996).



Figures 25–29. *Schizomyia diplocyclosae*. **25** Male terminalia **26** Larval spatula **27** Terminal larval segments dorsally **28** Ventral view of pupal head **29** Prothoracic spiracle in pupa. Scale bars: 50 µm (**25–27**), 100 µm (**28, 29**).

Remarks. *Schizomyia diplocyclosae* is morphologically very similar to *S. achyranthesae* but differs from it by the following characters: *S. diplocyclosae* has a shorter ovipositor (protrusible needle-like-portion three times as long as sternite VII while four times as long in *S. achyranthesae*), less developed dorsal lobes on the posterior margin of female tergite VIII, gonocoxite more pointed posteroapically, empodia longer than claws and larva with only two anal papillae (four in *S. achyranthesae*).

Schizomyia castanopsisae **Elsayed & Tokuda, sp. n.**
http://zoobank.org/30620DFD-56AD-4C7D-B1FE-035383F80721
Figs 30–42; Table S2

Characters as in *S. achyranthesae* except for the following:

Etymology. The species name, *castanopsisae,* is based on the generic name of the host plant, *Castanopsis sieboldii* (Fagaceae).

Type material. *Holotype*: 1♂ (KUEC): reared from a larva obtained from an inflorescence gall on *C. sieboldii* by A. K. Elsayed, collected from Hachijojima Island on 6.xii.2014, T. Kikuchi leg., emerged on 24.ii.2015. *Paratypes*: All paratypes were reared by A. K. Elsayed from inflorescence galls on *C. sieboldii* in Japan. 2 larvae: collected from Hachijojima Island on 6.xii.2014, T. Kikuchi leg., departed from galls on 22.xii.2014; 5 larvae: collected from Hachijojima Island on 6.xii.2014, T. Kikuchi leg., departed from galls on 25.xii.2014; 6 pupal exuviae, 2♀, 3♂: collected from Shikinejima Island on 10.xii.2014, M. Tokuda leg., emerged between 24.i–20.ii.2015; 3 pupal exuviae, 4♀, 2♂: collected from Hachijojima Island on 6.xii.2014, T. Kikuchi leg., emerged between 20.ii–5.iii.2015.

Description. *Head* (Fig. 30): Fronto-clypeus with 10–16 setae (n = 6). Palpus: first segment ca 43 μm, second 1.4 times as long as the first, third 1.5 as long as the second, fourth 1.2 as long as the third.

Thorax: Wing (Fig. 33) length 2.04–2.74 mm (n = 6) in female, 2.04–2.56 mm (n = 4) in male. Anepimeral setae 8–15 (n = 8); mesanepisternum scales 15–26 (n = 7); lateral scutum setae 15–27 (n = 8). Lengths of leg segments as in Suppl. material 1: Table S2.

Female abdomen (Figs 35, 36): Sternite VII about 3 times as long as preceding. Ovipositor: protrusible needle-like portion about 3.3 as long as sternite VII.

Male abdomen: Terminalia (Figs 37, 38): Gonocoxite length about 3.3 times as long as gonostylus.

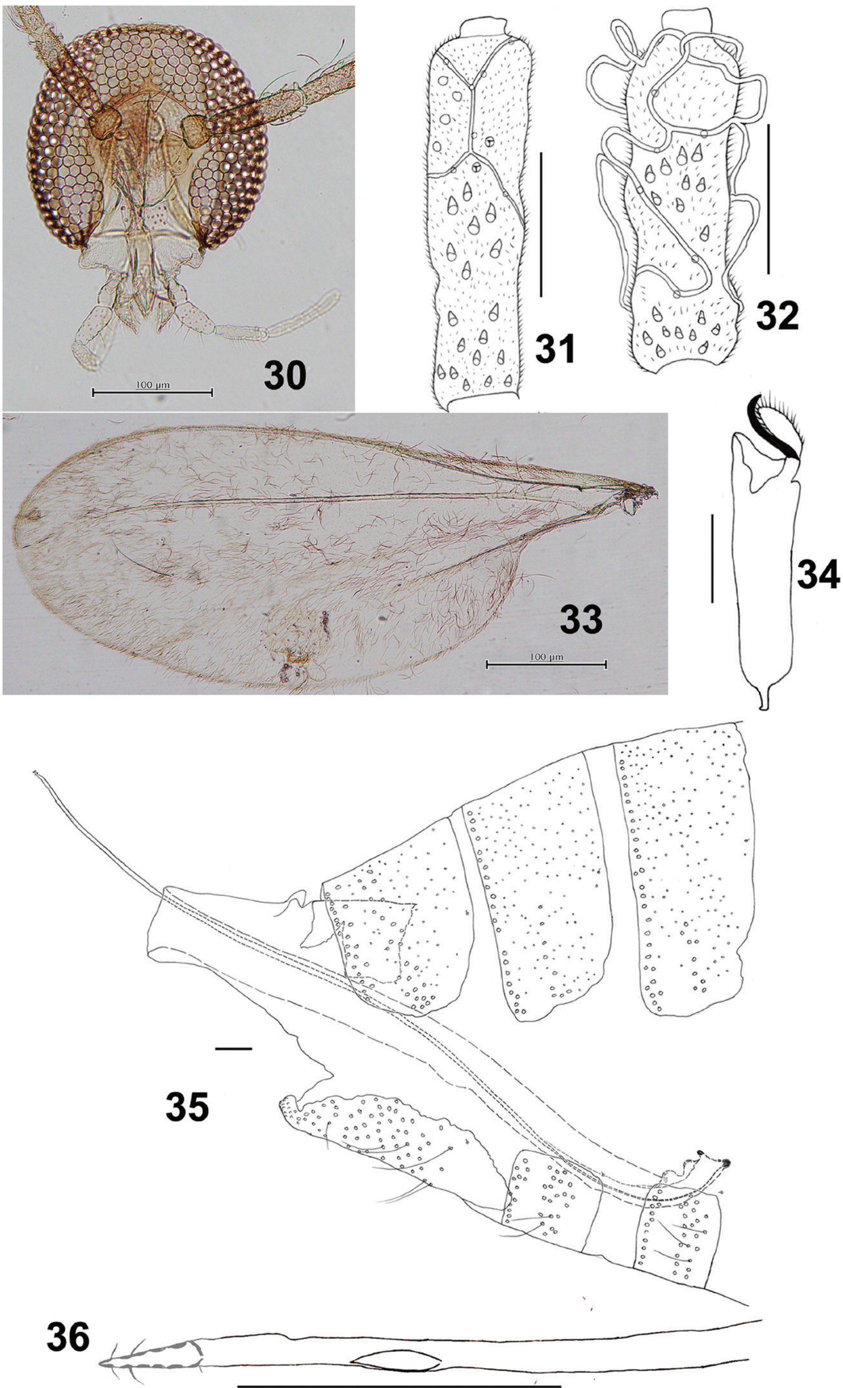
Mature larva: Sternal spatula (Fig. 39) with posterior portion about 2.8 times as wide as the base of the anterior free portion. Anus opening branched (Fig. 40).

Pupa (Fig. 42): Prothoracic spiracle 280–330 μm long (n = 6).

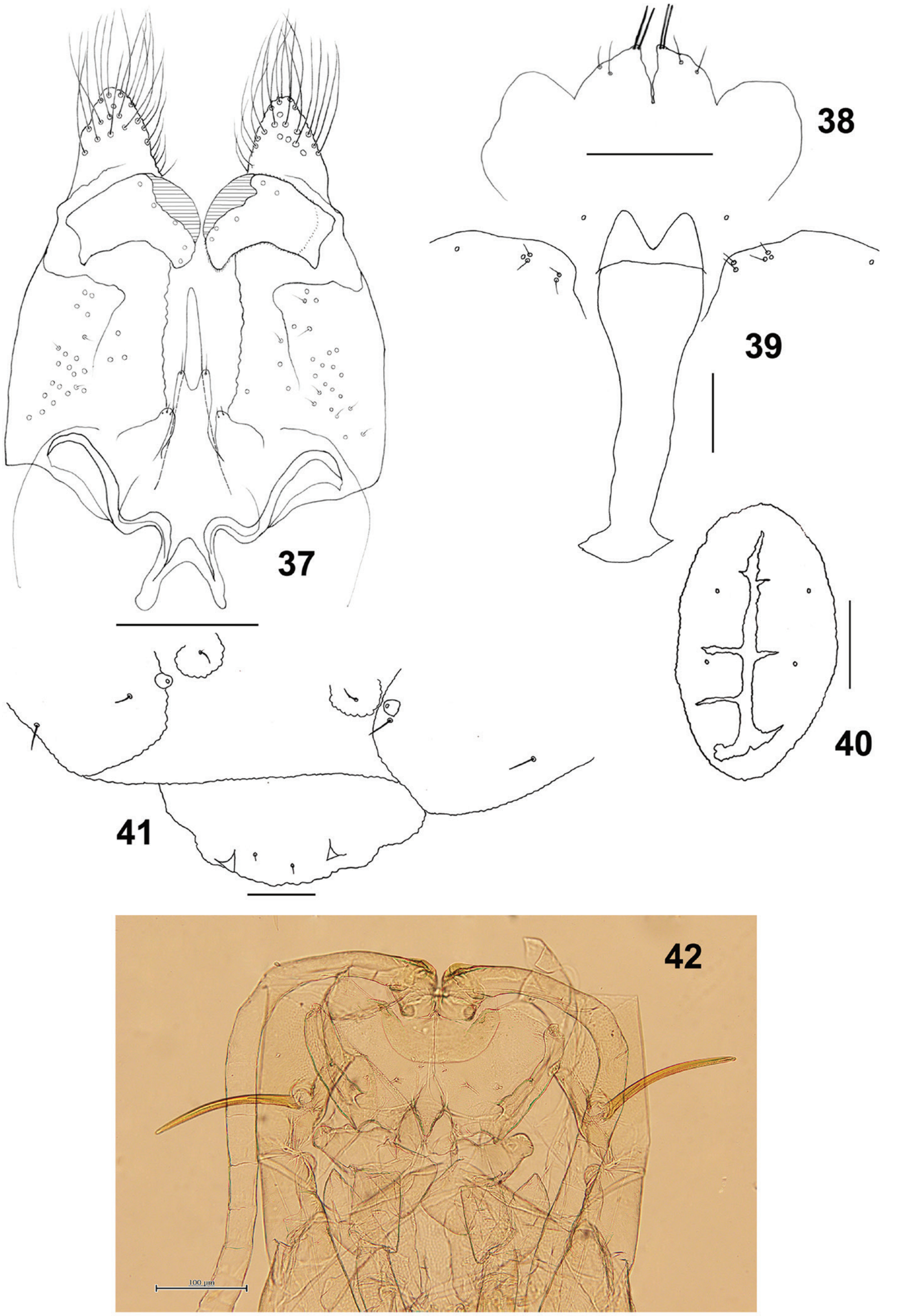
Distribution. Japan: The Izu Islands (from Niijima to Aogashima) (Tokuda et al. 2012a, b, 2013, 2015, Tokuda and Kawauchi 2013a), Kyushu (Kagoshima and Miyazaki Prefectures) (Nagai 2010, Tokuda and Kawauchi 2013b), Tanegashima Island (Yukawa et al. 2013) and Okinawa-honto Island (Yamauchi et al. 1982).

Gall and life history. *Castanopsis sieboldii* inflorescences galled by *S. castanopsisae* are rather irregularly swollen, 5.7–15.7 mm in diameter and 6.2–30.9 mm in length (Fig. 2) [Gall No. C-163 in Yukawa and Masuda (1996)]. Each gall consists of up to 30 chambers and each chamber contains a single larva. Mature larvae of *S. castanopsisae* left the galls collected in the Izu Islands in December within few days after the collection. Larvae were kept with soil in the laboratory and adults emerged the following January, February and March.

Remarks. *Schizomyia castanopsisae* is morphologically close to *S. asteris*, *S. achyranthesae* and *S. diplocyclosae*. *Schizomyia castanopsisae* can be separated from *S. asteris* by a



Figures 30–36. *Schizomyia castanopsisae.* **30** Head **31** Ventral view of female flagellomere V **32** Ventral view of male flagellomere V **33** Wing **34** Tarsomere V and acromere **35** Terminal part of female abdomen **36** Ovipositor apex. Scale bars: 50 µm (**31, 32, 34–36**), 100 µm (**30, 33**).



Figures 37–42. *Schizomyia castanopsisae*. **37** Male terminalia **38** Male cerci **39** Larval spatula **40** Larval anus **41** Terminal larval segments dorsally **42** Ventral view of pupal head and thorax. Scale bars: 50 µm (**37–41**), 100 µm (**42**).

shorter ovipositor (protrusible needle-like portion 3.3 times as long as sternite VII, while 5.7 times in *S. asteris*), the presence of dorsal setae on the gonostyli, and the tooth of gonostylus, which extends more dorsally than in that of *S. asteris*; from *S. achyranthesae* by a shorter ovipositor (four times as long as sternite VII in *S. achyranthesae*), more posteroapically pointed gonocoxite, and branched anal opening of larva; and from *S. diplocyclosae* by shorter empodia than tarsal claws (empodia are as long as claws in *S. diplocyclosae*) and the number of larval anal papillae (four in *S. castanopsisae* while two in *S. diplocyclosae*).

Schizomyia usubai **Elsayed & Tokuda, sp. n.**

http://zoobank.org/ACDD6BD7-7BAD-4330-8A79-03066DACA3F1
Figs 43–55; Table S2

Characters as in *S. achyranthesae* except for the following:

Etymology. The species name, *usubai*, honors the late Mr Shigeshi Usuba who reared adults of this species for the first time.

Type material. *Holotype*: 1♂ (KUEC): reared by A. K. Elsayed from a larva obtained from a fruit gall on *T. asiaticum*, collected from Torinosu, Tanabe City, Wakayama Prefecture, Japan, I. Matoba leg., emerged on 22.v.2017. *Paratypes*: All paratypes were reared from fruit galls on *T. asiaticum* in Japan. 4 larvae: collected from Mount Takakuma, Kagoshima Prefecture in 1969, J. Yukawa leg.; 4 larvae: galls collected from Imuta Lake-side, Kedouin, Satsuma-sendai City, Kagoshima Prefecture on 2.xi.1978, S. Sako leg.; 4 pupal exuviae, 2♂, 2♀: collected from Torinosu, Tanabe City, Wakayama Prefecture, I. Matoba leg., reared by A. K. Elsayed, emerged on 18.v.2017; 2 pupal exuviae, 1♀, 2♂: same data as holotype.

Description. *Head* (Fig. 43): Compound eyes separated on vertex by a diameter of 0.0–0.5 facets. Fronto-clypeal setae 15–16 setae (n = 4). Palpus: first segment ca 53.5 µm, second about as long as the first, third 1.6 as long as the second, fourth 1.4 as long as the third.

Thorax: Wing (Fig. 46) length 2.03–2.34 mm (n = 4) in female, 1.80–1.95 mm (n = 3) in male. Empodia as long as claws (Fig. 47). Anepimeral setae 10–20 (n = 4); mesanepisternum scales 17–40 (n = 4); lateral scutum setae 19–27 (n = 4). Lengths of leg segments as in Suppl. material 1: Table S2.

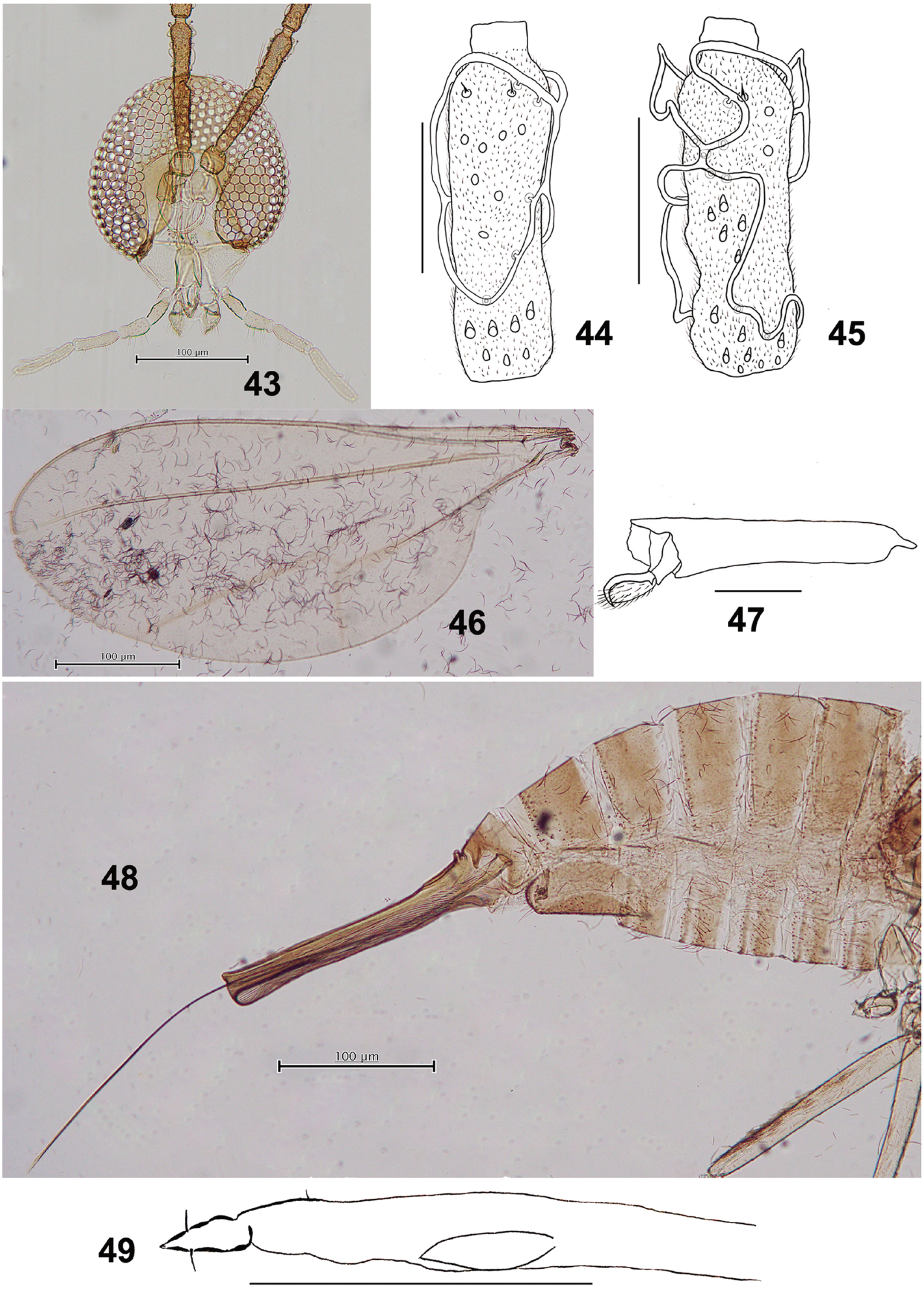
Female abdomen (Figs 48, 49): Sternite VII about 2.6 times as long as preceding. Ovipositor: protrusible needle-like portion about 4.5 as long as sternite VII.

Male abdomen: Terminalia (Fig. 50): Gonocoxite with developed, pointed apical lobe extending beyond gonostylus.

Mature larva: Sternal spatula (Fig. 51) with posterior portion about 3.5 times as wide as the base of the anterior free portion. Anus with branched opening (Fig. 52).

Pupa (Figs 54, 55): Prothoracic spiracle 250–350 µm long (n = 6).

Distribution. Japan: The Izu Islands (Tokuda et al. 2012b, 2013, Tokuda and Kawauchi 2013) Honshu, and Kyushu (Yukawa and Masuda 1996).



Figures 43–49. *Schizomyia usubai.* **43** Head **44** Ventral view of female flagellomere V **45** Ventral view of male flagellomere V **46** Wing **47** Tarsomere V and acromere **48** Terminal part of female abdomen **49** Ovipositor apex. Scale bars: 50 µm (**44, 45, 47, 49**), 100 µm (**43, 46, 48**).

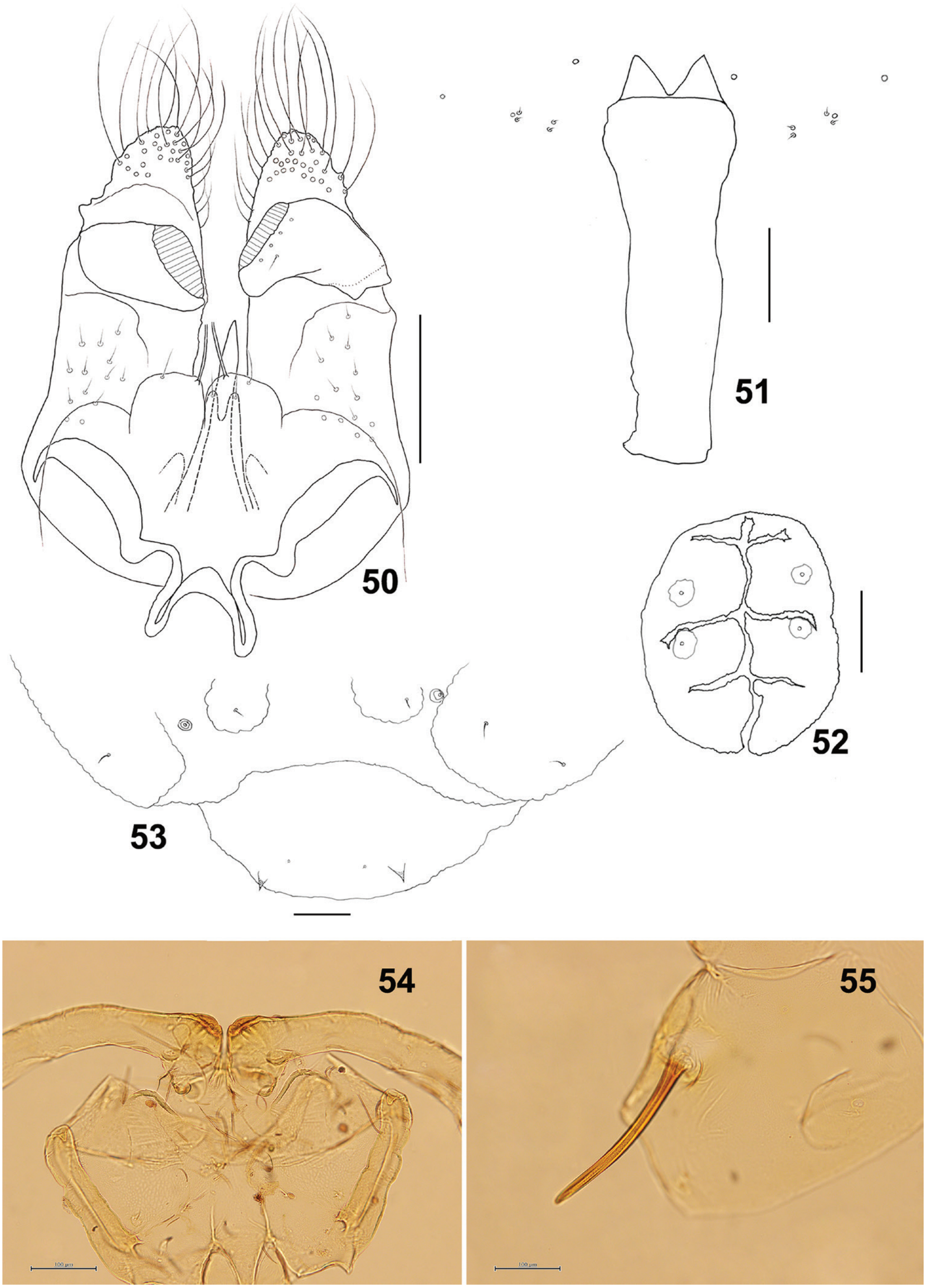


Figure 50–55. *Schizomyia usubai*. **50** Male terminalia **51** Larval spatula **52** Larval anus **53** Terminal larval segments dorsally **54** Ventral view of pupal head **55** Pupal prothoracic spiracle. Scale bars: 50 µm (**50–53**), 100 µm (**54, 55**).

Gall and life history. The normal fruit of *Trachelospermum asiaticum* (Apocynaceae) is V-shaped, consisting of a pair of very long and thin seed pods. When the fruits are galled by *S. usubai*, the apical parts of the fruit become fused and swollen, more or less cat-bell shaped (Fig. 3), about 12–18 mm in diameter and 27 mm in length [Gall No. D-033 in Yukawa and Masuda (1996)]. Each gall consists of 10–25 chambers and each chamber contains 10–25 larvae. Galls mature between late September and October and the larvae depart from galls to overwinter in soil. The adults of *S. usubai* emerge between late April and July (Yukawa 1978; Yukawa and Masuda 1996; present study). Similar galls probably induced by this species were found on *Trachelospermum gracilipes* Hook. f. var. *kiukiuense* (Hatus.) Kitam. on Tanegashima Island (Yukawa et al. 2013).

Remarks. *Schizomyia usubai* is close to *S. asteris*, *S. achyranthesae*, *S. diplocyclosae* and *S. castanopsisae*. *Schizomyia usubai* can be distinguished from *S. asteris* by a shorter ovipositor (protrusible needle-like portion about 4.5 times as long as sternite VII, while 5.5 times in *S. asteris*), longer empodia, and the presence of dorsal setae on gonostyli; from *S. achyranthesae* and *S. diplocyclosae* by a longer ovipositor (four and three times as long as sternite VII in *S. achyranthesae* and *S. diplocyclosae,* respectively), longer empodia, and branched opening of the larval anus. In addition, larva of *S. usubai* has four anal papillae, but two in *S. diplocyclosae*. *Schizomyia castanopsisae* is very similar to *S. usubai*, but can be separated by a shorter ovipositor (protrusible needle-like portion about 3.3 times as long as sternite VII, while 4.5 times in *S. usubai*), longer empodia, and less compressed circumfila of female flagellomeres.

Schizomyia paederiae Elsayed & Tokuda, sp. n.

http://zoobank.org/DE35F88A-484D-45CE-9BC2-4356E0A1DE97

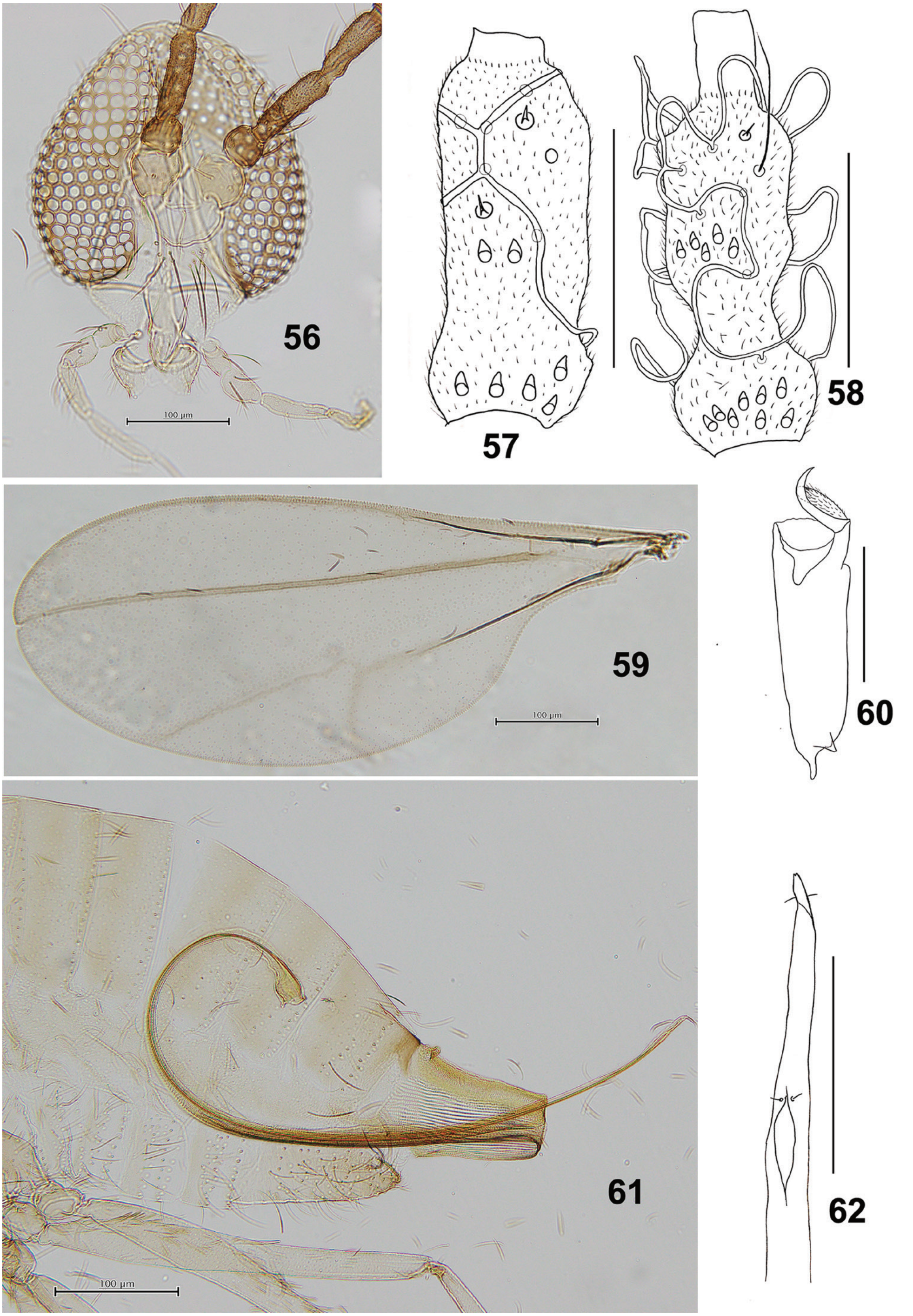
Figs 56–67; Tables S3

Characters as in *S. achyranthesae* except for the following:

Etymology. The species name, *paederiae,* is based on the generic name of the host plant, *Paederia foetida* (Rubiaceae).

Type material. *Holotype*: 1♂ (KUEC): reared from a larva obtained from a flower bud gall on *P. foetida*, collected from Misawa, Ogori City, Fukuoka Prefecture, Japan, K. Matsunaga leg., emerged between 11–15.viii.2017. *Paratypes*: All paratypes were reared from flower bud galls on *P. foetida* in Japan. 11 larvae: collected from Nishinoomote, Nishinoomote City, Kagoshima Prefecture, on 24.ix.2014, K. Ogata leg.; 4 pupal exuviae, 2♂, 7♀: same data as holotype.

Description. *Head* (Fig. 56): Compound eyes separated on vertex by a diameter of 0.0–1.5 facets, eye bridge consist of 6–7 facets long. Fronto-clypeus with 11–13 setae (n = 4). Palpus: first segment ca 28.6 µm, second 1.3 times as long as the first, third 1.4 as long as the second, fourth 1.2 as long as the third.Male flagellomeres with deep basal constriction and elongated necks (Fig. 58).



Figures 56–62. *Schizomyia paederiae*. **56** Head **57** Dorsal view of female flagellomere V **58** Dorsal view of female flagellomere V **59** Wing **60** Tarsomere V and acromere **61** Terminal part of female abdomen **62** Ovipositor apex. Scale bars: 50 µm (**57, 58, 60, 62**), 100 µm (**56, 59, 61**).

Thorax: Wing (Fig. 59) length 1.16–1.57 mm (*n* = 5) in female, 1.04–1.36 mm (*n* = 3) in male. Anepimeral setae 9 or 10 (*n* = 5); mesanepisternum scales 5–10 (*n* = 6); lateral scutum setae 23–28 (*n* = 5). Empodia as long as tarsal claws (Fig. 60). Lengths of leg segments as in Suppl. material 1: Table S3.

Female abdomen (Figs 61, 62): Anterior pair of trichoid sensilla situated medially on abdominal sternites II–VI; sternite VII about 3.4 times as long as preceding sternites. Ovipositor: protrusible needle-like portion about 4.8 times as long as sternite VII.

Male abdomen: Anterior pair of trichoid sensilla situated medially on sternites II–VI and laterally on sternite VIII, sternite VIII with scattered setae. Terminalia (Fig. 63): Gonostylus dorsally with several setae on distal half, with unfused and compressed denticles.

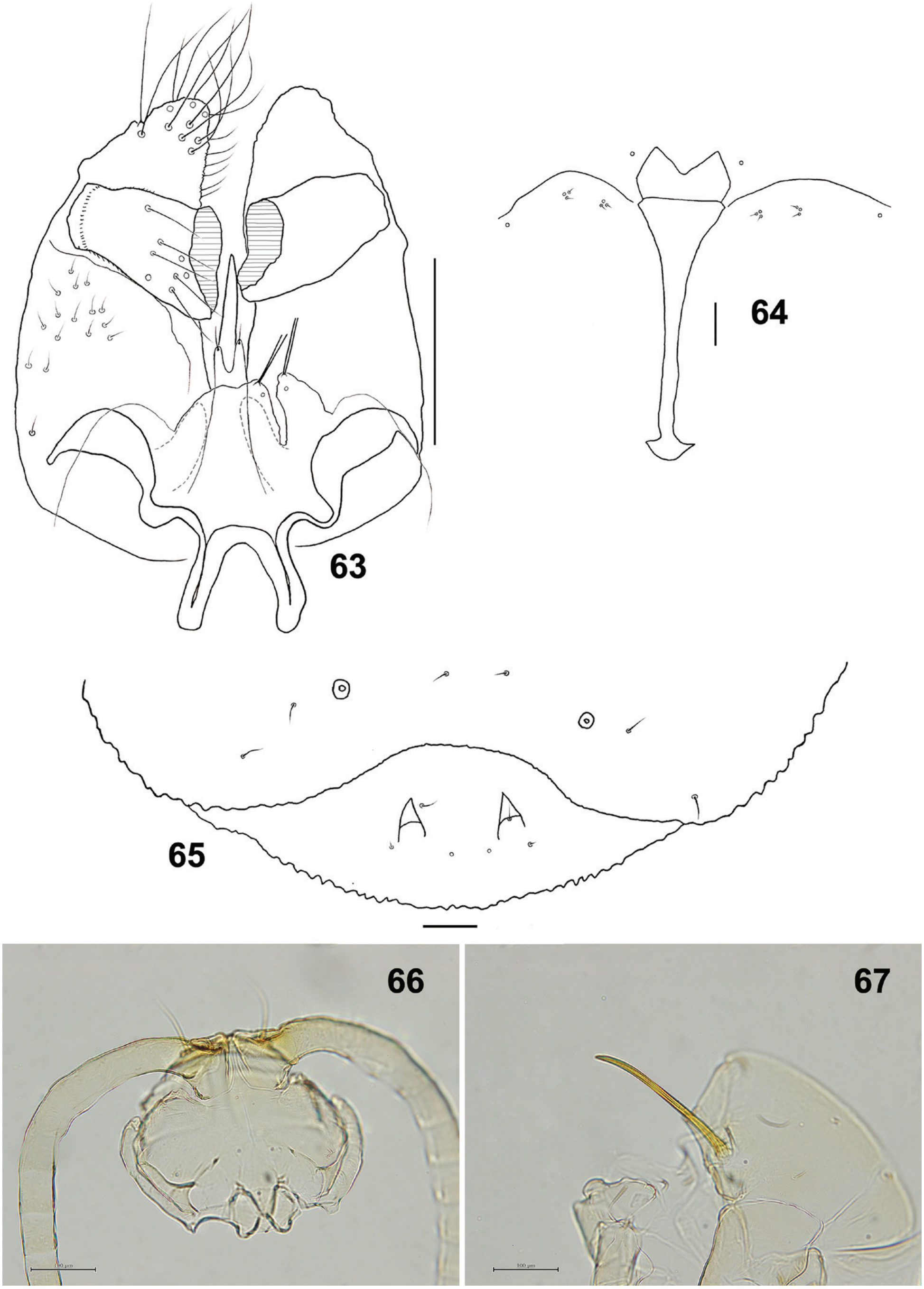
Mature larva: Abdominal segment VIII with 2 setose dorsal papillae. Posterior portion of sternal spatula about 3.3 times as wide as the base of the anterior free portion (Fig. 64); 2 groups of lateral papillae present on all thoracic segments, each consisting of 2 setose and 1 asetose papillae. Terminal segment with 8 terminal papillae, consisting of 4 setose, 2 asetose and 2 corniform ones (Fig. 65).

Pupa (Figs 66, 67): Prothoracic spiracle 230–290 μm long (*n* = 4).

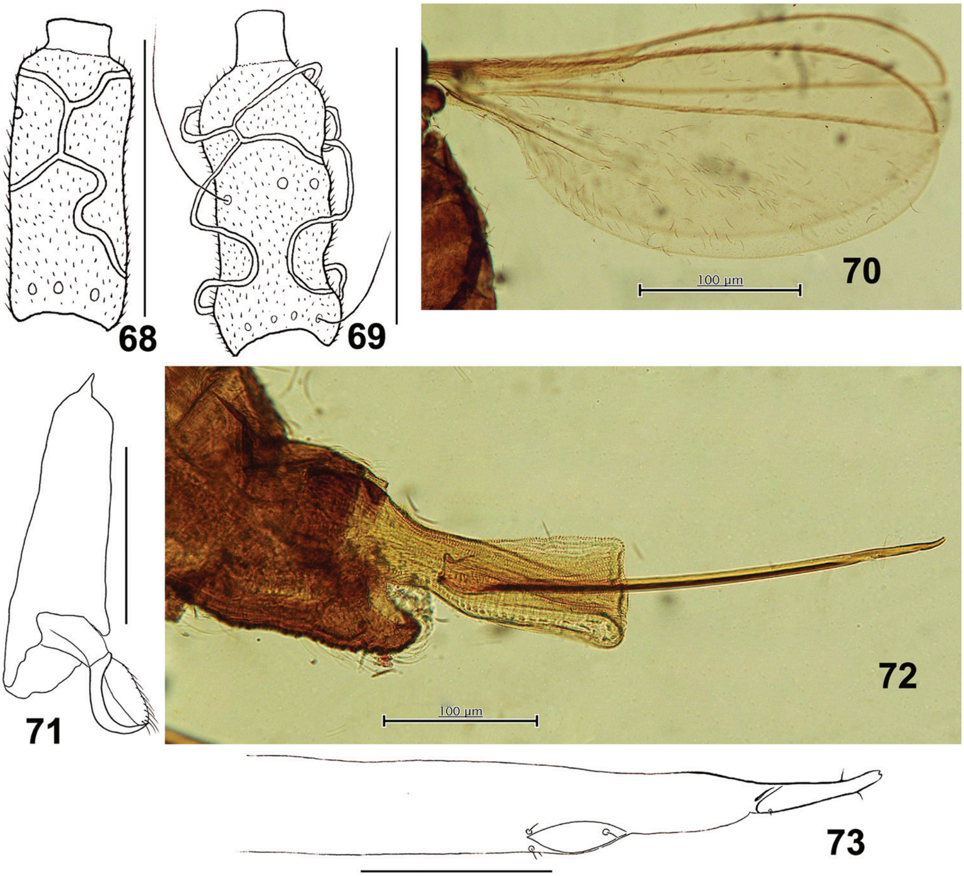
Distribution. Japan: Honshu, Shikoku, Kyushu, and Yakushima Island (Yukawa and Masuda 1996).

Gall and life history. *Schizomyia paederiae* induces flower bud galls on *P. foetida*. Basal parts of the galled flower buds are swollen, 3.0–5.6 mm in diameter and 4.0–6.1 mm in length (Fig. 4) [Gall No. D-037 in Yukawa and Masuda (1996)]. Galls are single-chambered and each gall contains 1–10 larvae. The larvae depart from mature galls from late August to September and overwinter in the soil. The adults of *S. paederiae* emerge in early August when the flower buds are available on the host plant (Yukawa and Masuda 1996).

Remarks. *Schizomyia paederiae* is distinguishable from other *Schizomyia* species, except four Russian species, i.e. *S. calathidiphaga*, *S. clematidis*, *S. spiraeae*, and *S. veronicastrum*, by its deeply constricted male flagellomeres (Kovalev 1964; Fedotova 2002). Firstly, the adults of *S. paederiae* differs from *S. calathidiphaga* by a slightly longer ovipositor (protrusible needle-like portion about 4.8 times as long as sternite VII, while 4.5 times in *S. calathidiphaga*), longer empodia (empodia are as long as claws in *S. paederiae*, but shorter in *S. calathidiphaga*), the position of gonostylus tooth (mostly covering only the apical margin in *S. paederiae*, but on the posteroapical margin in *S. calathidiphaga*), and the arrangement of papillae on the larval terminal segment (the two asetose terminal papillae are situated more posteriorly in *S. paederiae*, while more anteriorly in *S. clathidiphaga*). Then, the adults of *S. paederiae* can be separated from *S. clematidis*, *S. spiraeae* and *S. veronicastrum* by a longer neck of male flagellomere III, which is about 0.25 as long as node in *S. paederiae* but about 0.15 as long as node in other species, the position of gonostylus tooth (mostly covering the apical margin in *S. paederiae*, but on the posteroapical margin in the other species), and a much narrower hypoproct than *S. clematidis*.



Figures 63–67. *Schizomyia paederiae*. **63** Male terminalia **64** Larval spatula **65** Terminal larval segments dorsally **66** Ventral view of pupal head **67** Pupal prothoracic spiracle. Scale bars: 50 µm (**63–65**), 100 µm (**66, 67**).



Figures 68–73. *Schizomyia galiorum*. **68** Lateral view of female flagellomere V **69** Lateral view of male flagellomere V **70** Wing **71** Tarsomere V and acromere **72** Terminal part of female abdomen showing the ovipositor **73** Ovipositor apex. Scale bars: 50 µm (**68, 69, 71, 73**), 100 µm (**70, 72**).

Schizomyia galiorum Kieffer, 1889

Figs 68–76; Table S3

Characters as in *S. achyranthesae* except for the following:

Material examined. 2♂, 3♀ (Mamaev collection: slide no. B1-251369): collected from Rybatskij, Lithuania on 19.vii.1969; 1♀, 1 pupal exuviae (J. J. Kieffer's specimen in Felt collection).

Description. *Head*: Compound eyes with rounded facets; facets on vertex and eye bridge unobservable because the specimens mounted laterally. Palpus: first segment ca 23.4 µm, second 1.6 times as long as the first, third 1.4 as long as the second, fourth 1.4 as long as the third.

Thorax: Wing (Fig. 70) length 1.33–1.55 mm (n = 2) in male, 1.93 mm (n = 1) in female; R_5 joining C just before wing apex. Empodia as long as claws (Fig. 71).

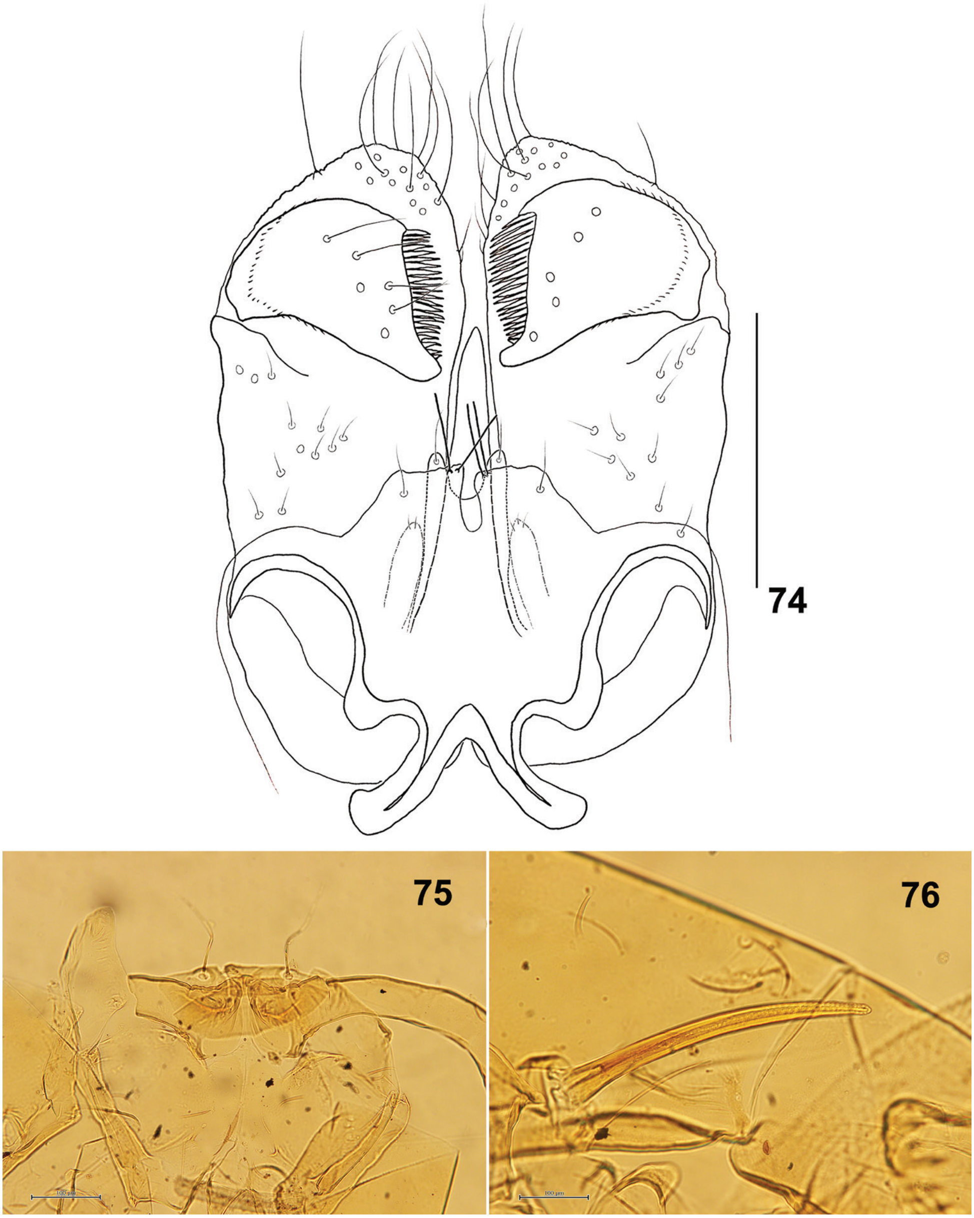


Figure 74–76. *Schizomyia galiorum*. **74** Male terminalia **75** Ventral view of pupal head **76** Pupal prothoracic spiracle. Scale bars: 50 µm (**74**), 100 µm (**75, 76**).

Anepimeral setae 9–15 (*n* = 5); mesanepisternum scales 14–18 (*n* = 4); lateral scutum setae 17–37 (*n* = 5). Lengths of leg segments as in Suppl. material 1: Table S3.

Female abdomen: Posterior margin of tergite VIII without dorsal lobes. Sternites with median pair of trichoid sensilla. Sternite VII about twice as long as VI. Ovipositor (Figs 72, 73): protrusible needle-like portion short, about 1.9 times as long as sternite VII; cerci fused, with few fine setae (Fig. 73).

Male abdomen: Sternites with median pair of trichoid sensilla. Sternite VII with two posterior rows of setae. Terminalia (Fig. 74): Gonocoxite with slightly developed apical lobe. Gonostylus with several setae on distal half dorsally, with group of setae on the basal half ventrally, and with distinctive unfused denticles. Hypoproct slightly longer than cerci.

Mature larva: Sternal spatula bilobed, the anterior free portion slightly wider than the posterior portion (Kieffer 1889). Two groups of lateral papillae present on each side of the spatula, each group of 2 setose and 1 asetose papillae. Terminal segment with 6 setose and 2 corniform terminal papillae (Möhn 1955).

Pupa (Figs 75, 76): Antennal horns slightly developed. Prothoracic spiracle 240 µm long (n = 1).

Distribution. Widespread Europe, Algeria and Kazakhstan (Gagné and Jaschhof 2017).

Remarks. This species is distinguished from eastern Holarctic congeners by the distinctly short ovipositor, the absence of dorsal lobes on the posterior margin of female tergite VIII, and the conjunction of wing vein C with R_5 before wing apex.

***Schizomyia patriniae* Shinji, 1938 comb. rev.**
Figs 77–85; Table S4

Schizomyia patriniae Shinji, 1938: 372.
Asphondylia partriniae Shinji, 1944: 376, missp. of *patriniae*.
Asteralobia patriniae (Shinji, 1938)

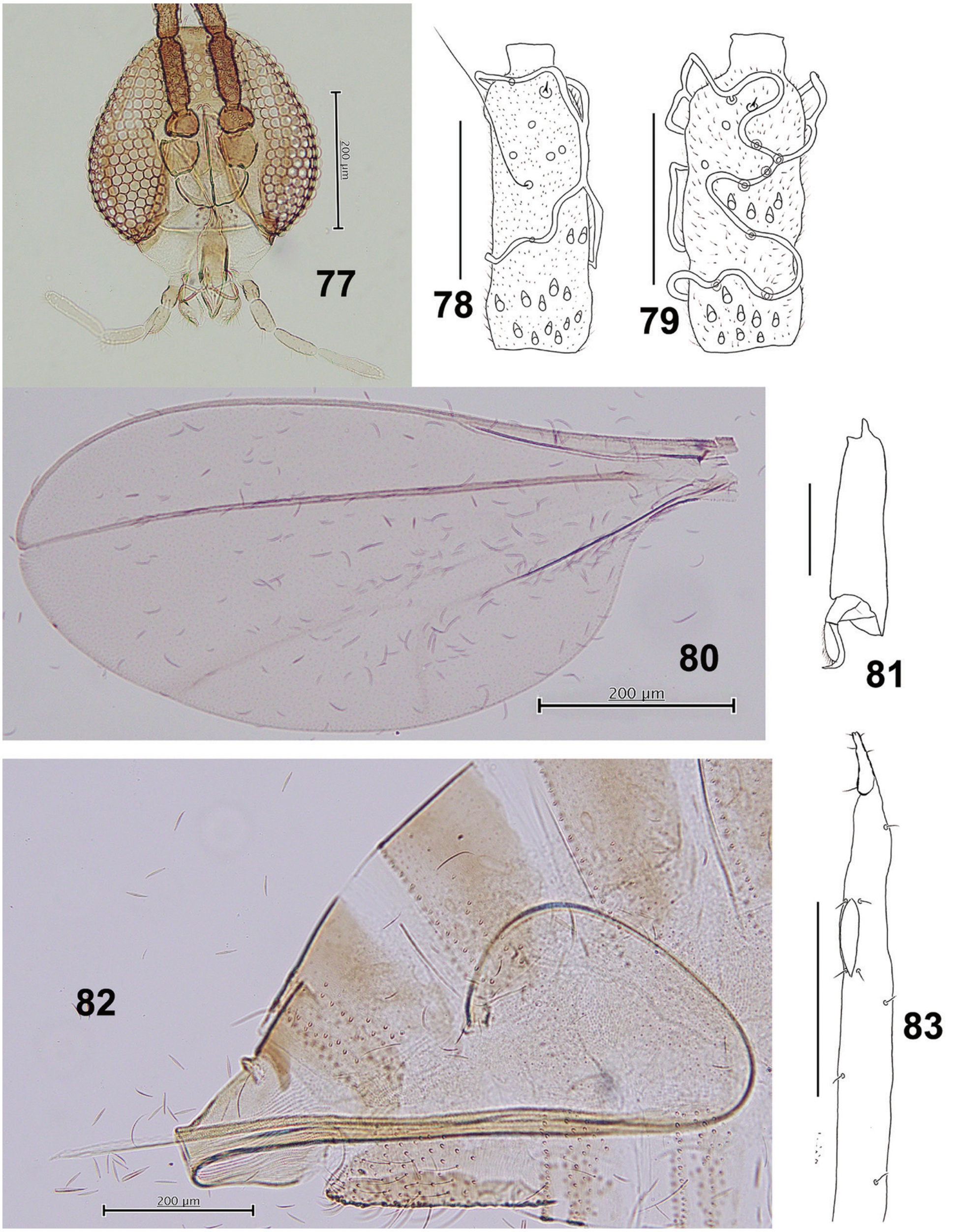
Characters as in *S. achyranthesae* except for the following:

Material examined. All obtained from flower bud galls on *Patrinia villosa* (Valerianaceae) in Japan. 6 larvae: collected from Maruyama, Ojiya City, Niigata Prefecture on 12.x.1981, K. Yamagishi leg. 1♂: Iozan, Kanazawa City, Ishikawa Prefecture on 17.x.1978, emerged on 22.iv.1979, J. Yukawa leg. 2♂, 4♀, 2 pupal exuviae: collected from Kyuragi, Karatsu City, Saga Prefecture on 12.x.2015, M. Tokuda leg., emerged on 23.viii.2016, reared by A. K. Elsayed. 2♂, 3♀, 2 pupal exuviae: collected from Kyuragi, Karatsu City, Saga Prefecture on 12.x.2015, M. Tokuda leg., emerged on 28.viii.2016, reared by A. K. Elsayed. 2♂: collected from Kyuragi, Karatsu City, Saga Prefecture on 12.x.2015, M. Tokuda leg., emerged on 29.viii.2016, reared by A. K. Elsayed.

Description. *Head* (Fig. 77): Compound eyes separated on vertex by a diameter of 0.25–1 facets, eye bridge consist of 5–6 facets long. Fronto-clypeus with 15–20 setae (n = 9). Palpus: first segment ca 34.1 µm, second 1.4 times as long as the first, third 1.4 as long as the second, fourth segment 1.5 as long as the third.

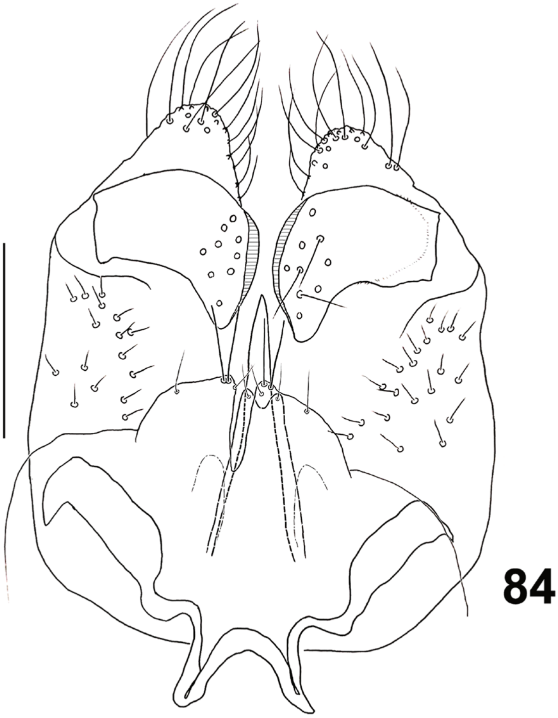
Thorax: Wing (Fig. 80) length 1.83–2.09 mm (n = 5) in female, 1.50–1.83 mm (n = 5) in male. Anepimeral setae 17–23 (n = 8); mesanepisternum scales 16–20 (n = 7); lateral scutum setae 32–46 (n = 8). Lengths of leg segments as Suppl. material 1: Table S4.

Female abdomen (Figs 82, 83): Median pair of trichoid sensilla present on sternites II–VII. Sternite VII about 3 times as long as preceding. Ovipositor: protrusible needle-like portion about 4.8 as long as sternite VII.



Figures 77–83. *Schizomyia patriniae*. **77** Head **78** Dorsal view of female flagellomere V **79** Ventral view of female flagellomere V **80** Wing **81** Tarsomere V and acromere **82** Terminal part of female abdomen **83** Ovipositor apex. Scale bars: 50 µm (**78, 79, 81, 83**), 200 µm (**77, 80, 82**).

Male abdomen: Trichoid sensilla present on sternites II–VIII in median position, except on VIII in lateral position. Terminalia (Fig. 84): Gonocoxite length about 3.3 times as long as gonostylus. Gonostylus with slim tooth in the dorsal and ventral views.



Figures 84–85. *Schizomyia paederiae*. **84** Male terminalia **85** Ventral view of pupal head and prothorax. Scale bars: 50 µm (**84**), 200 µm (**85**).

Mature larva: Abdominal segment VIII without dorsal papillae. Two groups of lateral papillae on all thoracic segments, each consisting of 2 setose and 1 asetose papillae. The terminal segment with 1 setose and 6 asetose terminal papillae (Yukawa 1983).

Pupa (Fig. 85): Prothoracic spiracle 260–290 µm long (n = 5).

Distribution. Japan: Hokkaido, Honshu and Shikoku (Yukawa and Masuda 1996).

Remarks. This species had been described by Shinji (1938) under the genus *Schizomyia*. Then, Yukawa (1983) combined the species with *Asteralobia* because of its shallowly constricted male flagellomeres. Because *Asteralobia* is synonymized under *Schizomyia* in this paper, *S. patriniae* is combined again with *Schizomyia*.

Schizomyia patriniae is distinguishable from known *Schizomyia* species, except three species that were previously treated as *Asteralobia* and newly combined here under *Schizomyia*, i.e. *S. sasakii*, *S. soyogo*, and *S. humuli*, by the presence of shallow constrictions on male flagellomeres and the absence of corniform papillae on the terminal larval segment. *S. patriniae* can be easily separated from *S. sasakii*, *S. soyogo* and *S. humuli* based on the number of papillae on the larval terminal segment: *S. patriniae* possesses two setose and six asetose terminal papillae, but *S. sasakii* and *S. soyogo* have only six setose terminal papillae, and *S. humuli* has four setose terminal papillae.

Schizomyia asteris **(Kovalev, 1964) comb. n.**

Figs 86–93; Table S4

Asteralobia asteris Kovalev, 1964

Characters as in *S. achyranthesae* except for the following:

Material examined. 2♂, 2♀: (Mamaev collection: slide no. B1-251363 & 251364), galls collected from *Aster* sp. in Kedrovaja Pad reserve, Russian Far East on 28.viii.1964. 4 larvae: galls collected from *A. tataricus* in Smolyaninovo, Primorsky Territory, Russian Far East on 13.ix.2002, M. Tokuda leg. 6 larvae: galls collected from *A. scaber* in Smolyaninovo, Primorsky Territory, Russian Far East on 13.ix.2002, M. Tokuda leg.

Description. *Head*: Compound eyes with rounded facets; facets on the vertex and eye bridge unobservable. Palpus: first segment ca 34.5 µm, second 1.8 times as long as the first, third 1.2 as long as the second, fourth 1.1 as long as the third.

Thorax: Wing length 1.93 mm (n = 1) in female, 1.55–2.02 mm (n = 2) in male. Anepimeral setae 14–15 (n = 3); mesanepisternum scales 17–23 (n = 7); lateral scutum setae 23–29 (n = 4). Lengths of leg segments as in Suppl. material 1: Table S4.

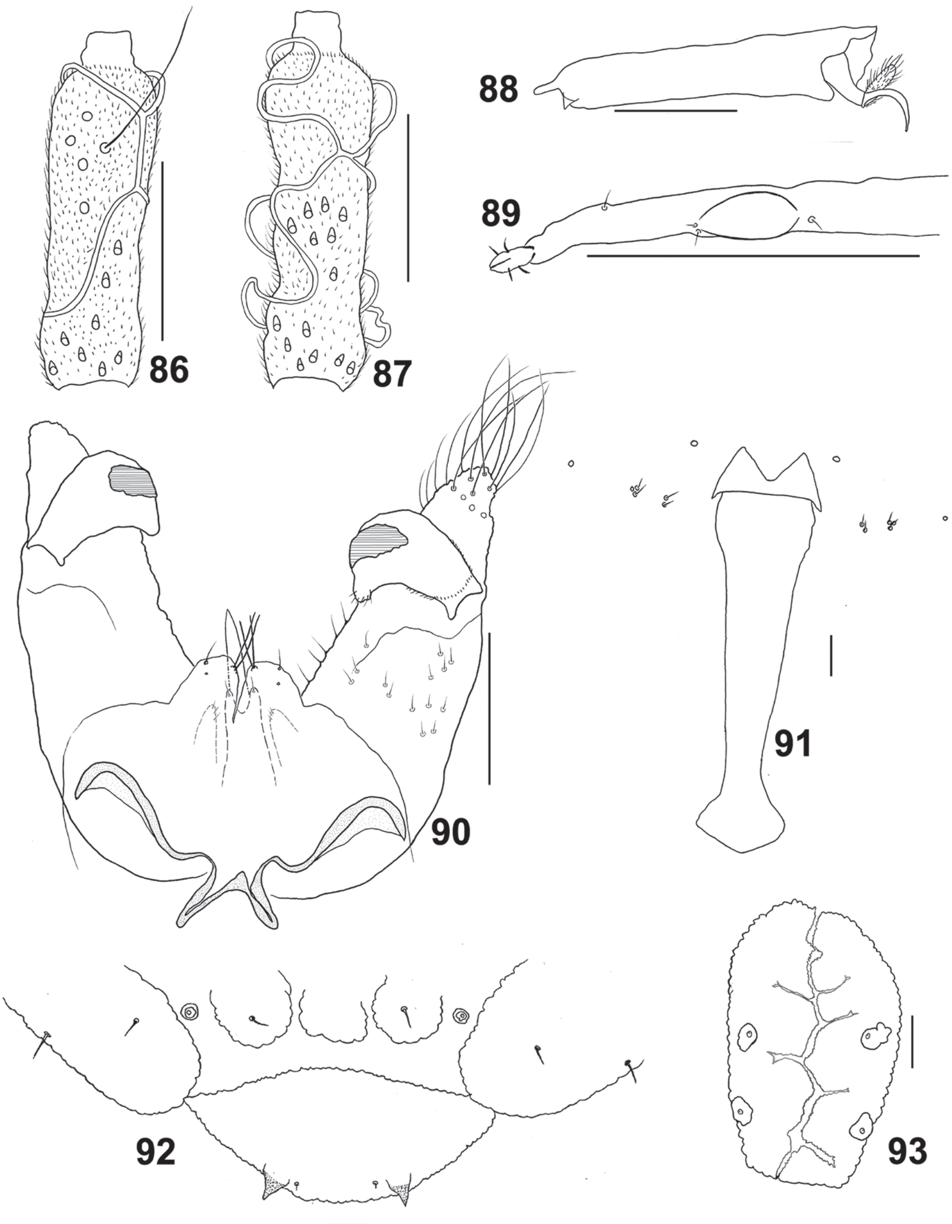
Female abdomen: Ovipositor: protrusible needle-like portion about 5.7 times as long as sternite VII; cerci divided medially, with sclerotized margins and few setae (Fig. 89).

Male abdomen: Terminalia (Fig. 90): Gonocoxite with pointed apical lobe extending beyond gonostylus. Gonocoxite length about 3.5 times as long as gonostylus. Gonostylus without setae ventrally and dorsally.

Mature larva: Sternal spatula with posterior portion about 3.3 times as wide as the base of the anterior free portion (Fig. 91). Abdominal segment VIII with three dorsal lobes, the outer two each with 1 setose dorsal papilla (Fig. 92). Anus with branched opening, and 4 asetose anal papillae (Fig. 93).

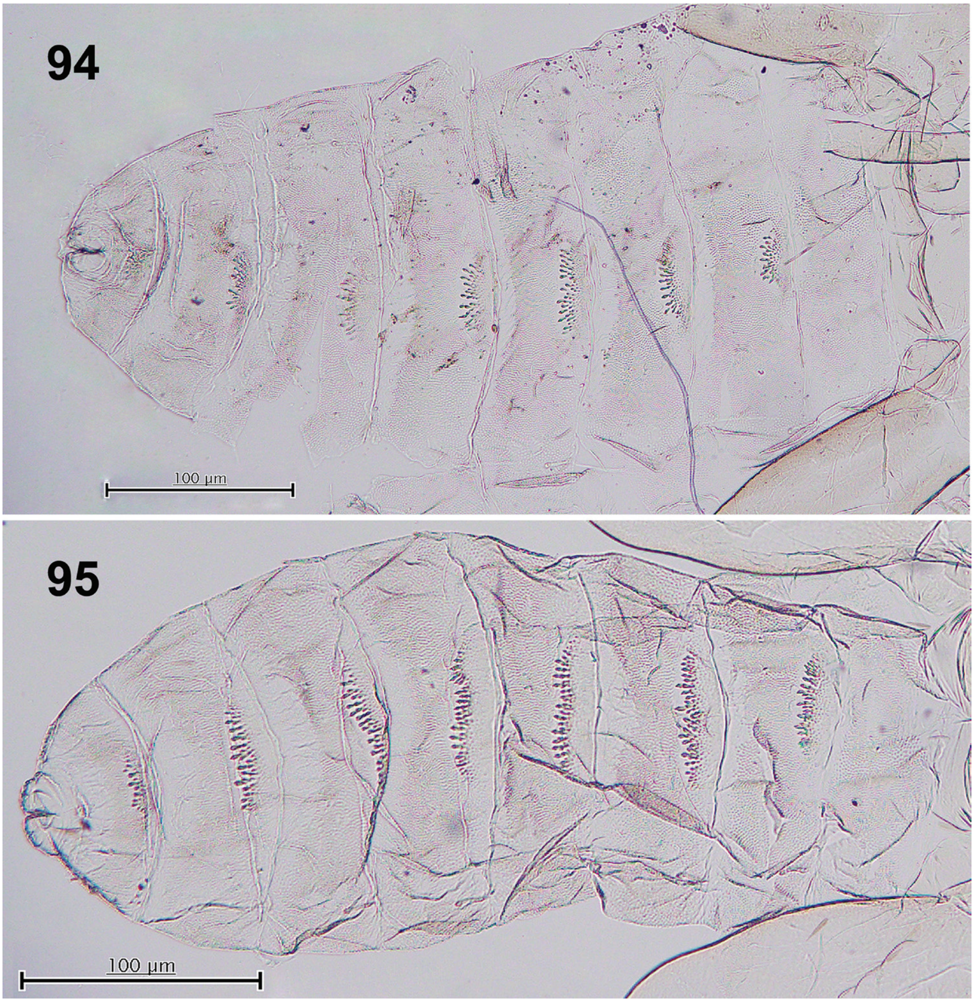
Pupa: Prothoracic spiracle about 220 µm long (n = 1).

Distribution. Russian Far East (Kovalev 1964; Tokuda et al. 2003).



Figures 86–93. *Schizomyia asteris*. **86** Lateral view of female flagellomere V **87** Lateral view of male flagellomere V **88** Tarsomere V and acromere **89** Ovipositor apex **90** Male terminalia **91** Larval spatula **92** Terminal larval segments dorsally **93** Larval anus. Scale bar: 50 µm.

Remarks. Although Kovalev (1964) mentioned that *S. asteris* is closest to *S. doellingeriae*, Tokuda et al. (2003) showed that they are distinguishable by the number of lateral papillae, terminal papillae, and the shape of anal opening.



Figures 94–95. Dorsal view of pupal abdominal segments. **94** *Schizomyia soyogo* **95** *Schizomyia sasaki*. Scale bar: 100 µm.

Taxonomic key to *Schizomyia* species in Japan

1	Male flagellomeres deeply constricted (Fig. 58)	***S. paederiae* sp. n.**
–	Male flagellomeres shallowly constricted (Fig. 7)	**2**
2	Trichoid sensilla present medially on adult sternites II–VI	**3**
–	Trichoid sensilla present laterally anterior to the sclerotized sternites II–VI in adults	**7**
3	Larval terminal segment with 8 terminal papillae	**4**
–	Larval terminal segment with fewer than 8 terminal papillae	**5**

4 Terminal papillae of 2 corniform and 6 setose papillae (Tokuda et al. 2003: fig. 1A) .... ***S. doellingeriae***
– Terminal papillae of 2 setose and 6 asetose (Yukawa 1983: fig. 4E) .... ***S. paterinia***
5 Terminal papillae made up of 4 setose papillae: 2 with long setae and 2 with tiny setae.. ***S. humuli***
– Terminal papillae made up of 6 setose papillae.. **6**
6 Pupal dorsal abdominal spines covering about 1/4 of the upper area of terga II–VIII (Fig. 94) (see Tokuda et al. 2004 for full-description of the species). .... ***S. soyogo***
– Pupal dorsal abdominal spines covering about 1/3 of the upper area of terga II–VIII (Fig. 95) (see Tokuda et al. 2004 for full-description of the species).. .... ***S. sasakii***
7 Larval anal opening simple (e.g. Fig. 14).. **8**
– Larval anal opening branched (e.g. Fig. 40) **9**
8 Protrusible needle-like portion of ovipositor about 4 times as long as sternite VII (Fig. 10) .... ***S. achyranthesae* sp. n.**
– Protrusible needle-like portion of ovipositor about 3 times as long as sternite VII (Fig. 23) .... ***S. diplocyclosae* sp. n.**
9 Protrusible needle-like portion of ovipositor about 3.3 times as long as sternite VII (Fig. 35) .... ***S. castanopsisae* sp. n.**
– Protrusible needle-like portion of ovipositor about 4.5 times as long as sternite VII (Fig. 49) .... ***S. usubai* sp. n.**

Molecular phylogenetic study

The complete molecular dataset of COI and 12S consisted of approximately 800 bp. The monophyly of *Schizomyia* was strongly supported with a 99% bootstrap value, and the genus was divided into two main clades. One clade with a 55% bootstrap support was subdivided into three subclades: one including *S. galiorum*, *S. doellingeriae*, *S. humuli*, *S. patriniae* and *S. kovelavi*; another including *S. sasakii, S. soyogo*, and *S. paederia*; and the third comprising *S. buboniae*. The second main clade contains six morphologically-close species: *S. diplocyclosae*, *S. castanopsisae*, *S. achyranthesae*, *S. solidaginis*, *S. asteris* and *S. usubai*, and gained a 95% bootstrap support.

Discussion

In the present study, we showed that constricted male flagellomeres, the only character used to separate *Asteralobia* from *Schizomyia* (Kovalev 1964), can be also observed in the type species of *Schizomyia*, *S. galiorum*, and hence *Asteralobia* is synonymized here

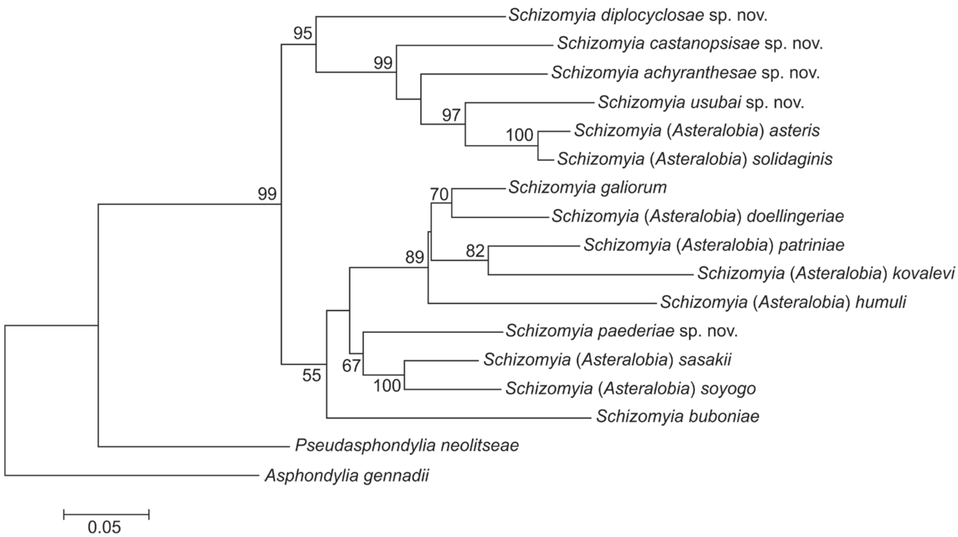


Figure 96. A phylogenetic reconstruction based on partial sequences of cytochrome oxidase subunit I (COI) and 12S small ribosomal subunit genes. The topology and branch length were produced by the maximum likelihood method (note the scale bar). Bootstrap values are indicated at branches gaining more than 50% support (10^3 replications).

under *Schizomyia*. Our molecular phylogenetic analysis strongly supported this conclusion with high bootstrap values.

Because of the broad definition of *Schizomyia*, which depends only on plesiotypic characters (Gagné and Marohasy 1997; Gagné 1994), some other genera of Schizomyiina, e.g., *Metasphondylia*, *Placochela* and *Schizandrobia*, are considered to fit easily into its definition (Gagné and Jaschhof 2017). Comprehensive taxonomic and molecular analyses including these genera are needed for further progress on the taxonomy of *Schizomyia*.

In the present study, six eastern Palearctic *Schizomyia* species, namely *S. achyranthesae, S. asteris*, *S. diplocyclosae*, *S. castanopsisae*, *S. usubai* and *S. solidaginis*, were shown to be close to each other and (although we have never examined the phylogenetic position of *S. solidaginis*) constructed a monophyletic clade in the molecular analysis. They differ from all known *Schizomyia* spp. by the laterally situated anterior pair of trichoid sensilla, which are present anterior to the sclerotized sternite. This character can be considered as derived because in other genera of Schizomyiina, the anterior pair of trichoid sensilla are usually located on the sternites. Future comprehensive taxonomic studies may treat these species as a natural cluster within *Schizomyia*.

Several important characters need to be re-evaluated in order to meet current taxonomic standards in many *Schizomyia* species. For example, the ovipositors of most known *Schizomyia* species were not described in detail, although they can be expected to be variable because of their adaptation for oviposition on different organs of hosts belonging to various, not related, families. Similarly, the pupa, which offers many diagnostic features for taxonomy in Schizomyiina (Möhn 1961), is still unknown and undescribed in many species, especially in those that develop in the soil and are not easily found. Descriptions of these unknown morphological features of *Schizomyia* species are essential to clarify the generic concept.

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Supplementary material 1

Supplementary data

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Data type: measurements

Explanation note: **Table S1.** Length (µm) of each leg segment in *Schizomyia achyranthesae* and *S. diplocyclosae*. **Table S2.** Length (µm) of each leg segment in *Schizomyia tokudai* and *S. usubai*. **Table S3.** Length (µm) of each leg segment in *Schizomyia paederiae* and *S. galiorum*. **Table S4.** Length (µm) of each leg segment in *Schizomyia patriniae* and *S. asteris*.

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