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A new genus and a new species of Schizomyiina (Diptera: Cecidomyiidae: Asphondyliini) inducing petiole galls on *Macaranga bancana* (Miq.) in Borneo, Malaysia

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Abstract

We describe a gall midge *Macarangamyia itiokai* Elsayed & Tokuda gen. n., sp. n. belonging to the subtribe Schizomyiina (Diptera: Cecidomyiidae: Asphondyliini) inducing petiole galls on *Macaranga bancana* (Miq.) in Lambir Hills National Park, Borneo, Malaysia. The new genus is distinguishable from all known genera of Schizomyiina by the unique dorsally-placed aedeagus slit, the short, membranous, protrusible ovipositor, with scattered strong setae ventrally and dorsally, and the presence of spiracles on all larval thoracic segments. It is compared and separated from its closely related Oriental genera of Schizomyiina.

Keywords: Gall midges, Cecidomyiidae, Asphondyliini, *Macaranga*, Sarawak

Introduction

The tree genus *Macaranga* Thouars (Euphorbiaceae) is widely distributed from Africa to the Pacific islands (Whitmore 2008) and has a wide spectrum of ant-plant mutualisms (e.g. Fiala *et al.* 1991, 1994, 1999). Many *Macaranga* species are true myrmecophytes harboring mutualistic species-specific ants of the genus *Crematogaster* Lund (Hymenoptera: Formicidae) that reduce the infestation of herbivores (Fiala *et al*. 1989; Itioka *et al*. 2000). Recently, some herbivorous insects have been revealed to overcome aggressive attacks of ants on *Macaranga* through behavioral, morphological, and/or chemical adaptations (e.g. Okubo *et al*. 2009; Inui *et al*. 2015; Shimizu-kaya & Itioka 2016; Yamasaki *et al*. 2016). Clarification of the whole herbivore fauna associated with *Macaranga* is important to understand the coevolutionary processes of plant-herbivore interactions as well as ant-plant mutualism.

Shimizu-kaya *et al.* (2015) found various galls induced by gall midges (Diptera: Cecidomyiidae) on several *Macaranga* species in Lambir Hills National Park, Borneo, Malaysia, whose taxonomic position and ecology have not yet been studied. They noticed that densities of galls induced by some gall midges on *Macaranga* spp. were higher on ant-inactive saplings than on ant-intact saplings. This suggests that ant-defense plays an important role to reduce the damage by gall midges. To understand their interactions with ants and how gall midges can survive on myrmecophytes, taxonomic studies on *Macaranga* gall midges are essential.

Currently, only two species of gall midges are known to induce galls on *Macaranga*. These are *Schizomyia macarangae* Nayar on *M. indica* Wight in India (Nayar 1953) and *S. novoguinensis* Kolesik on *M. aleuritoides*

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F.Muell. in Papua New Guinea (Kolesik & Butterill 2015). In the present study, we aim to contribute to the knowledge of tropical gall midge fauna and herbivorous insect fauna associated with myrmecophytic *Macaranga* by identifying a gall midge species that induces petiole galls on *Macaranga bancana* (Miq.) in Borneo, Malaysia.

Materials and methods

Collection of gall midges, morphological examination, and terminology. Petiole galls (Fig. 1) were collected by U. Shimizu-kaya from *M. bancana* in Lambir Hills National Park, Borneo, Malaysia, and transferred to the laboratory in the national park where some galls were dissected to obtain larvae and pupae, and others were kept in plastic bags to rear adults and obtain their pupal exuviae. Most specimens collected were preserved in 75% ethanol for morphological examinations while some were kept in 99.5% ethanol for photographing with a scanning electron microscope. The ethanol-preserved specimens were transferred to Japan under memoranda of understanding and the taxonomic study was conducted.

Gall midge specimens were mounted on slides in Canada balsam following Gagné (1994), except that the larva and adults were cleared separately in plastic tubes containing 10% NaOH at 50 ºC for four and two hours, respectively. Slide-mounted specimens were examined under a bright-field and phase-contrast microscope (H550L, Nikon, Tokyo) and the illustrations were made with the aid of a drawing tube. A semi-motorized fluorescence microscope (BX53, Olympus, Tokyo) was used to take pictures of some characters of mounted specimens with the aid of the microscope-attached color camera (DP22, Olympus, Tokyo). Some pupae were photographed with a scanning electronic microscope (Hitachi S-3400N) after mounting them in various positions on a copper stub using double face adhesive tape.

FIGURE 1. Petiole galls induced by *Macarangamyia itiokai* **n. sp.** on *Macaranga bancana* in Borneo, Malaysia.

Morphological terminology follows McAlpine *et al.* (1981) for adults, except for wing terminology that follows Yukawa (1971), and the pupal terminology follows Gagné (1994). The holotype and some of the paratypes of the newly described species are deposited in Research Development and Innovation Division, Forest Department Sarawak, Malaysia (FDSM). The remaining paratypes are deposited in the collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Japan (KUEC). Type specimens of *Luzonomyia symphoremae* Felt were borrowed from the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

Taxonomy

Macarangamyia Elsayed & Tokuda gen. nov.

Type species: *Macarangamyia itiokai* Elsayed &Tokuda sp. nov.

Diagnosis: *Macarangamyia* belongs to the tribe Asphondyliini because of the following synapomorphies: the female sternite VII is distinctly larger than preceding sternites, and the gonostyli are strongly sclerotized, dorsally situated, short and compact (Tokuda 2012). The tribe Asphondyliini is divided into two subtribes: Asphondyliina and Schizomyiina (Gagné & Jaschhof 2017). The new genus, *Macarangamyia,* belongs to the subtribe Schizomyiina because of the presence of unfused teeth of gonostyli (Tokuda 2012). *Macarangamyia* can be distinguished from other genera of Schizomyiina, such as the Oriental genera *Asphoxenomyia* Felt and *Luzonomyia* Felt, by the following combination of characters: palpi four-segmented; male flagellomeres with short necks; tarsal claws untoothed; ovipositor short, membranous, protrusible, with scattered strong setae ventrally and dorsally; female cerci fused, with some blunt-tipped setae; aedeagus cylindrical, with slit developed dorsally more than ventrally; larva with bidentate spatula; a pair of spiracles present on all thoracic and abdominal segments of larva, except the terminal segment.

Description. Adult: Head (Fig. 2): Compound eyes with octagonal facets. Mouthparts: palpi four-segmented; labrum triangular; labella well-developed. Antenna: flagellomeres cylindrical, with short necks; first and second flagellomeres partially fused; female flagellomeres with two connected rings of circumfila, except the terminal flagellomere with network-like circumfila, distal female flagellomeres successively shorter (Fig. 3); male flagellomeres with sinuous circumfila (Fig. 4).

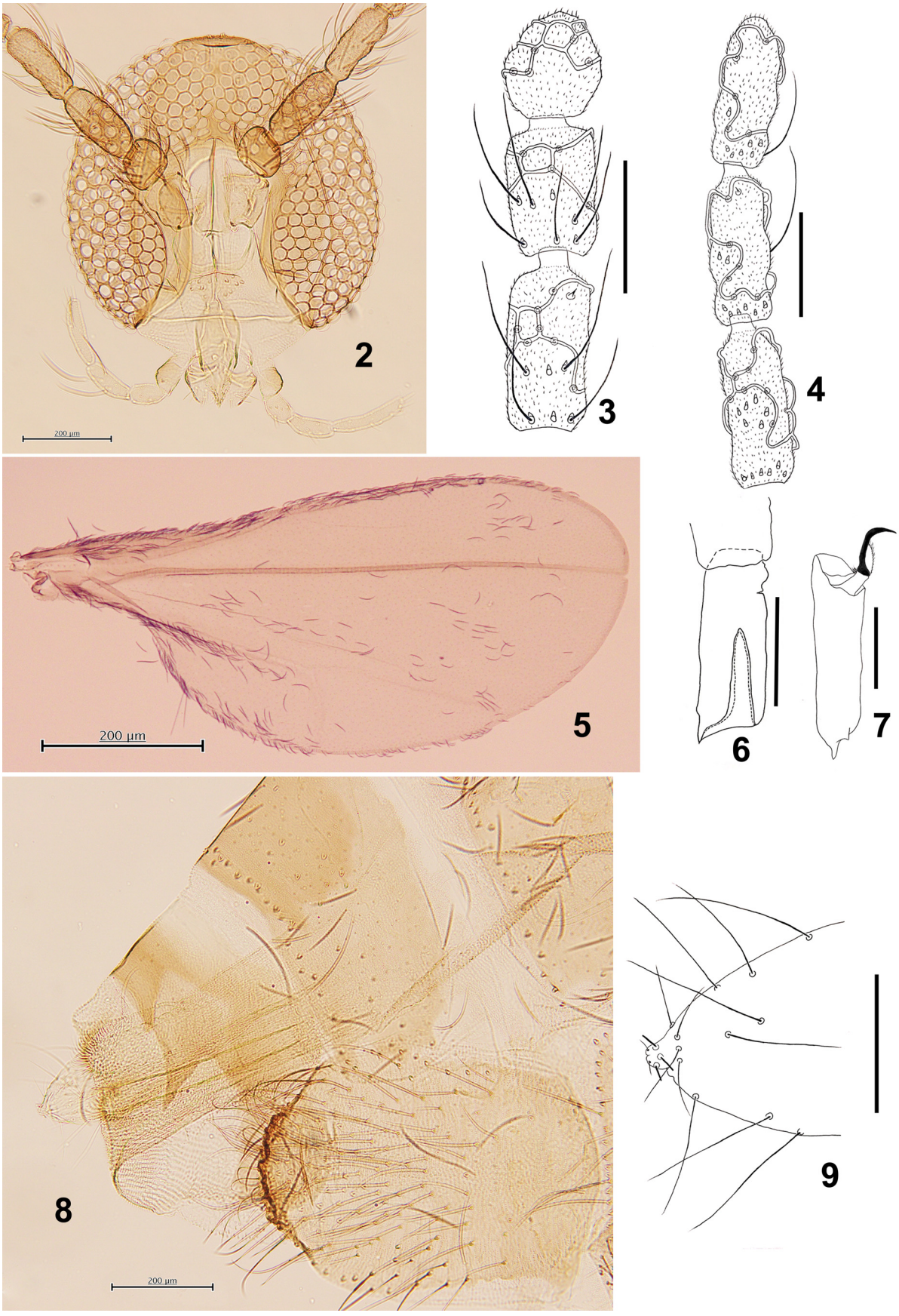
Thorax: Wing (Fig. 5): R_1 joining C before wing midlength, arculus present, R_5 joining C slightly after wing apex, C broken after the conjunction with R_5; M_{3+4} forked with Cu. Tarsomeres I simple (Fig. 6); tarsal claws (Fig. 7) curved, untoothed on all legs; empodia shorter than claws, covered with longer setulae apically than basally; pulvilli short.

Female abdomen: Tergites I–VII rectangular, with one posterior row of setae and some lateral setae; tergite VIII bare, notched laterally, posterior margin with a pair of well-developed dorsal lobs; trichoid sensilla absent from all tergites. Sternites II–VI each with several scattered setae anteriorly, one posterior row of setae; sternite VII elongated, completely sclerotized; posterior two thirds covered with scattered setae; no discernible trichoid sensilla on all sternites. Ovipositor (Figs. 8–9): protrusible, unpigmented, without microtrichiae, with scattered strong setae ventrally and dorsally; cerci tiny, fused, each bearing pair of thick blunt-tipped setae and several finer setae.

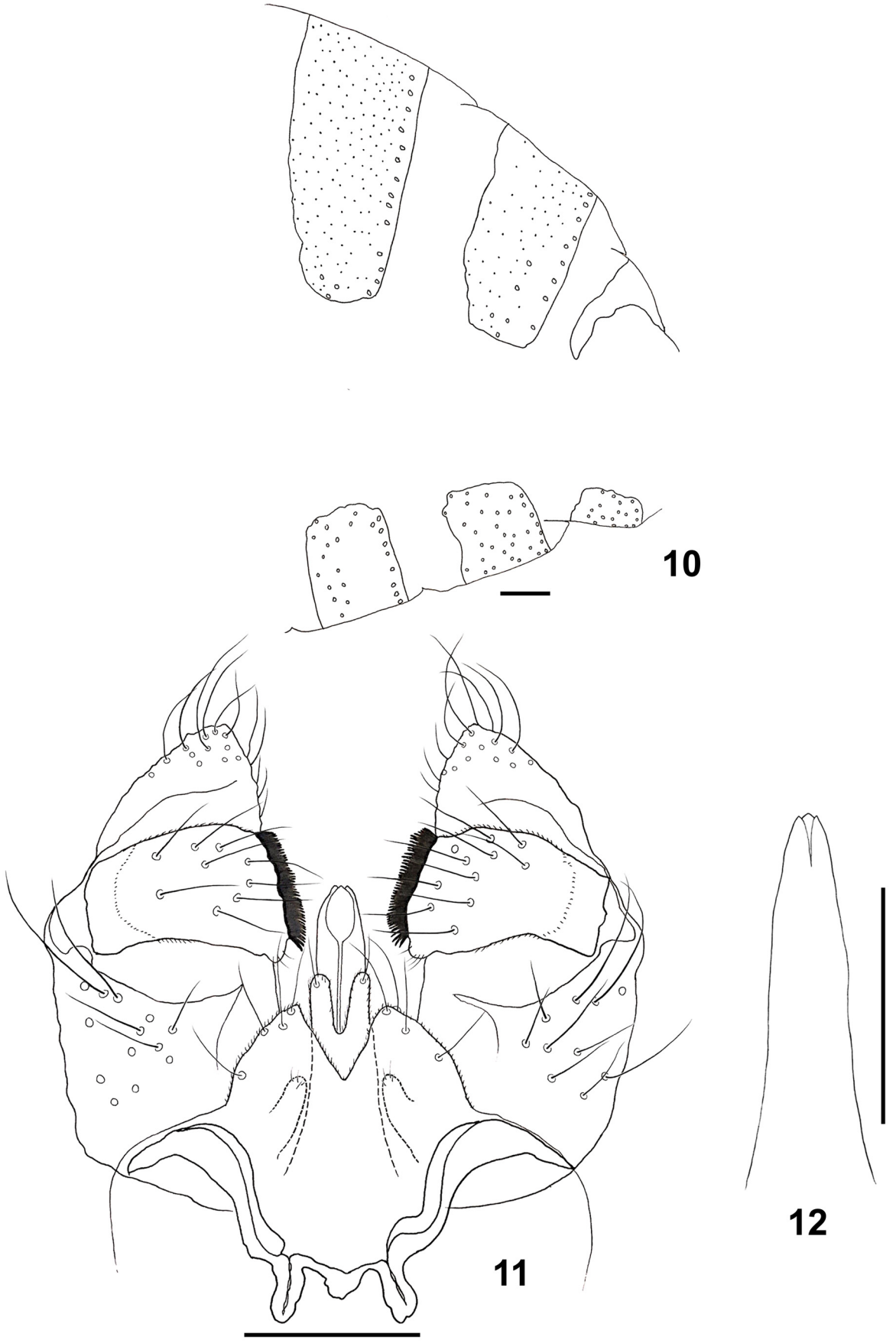
Male abdomen: Tergites I–VII as in female; tergite VIII bare, with median part longer and thicker than the lateral parts. Sternites II–VI as in female; sternite VII and VIII with several scattered setae (Fig. 10). Terminalia (Fig. 11–12): Gonocoxite massive, produced ventroapically. Gonostylus with unfused denticles. Cerci separated by V-shaped emargination. Hypoproct bilobed, with one seta at tip of each lobe. Parameres well-developed. Aedeagus cylindrical, with slit developed dorsally more than ventrally.

Larva: Sternal spatula bidentate. One group of lateral papillae on each side of spatula, with two setose and one asetose papillae. Two setose sternal papillae, two setose dorsal papillae, and a pair of spiracles present on all thoracic segments and abdominal segments I–VIII. Abdominal terminal segment elongated and tapered.

Pupa (Figs. 14–15): Exuviae not pigmented except the antennal and facial horns and prothoracic spiracle. Antennal and facial horns well-developed, each horn bidentate; two facial horns present, pointed. Prothoracic spiracle arched, pointed apically. Scutum medially without wrinkles surrounding the ecdysal line. Abdominal tergites without dorsal spines. Abdominal spiracles present on abdominal segments II–VI.



FIGURES 2–9. *Macarangamyia itiokai* **n. sp. 2.** Female head. **3.** Dorsal view of the male terminal flagellomeres. **4.** Ventral view of the female terminal flagellomeres. **5.** Wing. **6.** Tarsomere I. **7.** Terminal tarsomere. **8.** Terminal part of female abdomen. **9.** Dorsolateral view of the terminal part of the ovipositor. Scale bars = 50 µm, except of figures 2, 5 and 8 = 200 µm.



FIGURES 10–12. *Macarangamyia itiokai* **n. sp. 10.** Terminal part of male abdomen, excluding male terminalia. **11.** Male terminalia. **12.** Ventral view of aedeagus. Scale bars = 50 µm.

Etymology: The generic name is derived from the host-plant generic name, *Macaranga*, and the Greek noun *myia,* meaning fly. The gender is feminine.

Macarangamyia itiokai Elsayed & Tokuda sp. nov.

Head (Fig. 2): Compound eye bridge 6–7 facets long. Fronto-clypeal setae 9–13 (n = 13). Mouthparts: palpi four-segmented, first ca. 40.3 µm (28–47 µm), second ca. 35.7 µm (31–42 µm), third ca. 50.9 µm (40–63 µm), fourth ca. 78.3 µm (60–90 µm) (n = 9); labrum with microtrichous edges; labium setose.

Thorax: Wing (Fig. 5), length 1.79–1.96 mm in female (n = 4), 1.54–1.74 mm in male (n = 6); M_{3+4} and Cu very weak. Anepimeral setae 6–9 (n = 9); other pleural sclerites bare.

Female abdomen: Sternite VII about 3.3 times as long as preceding sternite. Ovipositor (Fig. 8–9): the protrusible portion about as long as sternite VII.

Male abdomen: Terminalia (Fig. 11): Gonocoxite about 2.2 times as long as width. Gonostylus with strong setae on the distal two thirds and unfused denticles covering most of the posterior margin. Cerci entirely microtrichous, setose. Hypoproct microtrichous. Parameres with several fine setae apically.

Full-grown larva: Sternal spatula broadened, length about 1.3 as long as width (Fig. 13). All thoracic segments and abdominal segments with ventral field of spinules, except prothoracic segment and terminal abdominal segment.

Pupa (Figs. 14–15): Antennal horns well-developed, each horn bidentate in the lateral view; two facial horns present, pointed; two lower facial papillae present between the facial horns, each with 24 to 43 µm (n = 6) long seta; two lateral facial papillae present on each side, each with very short seta; two pairs of cephalic papillae present, each pair consisting of one setose and one asetose papilla.. Prothoracic spiracle curved, about 135 to 165 µm (n = 7) in length, with trachea extending to the tip. Four dorsal papillae present on abdominal segment I–VII, only the outermost pair with setae. Abdominal segment VIII with only two setose papillae.

Etymology: This species is named in honor of Dr. Takao Itioka (Kyoto University, Japan) for his studies on the interactions between *Macaranga* spp., their symbiotic ants and other herbivorous insects in Borneo, Malaysia (e.g. Itioka *et al*. 2000; Itioka 2005; Shimizu-kaya & Itioka 2016).

Holotype: 1♂: Lambir Hills National Park, Borneo, Malaysia; collected on 26.vi.2013, Shimizu-kaya, U. leg., reared by Shimizu-kaya, U. from a petiole gall on *M. bancana,* deposited in FDSM.

Paratypes: All were collected and reared from petiole galls on *M. bancana* collected from Lambir Hills National Park, Borneo, Malaysia by Shimizu-kaya, U. Deposited in FDSM: 8♂, 3♀ & 6 pupal exuviae: collected on 26.vi.2013; 1♀ & 1 pupal exuviae: collected on 7.vii.2013. Deposited in KUEC: 10 pupal exuviae: collected on 26.vi.2013; 1 larva: collected on 7.vii.2013; 6♂, 4♀ & 3 pupal exuviae: collected on 1.iv.2014; 1♂: collected on 22.iv.2014.

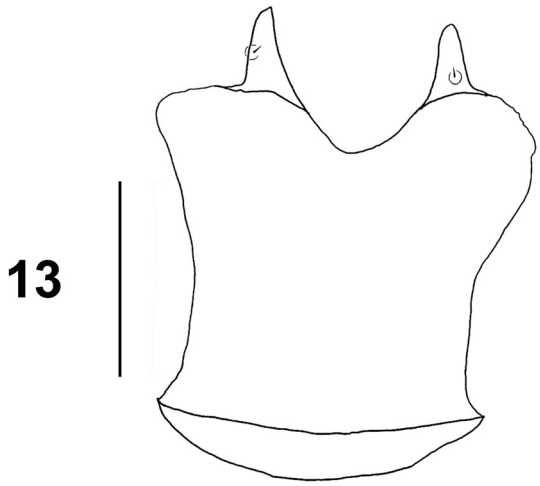
Distribution: Lambir Hills National Park, Borneo, Malaysia.

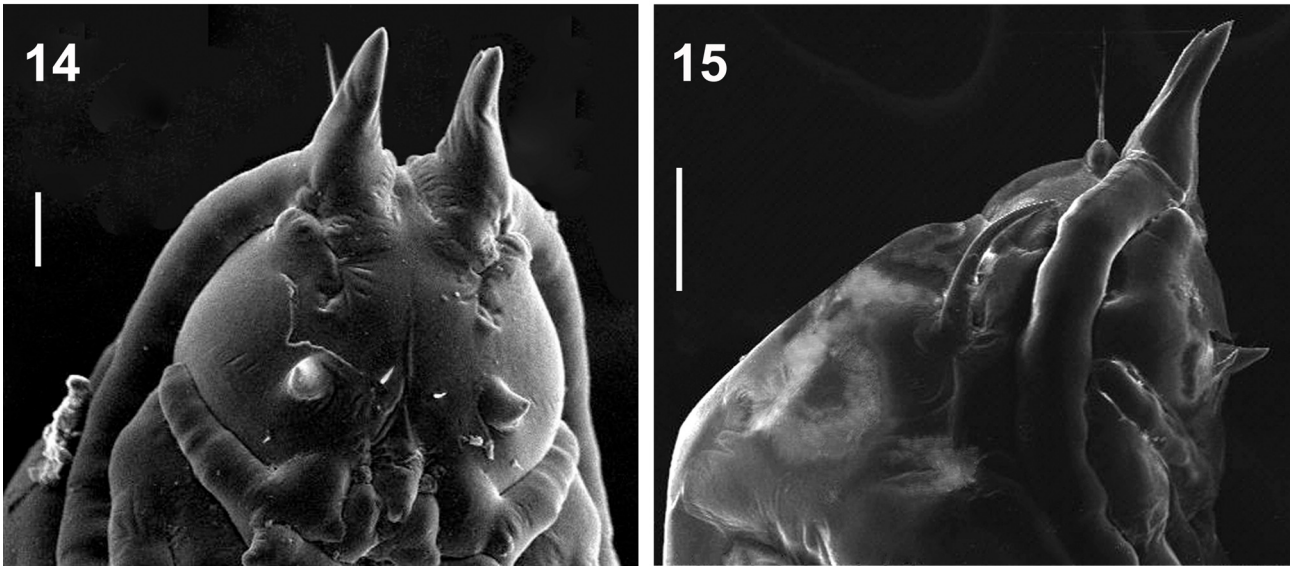
Biology: *Macarangamyia itiokai* induces spheroid galls on petioles of *M. bancana*. Two or more swellings are frequently fused together (1.2–3.0 mm in length, 0.8–1.5 mm in diameter, n = 6) and contain 2–15 (n = 12) small larval chambers. Each larval chamber contains only one larva. The pupation takes place inside galls.

Remarks. The new genus, *Macarangamyia* is distinguishable among all other Asphondyliini genera by the presence of well-developed dorsal and ventral aedeagus slit that is usually tiny and unnoticeable in Asphondyliini, except in some Australian species, namely *Schizomyia novoguineensis* Kolesik (Kolesik & Butterill 2015), *Okriomyia flabellidentata* Kolesik, *O. schwarzi* Kolesik (Kolesik 1998) and *Eocincticornia malarskii* Kolesik (Kolesik 1995), and the presence of spiracles on the larval meso- and metathoracic segments, which are usually absent in Cecidomyiidi (Gagné 1994), except for the larvae of *Paracalmonia paucula* Gagné that possesses spiracles only on metathorax (Gagné & Étienne 2009).

At present, only three genera of Schizomyiina are known from the Oriental region, i.e. *Asphoxenomyia* Felt, *Luzonomyia* Felt, and *Schizomyia* Kieffer (Gagné & Jaschhof 2017). *Schizomyia* is quite apart from *Macarangamyia* because female *Schizomyia* have needle-like ovipositors, while *Asphoxenomyia* and *Luzonomyia* are closer morphologically to *Macarangamyia* because of their short ovipositors. *Macarangamyia* can be distinguished from *Asphoxenomyia* as follows (Felt 1927; Peter Kolesik, personal communication): *Macarangamyia* has four-segmented palpi, while *Asphoxenomyia* have one-segmented palpi; tarsal claws of

Macarangamyia are simple, but toothed in *Asphoxenomyia*; ovipositor of *Macarangamyia* with tiny cerci distally, but that of *Asphoxenomyia* with large cerci (about 1/4 as long as the protrusible portion). The ovipositor of *Luzonomyia* (Fig. 16) is similar to the ovipositor of *Macarangamyia*, but the two genera can be separated from each other as follows: *Macarangamyia* has four-segmented palpi, while in *Luzonomyia* they are three-segmented; male flagellomeres of *Macarangamyia* have sinuous circumfila, but *Luzonomyia* has two connected rings of circumfila (Fig. 17); anterior pair of trichoid sensilla is absent on the abdominal tergites of both sexes of *Macarangamyia*, but present only in the female of *Luzonomyia*; *Macarangamyia* has a cylindrical aedeagus and broad gonostylus, but *Luzonomyia* with a broad aedeagus and pointed gonostylus (Gagné 1969; Felt 1918).

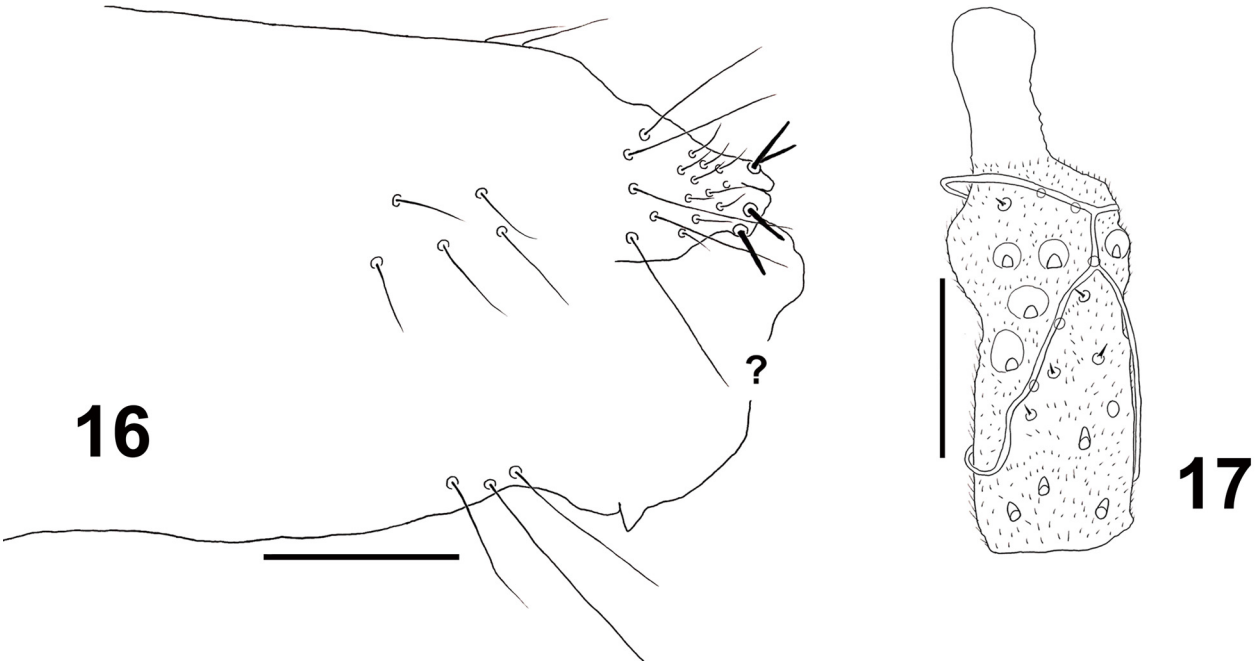




FIGURES 13–15. *Macarangamyia itiokai* **n. sp. 13.** Larval spatula. **14–15.** Pupal scanning electron micrographs **13.** Scanning electron micrograph showing ventral view of head. **14.** Scanning electron micrograph showing lateral view of anterior pupal segments. Scale bars = 100 µm, except of figure 13 = 50 µm.

The full-grown larva of *Macarangamyia* has a bidentate sternal spatula and elongated and tapered terminal abdominal segment. This feature is distinctly different from that of *Schizomyia* and rather similar to *Bruggmannia* Tavares, a Neotropical genus of Schizomyiina. However, *Bruggmannia* can be separated from *Macarangamyia* by many characters as follows according to the definition of *Bruggmannia* in Gagné (1994): *Macarangamyia* has four-segmented palpi, well-developed labrum and labium, while *Bruggmannia* has three-segmented palpi, and a reduced labrum and labium; male flagellomeres of *Macarangamyia* have slight constrictions and short necks, in contrast to those of *Bruggmannia*, which have deep constrictions and long necks; ovipositor of *Macarangamyia* with strong setae dorsally and ventrally, but that of *Bruggmannia* only ventrally; aedeagus is cylindrical in *Macarangamyia*, but broad in *Bruggmannia*; pupa of *Macarangamyia* has well-developed antennal and frontal horns and does not

have abdominal dorsal spines, while that of *Bruggmannia* has undeveloped or weakly-developed antennal and frontal horns and two rows of dorsal spines; larva of *Macarangamyia* has a bidentate spatula, but *Bruggmannia* larva lacks a spatula. For these reasons we regard *Macarangamyia* as a new genus to science.



FIGURES 16–17. *Luzonomyia symphoremae* Felt. **16**. Ovipositor, the question mark indicates unclear and damaged part. **17**. Dorsal view of male flagellomere V. Scale bars = 50 µm.

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