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Parampelomyia, another new gall midge genus (Diptera: Cecidomyiidae) associated with Vitaceae, with description of a new species developing in flower buds of the porcelain berry in Japan

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Abstract

We describe a gall midge *Parampelomyia yukawai* Elsayed and Tokuda **gen. nov. sp. nov.** belonging to the subtribe Schizomyiina (Diptera: Cecidomyiidae: Asphondyliini) based on an integrative taxonomic study. This species forms barely-swollen flower bud galls on the porcelain berry *Ampelopsis brevipedunculata* var. *heterophylla* (Vitaceae) in Japan. The new genus is distinguishable from all known schizomyiine genera by tarsomere I lacking a ventroapical extension, the bulbous base of the protrusible portion of the ovipositor, the fused and sclerotized female cerci, the bidentate gonostylus, and the larval terminal abdominal segment that bears two corniform, two asetose and six setose papillae. The new genus is compared with and separated from the similar genera *Schizomyia* and *Ampelomyia* morphologically and phylogenetically.

Keywords

Ampelopsis, Asphondyliini, grape, Schizomyiina, phylogeny, integrative taxonomy

1. Introduction

The grape family, Vitaceae, hosts at least 22 species and 12 genera of gall midges (Diptera: Cecidomyiidae: Cecidomyiinae) worldwide (Gagné and Jaschhof 2021; So et al. 2022). Of these species, four occur in Japan: *Asphondylia baca* Monzen, 1937 (Cecidomyiidi: Asphondyliini: Asphondyliina) that induces fruit galls on *Ampelopsis glandulosa* (Wallich) Momiyama var. *heterophylla* (Thunberg) Momiyama and *Vitis vinifera* L., and utilizes the leaf buds of *Weigela* Thunberg (Caprifoliaceae) as overwintering galling sites (Uechi et al. 2004); *Ampelomyia conicocoricis* Elsayed and Tokuda, 2019 (Cecidomyiidi: Asphondyliini: Schizomyiina) that forms conical leaf galls on cultivated *V. coignetiae* Pulliat ex Planchon (cultivar 'Suzumi-murasaki') as well as wild *V. coignetiae*, *V. flexuosa* Thunberg, *V. ficifolia* Bunge, and *V. saccharifera* Makino (Elsayed et al. 2019a; So et al. 2021); *Schizomyia uechii* Elsayed and Tokuda, 2019 (Cecidomyiidi: Asphondyliini: Schizomyiina) that induces flower bud galls on *A. glandulosa* var. *brevipedunculata* (Maxim.) Momiy (Elsayed et al. 2019b); and *Vitisiella gagnei* So, Elsayed and Tokuda, 2022 (Lasiopteridi: Dasineurini) that forms subglobular leaf galls on *V. vinifera* (So et al. 2022).

Yukawa and Sunose (1979) reported another gall midge species forming barely swollen flower bud galls on the porcelain berry *Ampelopsis brevipedunculata* var. *heterophylla* (Fig. 1A), but this species remained undescribed until now. Porcelain berry is a native vine to Japan and Southeast Asia (Del Tredici 2017) and was introduced to the United States in the 19th century as a landscape plant, but it became widely spread and is now regarded as an undesirable invasive species competing with native plants (Baranowski and Preisser 2018; USDA, NRCS 2022). For this reason, identification of the gall midge associated with flower buds of the porcelain berry in Japan is important to shed light on potential candidates for classical biological control.

In recent years, we have collected flower bud galls on porcelain berry and reared the gall midge adults (Fig. 1). Morphological examinations indicated that this species belongs to the subtribe Schizomyiina (Cecidomyiidi: Asphondyliini) based on the distinctly longer female sternite VII than preceding sternites, dorsally situated gonostyli of the male terminalia, unfused gonostylus tooth, and ventrally situated larval anus (Tokuda 2012). Morphological comparison with other Schizomyiina indicate that this gall midge does not fit the definition of any known genera. In the present study, we erect a new genus for this gall midge and report the species as new to science. We also evaluate the phylogenetic relationship between this gall midge and closely related genera.

2. Materials and methods

2.1. Collecting and rearing methods

Flower buds of *A. brevipedunculata* var. *heterophylla* (Vitaceae) with galls (Fig. 1A) were collected from Hokkaido and Honshu, Japan in late August 2014, 2019, 2020, and 2021. Some galls were cut open to obtain gall midge larvae, while the remaining were kept in plastic bags until the mature larvae emerged. Those mature larvae were transferred to plastic rearing cups designed following Elsayed et al. (2018a). The rearing cups were placed in the field in Autumn and were half-buried in the soil to allow the larvae to overwinter under natural conditions. These cups were brought back to the laboratory in late February and were kept at room temperature until adults emerged. The adults and their pupal exuviae were preserved in 99.5% ethanol.

2.2. Morphological examination and terminology

Gall midge specimens were mounted on slides in Canada balsam following the technique outlined in Gagné (1994), except for the clearing step for some larval and adult specimens following Elsayed et al. (2018b). The slide-mounted specimens were examined under an Olympus CX43 bright–field and phase–contrast microscope and the line illustrations were made following Elsayed et al. (2020a). Photos of gall, larva, pupal exuviae, and freshly emerged adults (Figs 1A–D, 6A) were taken with a Nikon D7500 APS-C DSLR camera and AF-S DX Micro NIKKOR 85mm f/3.5G ED VR lens. Freshly emerged adults were photographed after a cold-shock in a –30° freezer for one minute to reduce their activity. Photos showing the female dorsum and its terminal abdominal segments (Fig. 4A, B) were taken with a Leica MC170 HD camera attached to a Leica SAPO stereoscopic microscope. Photomicrographs of the head, wing, female abdomen, ovipositor, and pupal abdomen (Figs 2A, I, 3A, 4C, D, 6E) were taken with a digital camera (DP22, Olympus, Tokyo) attached to a semi-motorized fluorescence microscope (BX53, Olympus, Tokyo).

Morphological terminology mainly follows Gagné (2018) for adults. Terminology for larval and pupal morphology follows Gagné (1994), with the addition of the term "antennal papillae" in the pupa as in Elsayed et al. (2020b). The holotype and paratypes are deposited in the collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Japan (ELKU).

2.3. DNA extraction, sequencing, and alignment

Total DNA was extracted from three larvae obtained from flower bud galls of *A. brevipedunculata* var. *heterophylla* in Aomori, Japan in 2019 using DNeasy Blood and Tissue Kit (Qiagen, Tokyo, Japan) following the manufacturer's instructions. Fragments of the mitochondrial COI and ribosomal 12S genes were amplified and sequencing following Elsayed et al. (2017). The following sets of primers were used for the COI gene: J–1718 (5′–GGA GGA TTT GGA AAT TGA TTA GTT CC–3′) (Simon et al. 1994) and COIA (Funk et al. 1995); while the following sets were used for 12S 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), European Molecular Biology Laboratory (EMBL), and GenBank (www.ncbi.nlm.nih.gov/genbank) under the accession numbers shown in Table 1.

The resulting sequence data were analyzed with GenBank sequences of COI and 12S genes of two outgroup species of Asphondyliina and ingroup species of closely related genera *Ampelomyia* and *Schizomyia*, including the type species of both genera *A. conicocoricis* and *S. galiorum* (Table 1). The sequences were aligned using

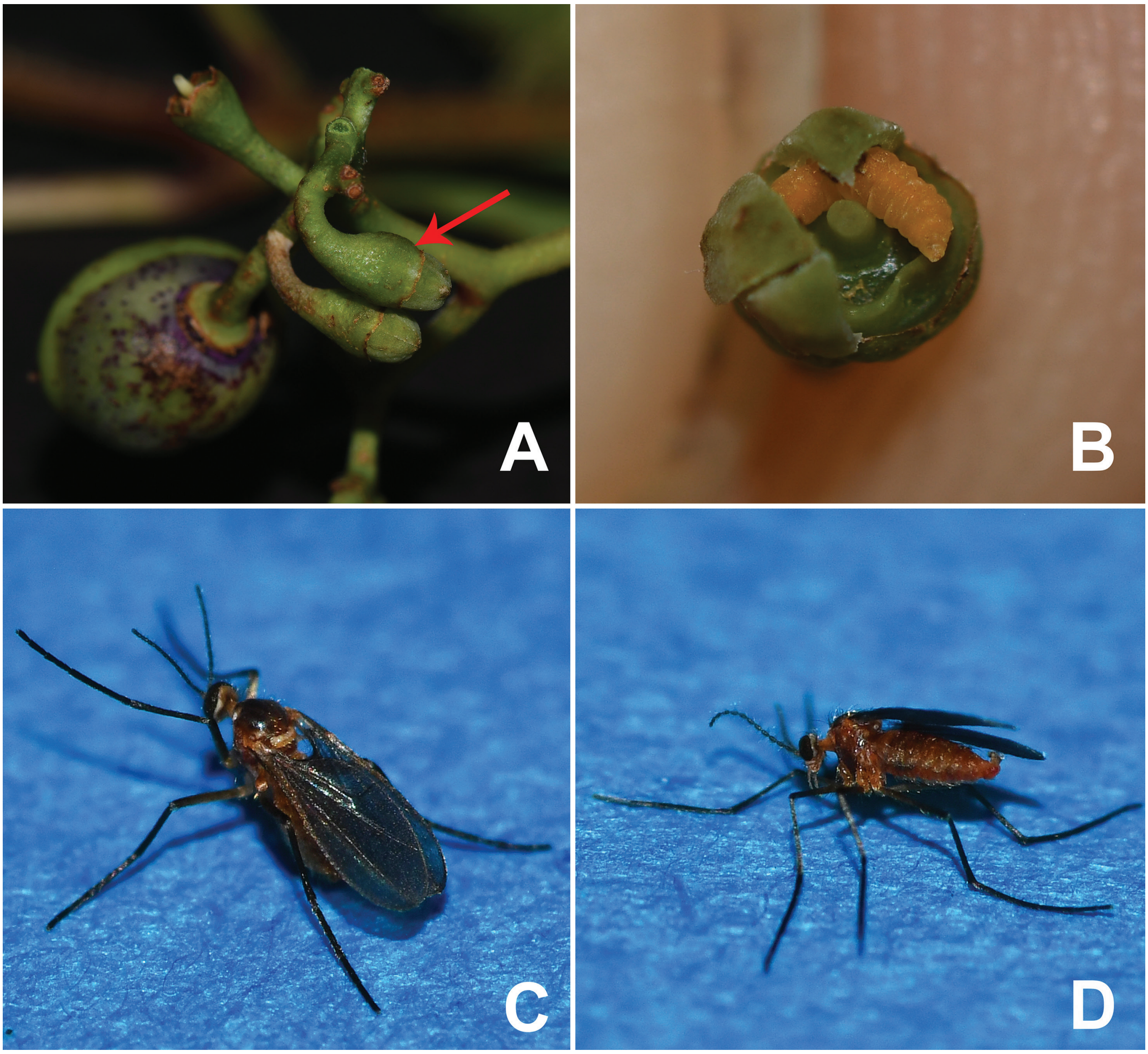


Figure 1. *Parampelomyia yukawai* sp. nov. (photographs by A. K. Elsayed). **A** Flower bud gall on *Ampelopsis brevipedunculata* var. *heterophylla* (Vitaceae). **B** Mature larva in a cut-open flower bud. **C** Freshly emerged female. **D** Freshly emerged male.

Table 1. Gall midge species used for genetic analysis.

	Gall midge species	COI	12S	References
Ingroup	*Parampelomyia yukawai* gen. nov. sp. nov.	LC709175–LC709177	LC709178–LC709180	This study
	Ampelomyia conicocoricis Elsayed and Tokuda, 2019	LC422078; LC422080; LC422083; LC422088	LC422109; LC422111; LC422114; LC422119	Elsayed et al. 2019a
	Schizomyia kovalevi (Skuhravá, 1986)	LC422068	LC422095	Elsayed et al. 2019a
	S. patriniae Shinji, 1938	AB176718	LC422105	Tokuda et al. 2004; Elsayed et al. 2019a
	S. galiorum Kieffer, 1889	AB213410	LC422108	Tokuda et al. 2005; Elsayed et al. 2019a
	S. doellingeriae (Kovalev, 1964)	LC422074	LC422101	Elsayed et al. 2019a
Outgroup	*Asphondylia gennadi* (Marchal, 1904)	AB115569	AB198012	Uechi et al. 2004
	Pseudasphondylia neolitseae Yukawa, 1974	AB334237	LC422092	Tokuda et al. 2008; Elsayed et al. 2019a

ClustalW algorithm in MEGA 11 (Tamura et al. 2021) and the final combined aligned sequence lengths including gaps were 887 bp. The phylogenetic relationships were inferred using the maximum likelihood (ML) method in RAxML GU 2.0.6 (Edler et al. 2021) based on the GTR+G model determined by jModelTest 2 (Guindon and Gascuel 2003; Darriba et al. 2012) and 1,000 bootstrap replications.

3. Results

3.1. Taxonomy

3.1.1. *Parampelomyia* Elsayed and Tokuda, 2023, gen. nov.

https://zoobank.org/5C6422FE-6ABE-4C11-B2D4-10790D960DC2

Type species. *Parampelomyia yukawai* Elsayed and Tokuda, sp. nov. here designated.

Description. ***Adult, head:*** Eyes with rounded facets (Fig. 2A). Antenna: flagellomeres I and II fused; flagellomeres cylindrical, but terminal female flagellomeres successively shorter, ending with spherical terminal flagellomere (Figs 1C, D, 2D, E). Mouthparts: labrum triangular; labella hemispherical; palpus 4-segmented, with palpiger (Fig. 2A, F). — ***Thorax:*** Anepimeron with setae; mesanepisternum with scales; katepisternum bare. Legs without ventroapical extension on tarsomere I (Fig. 2G); tarsal claws curved at midlength, untoothed (Fig. 2H). Wing (Fig. 2I): R_1 joining C before wing midlength; R_5 joining C slightly posteriad of wing apex; C broken after conjunction with R_5; wing fold present; M_4 forked with CuA. — ***Female abdomen:*** Ovipositor (Figs 3A, B, 4A–E): protrusible portion needle-like, pigmented, and bulbous at base, ending with fused and sclerotized cerci. — ***Male abdomen:*** Terminalia (Fig. 5B, C): gonocoxite extending ventrally as convex lobe beyond insertion of gonostylus, with long setae apically; gonostylus with 2 teeth, each of unfused denticles; cerci ellipsoid; hypoproct bilobed; aedeagus tapered apically. — ***Pupa:*** Exuviae hyaline, except projections of antennal bases, prothoracic spiracle and abdominal dorsal spines pigmented (Fig. 6A, B, E). Vertex with 2 cephalic papillae on each side, outermost papillae with long seta (Fig. 6C). Abdominal segments II–VI with spiracles. Abdominal terga I–VII with 6 dorsal papillae, outermost 2 with setae; tergum VIII with 2 setose dorsal papillae. — ***Mature larva:*** Cylindrical. Spatula bilobed (Fig. 7A). Two triplets of lateral papillae on each side of thoracic segments. Thoracic segments with 2 asetose sternal papillae and 2 asetose ventral papillae. Abdominal segments I–VIII with 4 asetose sternal papillae and 2 asetose ventral papillae. Six setose dorsal papillae on thoracic segments and abdominal segments I–VIII; abdominal segment VIII with 2 setose dorsal papillae. Anus located ventrally. Terminal segment with 2 corniform, 2 asetose and 6 setose papillae (Fig. 7B).

Diagnosis. This new genus can be distinguished from other known schizomyiine genera by the following combination of characters: palpus 4-segmented (Fig. 2A, F); tarsomere I without ventroapical extension (Fig. 2G); tarsal claws untoothed and bent at midlength (Fig. 2H); protrusible part of ovipositor bulbous at base, ending with fused sclerotized cerci (Figs 3A, B, 4C, D); gonostylus bidentate (Fig. 5B, C); larval abdominal segment VIII with 2 dorsal papillae; and larval terminal abdominal segment with 2 corniform, 2 asetose and 4 setose papillae (Fig. 7B).

Derivatio nominis. The generic name is composed of the Latinized Greek word, *para*, meaning near to, and the grape-associated gall midge genus name *Ampelomyia*.

3.1.2. *Parampelomyia yukawai* Elsayed and Tokuda, 2023, sp. nov.

http://zoobank.org/720CCE21-416F-4B99-B995-F1615AEB9BDF

Description and diagnosis. ***Adult*** (Fig. 1C, D)***, head:*** Eyes separated on vertex by diameter of 0–1 facet, eye bridge 6–7 facets long (Fig. 2A). Antenna: female and male flagellomeres with 2 connected rings of circumfila (Fig. 2B, C). Frons with 14–28 setae and no scales (n = 8). Mouthparts (Fig. 2A, F): palpal segments consecutively longer, with setae and no scales, segment IV ends with few stout short setae; labral edges covered with dense microtrichia; labella setose. — ***Thorax:*** Scutum pigmented, with 4 longitudinal lines of setae, not reaching scutellum posteriorly; median lines with 2–3 rows of setae; lateral lines with sparse setae near midlength of scutum; scutellum not pigmented, with setae laterally (Figs 1C, D, 4A). Anepimeral setae 16–21 (n = 9); mesanepisternal scales 17–27 (n = 8); and katepisternum bare. Empodia slightly longer than claws; pulvilli diminutive (Fig. 2H). Wing (Fig. 2I): 1.9–2.2 mm long in females (n = 6), 1.7–1.8 mm long in males (n = 2). — ***Female abdomen*** (Figs 3A, B, 4A–E)***:*** Tergites I–VII rectangular, with anterior pair of trichoid sensilla; tergites I–VI with sparse scales, lateral setae; tergites I–VI with mostly 1 posterior row of setae; tergite VII with 2 posterior rows of setae; tergite VIII differentiated from remainder of tergum only by anterior pair of trichoid sensilla and light pigmentation, posterior margin ends with pair of barely visible dorsal lobes. Sternites II–VI with scattered setae and scales on the anterior half; sternites III–VII with anterior pair of trichoid sensilla, placed medially except on VII placed laterally; sternites II–V with 1–2 posterior rows of setae; sternites VI with 2–3 posterior rows of setae; sternite VII about 2.4 times as long as preceding and 2 times as long as tergite VII, mostly covered with setae. Ovipositor (Figs 3A, B, 4A–E): protrusible portion about 4 times as long as sternite VII, dorsum of basal half portion more pigmented, distal half bent dorsoventrally (Figs 3A, 4B–D), with fine sensoria without visible setae (Fig. 4E). — ***Male abdomen:*** Tergites I–VI as in female; tergite VII with anterior pair of trichoid sensilla, mostly bare medially except few scales, with lateral setae and scales, and 1 row of posterolateral setae; tergite VIII band like, with pair of trichoid sensilla (Fig. 5A). Sternites II–V as in female; sternites VI–VII as VI in female; sternite VIII with anterolateral pair of trichoid sensilla placed intersegmentally, covered with scattered setae. Terminalia (Fig. 5B–D): gonocoxite about 2.4 times as long as width; gonostylus about 1.5 times lon-

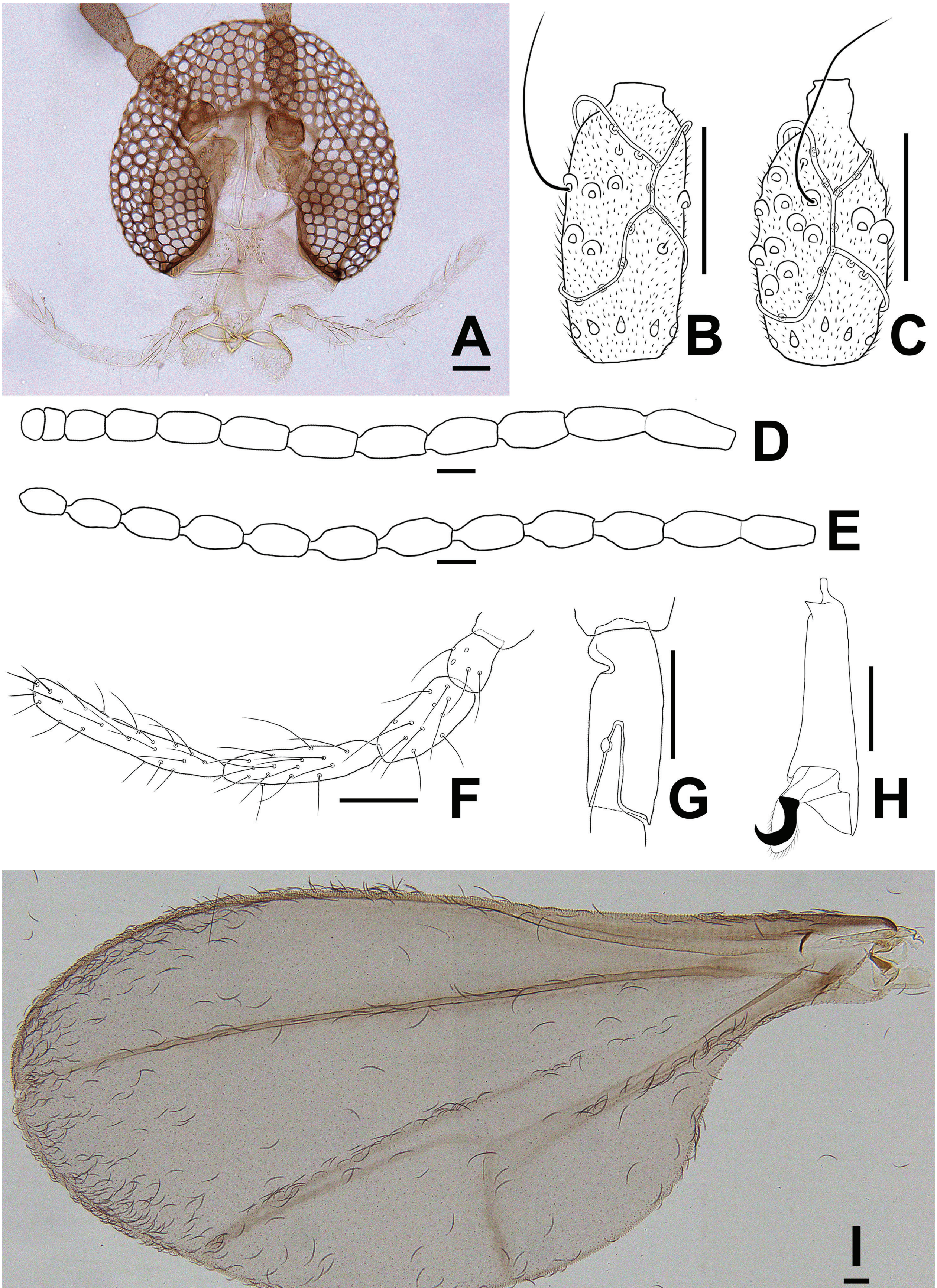


Figure 2. *Parampelomyia yukawai* sp. nov. **A** Head. **B** Dorsal view of female antennal flagellomere V. **C** Ventral view of male flagellomere V. **D** Female antennal flagellomeres. **E** Male antennal flagellomeres. **F** Palpus. **G** Tarsomere I. **H** Tarsomere V and acromere. **I** Wing. Scale bars = 50 µm.

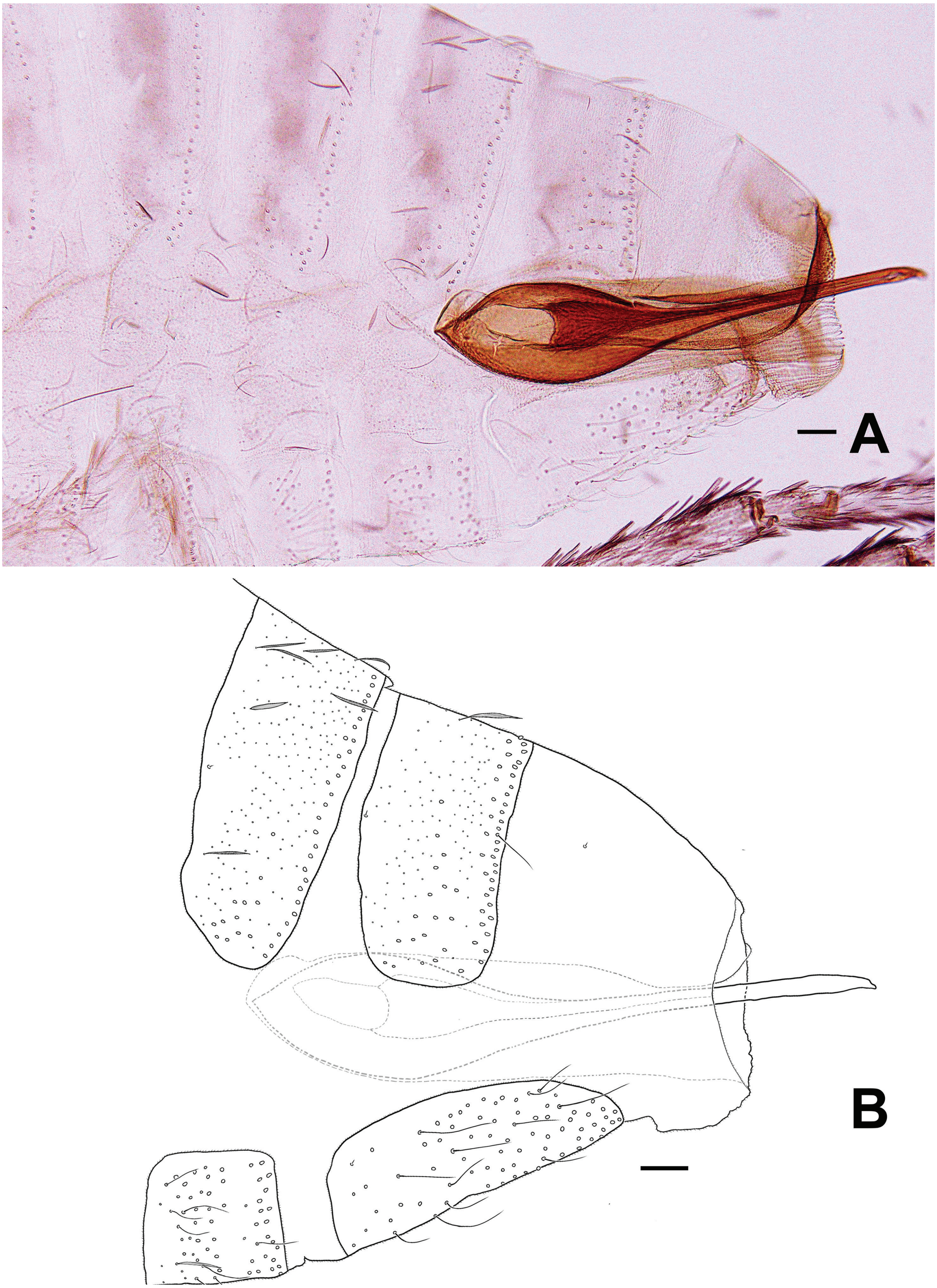
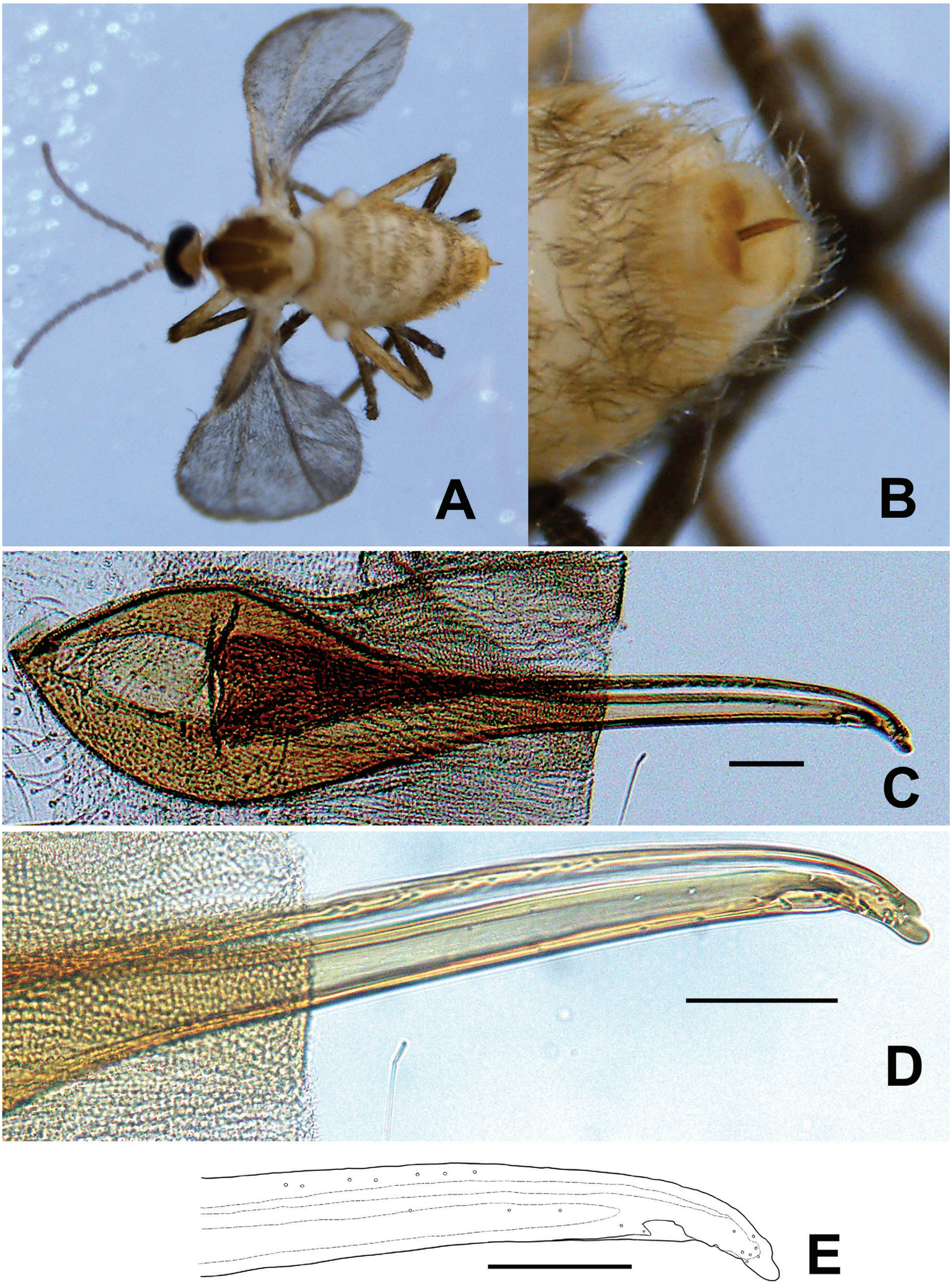


Figure 3. A, **B** Female terminal abdominal segments of *Parampelomyia yukawai* sp. nov. Scale bars = 50 µm.

ger than wide, with long setae dorsally on distal half and short setae ventrally; ventral gonostylar tooth shorter and more sclerotized than dorsal tooth (Fig. 5B, C); each cercus with strong seta and few fine setae along distal edge; hypoproct (Fig. 5D) shorter than cerci, with short lobes separated by shallow notch, each lobe with 1 short seta;



Figures 4. Female of *Parampelomyia yukawai* sp. nov. **A** Dorsal view of ethanol-preserved female body. **B** Dorsal view of terminal abdominal segments. **C** Protrusible part of ovipositor. **D–E** Terminal part of protrusible part of ovipositor. Scale bars = 50 µm.

gonocoxal lobes about 0.3 as long as aedeagus; aedeagus longer than cerci. — ***Pupa:*** Each antennal base with tiny anteroventral sclerotized projection and 1 asetose antennal papilla situated dorsoapically. Face with 2 setose and 2 asetose frontal papillae and triplet lateral papillae on each side, 1 setose and 2 asetose (Fig. 6C). Prothoracic

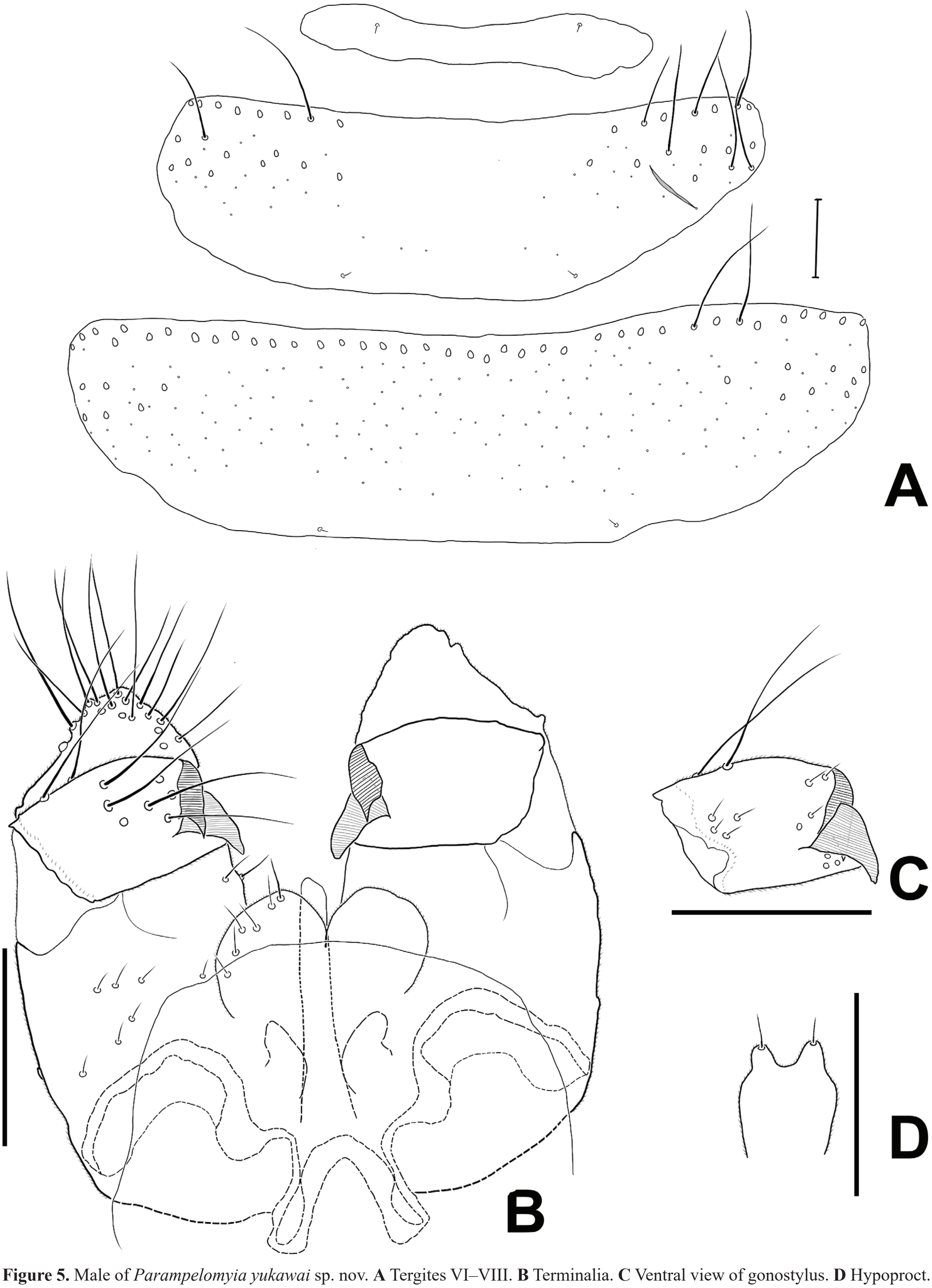


Figure 5. Male of *Parampelomyia yukawai* sp. nov. **A** Tergites VI–VIII. **B** Terminalia. **C** Ventral view of gonostylus. **D** Hypoproct. Scale bar = 50 µm.

spiracle with trachea extending to tip, ca. 3.2 times as long as cephalic seta and length ca. 8.2 times as long as wide at base (Fig. 6D). Terga II–VIII with 3–4 horizontal rows of dorsal spines on anteromedian third (Fig. 6E). — ***Mature larva:*** Orange in life (Fig. 1B). Spatular lobes shallowly separated (Fig. 7A). Abdominal segment VIII

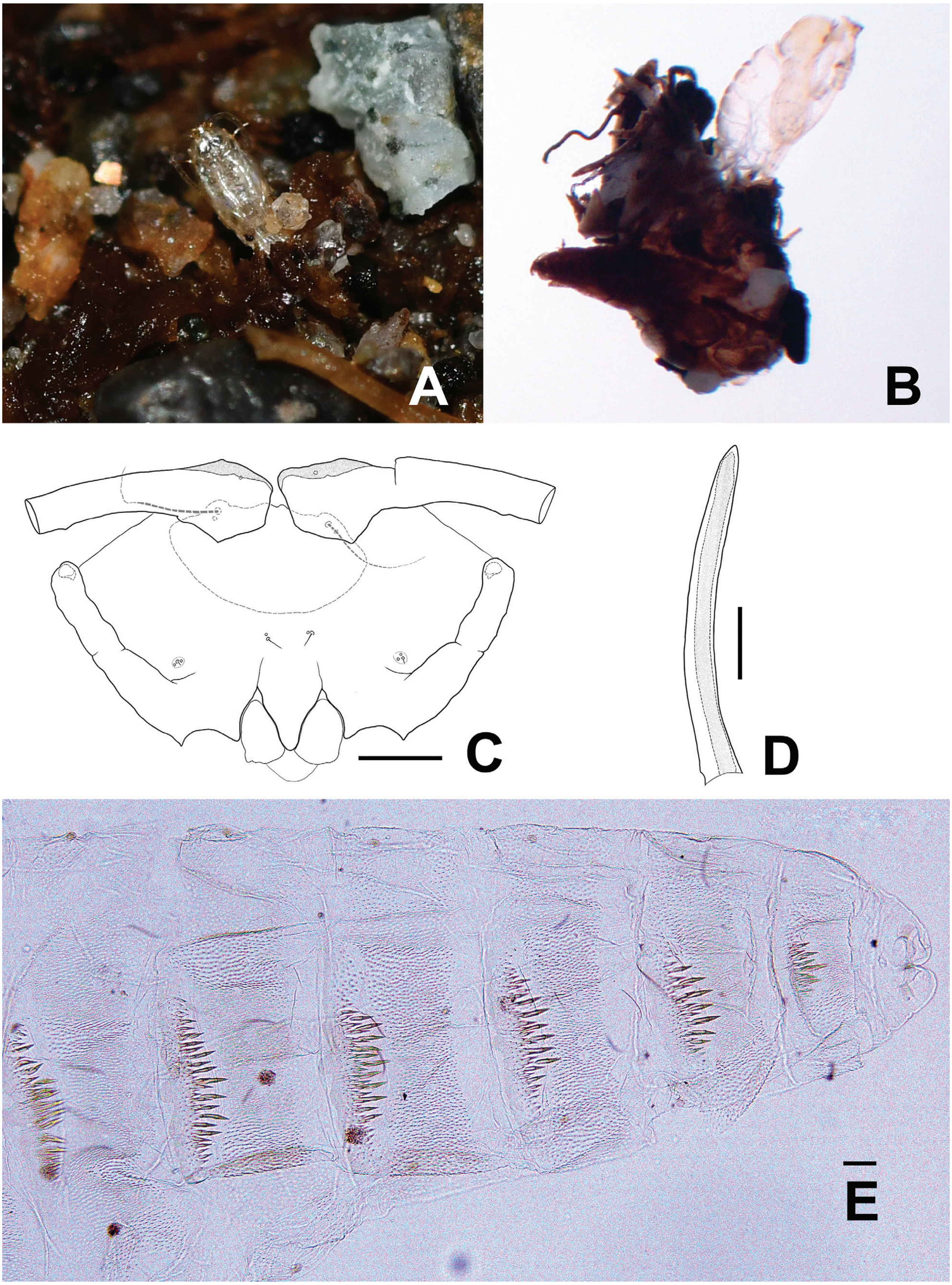


Figure 6. Pupa of *Parampelomyia yukawai* sp. nov. **A** Exuvia of fresh emerged adult in soil (photograph by A. K. Elsayed). **B** Exuvia protruding from cocoon. **C** Ventral view of pupal head. **D** Prothoracic spiracle. **E** Dorsal view of pupal abdomen.

with 2 dorsal papillae situated on mediodorsal lobe. Terminal segment with 2 large corniform papillae, 2 asetose papillae, and 6 setose papillae (Fig. 7B).

Derivatio nominis. It is our great pleasure to name this species in honor of the eminent emeritus professor Junichi Yukawa (Entomological Laboratory, Faculty of Agri-

Figure 7. Larva of *Parampelomyia yukawai* sp. **A** Larval spatula. **B** Larval abdominal segment VIII and terminal segment dorsally. Scale bars = 50 µm.

culture, Kyushu University) on the occasion of his decoration with The Order of the Sacred Treasure (Gold Rays with Neck Ribbon) by the Emperor of Japan in 2022. Professor J. Yukawa has outstanding contributions to the understanding of biodiversity, systematics and ecology of gall midges including species associated with Vitaceae (e.g. Yukawa 1971, 1974; Yukawa and Ohsaki 1988; Yukawa and Masuda 1996; Yukawa and Tokuda 2021; Yukawa et al. 2003, 2016, 2020) as well as other arthropods (e.g. Ngakan and Yukawa 2020; Yukawa et al. 2007, 2019).

Life history and remarks. *Parampelomyia yukawai* forms barely-swollen flower bud galls on *A. brevipedunculata* var. *heterophylla* (Vitaceae) (Fig. 1A, B). Galled flower buds remain unopened, and each gall consists of a single chamber containing a single larva (Fig. 1B). The corolla of galled flower bud dries and detaches after the mature larvae leave to overwinter in the ground in late August and early September. Larvae pupate in cocoons spun mostly from soil components (Fig. 6B). *Parampelomyia yukawai* has one generation per year, with adults emerging in the summer.

A similar sort of flower bud gall is known to be induced on the same host plant by *Schizomyia uechii*, but these galls are distinguishable from *P. yukawai* galls by their normal size and reddish corolla (Elsayed et al. 2019b; K. Matsunaga, personal communication).

Distribution. Japan: Hokkaido (Yukawa and Masuda 1996; Yukawa and Sunose 1997) and Honshu (Aomori and Shiga Prefectures).

Material examined. Holotype: JAPAN: Aomori Prefecture: 1 ♂, Nanatsutaki, Nakadomari, col. 29 Aug. 2020, A. Ichita leg., flower bud gall on *Ampelopsis brevipedunculata* var. *heterophylla* (Vitaceae), em. 23 Apr. 2021. Paratypes: JAPAN, all from flower bud galls on *Ampelopsis brevipedunculata* var. *heterophylla* (Vitaceae) • Hokkaido– 7 mature larvae, Etanbetsu, Asahikawa City, col. 1 Aug. 2014, T. Minami, leg. • 1♀, same collection data as for preceding, em. 06 Jun. 2021 • Aomori Pref.–1♂, 2♀♀ and 2 pupal exuviae, same collection data as for holotype, em. 21–29 Apr. 2021 • 1♂, 1♀ and 2 pupal exuviae, same type locality, col. 21 Aug. 2021, A. Ichita leg., em. 23 Apr.–02 May 2021 • 3 mature larvae, Minmaya, Sotogahama, col. 29 Aug. 2019, A. Ichita leg. • 3♀♀ and 2 pupal exuviae, same collection locality as for preceding, col. 27 Aug. 2019, A. Ichita leg. • Shiga Pref.– 1 mature larva, Omihachiman, Hachiman City, col. 6 Oct. 2020, A. Tominaga leg.

3.2. Molecular phylogenetic analysis

The monophyly of Schizomyiina was strongly supported by 100% bootstrap value (Fig. 8). The three specimens of *Parampelomyia yukawai* analyzed in this study were identical and constructed a separate clade from the clades of *Ampelomyia conicocoricis* and *Schizomyia* species. *Parampelomyia yukawai* differs from the clades of *Schizomyia* spp. and *Ampelomyia conicocoricis* with 19.9–24.1% (COI: 13.7–18.2%; 12S: 27.8–31.8%) and 26.0–26.6% (COI: 23.3–24.2%; 12S: 29.8–30.5%) of divergence distance, respectively.

4. Discussion

Among known schizomyiine genera, *Parampelomyia* is similar morphologically to the *Vitis*-associated genus, *Ampelomyia*, and the catch-all genus *Schizomyia*. However, *Parampelomyia* is distinctive from both genera by the following characters: male flagellomeres of *Parampelomyia* bear two simple connected whorls of circumfila, but sinuous circumfila in *Ampelomyia* and *Schizomyia*; the protrusible part of ovipositor is bulbous at the base in *Parampelomyia*, while wider at the base in *Ampelomyia* and extremely attenuate most known species of *Schizomyia*; and the gonostylus bears two teeth in *Parampelomyia*, but a single tooth in *Ampelomyia*

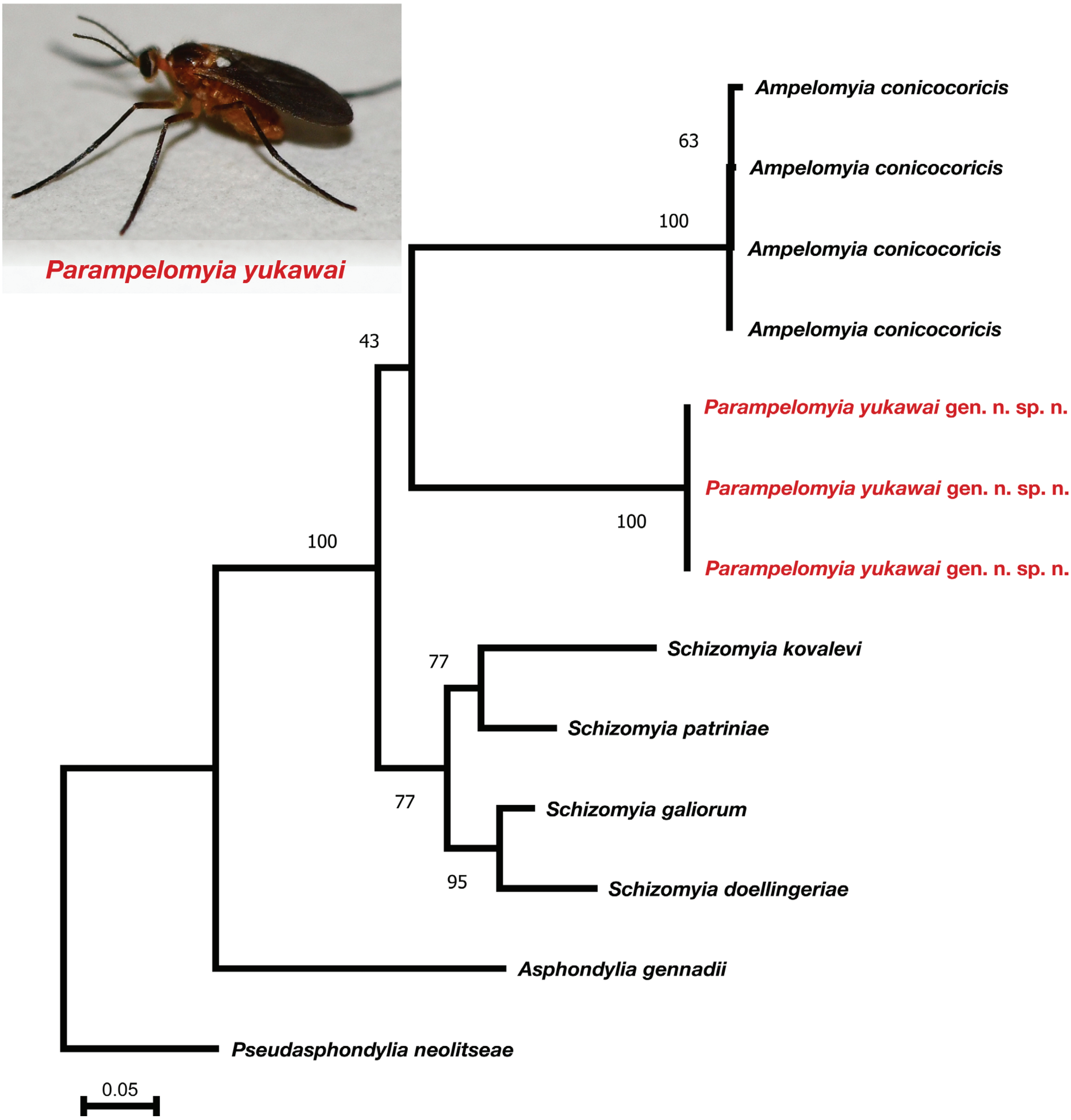


Figure 8. A Maximum Likelihood phylogenetic tree based on the amended alignment of 887 base pairs of COI and 12S partial sequences (including gaps).

and *Schizomyia* (Gagné and Marohasy 1997; Gagné and Menjivar 2008; Kolesik and Butterill 2015; Elsayed et al. 2018a, 2019a, 2019b, 2019c, 2020b, 2020c). Furthermore, *Parampelomyia* differs from *Ampelomyia* by the following characteristics: tarsomere I is simple in *Parampelomyia*, but possesses an apicoventral prolongation in *Ampelomyia*; tarsal claw is bent at midlength in *Parampelomyia*, while it is bent beyond midlength in *Ampelomyia*; female cerci are fused in *Parampelomyia*, but semi-fused in *Ampelomyia*; and the dorsalmost pleural papillae of larval abdominal segment VIII is not shifted to a dorsal position in *Parampelomyia* as in *Ampelomyia* (Elsayed et al. 2019).

Molecular phylogenetic analysis supports *Parampelomyia* is a distinct genus from the closely related genera within Schizomyiina, the catch-all genus *Schizomyia* and the *Vitis*-associated genus *Ampelomyia*. The high divergence distance indicates that their separation is rather old. *Schizomyia* comprise 75 species associated with wide diversity of host plants, and notability morphological variations within these species requires a worldwide revision (Elsayed et al. 2018a; Gagné and Jaschhof 2021). Since most *Schizomyia* species induce flower bud galls (Gagné 1989; A. K. Elsayed, unpublished data), a molecular analysis including all bud-associated *Schizomyia* may help clarify the evolutionary relationship between *Parampelomyia* and *Schizomyia*. On the other hand, known species of *Ampelomyia* form galls solely on leaves and stems of Vitaceae (Gagné 1994; Elsayed et al. 2019a), suggesting that *Parampelomyia* evolved separately on the flower buds. Further discoveries of *Ampelomyia* and *Parampelomyia* are necessary to track their co-evolutionary pathways on Vitaceae.

5. Acknowledgements

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