

Descriptions of two new species of the genus *Colocasiomyia* (Diptera, Drosophilidae) breeding on *Rhaphidophora* host plants in Yunnan, China

Run-Jie Jiao^{1,2}, Li-Hong Bai³, Jian-Jun Gao^{1,2}

1 Yunnan Key Laboratory of Plant Reproductive Adaptation and Evolutionary Ecology, School of Ecology and Environmental Science, Yunnan University, Kunming, Yunnan 650500, China **2** Laboratory of Ecology and Evolutionary Biology, Yunnan University, Kunming, Yunnan 650500, China **3** Huanglianshan National Nature Reserve, Lüchun, Yunnan 662500, China

Corresponding author: Jian-Jun Gao (gaojj@ynu.edu.cn)

Academic editor: Rudolf Meier | Received 18 July 2020 | Accepted 18 August 2020 | Published 16 September 2020

<http://zoobank.org/3F89CD8E-549D-42DE-94C8-618FCDB62D38>

Citation: Jiao R-J, Bai L-H, Gao J-J (2020) Descriptions of two new species of the genus *Colocasiomyia* (Diptera, Drosophilidae) breeding on *Rhaphidophora* host plants in Yunnan, China. ZooKeys 968: 127–141. <https://doi.org/10.3897/zookeys.968.56677>

Abstract

The genus *Colocasiomyia* de Meijere (Diptera, Drosophilidae) is known to include 30 described and nearly 60 undescribed species classified into six species groups. Among these, the *C. gigantea* group of seven known species (two Southeast Asian and five Chinese) proved to be peculiar for its specificity on monstroid (subfamily Monsteroideae, family Araceae) host plants. In this paper, two new species, *C. todai* Jiao & Gao, **sp. nov.** and *C. liae* Jiao & Gao, **sp. nov.**, are described as members of the *C. gigantea* group with specimens collected from inflorescences of the monstroid host species *Rhaphidophora peepla* (Roxb.) Schott and *R. crassicaulis* Engl. & Krause, respectively, in Yunnan, China. The two new species are delimited, in comparison with all known species, based on not only morphological but also DNA barcode (partial sequence of the mitochondrial *COI*, i.e., cytochrome *c* oxidase subunit I, gene) data. A revised key to all the nine species of the *C. gigantea* species group is provided.

Keywords

DNA barcoding, key to species, morphology, neighbor-joining tree, taxonomy

Introduction

The genus *Colocasiomyia* de Meijere, 1914 is among a few well known anthophilic genera in the family Drosophilidae (Brncic 1983; Grimaldi et al. 2003; Fu et al. 2016). Species in this genus (30 described and nearly 60 undescribed ones) are all discovered from tropical and subtropical regions of the Old World, and taxonomically classified into six species groups: i.e., the *C. crassipes* group of two (2 described + 0 undescribed) species associated with hosts from the family Magnoliaceae, the *C. zeylanica* group of six (2 + 4) species associated with hosts from the family Arecaceae, three species groups [*C. toshiokai* group of six (6 + 0) species, *C. baechlii* group of thirty (2 + 28) species, and *C. cristata* group of thirty three (11 + 22) species] associated with aroid hosts from the subfamily Aroideae, and the *C. gigantea* group of seven known species associated with aroid hosts from the subfamily Monsteroideae (e.g., Sultana et al. 2006; Fartyal et al. 2013; Li et al. 2014; Shi et al. 2019). The *C. gigantea* group was erected by Fartyal et al. (2013) for three species: *C. gigantea* (Okada) using *Epipremnum pinnatum* in Java, Indonesia and Solomon Is., *C. rhabdophorae* Gao & Toda using *Rhabdophora hookeri* in Yunnan, southwestern China, and *C. scindapsae* Fartyal & Toda using *Scindapsus coriaceus* in Sabah, Malaysia. Li et al. (2014) subsequently described four additional species, i.e., *C. longifilamentata* Li & Gao, *C. longivalva* Li & Gao, *C. hailini* Li & Gao and *C. yini* Li & Gao from western Yunnan with specimens collected from inflorescences of *Rhabdophora decursiva* (Roxb.) Schott. Here we add two new, Chinese species, i.e., *C. todai* Jiao & Gao, sp. nov. and *C. liae* Jiao & Gao, sp. nov., to the *C. gigantea* group, with specimens collected from inflorescences of *Rhabdophora peepla* (Roxb.) Schott and *R. crassicaulis* Engl. & Krause, respectively, in Yunnan (Fig. 1).

Material and methods

Specimens and morphological observation

Colocasiomyia specimens were collected in western (Baoshan) and southern (Lüchun) Yunnan using an insect net (for adults) or by dissecting host inflorescences or infructescences (for eggs or dormant larvae within egg capsules on host infructescences), and immediately preserved in 70% (for morphological observation) or 100% (for DNA sequencing) ethanol (Table 1).

We observed external morphology and detailed structures of dissected organs following the methods in Fartyal et al. (2013) and Li et al. (2014). For species illustration, we used a DinoLite Digital Eyepiece Camera to photograph the entire body, the wing, foreleg, and male/female genitalia for representative specimens. We followed McAlpine (1981) for the morphological terminology and Zhang and Toda (1992) for the definitions of measurements and indices. The type specimens are deposited in Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China (KIZ).



Figure 1. Two host plant species of *Colocasiomyia* flies **A–D** *Rhabdophora peepla* (Roxb.) Schott (Ertaipo, Mt. Gaoligongshan, Yunnan, China) **E–H** *R. crassicaulis* Engl. & Karase (Qimaba, Lüchun, Yunnan, China) **A, E** plants climbing on tall trees **B, F** inflorescence buds **C, G** inflorescences **D, H** infructescences (with leaves shown together in **H**).

Species delimitation

Adult *Colocasiomyia* specimens, either newly obtained or collected previously, were first identified and sorted into species of the *C. gigantea* group in light of morphology. DNA barcodes (i.e., the 658-bp barcoding region of the mitochondrial cytochrome *c* oxidase I, *COI*, gene) were determined for representing specimens (adults, eggs, or

Table 1. *Colocasiomyia* specimens (noted in cases of egg or larval stage) used for DNA sequencing in the present study.

Species	Voucher specimens (voucher #/Stage/Gender) and collection data
<i>C. hailini</i>	01518–01522/larva/-, 01727–01731/egg/-, 01295/egg/-, 01296/egg/-; Laomengshan, Baihualing, Baoshan, Yunnan, China; ex. spadices or spathes of <i>R. decursiva</i>
<i>C. liae</i> sp. nov.	10485/adult/♂, 10486/adult/♀; Qimaba, Lüchun, Yunnan, China; ex. inflorescences of <i>R. crassicaulis</i> 09562–09267/adult/♂, 09568–09272/adult/♀; Qimaba, Lüchun, Yunnan, China; reared from infructescences of <i>R. crassicaulis</i>
<i>C. longifilamentata</i>	01133/adult/♂, 01252/egg/-, 0158/egg/-, 01588/larva/-; Laomengshan, Baihualing, Baoshan, Yunnan, China; ex. inflorescences of <i>R. decursiva</i>
<i>C. longivalva</i>	01722/adult/♀; Laomengshan, Baihualing, Baoshan, Yunnan, China; ex. inflorescences of <i>R. decursiva</i> 10103/adult/♂, 10111/adult/♂, 10114/adult/♂, 10120/adult/♀, 10121/adult/♂, 10124/adult/♀, 10127/adult/♂, 10132/adult/♀, 10133/adult/♂, 10134/adult/♀, 10135/adult/♀, 10139/adult/♂, 10143/adult/♀, 10145/adult/♀, 10146/adult/♀; Ertaiipo, Baihualing, Baoshan, Yunnan, China; ex. inflorescences of <i>R. peepla</i>
<i>C. todai</i> sp. nov.	10100/adult/♀, 10102/adult/♀, 10105–10110/adult/♀, 10112/adult/♀, 10113/adult/♀, 10115–10118/adult/♂, 10122/adult/♂, 10128/adult/♀, 10129/adult/♂, 10130/adult/♀, 10131/adult/♂, 10136–10138/adult/♀, 10140–10142/adult/♀, 10144/adult/♀; same collection data as above
<i>C. yini</i>	10123/adult/♂; same collection data as above

larvae) of each morpho-species (Table 1). We followed the methods of Li et al. (2014) for DNA extraction, PCR amplification and DNA sequencing, using Folmer et al.’s (1994) primer pair LCO1490 (5'- GGTC ACAA TCATA AAGAT ATTGG -3') and HCO2198 (5'- TAAAC TTCAG GGTGA CAAA AAATC A -3'). Sequences obtained were edited in the SeqMan module of the DNASTar package version 7.1.0 (DNASTar Inc., Madison, WI). The newly determined sequences were then aligned with 45 previously determined barcodes of the *C. gigantea* group (Table 1) using the software MEGA7 (Kumar et al. 2016). Neighbor-joining (NJ) trees were constructed in MEGA7 with the sequence alignment based on *p*-distances and also Kimura 2-parameter (K2P) divergences for comparison, with node supports (bootstrap percentages) calculated with 1000 replicates. The intra- and interspecific *p*- and K2P-distances were calculated for all the species in MEGA7, and then the barcoding “gap” (Meyer and Paulay 2005) was evaluated with intra- and interspecific *p*-distances, following the methods in Meier et al. (2006, 2008).

Results

DNA barcoding

A total of 60 adult specimens of the *C. gigantea* group were morphologically sorted into five species, including three known (*C. longifilamentata*, *C. longivalva*, and *C. yini*) and two new ones (*C. todai* sp. nov. and *C. liae* sp. nov.) (Table 1). *COI* barcodes were determined for all 60 adults and 15 immature specimens (12 of *C. hailini*, 3 of *C. longifilamentata*) (Table 1). The alignment of the 75 newly determined barcodes (GenBank accession numbers: MT916851–MT916925) and the 45 previously determined ones spans 658 (494 conserved, 164 variable including 154 parsimony-informative) nucleotide sites.

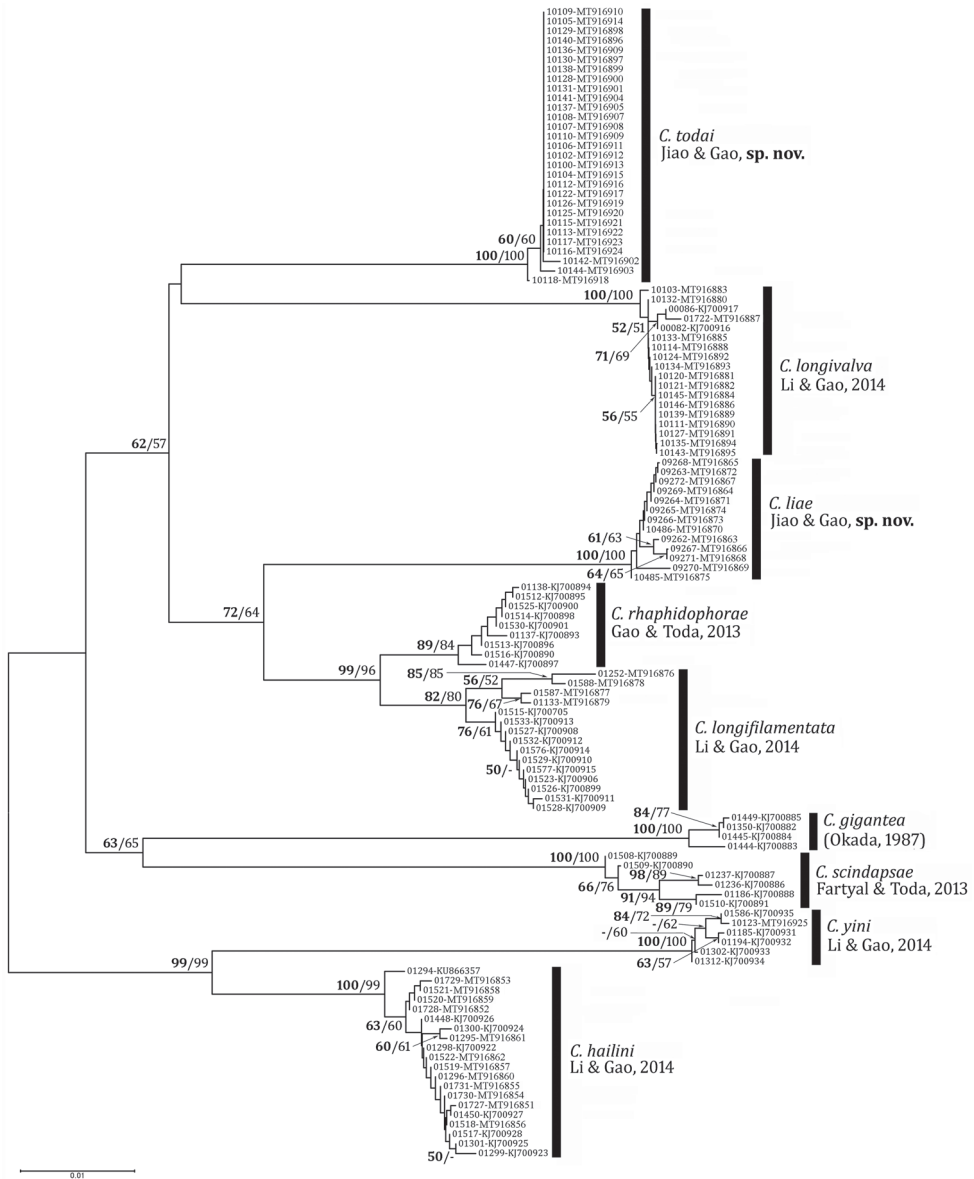


Figure 2. Unrooted neighbor-joining tree of the *C. gigantea* species group built based on *p*-distances between *COI* sequences. Label of each operational taxonomic unit (OTU) is given in the form of “voucher number-GenBank accession number”. Numbers beside nodes are bootstrap percentages (shown when ≥ 50 ; BP based on *p*-distance/BP based on K2P-distance).

The two NJ trees based on *p*- and K2P-distances showed the identical topology, but slightly differed in terms of BP: the tree based on *p*-distances yielded overall higher BPs (Fig. 2; but the K2P-distance tree not shown), confirming Srivathsan and Meier’s (2012)

observation that *p*-distance performs better than K2P-distance in NJ-tree construction for DNA barcoding. The sequence clusters corresponding to the morpho-species are all compact and supported with high BPs (≥ 80 in both of the *p*- and K2P-distance methods). The grouping of two small-bodied species, *C. hailini* and *C. yini*, was strongly supported (BP = 99 in both methods). In addition, the sister-relationship between *C. rhabdophorae* and *C. longifilamentata* was strongly supported (BP = 99 and 96, respectively).

Table 2 shows the intra- and interspecific *p*-distances in the *C. gigantea* group. The observed maximal intraspecific distance (0.0130 in *C. longifilamentata*) was the same as the minimal interspecific one (0.0130 between *C. longifilamentata* and *C. rhabdophorae*). There was a gap (size = 0.0117 *p*-distance) between the overall mean intraspecific (0.0013) and the minimal interspecific *p*-distances, while by deleting the 5% largest intraspecific and the 5% smallest interspecific distances, a wider gap (size = 0.0491 *p*-distance) was observed.

Taxonomy

Colocasiomyia gigantea species group Fartyal et al. (2013)

Included species. *C. gigantea* (Okada, 1987); *C. rhabdophorae* Gao & Toda and *C. scindapsae* Fartyal & Toda in Fartyal et al. (2013); *C. hailini* Li & Gao, *C. longifilamentata* Li & Gao, *C. longivalva* Li & Gao, and *C. yini* Li & Gao in Li et al. (2014); *C. todai* Jiao & Gao, sp. nov. and *C. liae* Jiao & Gao, sp. nov.

Key to species of the *C. gigantea* species group

This key is updated from that of Li et al. (2014), referring to some figures in Okada (1987), Fartyal et al. (2013), and Li et al. (2014) which are indicated with the subscripts “O87”, “F13”, and “L14”, respectively.

- 1 Aedeagus not pubescent; aedeagal apodeme as long as or longer than aedeagus (fig. 4C_{F13}, fig. 40_{L14}, fig. 47_{L14}) **2**
- Aedeagus pubescent (except for *C. liae* sp. nov.); aedeagal apodeme distinctly shorter than aedeagus **4**
- 2 Foreleg tarsomere II with seven or eight pegs (fig. 4B_{F13}). Epandrial apodeme medially narrower than epandrium (fig. 4C_{F13}). Aedeagal apodeme much longer than aedeagus (fig. 4C_{F13}). Distal, narrow part of oviscapt much shorter than proximal, broad part, apically shaped like arrowhead, with a pair of stout, peg-like ovisensilla at apex (fig. 4B_{F13}) ***C. scindapsae* Fartyal & Toda**
- Foreleg tarsomere II with six pegs (fig. 14_{L14}, fig. 15_{L14}). Epandrial apodeme well developed into distally tapering, triangular extension strongly projected anteriorly, twice as long as epandrial width (fig. 38_{L14}, fig. 45_{L14}). Aedeagal apodeme slightly longer than aedeagus (fig. 40_{L14}, fig. 47_{L14}). Distal, narrow part of oviscapt rod-shaped, slightly shorter than proximal, broad part, with only trichoid ovisensilla (fig. 43_{L14}, fig. 50_{L14}) **3**

- 3 Wing C3F index $< 2/3$. Distance between antennal sockets same as socket width. Distal, narrow part of oviscapt constricted subbasally on dorsal margin (fig. 43_{L14}) ***C. hailini* Li & Gao**
- Wing C3F index $> 2/3$. Distance between antennal sockets larger than socket width. Distal, narrow part of oviscapt finger-like, not constricted subbasally on dorsal margin (fig. 50_{L14}) ***C. yini* Li & Gao**
- 4 Labellum with 14 pseudotracheae per side. Distal, narrow part of oviscapt broadly truncate apically, much shorter than proximal, broad part (fig. 1F_{O87}, fig 2H_{F13}) ***C. gigantea* (Okada)**
- Labellum with 17 or more pseudotracheae per side. Distal, narrow part of oviscapt not or only slightly truncate apically, ca $2/5$ or longer the length of proximal, broad part. **5**
- 5 Epandrium notched above basal corner of epandrial ventral lobe (fig. 30_{L14}). Ventral lobe apically with a grooved, finger-like peg (fig. 32_{L14}). Distal, narrow part of oviscapt twice as long as proximal, broad part (fig. 36_{L14}) ***C. longivalva* Li & Gao**
- Epandrium not notched along posterior margin. Ventral lobe apically with an ungrooved, inward-curved peg or thick spine. Distal, narrow part of oviscapt as long as or shorter than proximal, broad part **6**
- 6 Labellum with 17 pseudotracheae per side. Epandrium broad, with short ventral lobe apically inlaid with thick, long, inward-curved, spine-like seta (Fig. 4A, B) ***C. todai* Jiao & Gao, sp. nov.**
- Labellum with ≥ 21 pseudotracheae per side. Epandrium with long ventral lobe apically inlaid with short peg. **7**
- 7 Epandrium with setae on posterior margin in addition to those on ventral lobe (fig. 24_{L14}). Distal, narrow part of oviscapt nearly as long as proximal, broad part (fig. 28_{L14}) ***C. longiflamentata* Li & Gao**
- Epandrium with no setae on posterior margin above ventral lobe (fig. 3C_{F13}, Figs 5A, B); distal, narrow part of oviscapt distinctly shorter than proximal, broad part (fig. 3G_{F13}, Fig. 5F) **8**
- 8 Labellum with 21–22 pseudotracheae per side. Ventral lobe of epandrium well developed, narrowing distally in lateral view, scabbard-like, with 3 long setae on dorsosubbasal margin, apically inlaid with a short peg; cercus with slightly projected ventral apex (fig. 3C_{F13}). Aedeagal apodeme ca $3/4$ the length of aedeagus (fig. 3D_{F13}). Distal, narrow portion of oviscapt narrowing distally, gently curved dorsad (fig. 3G_{F13}) ***C. rhaphidophorae* Gao & Toda**
- Labellum with 34 pseudotracheae per side. Ventral lobe of epandrium prolonged like a rod, distally slightly broadened in lateral view, with 2 long and 1 medium-length setae on its insertion, apically inlaid with a relatively long, claw-like peg; circus ventrally lacking projected apex (Fig. 5A, B). Aedeagal apodeme short than $1/2$ the length of aedeagus (Fig. 5D, E). Distal, narrow portion of oviscapt extended with nearly even width, slightly sinuate (Fig. 5F) ***C. liae* Jiao & Gao, sp. nov.**

Table 2. Intra- and interspecific *p*-distances in the *Colocasiomyia gigantea* species group.

#	Species name	Number of sequences	Intraspecific distances ([Minimal, Maximal], Mean ± SE ^a)	Interspecific distances ^b								
				1	2	3	4	5	6	7	8	9
1	<i>C. gigantea</i>	4	[0.0000, 0.0058], 0.0029 ± 0.0016		0.0898	0.1020	0.0940	0.1120	0.0912	0.0890	0.0907	0.1170
2	<i>C. hailini</i>	20	[0.0000, 0.0065], 0.0017 ± 0.0007	0.1006		0.0807	0.0802	0.0904	0.0797	0.0846	0.0768	0.0560
3	<i>C. liae</i> sp. nov.	13	[0.0000, 0.0063], 0.0015 ± 0.0008	0.1178	0.0912		0.0857	0.0822	0.0802	0.0923	0.0715	0.0938
4	<i>C. longifilamentata</i>	15	[0.0000, 0.0130], 0.0047 ± 0.0015	0.1081	0.0975	0.0990		0.0706	0.0130	0.0802	0.0595	0.1057
5	<i>C. longivalva</i>	18	[0.0000, 0.0049], 0.0006 ± 0.0003	0.1186	0.1037	0.0952	0.0796		0.0706	0.0822	0.0711	0.1174
6	<i>C. raphidophorae</i>	9	[0.0000, 0.0053], 0.0017 ± 0.0008	0.0985	0.0912	0.0978	0.0268	0.0796		0.0857	0.0579	0.1017
7	<i>C. scindapsae</i>	6	[0.0000, 0.0100], 0.0059 ± 0.0021	0.1042	0.1053	0.1090	0.0978	0.0952	0.0990		0.0819	0.1177
8	<i>C. todai</i> sp. nov.	29	[0.0000, 0.0030], 0.0003 ± 0.0002	0.0983	0.0896	0.0791	0.0729	0.0785	0.0658	0.1058		0.1057
9	<i>C. yini</i>	6	[0.0000, 0.0000], 0.0016 ± 0.0010	0.1217	0.0657	0.1047	0.1217	0.1258	0.1141	0.1342	0.1139	

^a SE, standard error.
^b Maximal values below diagonal, minimal values above diagonal.

Descriptions of species

***Colocasiomyia todai* Jiao & Gao, sp. nov.**
<http://zoobank.org/A34F80B7-8EB2-4686-BE1F-54AD9364A8CF>
Figures 3A–E, 4

Diagnosis. This species closely resembles *C. longivalva* in external morphology and structure of male genitalia, but can be distinguished from the latter by epandrium with short ventral lobe apically inlaid with a thick, long, inward-curved, spine-like seta (Fig. 4A, B); surstylus with 2 small, peg-like sensilla on inner, apical surface, 2 tiny, tooth-like setae on inner, ventrosubapical surface, and 1 tiny, trichoid seta on inner, dorsosubapical surface (Fig. 4C); and distal, narrow portion of oviscapt shorter than proximal, broad portion (Fig. 4F).

Description. (♂, ♀). **Head:** Supracervical setae about 17 per side. Dorsomedial arm of tentorial apodeme about 2/3 as long as dorsolateral arm. Eye red, somewhat roundish, lacking interfacetal setulae. Frontal vitta mat, black. First flagellomere not concave on inner margin. Facial carina trapeziform, medially twice as wide as first flagellomere, as long as pedicel and first flagellomere combined. Palpus convex on ventrodistal portion. Cibarium posterior sensilla minute, 1 or 2 per side. Labellum with 19 pseudotracheae per side.

Thorax (Fig. 3A, C): Scutum, scutellum and thoracic pleura glossy, blackish brown to black. Acrostichal setulae in 6 rows.

Wing (Fig. 3D) hyaline, veins yellow. Halter grayish brown except for grayish yellow stalk.

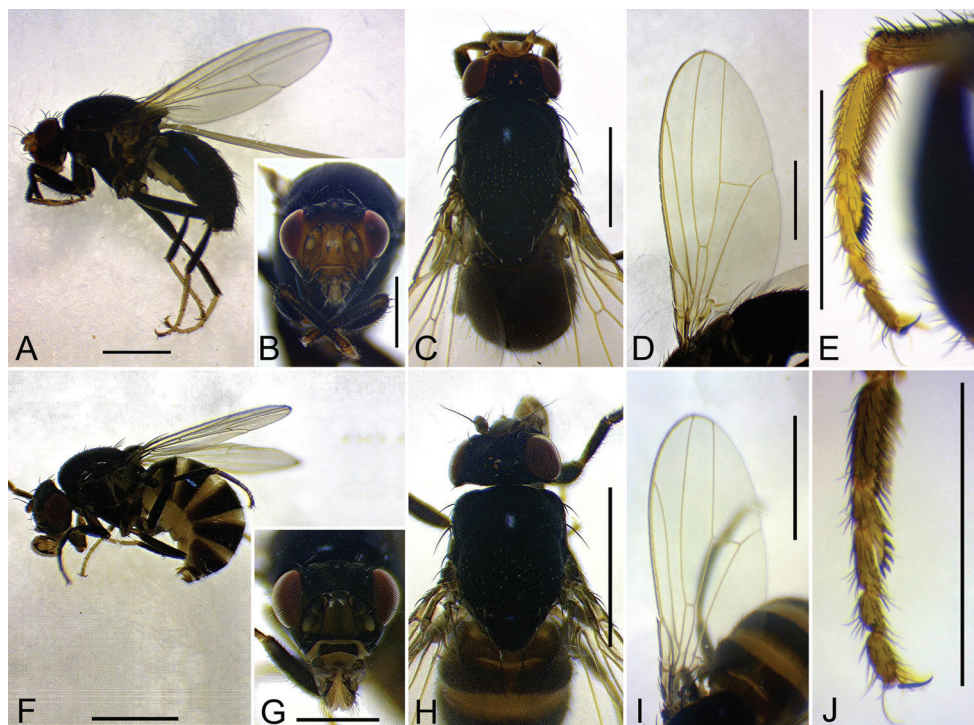


Figure 3. Adult males of the new species: lateral habitus, head (anterior view), head and thorax (dorsal view), wing (ventral view of left one in **D** dorsal view of right one in **I**), and fore leg (right one, inner view) **A–E** *Colocasiomyia todai* Jiao & Gao, sp. nov. (#10122) **F–J** *C. liae* Jiao & Gao, sp. nov. (#10485). Scale bars: 1.0 mm except for **B**, **E**, **G** and **J** (0.5 mm).

Legs (Fig. 3A, E) blackish brown to black except for grayish yellow tarsi. Foreleg second tarsomere with 10–12 pegs. Foreleg coxa large, with 1–2 long setae on underside near attachment to trochanter. Small preapical dorsal seta present only on hindleg tibia.

Abdomen (Fig. 3A): Tergites glossy, entirely black; II to VI+VII each bearing setulae and setae in approximately 3–4 transverse rows; setae of posteriormost row largest. Sternites yellowish brown to blackish brown; VI somewhat triangular, posteriorly not bilobed.

Male terminalia (Fig. 4A–E): Epandrium broad, with large, prominent apodeme lobe on anteromedial to subventral margin, pubescent except for anterior and ventral margins; anteroventral portion curved inward, apically articulated to lateral arm of hypandrium (Fig. 4A, B). Cercus semilunar, narrowly projected at ventral apex, pubescent except for anterior margin and ventral 1/3, with ca 52 setae (Fig. 4A). Surstylus entirely narrow sclerite, elongated downward, basally articulated with epandrial ventral lobe (Fig. 4A–C). Tenth sternite less sclerotized, folded into two lateral lobes caudo-dorsally connected with each other (Fig. 4B). Hypandrium long, thin plate, distal 1/3 constricted, posteriorly T-shaped, with lateral arms fused to aedeagal basal processes (Fig. 4D, E). Paramere broad sword-shaped in lateral view, coalescent to hypandrium,

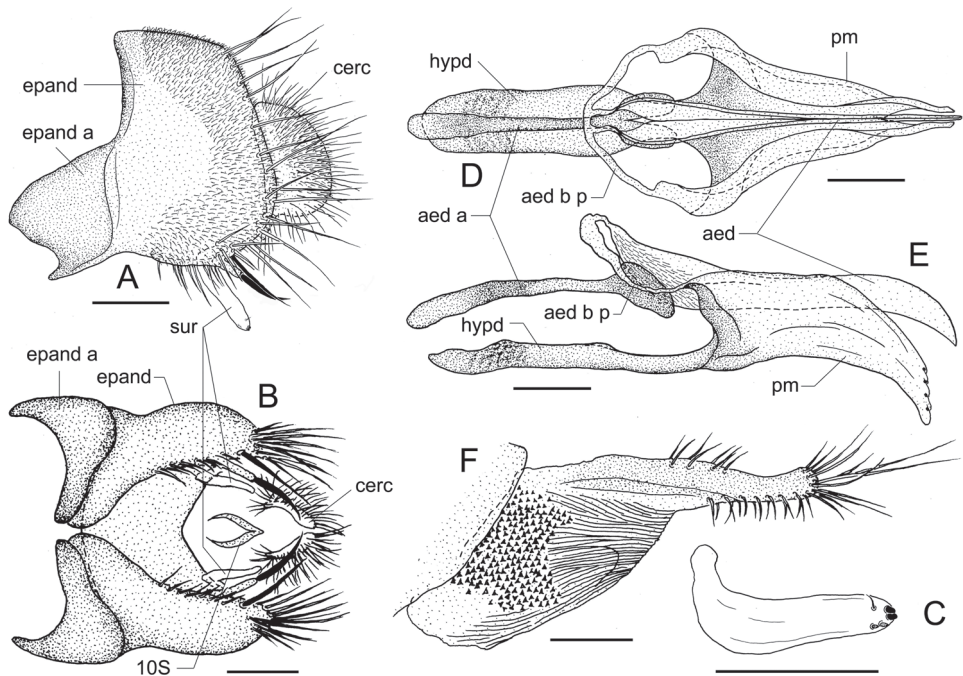


Figure 4. *Colocasiomyia todai* Jiao & Gao, sp. nov. Adult male (holotype #10122) and female (paratype, #10100) from Ertaiipo, Gaoligong Mountains, Baoshan, Yunnan, China **A** periphallal organs (lateral view) **B** periphallal organs (ventral view) **C** surstylus (right one, inner view) **D** phallic organs (dorsal view) **E** phallic organs (lateral view) **F** oviscapt (lateral view). Abbreviations: aed = aedeagus, aed a = aedeagal apodeme, aed b p = aedeagal basal process, cerc = cercus, epand = epandrium, epand a = epandrial apodeme, hypd = hypandrium, pm = paramere, 10S = tenth sternite. Scale bars: 0.1 mm.

triangular in ventral view, distally curved ventrad, with ca 4 sensilla arranged in a row (Fig. 4D, E). Aedeagus separated into a pair of lobes ventrally connected with each other, pubescent basally, subapically bent ventrad, pointed at apex; aedeagal basal processes somewhat membranous, connecting dorsobasal corners of aedeagus and lateral arms of hypandrium (Fig. 4D, E).

Female terminalia (Fig. 4F): Tergite VII mid-dorsally not constricted; VIII pubescent nearly entirely, with 3 setae in a vertical row on discolored, posteroventral portion. Oviscapt with distal, narrow elongation in addition to proximal, broad portion; proximal portion with large patch of dense, distinct warts; distal portion apically more or less truncated, with ca 3–4, 7–8 and 6 trichoid ovisensilla per side on basal 2/5 of dorsal margin, entire ventral margin, and at apex, respectively, but lacking any peg-like ovisensillum.

Measurements: BL = 3.53 (range in 5♂ paratypes: 3.27–3.38; range in 5♀ paratypes: 3.25–3.70) mm, ThL = 1.67 (1.56–1.72; 1.49–1.73) mm, WL = 3.38 (3.09–3.45; 3.15–3.45) mm, WW = 1.50 (1.25–1.48; 1.30–1.50) mm.

Indices: arb = 0/0 (5♂, 5♀, or less if noted, paratypes: 0/0), FW/HW = 0.56 (0.56–0.59), ch/o = 0.59 (0.45–0.57), prorb = 0.87 (0.75–0.99), rcorb = 0.38 (0.35–0.49), orbito = 0.68 (0.64–0.95), vb = 0.46 (0.34–0.49), dcl = 0.51 (4♂, 5♀:

0.49–0.56), dcp = 1.09 (4♂, 5♀: 1.02–1.19), sterno = 0.97 (0.75–0.98), sctl = 0.75 (4♂, 5♀: 0.73–0.84), sctlp = 1.47 (1.28–1.47), C = 1.82 (1.82–2.11), 4c = 1.26 (1.05–1.25), 4v = 2.02 (1.76–2.04), 5x = 0.91 (0.89–1.06), ac = 3.45 (3.09–3.82), M = 0.46 (0.38–0.48), C3F = 0.80 (0.73–0.85).

Material examined. *Holotype* ♂ (#10122): CHINA: *ex* inflorescence of *Rhaphidophora peepla* (Roxb.) Schott, Ertaiipo, Gaoligong Mountains, Baoshan, Yunnan, China, 25°18.0'N, 98°47.0'E, ca 2200 m, 31.vii.2019, Jian-Jun Gao and Xue-Lin Ye (KIZ). *Paratypes*: same data as holotype except for ca 2000–2250 m (5♂: #10115–18, #10131; 13♀: #10100, #10102, #101004, #10106–08, #10110, #10112, #10113, #10125, #10126, #10128, #10130) (KIZ).

Distribution. China (Yunnan).

Host plant. *Rhaphidophora peepla* (Roxb.) Schott (Fig. 1A–D).

Etymology. Patronym, in honor of Professor Masanori J. Toda (Hokkaido University), who dedicated himself to the studies of taxonomy and flower-visiting/breeding behaviors of *Colocasiomyia* flies.

Remarks. Li et al. (2014) described *C. longifilamentata*, *C. hailini*, *C. yini* and *C. longivalva* with specimens collected exclusively from inflorescences of *R. decursiva* at Baihualing, Baoshan, Western Yunnan, but mentioned that very few adults of the last two species, especially *C. longivalva*, were collected from inflorescences of *R. decursiva*. Our subsequent field work there has revealed that *C. longifilamentata* and *C. hailini*, rarely together with *C. yini*, share inflorescences/infructescences of *R. decursiva* as their breeding resources but that *C. longivalva* does not breed on this plant at all (data not shown). Recently, we have found that *C. longivalva*, together with *C. todayi* sp. nov., use *R. peepla* as a host plant: adults of both species were abundantly collected from inflorescences of this plant (Table 1); and a large number of *Colocasiomyia* 1st-instar larvae were found overwintering within egg capsules between growing pistils of infructescences, and they were later identified as *C. longivalva* or *C. todayi* sp. nov. by DNA barcoding or by examining the morphology of adults obtained from rearing the 1st-instars (data not shown).

Colocasiomyia liae Jiao & Gao, sp. nov.

<http://zoobank.org/D074B28A-894D-4B01-9D8E-537EE4140D29>

Figures 3F–J, 5

Diagnosis. This species closely resembles *C. rhaphidophorae* in external morphology and structure of male and female genitalia, but can be distinguished from the latter by epandrial ventral lobe rod-like, distally slightly broadened in lateral view, apically inlaid with a relatively long, claw-like peg (Fig. 5A, B); aedeagus broader in distal half in ventral view (Fig. 5D); surstylus apically expanded, with 1 dorsosubapical, 2 ventrosobapical, minute sensilla in addition to three small setulae at apex (Fig. 5A); distal, narrow elongation of oviscapt somewhat sinuate in lateral view (Fig. 5F).

Description. (♂, ♀). **Head:** Supracervical setae about 9–10 per side. Dorsomedial arm of tentorial apodeme about 1/3 as long as dorsolateral arm. Eye red, somewhat

roundish, with very sparse interfacetal setulae. Frontal vitta mat, black. First flagellomere not concave on inner margin. Facial carina broad trapeziform, medially twice as wide as first flagellomere, as long as pedicel and first flagellomere combined. Palpus convex on ventrodistal portion. Cibarium posterior sensilla minute, 2 or 3 per side. Labellum with 34 pseudotracheae per side.

Thorax (Fig. 3F, H): Scutum, scutellum and thoracic pleura glossy, black. Acrostichal setulae in 6 rows.

Wing (Fig. 3I) hyaline, veins yellow. Halter grayish brown except for grayish yellow stalk.

Legs (Fig. 3F, J) blackish brow to black except for grayish yellow knee joints and tarsi: Foreleg second tarsomere with 10 pegs (Fig. 3J). Foreleg coxa large, with 1–2 long setae on underside near attachment to trochanter. Small preapical dorsal seta present only on hindleg tibiae.

Abdomen (Fig. 3F): Tergites glossy, entirely black except for anterior, narrow, grayish margins on III–VI; II to VI+VII each bearing setulae and setae in approximately 3–4 transverse rows; setae of posteriormost row largest. Sternites yellowish brown to blackish brown; VI posteriorly bilobed.

Male terminalia (Fig. 5A–E): Epandrium dorsally narrow, with prominent apodeme on anteromedial to ventral margin, unpubescent on medial and anteroventral portions; ventral portion curved inward, apically articulated to lateral arm of hypandrium; ventral lobe well developed, with two long and one medium-length, thick setae on its insertion, and 6–7 setae along ventral margin (Fig. 5A, B). Cercus somewhat trapeziform, pubescent on dorsal 2/3, with ca 29 setae on dorsal 1/3 and posterior margin (Fig. 5A). Surstylus entirely narrow sclerite, grayish yellow, elongated downward, basally articulated with epandrial ventral lobe (Fig. 5A, B). Tenth sternite medially forms vertical ridge, ventrally folded upwardly, forming a large, peripheral lobe (Fig. 5C). Hypandrium long, thin plate, distal 1/2 constricted, posteriorly T-shaped, with lateral arms fused to aedeagal basal processes (Fig. 5D, E). Paramere broad, double-layered, coalescent to hypandrium, gently curved ventrad at distal 1/3, ventrosubapically with a minute sensillum, distally hirsute (Fig. 5D, E). Aedeagus entirely unpubescent, bent dorsad gently (Fig. 5D, E); aedeagal basal processes somewhat membranous, connecting dorsobasal corners of aedeagus and lateral arms of inner and outer layers of hypandrium (Fig. 5D, E).

Female terminalia (Fig. 5F): Tergite VII mid-dorsally not constricted; VIII pubescent nearly entirely, with 3 setae in a vertical row on discolored, posteroventral portion. Oviscapit with distal, narrow elongation in addition to proximal, broad portion; proximal portion with large patch of dense, distinct warts; distal, narrow portion, with ca 2, 8 and 5 trichoid ovisensilla per side on basal 1/3 of dorsal margin, entire ventral margin, and at apex, respectively, and a tiny, peg-like ovisensillum near subapical, dorsal margin.

Measurements: BL = 2.45 (1♀ paratype: 2.60) mm, ThL = 1.10 (0.93) mm, WL = 2.08 (1.80) mm, WW = 0.91 (0.75) mm.

Indices: arb = 0/0 (1♀ paratype: 0/0), FW/HW = 0.57 (0.58), ch/o = 0.47 (0.51), prorb = 1.14 (1.05), rcorb = 0.28 (0.45), orbito = 0.67 (0.86), vb = 0.34

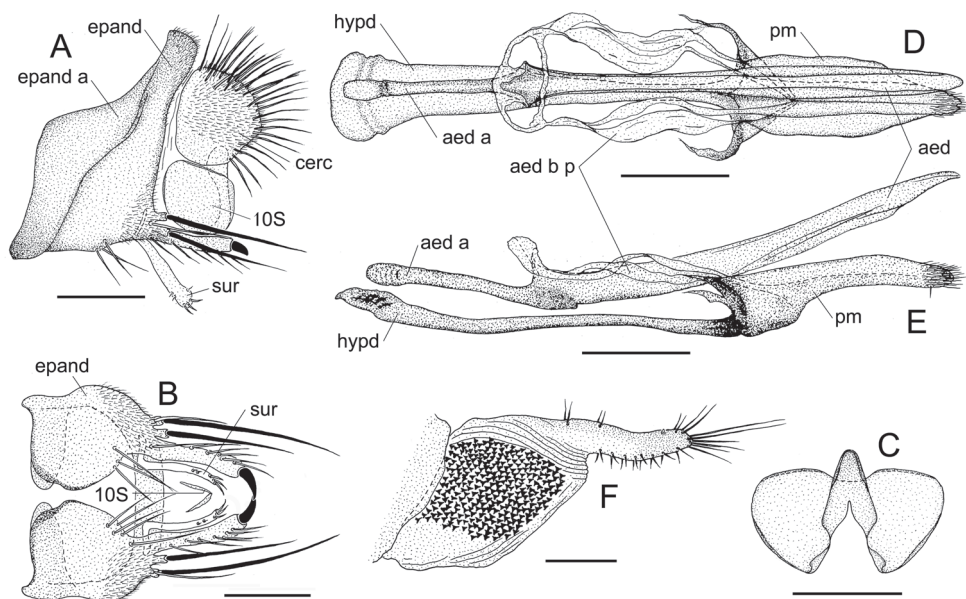


Figure 5. *Colocasiomyia liae* Jiao & Gao, sp. nov. Adult male (holotype #10485) and female (paratype, #10486) from Qimaba, Lüchun, Yunnan, China. **A** peripheral organs (lateral view) **B** peripheral organs except cerci (ventral view) **C** tenth sternite (posteroventral view) **D** phallic organs (dorsal view) **E** phallic organs (lateral view) **F** ovipositor (lateral view). Abbreviations: aed = aedeagus, aed a = aedeagal apodeme, aed b p = aedeagal basal process, cerc = cercus, epand = epandrium, epand a = epandrial apodeme, hypd = hypandrium, pm = paramere, 10S = tenth sternite. Scale lines: 0.1 mm.

(0.35), dcl = 0.53 (0.53), dcp = 0.97 (0.96), sterno = 0.75 (0.68), sclt = 0.56 (0.59), scltp = 1.20 (1.03), C = 1.75 (1.64), 4c = 1.12 (1.18), 4v = 1.58 (1.70), 5x = 0.88 (0.92), ac = 3.94 (3.68), M = 0.36 (0.34), C3F = 0.82 (0.74).

Material examined. *Holotype* ♂ (#10485): CHINA: *ex* inflorescence of *Rhaphidophora crassicaulis* Engl. & Krause, Qimaba, Lüchun, Yunnan, China, 22°48.0'N, 102°15.0'E, ca 750 m, 6.vii.2020, Jian-Jun Gao and Run-Jie Jiao (KIZ). *Paratype*: same data as holotype (1 ♀: #10486) (KIZ).

Distribution. China (Yunnan).

Host plants. *Rhaphidophora crassicaulis* Engl. & Krause (Fig. 1E–H).

Etymology. Patronym, in honor of Professor Heng Li (Kunming Institute of Botany, Chinese Academy of Sciences), who helped us with the identifications of various aroid host plants of *Colocasiomyia* flies.

Remarks. Numbers of adults of this species were obtained by rearing infructescences of *R. crassicaulis* collected on November 1, 2018 from the type locality, indicating that this species breeds on inflorescences/infructescences of this host plant. These adults were not defined as type specimens due to obviously insufficient body pigmentation and sclerotization, though some of them were used for DNA barcoding.

Acknowledgements

We thank the Administrations of the Huanglianshan National Nature Reserve and the Gaoligongshan National Nature Reserve for permission and supports of the field works within the Reserves. We also thank Mr Shi-Bin Jiao and Mr Xue-Lin Ye for their help in the field. This work was supported by the National Natural Science Foundation of China (Nos. 31572238).

References

- Brcic D (1983) Ecology of flower-breeding *Drosophila*. In: Ashburner M, Carson HL, Thompson Jr JN (Eds) *The Genetics and Biology of Drosophila* (Vol 3d). Academic Press Inc, London, 333–382.
- Fartal RS, Gao JJ, Toda MJ, Hu YG, Takano KT, Suwito A, Katoh T, Takigahira T, Yin JT (2013) *Colocasiomyia* (Diptera: Drosophilidae) revised phylogenetically, with a new species group having peculiar lifecycles on monstroid (Araceae) host plants. *Systematic Entomology* 38: 763–782. <https://doi.org/10.1111/syen.12027>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I for diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299. https://www.academia.edu/9100684/DNA_primers_for_amplification_of_mitochondrial_cytochrome_c_oxidase_subunit_I_from_diverse_metazoan_invertebrates [Accessed on: 2020-8-11]
- Fu Z, Toda MJ, Li NN, Zhang YP, Gao JJ (2016) A new genus of anthophilous drosophilids, *Impatiophila* (Diptera, Drosophilidae): morphology, DNA barcoding and molecular phylogeny, with descriptions of thirty-nine new species. *Zootaxa* 4120(1): 1–100. <https://doi.org/10.11646/zootaxa.4120.1.1>
- Grimaldi D, Ervik F, Bernal R (2003) Two new Neotropical genera of Drosophilidae (Diptera) visiting palm flowers. *Journal of the Kansas Entomological Society* 76: 109–124. https://www.researchgate.net/publication/287001289_Two_new_neotropical_genera_of_Drosophilidae_Diptera_visiting_palm_flowers [Accessed on: 2020-8-11]
- Li NN, Toda MJ, Fu Z, Li SH, Gao JJ (2014) Taxonomy of the *Colocasiomyia gigantea* species group (Diptera, Drosophilidae), with descriptions of four new species from Yunnan, China. *ZooKeys* 406: 41–64. <https://doi.org/10.3897/zookeys.406.7176>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33(7): 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- McAlpine JF (1981) Morphology and terminology: adults. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Eds) *Manual of Nearctic Diptera* (Vol. 1). Biosystematics Research Institute, Ottawa, 9–63.
- Meier R, Shiyang K, Vaidya G, Peter KL Ng (2006) DNA barcoding and taxonomy in Diptera: A tail of high intraspecific variability and low identification success. *Systematic Biology* 55: 715–728. <https://doi.org/10.1080/10635150600969864>

- Meier R, Zhang G, Ali F (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the “barcoding gap” and leads to misidentification. *Systematic Biology* 57: 809–813. <https://doi.org/10.1080/10635150802406343>
- Meyer CP, Paulay G (2005) DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology* 3: 2229–2238. <https://doi.org/10.1371/journal.pbio.0030422>
- Okada T (1987) Further notes on the genus *Drosophilella* Duda with descriptions of two new species from Indonesia (Diptera, Drosophilidae). *Proceedings of the Japanese Society of Systematic Zoology* 36: 38–45.
- Shi T, Toda MJ, Takano KT, Yafuso M, Suwito A, Wong SY, Shang SQ, Gao JJ (2019) A review of taxonomy and flower-breeding ecology of the *Colocasiomyia toshiokai* species group (Diptera: Drosophilidae), with description of a new species from Indonesia. *European Journal of Entomology* 116: 341–361. <https://doi.org/10.14411/eje.2019.037>
- Srivathsan A, Meier R (2012) On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics* 28: 190–194. <https://doi.org/10.1111/j.1096-0031.2011.00370.x>
- Sultana F, Hu YG, Toda MJ, Takenaka K, Yafuso M (2006) Phylogeny and classification of *Colocasiomyia* (Diptera, Drosophilidae), and its evolution of pollination mutualism with aroid plants. *Systematic Entomology* 31: 684–702. <https://doi.org/10.1111/j.1365-3113.2006.00344.x>
- Zhang WX, Toda MJ (1992) A new species-subgroup of the *Drosophila immigrans* species-group (Diptera, Drosophilidae), with description of two new species from China and revision of taxonomic terminology. *Japanese Journal of Entomology* 60: 839–850.