

**Molecular Phylogeny Revealing the Single Origin of *Cinnamomum*-associated
Bruggmanniella (Diptera: Cecidomyiidae) in Asia, and Descriptions of Three New and One
Newly Recorded Species from Taiwan**

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Cecidomyiid genus *Bruggmanniella* contains four Lauraceae-associated species in Asia and 13 species associated with various plant families in Latin American. In this article, three new species, *B. sanlianensis* sp. nov., *B. turoguei* sp. nov. and *B. shianguei* sp. nov., and one newly recorded species, *B. cinnamomi*, are reported on *Cinnamomum* plant species (Lauraceae) from Taiwan. Molecular phylogenetic analysis was conducted for the four *Cinnamomum*-associated *Bruggmanniella*, together with *B. brevipes* Lin, Yang and Tokuda, *B. actinodaphnes* Tokuda and Yukawa, three *Pseudasphondylia* and two *Daphnephila* species. Monophyly of Asian *Bruggmanniella* and the single origin of *Cinnamomum*-associated *Bruggmanniella* were supported based on Neighbor-joining, Maximum-likelihood, and Bayesian inference. The Additionally, *Cinnamomum*-associated *Bruggmanniella* species have the closest sistership with *B. brevipes* that are associated with the plant genus *Neolitsea* (Lauraceae). These result suggesting *B. brevipes*, *B. actinodaphnes* and *B. cinnamomi* are not members of genus *Bruggmanniella* that was not consistent with a recent morphology-based phylogenetic study. Among the *Cinnamomum*-associated lineage, a leaf galler *B. sanlianensis* sp. nov. is a sister to the clade of stem gallers, suggesting that host organ shift from leaf to stem occurred prior to host shift. Besides, paraphyly of the Taiwanese stem galler

with respected to Japanese *B. cinnamomi* implies that the distributional range expansion of *B. cinnamomi* from Taiwan to Japan.

Key Words: *Bruggmanniella*, Taiwan, Japan, Divergence, Biogeography.

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BACKGROUND

The gall midge genus *Bruggmanniella* Tavares comprises 17 species that exhibit amphi-Pacific distribution and special patterns of host spectrum. Thirteen New World species are associated with various host families, while four East Asian species are with Lauraceae, *i.e.*, *B. cinnamomi* Tokuda & Yukawa, 2005 with *Cinnamomum yabunikkei* H. Ohba (= *C. japonicum* Sebold ex. Nakai), *B. actinodaphnes* Tokuda & Yukawa, 2005 and *B. litseae* Lin, Yang & Tokuda, 2020 with *Litsea* species (including *Actinodaphne*) and *B. brevipes* Lin, Yang & Tokuda, 2019 with *Neolitsea parvigemma* (Hayata) Kanehira & Sasaki (Tokuda and Yukawa 2006; Maia et al. 2010; Tokuda 2012; Gagné and Joffhorn 2017; Lin et al. 2019, 2020; Garcia et al. 2020; Rodrigues et al. 2020). Furthermore, several undescribed species were found on Lauraceae in Asia. Tokuda and Yukawa (2006) noted one unnamed species on *L. acuminata* (Blume) Sh. Kurata (= *Actinodaphne acuminata* (Blume) Meissn.) in Okinawa, Japan. Many other galls possibly induced by *Bruggmanniella* are known on *Cinnamomum* and *Litsea* in Taiwan (Tung et al. 2018), which suggests a high diversification of *Bruggmanniella* on Asian Lauraceae.

From a systematics viewpoint, the identity of *Bruggmanniella* is controversial due to its heterogeneity on ecological aspects and morphological features. Tokuda and Yukawa (2006) described two *Bruggmanniella* species from Japan and mentioned that Asian *Bruggmanniella* species are similar to the Palaearctic genus *Pseudasphondylia* Monzen, 1955 in male morphological

features such as the presence of parameres and separate teeth on the gonostylus. However, *Bruggmanniella* can be distinguished from *Pseudasphondylia* by the presence of setae on larval cervical papillae and on all ventral papillae. Recently, Garcia et al. (2020) provided a cladistics hypothesis based on 57 characters and 27 species belonging to genera *Asphondylia* Loew, *Bruggmannia* Tavares, *Bruggmanniella* Tavares, *Illiciomyia* Tokuda, *Parazalepidota* Maia, *Pseudasphondylia* Monzen, *Schizomyia* Kieffer, and *Lopesia* Rübsaamen. Most *Bruggmanniella* species are included in this analysis except *B. litseae* and *B. byrsonimae*. Based on their analysis, *B. brevipes* is situated in the basal clade of *Pseudasphondylia* and *Bruggmanniella*, and two Japanese *Bruggmanniella* species were subsumed in the clade of *Pseudasphondylia*. Thus, Garcia et al. erected the new genus *Odontokeros* Garcia, Lamas and Urso-Guimarães, 2020 for *B. brevipes* and combined two Japanese *Bruggmanniella* with *Pseudasphondylia*. However, bootstrap of key nodes on the topology are poorly supported (less than 30%), especially on node of *B. brevipes* + (*Pseudasphondylia* + *Bruggmanniella*), and among *Pseudasphondylia* and two Asian *Bruggmanniella* species (*B. cinnamomi* and *B. actinodaphnes*). In addition, their morphological definition of the new genus is obscure, consisting of only a slight difference in the larval sternal spatula and “shorter adult legs.” In this study, we show that the hypothetical system proposed by Garcia et al. (2020) cannot be supported.

We here described three new *Bruggmanniella* species from Taiwan that induce stem and leaf galls on *Cinnamomum* (Fig. 1), two on *C. osmophloeum* and one on *C. subavenium*; additionally, *B. cinnamomi* that induces stem galls on *C. insularimontanum* is newly recorded from Taiwan. Furthermore, we conducted molecular phylogenetic analyses of *Cinnamomum*-associated *Bruggmanniella* to infer its possible divergence processes.

MATERIALS AND METHODS

Collecting galls and gall midges

Galls were collected from various localities of Taiwan from 2014 to 2020. Larval and pupal

specimens were obtained from galls and dissected under a stereoscopic microscope. Adults were reared in the laboratory and pupal exuviae were saved. Specimens were preserved in 70% ethanol for morphological studies or in 99.5% ethanol for molecular works.

Slide preparation

Specimens were mounted on slides following Gagné (1994). Specimens were examined under a microscope (Leica DM 750, Germany) and illustrated with a drawing tube. Morphological terminology of adult thoracic plates follows Tokuda (2004) and of others according to McAlpine (1981). Larval and pupal morphological terminology follows Gagné (1994). Type specimens were deposited in the Laboratory of Insect Systematics and Evolution, National Cheng-Hsing University, Taichung, Taiwan (NCHU). Type materials of *B. cinnamomi* were examined (ELKU: Collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan).

Molecular analysis

A partial region of mitochondrial DNA cytochrome oxidase subunit I (*COI*) was sequenced following Pan et al. (2015) and Lin et al. (2020). Primers are designed based on Cameron et al. (2007) as follows; forward: Diptera-49F (5'-AAT CAT AAA GAT ATT GGA AC-3') and reverse: Diptera-734R (5'-CAA CAT TTA TTT TGA TTT TTT GG-3'). DNA sequences were aligned by ClustalW (Thompson et al. 1994) via software BioEdit (Hall 1999). Then 19 new and two prepared sequences (*B. cinnamomi* and *P. kiritanii*) were uploaded to DNA Data Bank of Japan (DDBJ) (Table 1).

Sequences of two *Daphnephila* species, *D. urnicola* Chiang, Yang & Tokuda, 2015 (AB857360) and *D. truncicola* Tokuda, Yang & Yukawa, 2008 (AB334228) and one *Pseudasphondylia* species, *P. matatabi* Yuasa & Kumazawa, 1938 (AB085873) were downloaded from National Center for Biotechnology Information (NCBI) (Table 1). Sequences of *D. truncicola* was selected as the root taxon in phylogenetic analyses according to the result of morphology-based phylogeny shown in Tokuda and Yukawa (2007) and Tokuda et al. (2008).

Phylogenetic clustering was performed by Maximum Likelihood (ML) and Neighbor-joining (NJ) methods with MEGA7 (Kumar et al. 2016), and Bayesian inference (BI) via MrBayes 3.2 (Ronquist et al. 2012). In ML inference, GTR+I+G model were determined based on jModelTest 2.1.10 (Darriba et al. 2012) and pairwise deletion in proportional distance were selected with 1000 replication for bootstrap. As for NJ method, same setting as in ML method except model using Kimura's two-parameter divergence (Kimura 1980). As to BI, the best-fit substitution model TIM2+I was determined via jModelTest 2.1.10 (Darriba et al. 2012) using Bayesian Information Criterion (BIC). Markov chain Monte Carlo (MCMC) chain length was 5×10^5 generations with sampling every 1000 generations with the first 25% of steps discarded as burn-in by TreeAnnotator. Posterior clade probabilities were summarized on a maximum clade credibility tree. Divergences distance between *Bruggmanniella* species were calculated via P-distance with MEGA7 (Kumar et al. 2008).

The LSID (Life Science Identifier) numbers of new species have been registered in ZooBank (www.zoobank.org) and are shown in the taxonomy section below.

RESULTS

TAXONOMY

Order Diptera Linnaeus, 1758

Family Cecidomyiidae Newman, 1834

Tribe Asphondyliini Gagné, 1994

Genus *Bruggmanniella* Tavares, 1909

Bruggmanniella Tavares, 1909: 19.

Type species: *Bruggmanniella braziliensis* Tavares, 1909.

Hemibruggmanniella Möhn, 1961b: 6.

Type species: *Bruggmanniella obita* Tavares, 1920.

Odontokeros Garcia, Lamas and Urso- Guimarães, 2020: 10. Syn. nov.

Genus *Bruggmanniella* belongs to the subtribe Asphondyliina and comprises 17 known species worldwide (Gagné and Jaschhof 2017; Lin et al. 2019, 2020; Garcia et al. 2020; Rodrigues et al. 2020). In this article, three new species are described and one known species is newly recorded from Taiwan, all of which are associated with *Cinnamomum* species.

Morphological features of *Bruggmanniella* were summarized in Gagné (1994) and Gagné et al. (2004), and they were compared with related Palearctic genera in Tokuda and Yukawa (2006). Briefly, *Bruggmanniella* species are separated from *Pseudasphondylia* Monzen, 1955 and *Probruggmanniella* Möhn, 1961 by the presence of setae on the larval cervical papillae and on all papillae of the ventral surface, less constricted male flagellomeres, convolute male circumfila, presense of an apical spur on the first tarsomeres, and the absense of setae on lateral papillae on larval thoracic segments (Tokuda 2004; Möhn 1961a; Tokuda and Yukawa 2006). In addition, two separate teeth on the gonostylus are regarded as a common feature of *Bruggmanniella* (Tokuda and Yukawa 2006). However, in this article we describe a new species which possesses a bidentate tooth of the gonostylus because other morphological features fit well with the genus and our molecular phylogenetic analysis clearly indicates that the species is included in the clade of *Bruggmanniella* (see below).

***Bruggmanniella turoguei* sp. nov. Lin, Yang and Tokuda**

(Figs. 2, 5A, 6A, D; Tables 2)

urn:lsid:zoobank.org:act:369831A8-5701-4D23-A7A1-B6403FCCEA8F

Type materials: *Holotype*. Male (on slide, NCHU), TAIWAN: Taichung City, Shalian Ln, adult reared from galls that collected on 28.iv.2016 and emerged on 2.v.2016, S.F. Lin leg. *Paratypes*. TAIWAN: 7♂7♀ (on slides, NCHU) collected data same as holotype; 2 pupal exuviae (on slides, NCHU) Taichung City, Shalian Ln, 28.iv.2018, S.F. Lin leg.; 1 pupa (in ethanol, NCHU), 2 pupal exuviae (on slide, NCHU) Taichung City, Shalian Ln, 1.iv.2018, S.F. Lin leg.; 10 larvae (6 on slides, 4 in ethanol, NCHU), Nantou Co., Lianhuachi Research Center, 20.iii.2020, S.F. Lin leg.

Other material examined: TAIWAN: 1 larval skin (on slide, NCHU), Nantou, Lianhuachih Research Center, 2.iv.2019, S.F. Lin leg.

Etymology: The species name is derived from transliteration of the Chinese common name of the host species.

Host: *Cinnamomum osmophloeum* Kaneh is endemic to Taiwan (Lu et al. 2000).

Gall: Multi-chambered stem galls with irregular swellings; approximately 1cm in diameter and 1 to 12cm long.

Distribution: Mountain areas of central Taiwan

Life history: Immature larvae are found in the galls from January to early April; larvae mature and pupate during April; adults emerge from galls in early May. One or two years are required to complete one generation.

Adult: Head. Eye bridge three to four facets long; frontoclypeal setal count as in table 2; palpus four-segmented, first segment globose, second to fourth successively elongated; twelve flagellomeres all with flexuous circumfila, gradually becoming shorter from first to tenth, distal three subequal in length in male, gradually shortened and the terminal one subglobular in female (Fig. 2B, C). Thorax. Thoracic setal and scale counts as in table 2; legs with dense blackish scales; first tarsomeres of all legs with apical spur (Fig. 2D); female fifth tarsomere shorter than male; claws strongly curved on all legs; pulvilli shorter than claws; empodia as long as claws (Fig. 2E); Wing densely covered with dark grayish hairs; wing length 2.5–2.8 mm in male ($n = 8$) and 3.1–3.4 mm in female ($n = 7$), 2.6 times as long as wide in male and 2.2–2.5 times as long as wide in female, R_5 joining costa posterior to wing apex (Fig. 2G, H). *Male abdomen.* First through seventh abdominal tergites rectangular, with one or two rows of posterior setae; both tergites and sternites without anterior pair of trichoid sensilla. Terminalia (Fig. 5A): cerci setose, each rounded at apex; hypoproct deeply incised V-shaped emargination, each lobe with an apical seta; gonostylus suboval, distally with 2 very closely situated solid teeth; gonocoxite massive, with mediobasal lobe; aedeagus longer than hypoproct, distally tapering, basal part laterally sclerotized. *Female abdomen.* Seventh sternite 448–680 μm long ($n = 7$), 2.1 times as long as sixth sternite; ovipositor protractile, slender, aciculate, basally with a bilobed cerci-like structure; needle part of ovipositor 0.88–0.96 mm long ($n = 7$), 1.4–2.1 times as long as seventh sternite. Otherwise as in male.

Pupa: Body length 3.2–3.4 mm, pupal skin not pigmented except for antennal horn. Antennal horn 450–500 µm long, dorsoventrally flattened, anterior margin narrowed, posterolaterally with small lobe (Fig. 6A); cephalic seta 80–90 µm long; frons without horns; facial papillae not visible; prothoracic spiracle 280–320 µm long; spiracles on second to sixth abdominal segments 25–30 µm long; second to seventh abdominal segments with 9 to 10 transverse rows of spines; eight dorsal papillae on first to seventh abdominal segments, most outer and second inner pairs with seta; two dorsal papillae on eighth abdominal segment, each with seta; each segment with pleural papilla, each with seta.

Full growth larva: Body color yellow, body length 2.8–3.0 mm. Second antennal segment short, conical; cervical papillae with seta. Sternal spatula approximately 300 µm long (Fig. 6D), anteriorly with two lobes; four lateral papillae and a sternal papillae present on each side of all thoracic segments, each with seta; four dorsal papillae on all thoracic and first through seventh abdominal segments, 2 dorsal papillae on eighth abdominal segment, each with seta; one pleural papilla present on each side, each with seta; terminal papillae not apparent. Each abdominal segment, except terminal one, ventrally with many transverse rows of minute spines and covered with small triangular spines.

***Bruggmanniella shianguei* sp. nov. Lin, Yang and Tokuda**

(Figs 3 and 5B, 6B, E; Table 3)

urn:lsid:zoobank.org:act:151A1E27-B237-44CC-9EEC-41BD4CA08E66

Type material: *Holotype*. Male (on slide, NCHU), TAIWAN: Nantou Co., Huisun Forest Area, adult emerged on 27.iv.2016 reared from stem gall collected on 21.iv.2016, S.F. Lin leg. *Paratypes*. TAIWAN: 5♂4♀ (on slides, NCHU), same data as holotype; 2♂4♀ (on slides, NCHU) same data as holotype except emergence date on 2.v.2016, S.F. Lin leg.; 2♀ (on slides, NCHU) same data as holotype except emergence date on 23.iv.2016, S.F. Lin leg.; 1 larva, 6 pupal exuviae (on slides, NCHU), same collection data as holotype, S.F. Lin leg.; 2 larvae (on slides, NCHU), 13 pupae (4 on slides and 9 in ethanol, NCHU), Taichung City, Mt. Tungma, 25.iv.2017, S.F. Lin leg.

Etymology: The species name is derived from Chinese pronunciation of host plant species.

Host: Cinnamomum subavenium Miq. is distributed in Borneo, Cambodia, China South-Central, China Southeast, Malaya, Myanmar, Sulawesi, Taiwan, and Vietnam (Lu et al. 2000; Hassler 2019).

Gall: Multi-chambered ellipsoid galls are induced on stem. The size is smaller than 1 cm long and wide.

Distribution: Mountain areas of Taiwan.

Life history: Immature larvae are found in the gall from October to the following February and mature larvae in March. Pupae are found from late March to early April and adults emerge in early May. This species does not seem to be univoltine, because well-developed galls are not found every year in the same locality. Instead, fresh galls are found biennially, suggesting that the species has a two-year life type of cycle.

Adult: Head. Eye bridge two or three facets long; frontoclypeal setal counts as in table 3; palpus 4-segmented (Fig. 3A), first globose, second to fourth elongated and subequal in length; distal three flagellomeres as in figure 3B and 3C. *Thorax.* First tarsomeres as in figure 3D; empodia equal length to claws in male, shorter than claw in female (Fig. 3E, F). Wing 2.0–2.8 mm long in male ($n = 7$, Fig. 3G) and 2.8–3.2 mm long in female ($n = 4$, Fig. 3H); thoracic setal and scale counts as in table 3. *Male abdomen.* Terminalia (Fig. 6B): hypoproct shorter than cerci, shallowly incised V-shaped emargination; 2 solid teeth of gonostylus rather apart from each other; gonocoxite slightly extended beyond gonostylus. *Female abdomen.* Seventh sternite 469–538 μm long ($n = 8$), 2.0 times as long as sixth sternite; needle part of ovipositor 0.85–0.92 mm long, 1.8 times as long as seventh sternite. Otherwise as in *B. turoguei*.

Pupa: Body length 1.8–2.4 mm. Antennal horn dorsoventrally flattened, anterior margin narrowed, posterolaterally with small lobe, antennal horn 460–480 μm long (Fig. 6B); cephalic papilla with seta, 75–85 μm long; prothoracic horn 280–310 μm long; spiracles on second to sixth abdominal segments 25–30 μm long; second to seventh abdominal segments with nine to 10 transverse rows of spines. Otherwise as in *B. turoguei*.

Full growth larva: Body color yellow, body length 1.6–1.8 mm. Second antennal segment short, conical; cervical papillae with seta. Sternal spatula 260–300 μm long (Fig. 6E), anteriorly with two lobes; four lateral papillae and a sternal papillae present on each side of all thoracic

segments (Fig. 6E), each with seta. Otherwise as in *B. turoguei* sp. nov.

***Bruggmanniella sanlianensis* sp. nov. Lin, Yang and Tokuda**

(Figs. 4, 5C, 6C, F; Table 4)

urn:lsid:zoobank.org:act:9F8D07AA-13AF-4335-BCA9-822D12240DF4

Type material: Holotype. Male (on slide, NCHU), TAIWAN: Taichung City, Shalian Ln, adult emerged on 15.v.2017 and reared from collected galls on 4.v.2017, S.F. Lin leg. *Paratypes.* TAIWAN: 3♂3♀ (on slides, NCHU), data are same as holotype; 1♂1♀ (on slide, NCHU) Taichung City, Shalian Ln, adult emerged on 5.v.2016 and reared from collected galls on 25.iv.2017, S.F. Lin leg. (NCHU); 5 larvae, 2 pupa, 7 pupal exuviae (on slides, NCHU) Taichung City, Shalian Ln, 1.iv.2018, S.F. Lin leg.; 2 larvae (on slides, NCHU), Taichung City, Shalian Ln, 25.iv.2017, S.F. Lin leg.

Etymology: The species name is derived from the type locality, Sanlian Ln. ost. *Cinnamomum osmophloeum* Kaneh is an endemic plant species to Taiwan.

Gall: Tear-shaped and single-chambered gall on leaf veins (Fig. 1D), 2–3 mm long and 1–2 mm wide. Possibly 1–20 galls occur on one leaf.

Distribution: Mountain areas of Taiwan.

Life history: This gall midge is univoltine. Immature larvae are found in the galls from December to the following March. Mature larvae are found in April and pupate from middle to late April and adults emerged in early May.

Adult: Head. Frontoclypeal setal counts as in table 4; palpus as in figure 4A, first segment round, second to fourth gradually becoming longer. *Thorax.* First tarsomere as in figure 4D; thoracic setal and scale counts as in table 4; empodia equal length to claws in male and shorter than claws in female (Fig. 4E, F); wing 2.2–2.4 mm long in male ($n = 5$) and 2.2–2.7 mm ($n = 4$) in female (Fig. 4G, H). Otherwise as in *B. turoguei*. *Male abdomen.* Terminalia (Fig. 5C): hypoproct entire or slightly emarginated, slightly shorter than cerci; gonostylus distally with a bidentate tooth. *Female abdomen.* Seventh sternite 365–410 μm long ($n = 4$), 2.5–3.0 times as long as sixth sternite; needle part of ovipositor 0.64–0.74 mm long, 1.8 times as long as seventh sternite. Otherwise as in

B. turoguei.

Pupa: Body length 1.5–1.8 mm. Antennal horn 260–290 μm long (Fig. 6C), anteriorly narrowed, posterolaterally with small lobe; cephalic setae 50–60 μm long; frons without horns; prothoracic spiracle 150–160 μm long; spiracles on second to sixth abdominal segments 30–35 μm long; second to seventh cover with six to eight and eighth cover with two to four transverse rows spines. Otherwise as in *B. turoguei*.

Full growth larva: Body length 1.2–1.6 mm. Sternal spatula 200–225 μm long, anteriorly with two angular lobes; four lateral papillae and one sternal papillae of each sides on all thoracic segments, each with seta (Fig. 6F). Otherwise as in *B. turoguei* sp. nov.

***Bruggmanniella cinnamomi* Tokuda & Yukawa**

(Fig. 7; Table 5)

Description: see Tokuda and Yukawa (2006). Number of palpal segment is four in both sex (Fig. 7A) that was misidentified in Tokuda and Yukawa (2006). Additional descriptions are as follows: female tarsal claws thicker than male (Fig. 7B–C); Wing 2.6–2.9 mm long in male ($n = 8$, Fig. 7D) and 3.1–3.6 mm long in female ($n = 8$); Frontoclypeal and thoracic setal counts as in table 5.

Specimen examined: *Holotype*. Male (on slide, ELKU), JAPAN: Nakagusuku, Okinawa, adult reared by M. Tokuda and emerged on 15.iii.2001 from stem galls that collected on 25.ii.2001, leg. J. Yukawa, S. Yamauchi. *Paratypes*. JAPAN: (Cecid. Nos. C7101–7114; C7151–C7164; see Tokuda and Yukawa (2006) for detailed information).

Other specimens: TAIWAN: 8♂, 8♀, 2 pupal exuviae (on slide, NCHU), Pingtung Co., Dahan forest road, adult emerged on 25–27.iv.2016 from galls that collected on 19.v.2016, S.F. Lin leg.; 4 pupal exuviae (on slide, NCHU) Pingtung Co., Dahan forest road, 28.iii.2014, S.F. Lin leg.; 5 larvae (on slide, NCHU) Nantou Co., Gaofeng Ln, 19.iv.2016, S.F. Lin leg.

Host and Distribution: The host species is *C. yabunikkei* (= *C. japonica*) in south-western islands of Japan (Tokuda and Yukawa 2005) and *C. insularimontanum* in Taiwan (new host and distribution records).

Gall: Swollen multi-chambered stem gall, which is 1 to 12 cm long and 1 cm wide.

Life history: Adults emerge in mid-March and early April in Japan (Tokuda and Yukawa 2006) and in early May in Taiwan. Further details of the life history remain unclear.

Taxonomic remarks

As mentioned earlier, *B. sanlianensis* sp. nov. possesses a bidentate apical tooth of gonostylus, which does not fit the previous definition of the genus *Bruggmanniella*. However, other morphological features and current molecular phylogenetic works (see below) support close relationships with other *Cinnamomum*-associated *Bruggmanniella* species. Therefore, *B. sanlianensis* sp. nov. should be treated as a member of *Bruggmanniella* (Fig. 8).

The two other new species, *B. turoguei* sp. nov. and *B. shianguei* sp. nov., both possess finger-like pupal antennal horns (Fig. 6A–B), which are similar to *B. cinnamomi*. Within these three species, the gonostylus tooth is well separated in *B. shianguei* sp. nov. (Fig. 5B), but closely situated in *B. turoguei* sp. nov. (Fig. 5A) and *B. cinnamomi*. Then *B. turoguei* sp. nov. and *B. cinnamomi* are distinguishable by the shape of hypoproct, which is deeply emarginated in the former but only slightly in the latter. In addition, pupa and larva differ in size among them: pupae of *B. turoguei* sp. nov., *B. shianguei* sp. nov. and *B. cinnamomi* are 3.2–3.4, 1.8–2.4 and 3.4–4.3 mm long, respectively, and larvae of *B. shianguei* sp. nov. and *B. cinnamomi* are 1.6–1.8 mm and 2.7–3.5 mm (Tokuda and Yukawa 2006), respectively.

In this paper, we re-combine two species *B. cinnamomi* comb. rev. and *B. actinodaphnes* comb. rev. with *Bruggmanniella* because of morphological and molecular similarities (Fig. 8). Although these two species were transferred to *Pseudasphondylia* based on an unstable phylogenetic analysis in Garcia et al. (2020), they are definitely the relatives of Lauraceae-associated Asian *Bruggmanniella* (Tokuda and Yukawa 2005 2006). We recognize that further studies are needed to determine the relationships between Asian and Neotropical (plus Nearctic) *Bruggmanniella*, and the species might be divided into several genera in future. However, we consider that more comprehensive studies are needed to conclude the detailed relationship and at

least for the present, we prefer to retain all of them as members of *Bruggmanniella*.

Molecular phylogeny and genetic distance

Phylogenetic tree of three methods represented similar topology. Monophyly of the Asian *Bruggmanniella* and genus *Pseudasphondylia* were supported in NJ (Fig. 8), ML and BI tree that were supported by bootstrap value more than 50% in ML and NJ tree and 0.5 in BI topology. Among *Bruggmanniella* species, the basal lineage is *Litsea*-associated species, *B. actinodaphnes* that is a sister group to *Neolitsea*-associated species *B. brevipes* plus *Cinnamomum*-associated species. Within *Cinnamomum*-associated taxa, three stem gallers belong to a single clade whose monophyly was highly supported by bootstrap values (99%, 94% and 1 in NJ, ML, and BI tree respectively). Then the leaf gall midge, *B. sanlianensis* sp. nov., is situated at the basal part of stem gallers' clade. Among stem gallers, *B. shianguei* sp. nov. occupies the basal position and received high supported value in three phylogenetic methods. However, the relationship between *B. turoguei* sp. nov. and *B. cinnamomi* was discordant in three phylogenetic methods. Monophyly of *B. cinnamomi* was supported in both NJ and ML but undetermined due to unsolved relationship with *B. turoguei* sp. nov. in BI tree. In *B. cinnamomi*, Taiwanese taxa is monophyletic and sister to Japan taxa in ML method but become a paraphyletic group with respect to Japan taxa in NJ tree (Fig. 8).

The genetic distances within species are 0.4%, 1.9%, 1.9%, 2.0% in *B. cinnamomi*, *B. turoguei* sp. nov., *B. shianguei* sp. nov. and *B. brevipes*, respectively. At the interspecific level within *Cinnamomum*-associated *Bruggmanniella*, genetic distance of most species pair doesn't higher than 7.0%. The lowest genetic distance is between taxa of *B. cinnamomi* (Taiwanese population) and *B. turoguei* sp. nov. (1.8%) (Table 6). At the interspecific level among *B. actinodaphnes*, *B. brevipes* and *Cinnamomum*-associated species the genetic distance are 12.8–16.4%.

DISCUSSION

Pseudasphondylia and Asian *Bruggmanniella*

Garcia et al. (2020) proposed that most Asian *Bruggmanniella* species do not belong to the genus *Bruggmanniella*. In their scheme, *B. cinnamomi* and *B. actinadaphnes* are members of *Pseudasphondylia* and become a sister group to each. Besides, *B. brevipes* was treated as a distinct genus (*Odontokeros*) and became a sister group of *Pseudasphondylia* + *Bruggmanniella*. However, our tree (Fig. 8) suggested monophyly of *Pseudasphondylia* and Asian *Bruggmanniella*, and two groups have a sister relationship. In addition, our topology suggests *B. brevipes* is a sister to *Cinnamomum*-associated taxa with strongly supported score that indicate more close relationship with *Cinnamomum*-associated taxa than *Pseudasphondylia*. That is, *B. cinnamomi* by no means belongs to genus *Pseudasphondylia* and *B. brevipes* should be treated under Asian *Bruggmanniella*. Characteristics of *Bruggmanniella* larvae and pupae have high heterogeneity in both Neotropical and Asian groups. To erect new genus or genera is not suitable under the current taxonomic framework because it would lead to chaos in further taxonomic works. At present, the phylogeny of *Bruggmanniella* is still inconclusive due to nodes with lower supported value of morphological-based cladogram (Garcia et al. 2020) and currently insufficient sampling of DNA fragments and taxa, especially DNA of type species *B. braziliensis*. Thus, comprehensiveness of data, including taxonomic unit, species, morphological features and DNA information of *Bruggmanniella* is essential in resolving this situation.

Molecular information is a useful tool for species identification, especially for gall midges whose adult specimens are sometimes very difficult to obtain and adults share similar morphological features in some genera such as *Asphondylia* (Yukawa et al. 2003; Uechi and Yukawa 2004; Elsayed et al. 2018). Three new of four *Bruggmanniella* species, *B. turoguei* sp. nov., *B. shianguei* sp. nov. and *B. sanlianensis* sp. nov., can be discriminated not only by morphological traits but also based on *COI* sequences, even their galls are induced on the same host species or galling organ. Our research is the first paper addressing the molecular phylogeny of Asian *Bruggmanniella* and confirming the single origin of *Cinnamomum*-association in the genus. Their pairwise genetic distances in a partial *COI* gene were rather large: for example, the maximum distance of the region was 7.0% within the species pair of *Cinnamomum*-associated *Bruggmanniella*, but were 13.5–16.4% compare with *Neolitsea*-associated *Bruggmanniella* (*B.*

brevipes) and 12.4–14.1%. These variations are higher than the distance at the general generic level (9.3%) of dipteran group (Hebert et al. 2003). This could correspond to highly morphological heterogeneity between these two groups and implies that Asian *Bruggmanniella* species have diverged on different host genera rather early in the history.

Divergence among *Cinnamomum*-associated *Bruggmanniella*

Diversification of gall inducers could be triggered by various biotic and/or abiotic factors. In gall inducing cecidomyiids, host plant and organ shift are considered as important mechanisms of divergence (Tokuda et al. 2004; Yukawa et al. 2005; Joy and Crespi 2006; Dorchin et al. 2009; Stireman et al. 2010; Mathur et al. 2012). For instance, the host shift of *Dasineura folliculi* (Felt), 1908 within the host genus *Solidago* was reported via the behavioral, ecological and genetic evidences (Dorchin et al. 2009). In addition, Joy and Crespi (2006) revealed that organ shifts are critical for divergence events in 14 *Asphondylia* species on their single host plant, creosote bush *Larrea tridentata* (DC.) Coville.

In our study system of *Cinnamomum*-associated *Bruggmanniella*, the evolutionary processes and speciation events seem to involve three different mechanisms: organ shift, host shift, and geographical isolation. We propose an evolutionary scenario based on the phylogenetic relationships. Leaf-galling habit is an ancestral trait, and the organ shift from leaf to stem is likely to have occurred later in the group. Among the stem gallers, the three species seem to have diverged via host plant shifts, but we need to further confirmation whether the divergent time among the stem gallers is later than the speciation of their host plants. A later event happened in *B. cinnamomi* populations that diverged between Taiwan and the Ryukyu Islands, Japan, via geographical isolation and host plant shift. The *COI* genetic distance of Taiwanese and Japanese taxa of *B. cinnamomi* is approximately 0.8%, revealing that the divergence time is around 0.35–0.4 Mya based on divergence distance of 2.0 or 2.3% per million years (DeSalle et al. 1987; Brower 1994). It corresponds to the time of origination of Ryukyu Islands in the last connection to Taiwan (Kimura 1996; Osozawa et al. 2012), suggesting that *B. cinnamomi* expanded its range from Taiwan to the Ryukyu Islands following by a host shift to an alternative one *C. yabunikkei* (= *C. japonica*) in

Okinawa. As a whole, the evidence strongly suggests that *B. cinnamomi* in the Ryukyus has a southern origin.

Geographical distribution and host association

The host spectrum and geographical isolation are key factors to understand divergence pathways of cecidomyiids. *Bruggmanniella cinnamomi* utilizes *C. yabunikkei* on the Southwest Islands of Japan but *C. insularimontanum* in Taiwan. Interestingly, *C. yabunikkei* is also distributed in Taiwan but *B. cinnamomi* does not induce galls on it. Two factors could be responsible for this phenomenon. From the host spectrum point of view, phenological asynchrony may be a key factor, because adult females need to oviposit in a suitable developmental stage of host organs (Yukawa 2000). Alternatively, Taiwanese populations of *C. yabunikkei* may be resistant to *B. cinnamomi*. From the biogeographical point of view, *B. cinnamomi* has never found in the Palearctic Region of Japan (*i.e.*, Kyushu or northern areas) as well as in Korean Peninsula up to date (Yukawa and Masuda 1996; Paik et al. 2004; Tokuda and Yukawa 2006; Kim et al. 2015), even though the host plant of *B. cinnamomi* is distributed in these areas. The mechanism inhibiting the distributional expansion of *B. cinnamomi* to northward would be an interesting study subject, because the Tokara Strait, which is the geographical border between Palearctic and Oriental Regions, does not seem to act as a barrier for northward expansion by gall midges (Tokuda 2018).

The other three *Bruggmanniella* species are known only from Taiwan at present. *Bruggmanniella turoguei* sp. nov. and *B. sanlianensis* sp. nov. are associated with Taiwanese endemic species, *C. osmophloeum*, while *B. shainguei* sp. nov. induces galls on *C. subavenium*, which is distributed widely in southern China (Lu et al. 1999). Although *Bruggmanniella* species and their galls have never been found in China, it is assumed that *Cinnamomum*-associated *Bruggmanniella* will be found there and Southeast Asian countries because these regions are parts of origins of Taiwanese fauna and flora, and also species-rich areas of the genus *Cinnamomum* (Huang et al. 2016).

CONCLUSIONS

Three new species of *Bruggmanniella* were described from Taiwan, which increased species number of the genus to seven and 20 in Asia and the World, respectively. In this article, molecular phylogeny of Asian *Bruggmanniella* species suggests its monophyletic and single origin of *Cinnamomum*-associated species, which do not consistent with morphological-based phylogenetic concept in Garcia et al. (2020). Considering of genetic distance of the Asian species and morphological heterogeneity among *Bruggmanniella* species, Asian species will be divided into several genera. However, comprehensive data of *Bruggmanniella* species, including taxonomic unit, species, morphological features, and DNA information, are essential in resolving this situation.

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Authors' contributions: SFL worked on collected specimens, illustrations, description and molecular work; MMY got funds for this work; All authors wrote the manuscript and approved the final manuscript and consent to publication.

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Availability of data and materials: DNA sequences generated in the study have been deposited in the DNA Data Bank of Japan (DDBJ) database.

Consent for publication: All the authors consent to the publication of this manuscript.

Ethics approval consent to participate: This research followed the guidelines specified by the research permits from the Forestry bureau (of Nantou and Pingtung), council of agriculture in Taiwan for collection permission.

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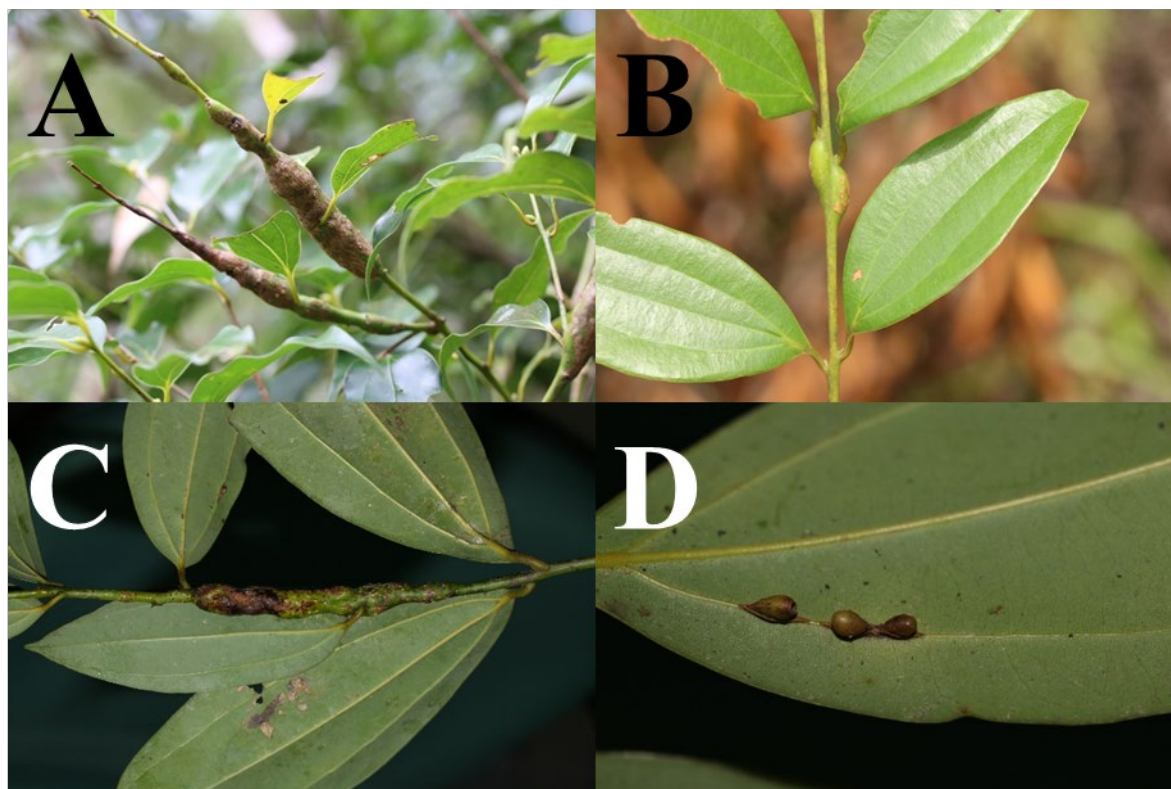
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2 **Fig 1.** Plant galls induced by Taiwanese *Bruggmanniella* species on *Cinnamomum* species. (A)
3 Stem galls on *C. insularimontanum*. (B) Stem galls on *C. subavenium*. (C) Stem galls on *C.*
4 *osmophloeum*. (D) Leaf galls on *C. osmophloeum*.

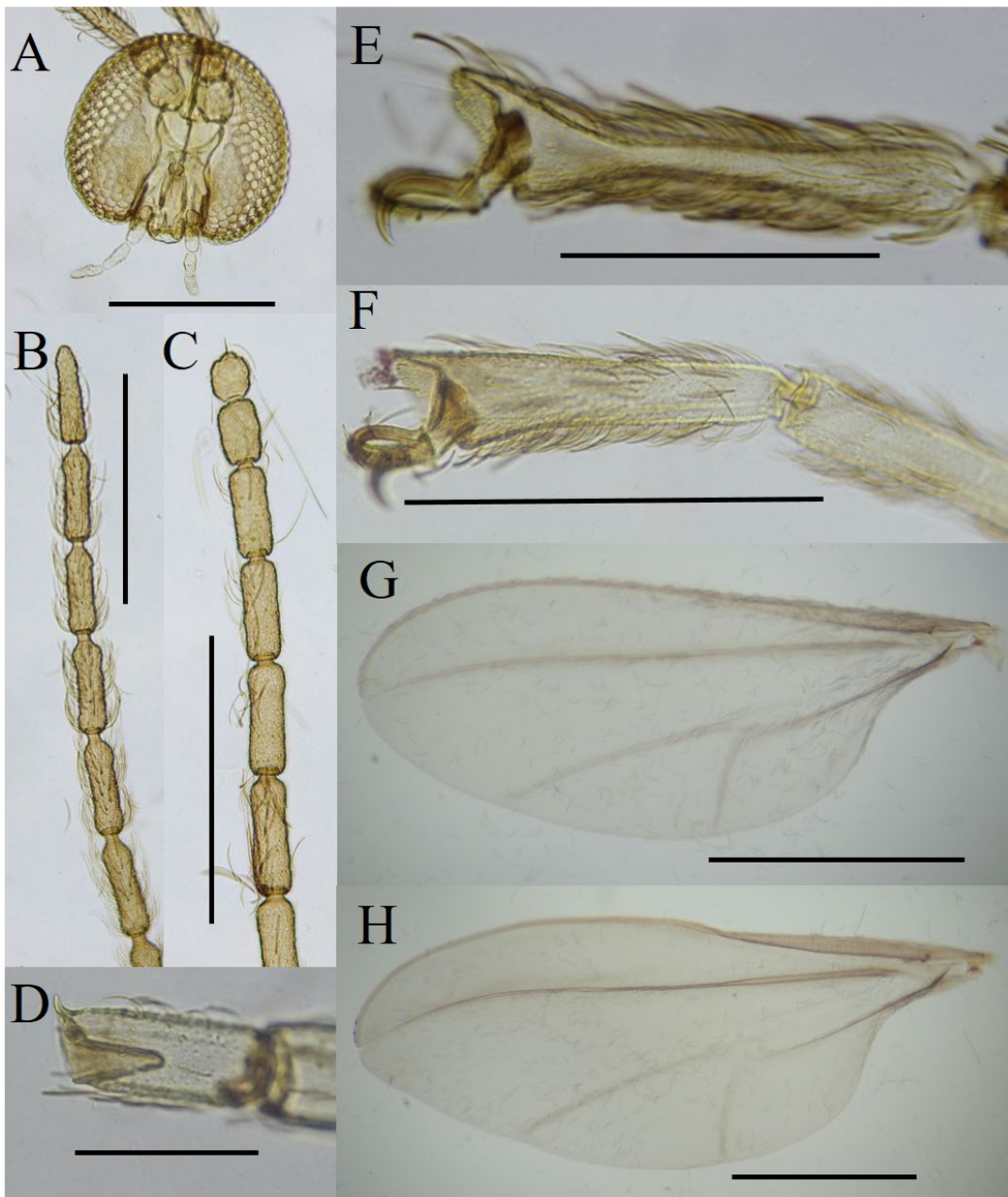


Fig. 2. *Bruggmanniella turoguei* sp. nov. (A) Male head (ventral view) (B) Male antenna (7-12 segment). (C) Female antenna (7-12 segment). (D) Male 1st tarsomere. (E) Male 5th tarsomere. (F) Female 5th tarsomere. (G) Male wing. (H) Female wing. Scale bars: A–C = 0.03 mm, D–F = 0.1 mm and for G–H = 1 mm.

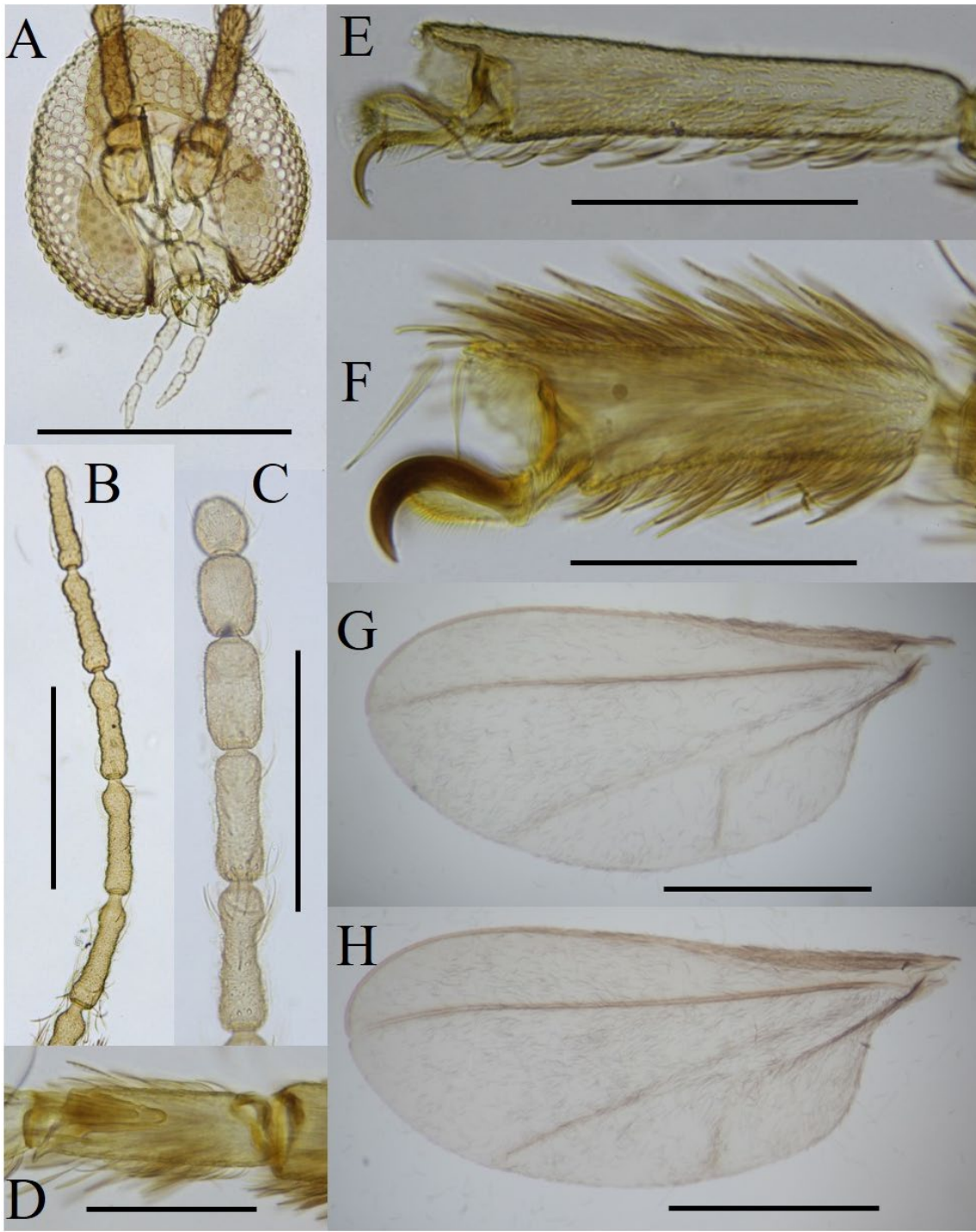


Fig. 3. *Bruggmanniella shianguei* sp. nov. (A) Male head (ventral view) (B) Male antenna (8–12 segment). (C) Female antenna (8–12 segment). (D) Male 1st tarsomere. (E) Male 5th tarsomere. (F) Female 5th tarsomere. (G) Male wing. (H) Female wing. Scale bars: A–C = 0.03 mm, D–F = 0.1 mm, G–H = 1 mm.

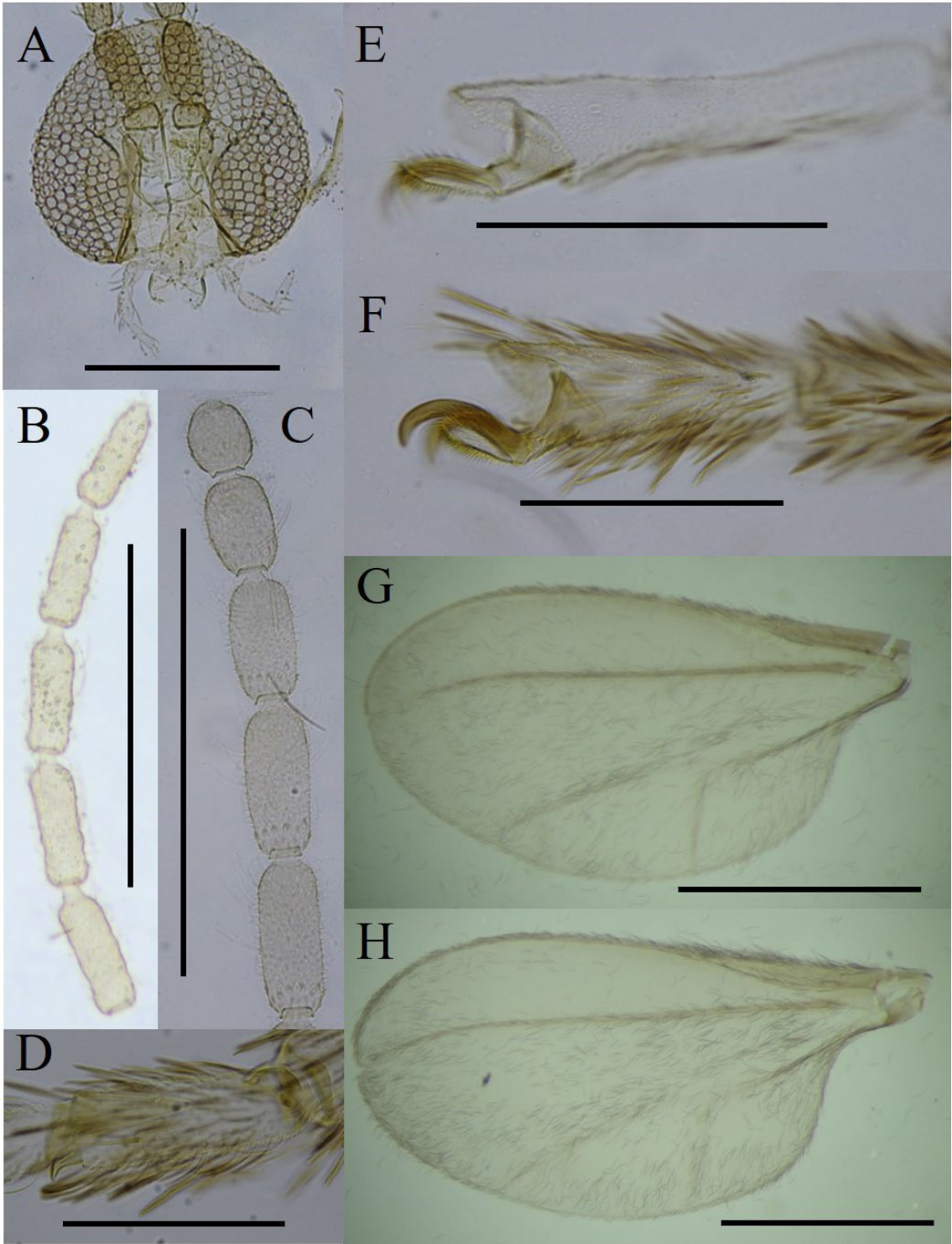


Fig. 4. *Bruggmanniella sanlianensis* sp. nov. (A) Male head (ventral view) (B) Male antenna (8–12 segment). (C) Female antenna (8–12 segment). (D) Male 1st tarsomere. (E) Male 5th tarsomere. (F) Female 5th tarsomere. (G) Male wing. (H) Female wing. Scale bars: A–C = 0.03 mm, D–F = 0.1 mm, G–H = 1 mm.

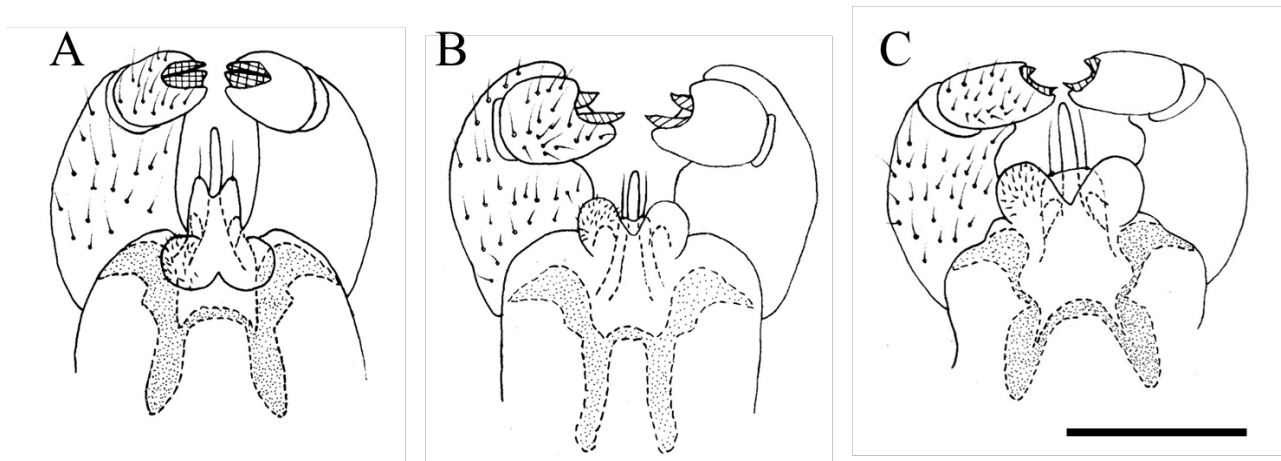


Fig. 5. Male genitalia of Taiwanese *Cinnamomum*–associated *Bruggmanniella*. (A) *B. turoguei* sp. nov., (B) *B. shianguei* sp. nov., and (C) *B. sanlianensis* sp. nov. Scale bar = 0.1 mm.

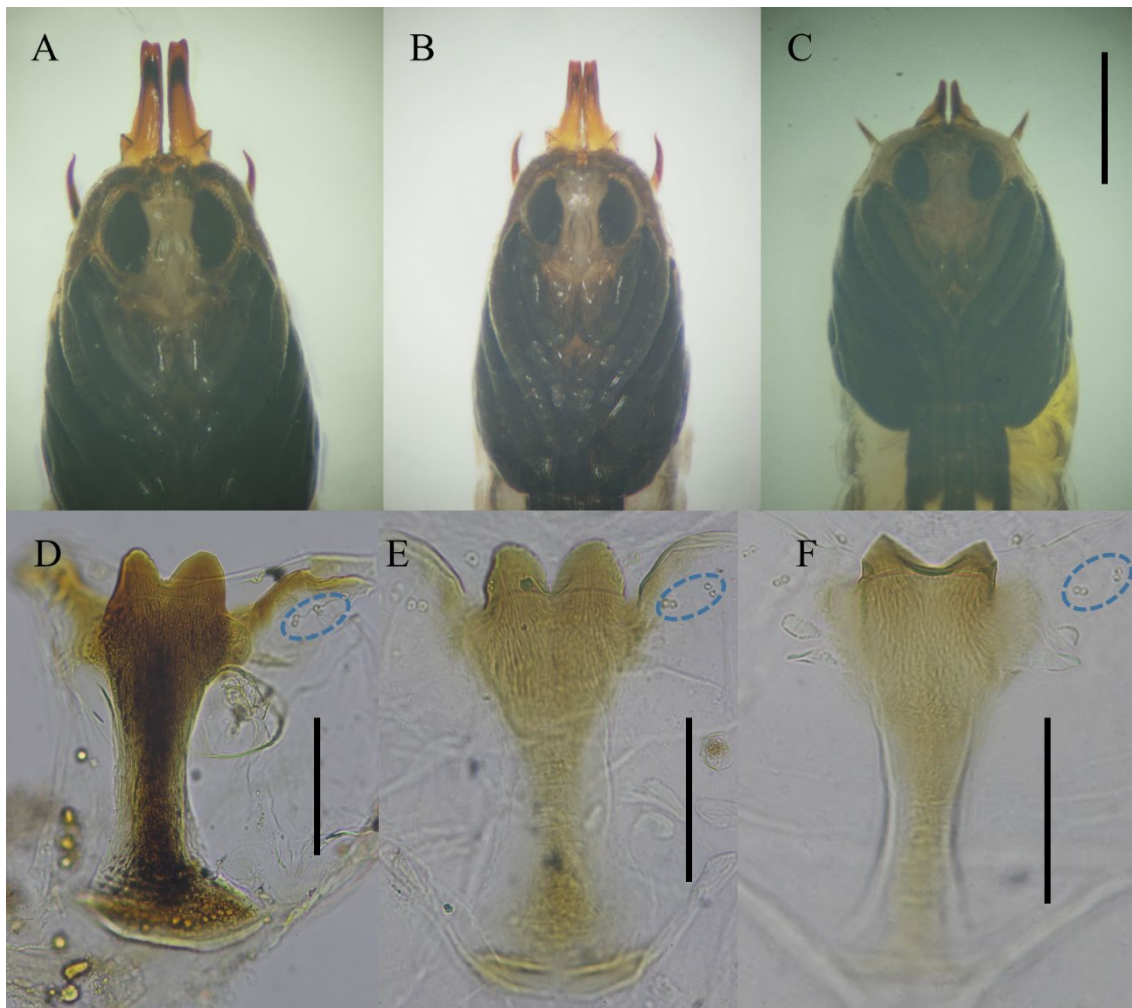
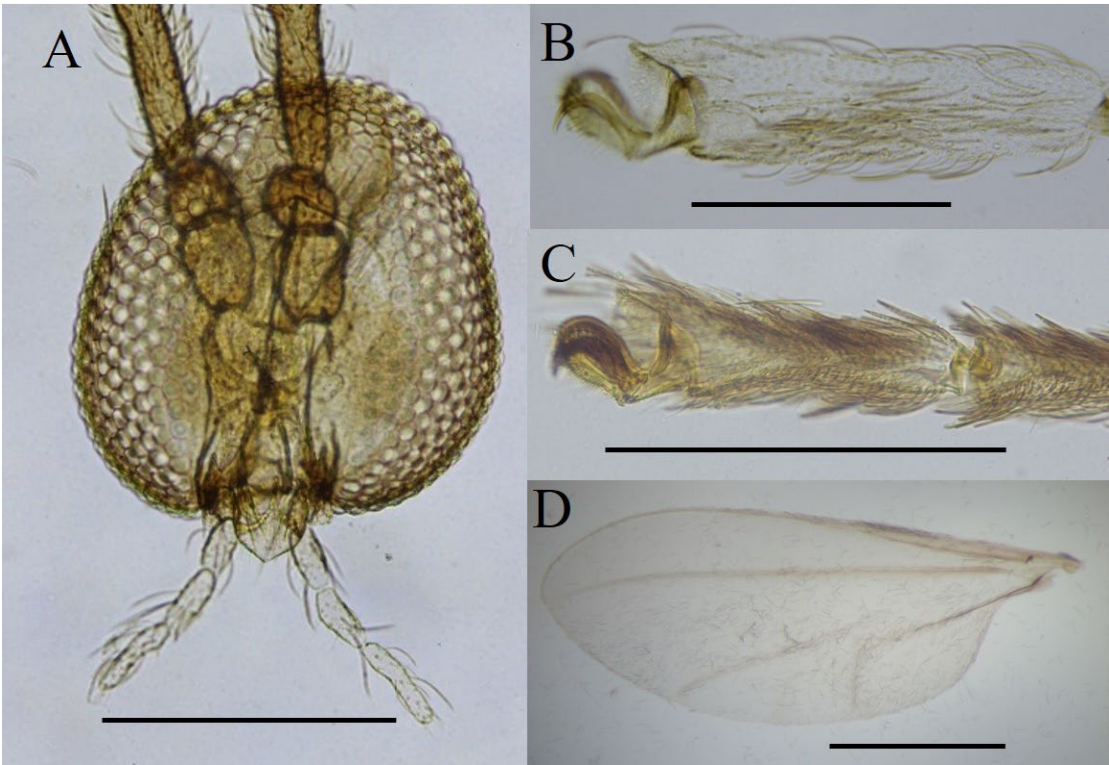


Fig. 6. Taiwanese *Cinnamomum*–associated *Bruggmanniella*. *B. turoguei* sp. nov. (A, D), *B. shianguei* sp. nov. (B, E), *B. sanlianensis* sp. nov. (C, F). Scale bars: pupal head (A–C) = 0.5 mm and for larval sternal spatula (D–F) = 0.1 mm. Larval lateral papillae showing in broken blue

1 circles.
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4 **Fig. 7.** *Bruggmanniella cinnamomi* (A) Male head (ventral view). (B) Male 5th tarsomere. (C)
5 Female 5th tarsomere. (D) Male wing. Scale bars: A = 0.03 mm; B–C = 0.1 mm; D = 1 mm.

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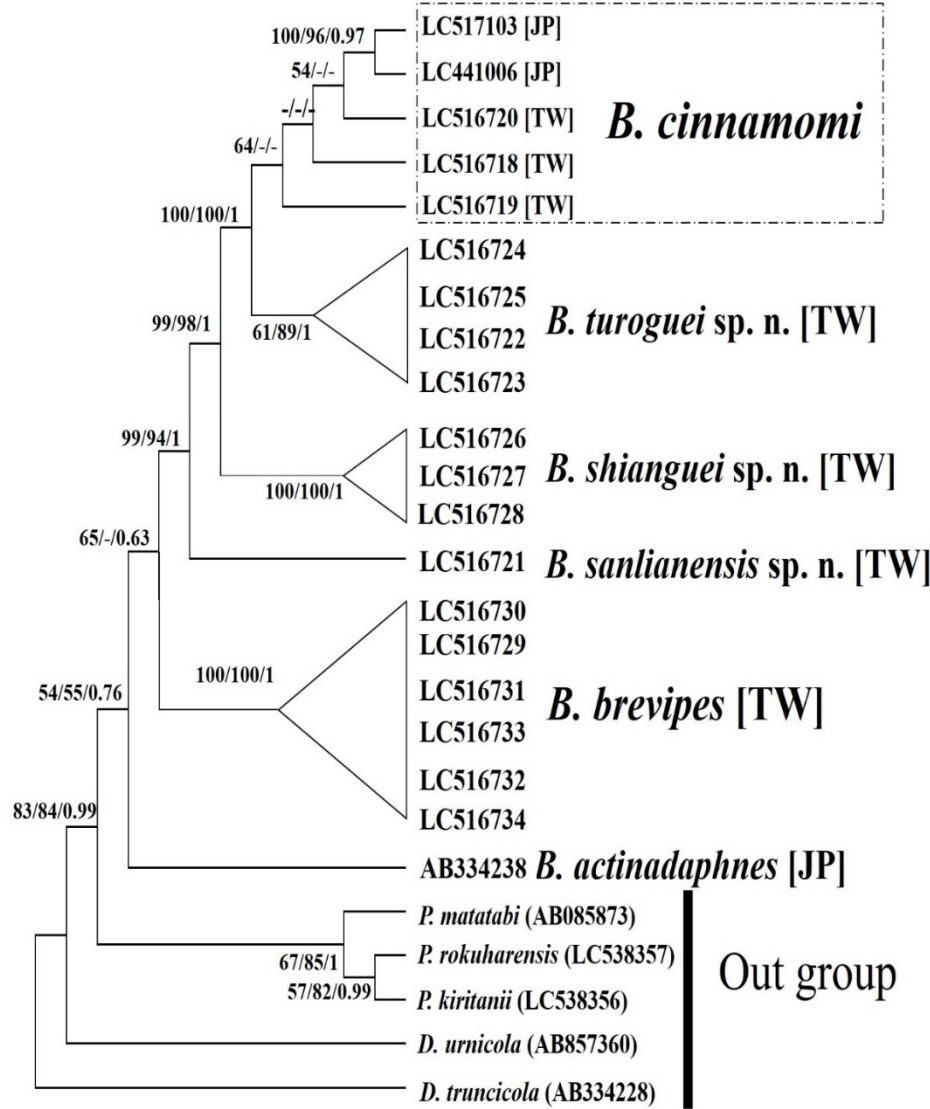


Fig. 8. Cladogram of East Asian *Bruggmanniella* based on Neighbor-joining method with DNA COI region. (Support values beside nodes are Neighbor-joining (left)/ Maximum-likelihood (media)/ Bayesian inference (right). “-” indicate supported values less than 50% in ML and NJ, and 0.5 in BI inference.

Table 1. DNA information of *Bruggmanniella* species and relative species

| Scientific name | Host | Gall organ | Locality | Collector | Accession Number |
|----------------------------------|------------------------------|------------|--------------------------|---------------------------|--------------------------|
| <i>B. cinnamomi</i> | <i>Cinnamomum yabunikkei</i> | Stem | Okinawa , Japan | M Tokuda | LC441006*, LC517103** |
| <i>B. cinnamomi</i> | <i>C. insularimontanum</i> | Stem | Pingtung, Taiwan | SF Lin | LC516718-20 |
| <i>B. shianguei</i> sp. nov. | <i>C. subavenium</i> | Stem | Nantou, Taiwan | SF Lin | LC516726-28 |
| <i>B. turoguei</i> sp. nov. | <i>C. osmophloeum</i> | Stem | Taichung, Taiwan | SF Lin | LC516722-25 |
| <i>B. sanlianensis</i> sp. nov. | <i>C. osmophloeum</i> | Leaf | Taichung, Taiwan | SF Lin | LC516721 |
| <i>B. brevipes</i> | <i>Neolitsea parvigemma</i> | Bud | Pingtung, Taiwan | SF Lin | LC516729-34 |
| <i>B. actindaphnes</i> | <i>Litsea coreana</i> | Stem | Mt. Mukabaki, Japan | M Tokuda | AB334238* |
| <i>Pseudasphondylia matatabi</i> | <i>Actinidia polygama</i> | Flower bud | Hisayama, Fukuoka, Japan | J Yukawa, N Uechi, M Tuda | AB085873* |
| <i>P. rokuharensis</i> | <i>Viburnum dilalatum</i> | Fruit | Hanayama, Honshu, Japan | M Tokuda, J Yukawa | LC538357 |
| <i>P. kiritanii</i> | <i>Cornus controversa</i> | Flower bud | Futo, Honshu, Japan | J Yukawa, K Kiritani | LC538356** |
| <i>Daphnephila urnicola</i> | <i>Machilus zuihonensis</i> | Leaf | Nantou, Taiwan | TC Chiang | AB857360* |
| <i>Daphnephila truncicola</i> | <i>Machilus thunbergii</i> | Stem | Taipei, Taiwan | TC Chiang | AB334228* |

“*” indicate DNA sequences download from National Center for Biotechnology Information; “**” indicate DNA sequences were prepared by M

Tokuda and upload in the present study.

Table 2. Frontoclypeal and thoracic setal counts of *Bruggmanniella turoguei* sp. nov.

| Specimens | Male | | | Female | | |
|------------------------------|------|----------------|-------|--------|----------------|-------|
| | n | Mean \pm SD | Range | n | Mean \pm SD | Range |
| Frontoclypeal setae | 3 | 18.0 \pm 2.0 | 16-21 | 5 | 18.6 \pm 1.3 | 17-20 |
| Anterior dorsolateral setae | 8 | 31.4 \pm 4.5 | 21-35 | 7 | 38.9 \pm 5.2 | 29-45 |
| Posterior dorsolateral setae | 4 | 25.5 \pm 1.3 | 24-27 | 4 | 36.5 \pm 1.0 | 36-38 |
| Mesopleural scales | 6 | 26.0 \pm 3.7 | 21-31 | 7 | 26.7 \pm 6.4 | 16-35 |
| Mesepimeral setae | 8 | 31.9 \pm 3.8 | 27-37 | 7 | 36.6 \pm 6.2 | 27-44 |

Table 3. Frontoclypeal and thoracic setal counts of *Bruggmanniella shianguei* sp. nov.

| Specimens | Male | | | Female | | |
|------------------------------|------|----------------|-------|--------|----------------|-------|
| | n | Mean \pm SD | Range | n | Mean \pm SD | Range |
| Frontoclypeal setae | 4 | 19.3 \pm 4.2 | 15-25 | 9 | 17.9 \pm 1.7 | 15-20 |
| Anterior dorsolateral setae | 8 | 33.9 \pm 4.5 | 24-38 | 8 | 42.5 \pm 5.2 | 37-54 |
| Posterior dorsolateral setae | 6 | 29.0 \pm 3.9 | 23-35 | 7 | 37.1 \pm 3.6 | 32-43 |
| Mesopleural scales | 7 | 19.9 \pm 3.1 | 17-26 | 9 | 27.4 \pm 6.3 | 19-38 |
| Mesepimeral setae | 8 | 32.4 \pm 6.0 | 21-38 | 9 | 35.2 \pm 4.4 | 29-42 |

Table 4. Frontoclypeal and thoracic setal counts of *Bruggmanniella sanlianensis* sp. nov.

| Specimens | Male | | | Female | | |
|------------------------------|------|-----------------|-------|--------|----------------|-------|
| | n | Mean \pm SD | Range | n | Mean \pm SD | Range |
| Frontoclypeal setae | 4 | 20.5 \pm 1.7 | 19-23 | 2 | 19.0 \pm 0.0 | 19 |
| Anterior dorsolateral setae | 1 | 32.0 | 32 | 2 | 36.0 \pm 1.4 | 35-37 |
| Posterior dorsolateral setae | 2 | 41.5 \pm 10.6 | 34-49 | 0 | NA | NA |
| Mesopleural scales | 1 | 14.0 | 14 | 1 | 24.0 | 24 |
| Mesepimeral setae | 2 | 35.0 \pm 2.8 | 33-37 | 4 | 25.8 \pm 2.1 | 23-28 |

“NA” indicate no data.

Table 5. Frontoclypeal and thoracic setal counts of *Bruggmanniella cinnamomi*

| Specimens | Male | | | Female | | |
|------------------------------|------|----------------|-------|--------|----------------|-------|
| | n | Mean \pm SD | Range | n | Mean \pm SD | Range |
| Frontoclypeal setae | 4 | 19.0 \pm 1.6 | 17-21 | 4 | 21.8 \pm 2.5 | 19-25 |
| Anterior dorsolateral setae | 8 | 37.1 \pm 4.2 | 32-43 | 7 | 42.4 \pm 3.6 | 37-49 |
| Posterior dorsolateral setae | 5 | 29.6 \pm 3.8 | 25-34 | 2 | 39.0 \pm 5.7 | 35-43 |
| Mesopleural scales | 8 | 28.8 \pm 7.0 | 19-39 | 5 | 25.8 \pm 6.1 | 18-33 |
| Mesepimeral setae | 8 | 39.3 \pm 3.3 | 31-45 | 7 | 41.6 \pm 8.0 | 28-54 |

Table 6. Genetic distance of *COI* sequence among Asian *Bruggmanniella*

| | <i>B. cinnamomi</i> | | <i>B. turoguei</i> sp. nov. | <i>B. shianguei</i> sp. nov. | <i>B. sanlianensis</i> sp. nov. | <i>B. actindaphnes</i> |
|---------------------------------|---------------------|---------|-----------------------------|------------------------------|---------------------------------|------------------------|
| | JP taxa | TW taxa | | | | |
| <i>B. cinnamomi</i> (TW taxa) | 0.8% | | | | | |
| <i>B. turoguei</i> sp. nov. | 2.2% | 1.8% | | | | |
| <i>B. shianguei</i> sp. nov. | 5.7% | 4.3% | 5.2% | | | |
| <i>B. sanlianensis</i> sp. nov. | 7.0% | 5.7% | 6.5% | 6.4% | | |
| <i>B. actindaphnes</i> | 12.7% | 13.1% | 14.1% | 13.3% | 12.4% | |
| <i>B. brevipes</i> | 16.4% | 14.3% | 14.7% | 14.5% | 13.5% | 15.5% |

*TW and JP refer to Taiwanese and Japanese.