Zoological Studies

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Molecular Phylogeny Revealing the Single Origin of *Cinnamomum*-associated *Bruggmanniella* (Diptera: Cecidomyiidae) in Asia, with 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. The Asian *Bruggmanniella* and the *Cinnamomum*-associated *Bruggmanniella* were monophyletic on the Neighbor-joining, Maximum-likelihood, and Bayesian inference trees. In addition, *Cinnamomum*-associated *Bruggmanniella* species had the closest sistership with *B. brevipes*, which are associated with the plant genus *Neolitsea* (Lauraceae). These results suggest that *B. brevipes*, *B. actinodaphnes* and *B. cinnamomi* are members of genus *Bruggmanniella*, a finding that is not consistent with another recent morphology-based phylogenetic study. Among the *Cinnamomum*-associated lineages, the 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. Additionally, the paraphyly of the Taiwanese stem galler with respect to Japanese *B. cinnamomi* suggests that the distributional range of *B. cinnamomi* be expanded from Taiwan to Japan.

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

BACKGROUND

The gall midge genus *Bruggmanniella* Tavares comprises 17 species, which 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 with Lauraceae: *B. cinnamomi* Tokuda & Yukawa, 2006 with *Cinnamomum yabunikkei* H. Ohba (= *C. japonicum* Sebold ex. Nakai), *B. actinodaphnes* Tokuda & Yukawa, 2006 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 have been 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 that may be 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 this analysis by Garcia et al. (2020), *B. brevipes* is situated in the basal clade of *Pseudasphondylia* and *Bruggmanniella*, and two
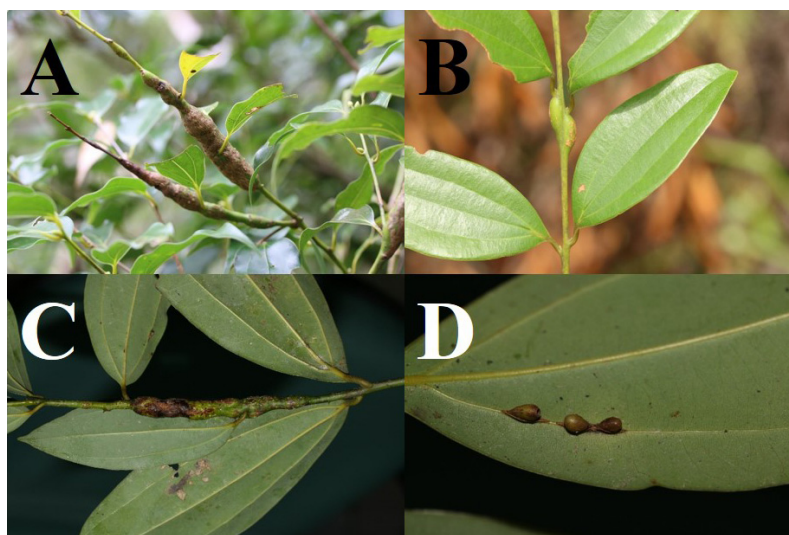
Japanese *Bruggmanniella* species were subsumed in the clade of *Pseudasphondylia*. Thus, Garcia et al. (2020) erected the new genus *Odontokeros* Garcia, Lamas and Urso-Guimarães, 2020 for *B. brevipes* and combined two Japanese *Bruggmanniella* with *Pseudasphondylia*. However, bootstraps of key nodes on the topology are poorly supported (less than 30%), especially on the 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) is not supported.

We 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, we record *B. cinnamomi*, which induces stem galls on *C. insularimontanum*, in Taiwan for the first time. Furthermore, we conducted molecular phylogenetic analyses of *Cinnamomum*-associated *Bruggmmanniella* 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

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

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 work.

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 the morphology of other parts follows 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 were designed based on Cameron et al. (2007); 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 the DNA Data Bank of Japan (DDBJ) (Table 1).

Sequences of two *Daphnephila* species, *D. urnicola* Chiang, Yang & Tokuda, 2015 (in Pan et al. 2015) (AB857360) and *D. truncicola* Tokuda, Yang & Yukawa, 2008 (AB334228), and one *Pseudasphondylia* species, *P. matatabi* Yuasa & Kumazawa, 1938 (AB085873), were downloaded from the National Center for Biotechnology Information (NCBI) (Table 1). Sequences of *D. truncicola* was selected as the root taxon in phylogenetic analyses according to the results of the morphology-based phylogeny 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). For ML inference, the GTR+I+G model was determined based on jModelTest 2.1.10 (Darriba et al. 2012) and pairwise deletion in proportional distance was selected with 1000 bootstrap replicates. The NJ method was performed using the same settings and in the ML method, except that the model used was Kimura's two-parameter divergence (Kimura 1980). As for 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. Divergence distances between *Bruggmanniella* species were calculated via P-distance with MEGA7 (Kumar et al. 2016).

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

Table 1. DNA information on *Bruggmanniella* species and relatives

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

"*" indicates DNA sequences downloaded from the National Center for Biotechnology Information; "**" indicates DNA sequences prepared by M Tokuda and used in the present study.

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 recorded from Taiwan for the first time; all the species are associated with *Cinnamomum* species.

Morphological features of *Bruggmanniella* are summarized in Gagné (1994) and Gagné et al. (2004), and they were compared to 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 based on its bidentate tooth of the gonostylus and other morphological features that fit well with the genus; in addition, our molecular phylogenetic analysis clearly indicates that the species is in the clade of *Bruggmanniella* (see below).

Bruggmanniella turoguei sp. nov. Lin, Yang and Tokuda
(Figs. 2, 5A, 6A, D; Table 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 1 cm in diameter and 1 to 12 cm 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, R5 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.
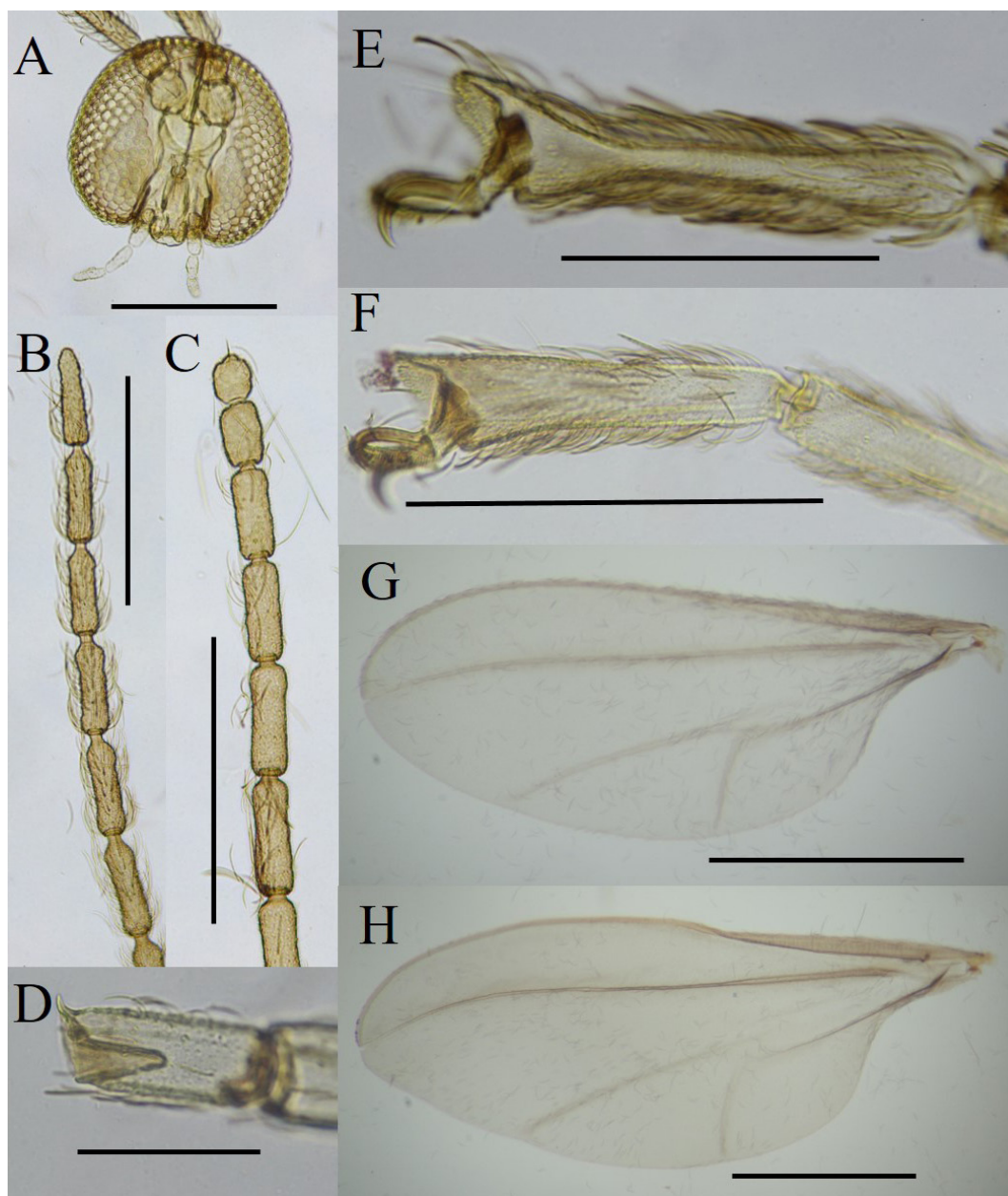
Fig. 2. *Bruggmanniella turoguei* sp. nov. (A) Male head (ventral view) (B) Male antenna (7–12 segment). (C) Female antenna (7–12 segment). (D) Male 1st tarsomere. (E) Male 5th tarsomere. (F) Female 5th tarsomere. (G) Male wing. (H) Female wing. Scale bars: A–C = 0.03 mm; D–F = 0.1 mm; G–H = 1 mm.

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

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

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 sterminte; 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

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

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

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.

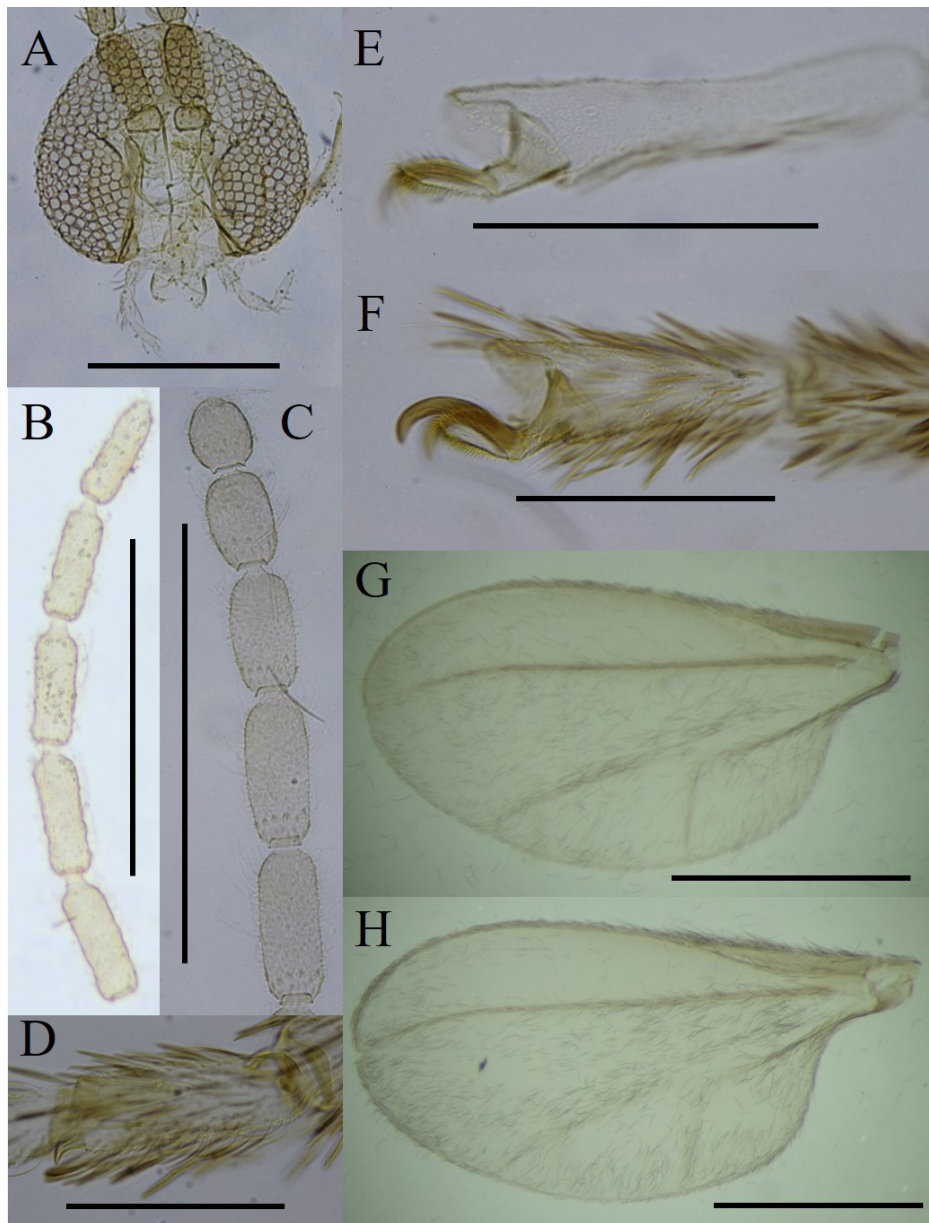### Bruggmanniella sanlianensis sp. nov. Lin, Yang and Tokuda

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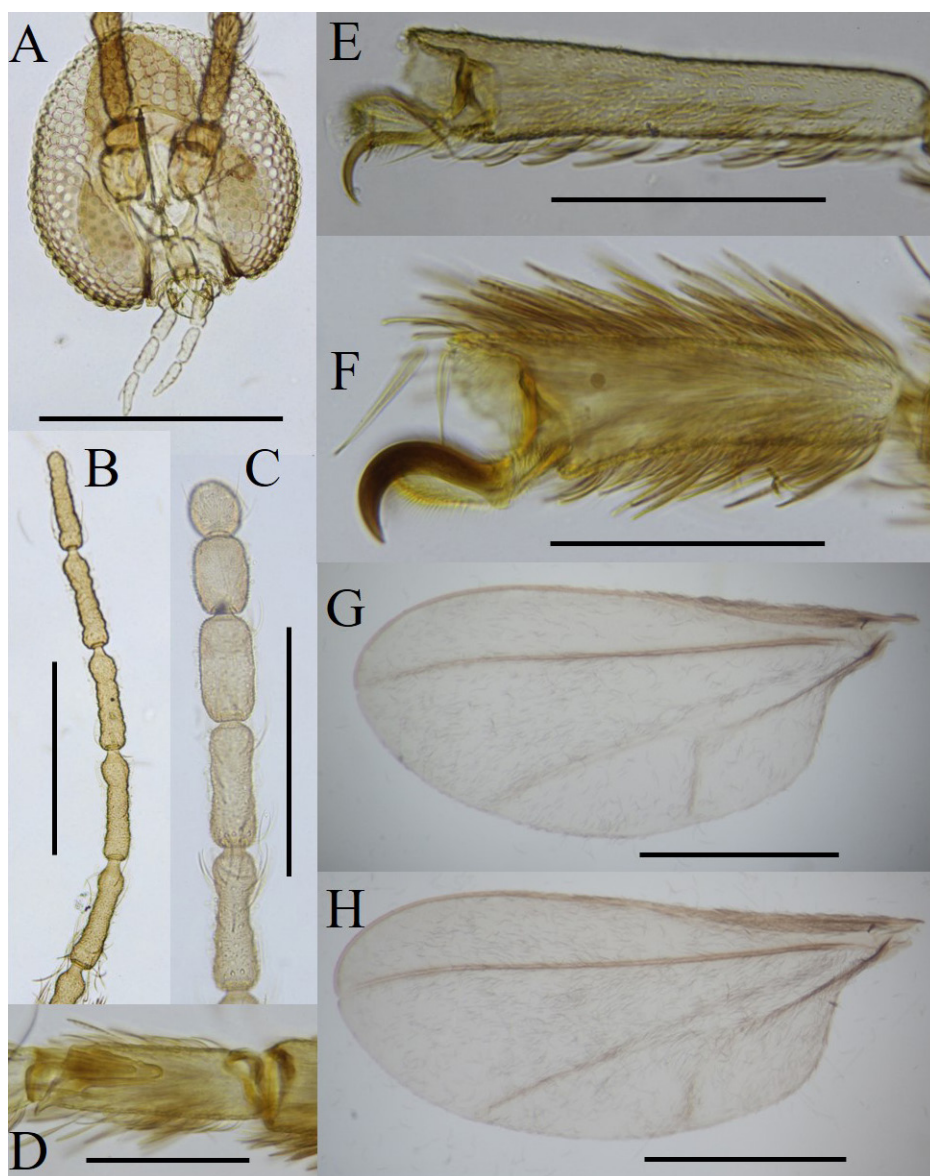
Fig. 3. *Bruggmanniella shianguei* sp. nov. (A) Male head (ventral view) (B) Male antenna (8–12 segment). (C) Female antenna (8–12 segment). (D) Male 1st tarsomere. (E) Male 5th tarsomere. (F) Female 5th tarsomere. (G) Male wing. (H) Female wing. Scale bars: A–C = 0.03 mm; D–F = 0.1 mm; G–H = 1 mm.

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.

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

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

"NA" indicate no data.

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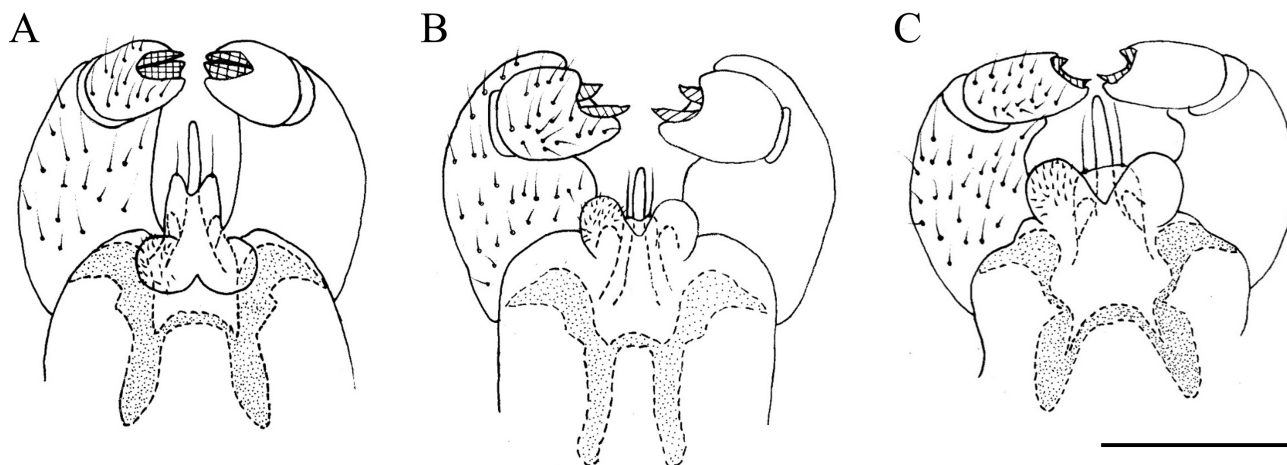
Fig. 5. Male genitalia of Taiwanese *Cinnamomum*–associated *Bruggmanniella*. (A) *Bruggmanniella turoguei* sp. nov., (B) *B. shianguei* sp. nov., and (C) *B. sanlianensis* sp. nov. Scale bar = 0.1 mm.

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 2006) 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.
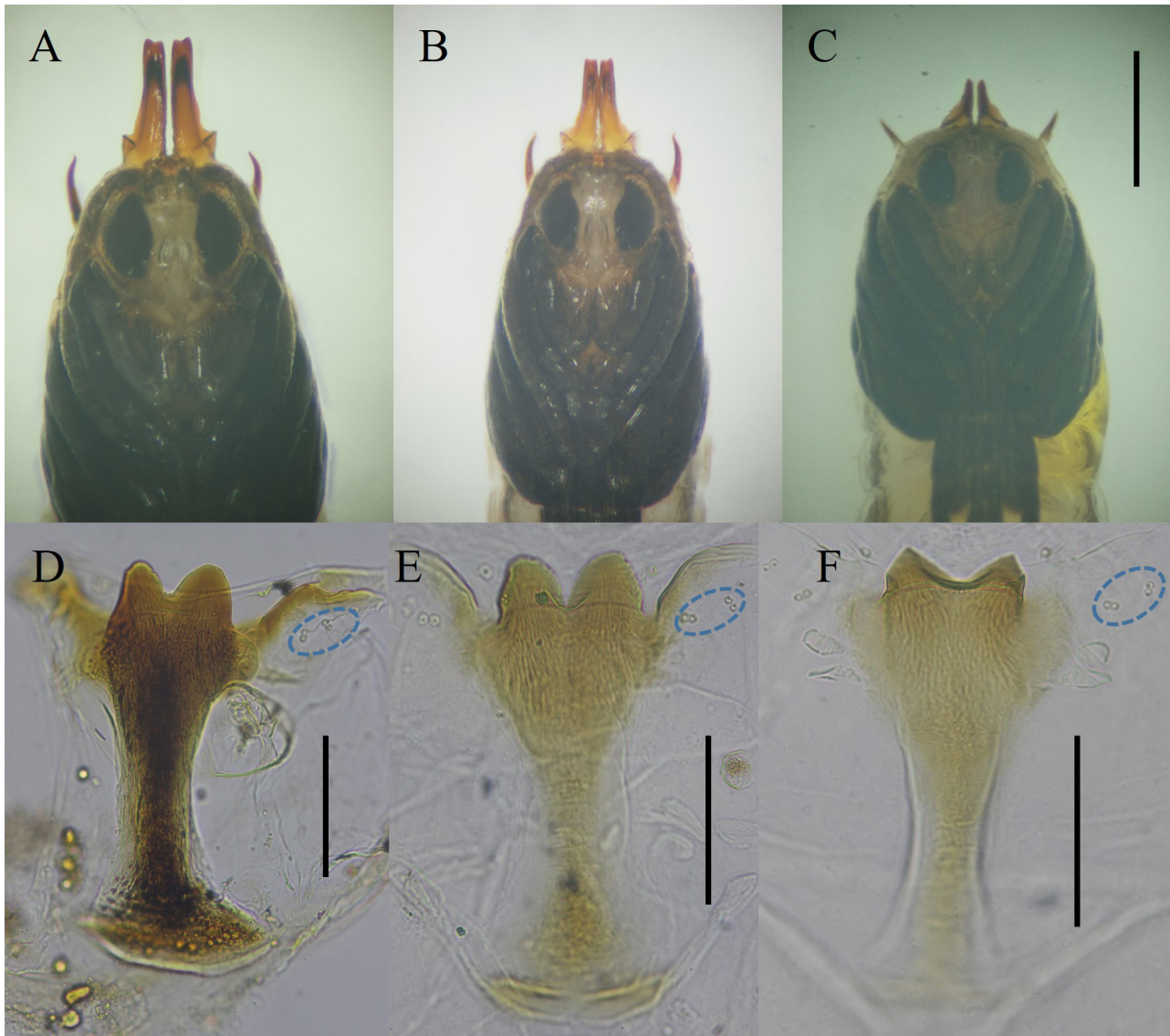
Fig. 6. Taiwanese *Cinnamomum*–associated *Bruggmanniella*. *Bruggmanniella turoguei* sp. nov. (A, D), *B. shianguei* sp. nov. (B, E), *B. sanlianensis* sp. nov. (C, F). Scale bars: pupal head (A–C) = 0.5 mm and for larval sternal spatula (D–F) = 0.1 mm. Larval lateral papillae shown in dotted blue circles.

and *B. shianguei* sp. nov., both possess finger-like pupal antennal horns (Fig. 6A–B) similar to those of *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*. *Bruggmanniella turoguei* sp. nov. and *B. cinnamomi* are distinguishable by the shape of the 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 similarites (Fig. 8). Although these two species were transferred to *Pseudasphondylia* based on
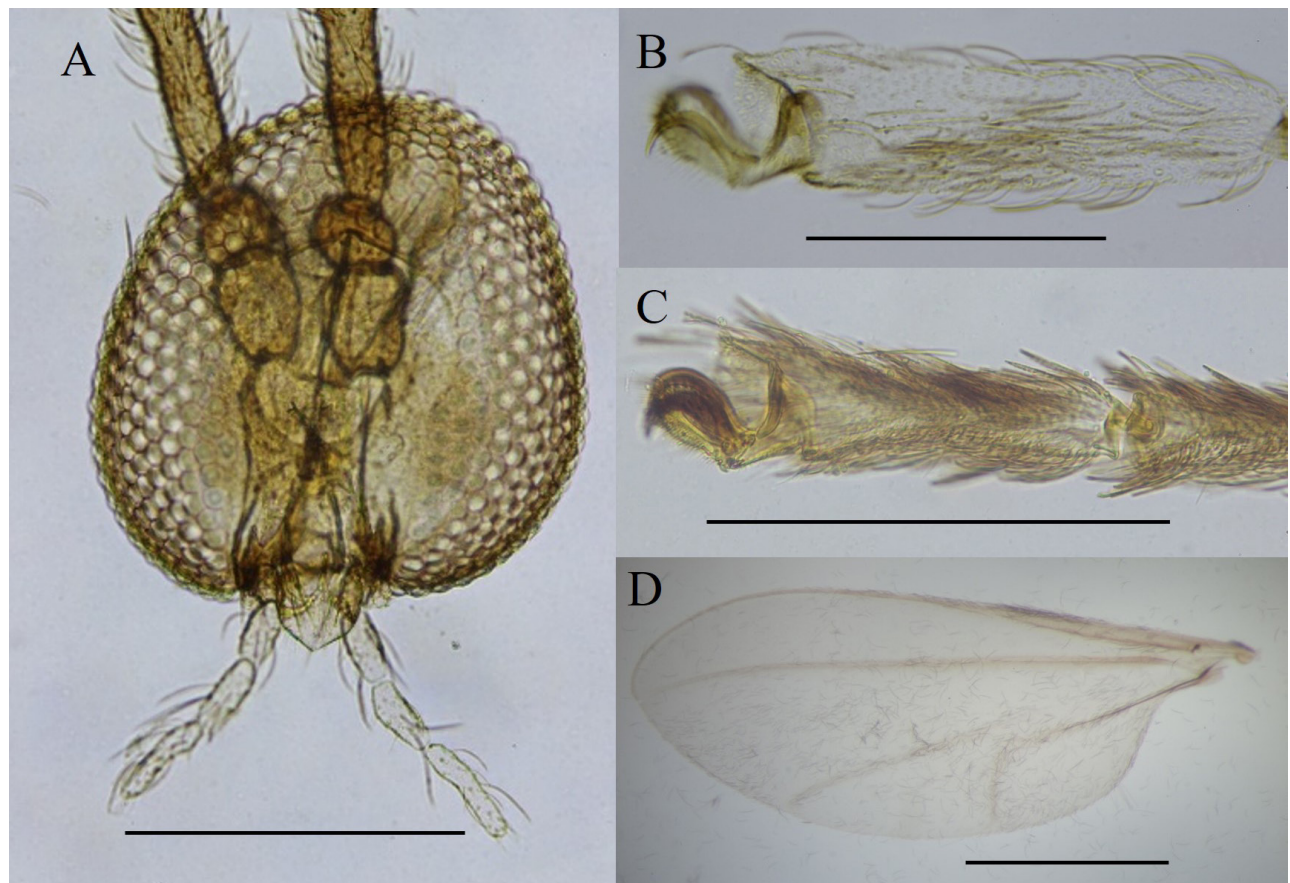
Fig. 7. *Bruggmanniella cinnamomi* (A) Male head (ventral view). (B) Male 5th tarsomere. (C) Female 5th tarsomere. (D) Male wing. Scale bars: A = 0.03 mm; B–C = 0.1 mm; D = 1 mm.

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

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

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 believe that more comprehensive studies are needed to conclusively determine the detailed relationship and, at least for the present, we prefer to retain all of them as members of *Bruggmanniella*.

Molecular phylogeny and genetic distances

The phylogenetic trees created from the three methods had similar topologies. Asian *Bruggmanniella* and genus *Pseudasphondylia* were monophyletic in

the NJ (Fig. 8), ML and BI trees, with more than 50% bootstrap support in the ML and NJ trees and 0.5 support in the BI topology. Among *Bruggmanniella* species, the basal lineage is a *Litsea*-associated species, *B. actinodaphnes*, which 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 is highly supported by bootstrap values (99%, 94% and 1 in NJ, ML and BI trees, respectively). 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. The monophyly of *B.*
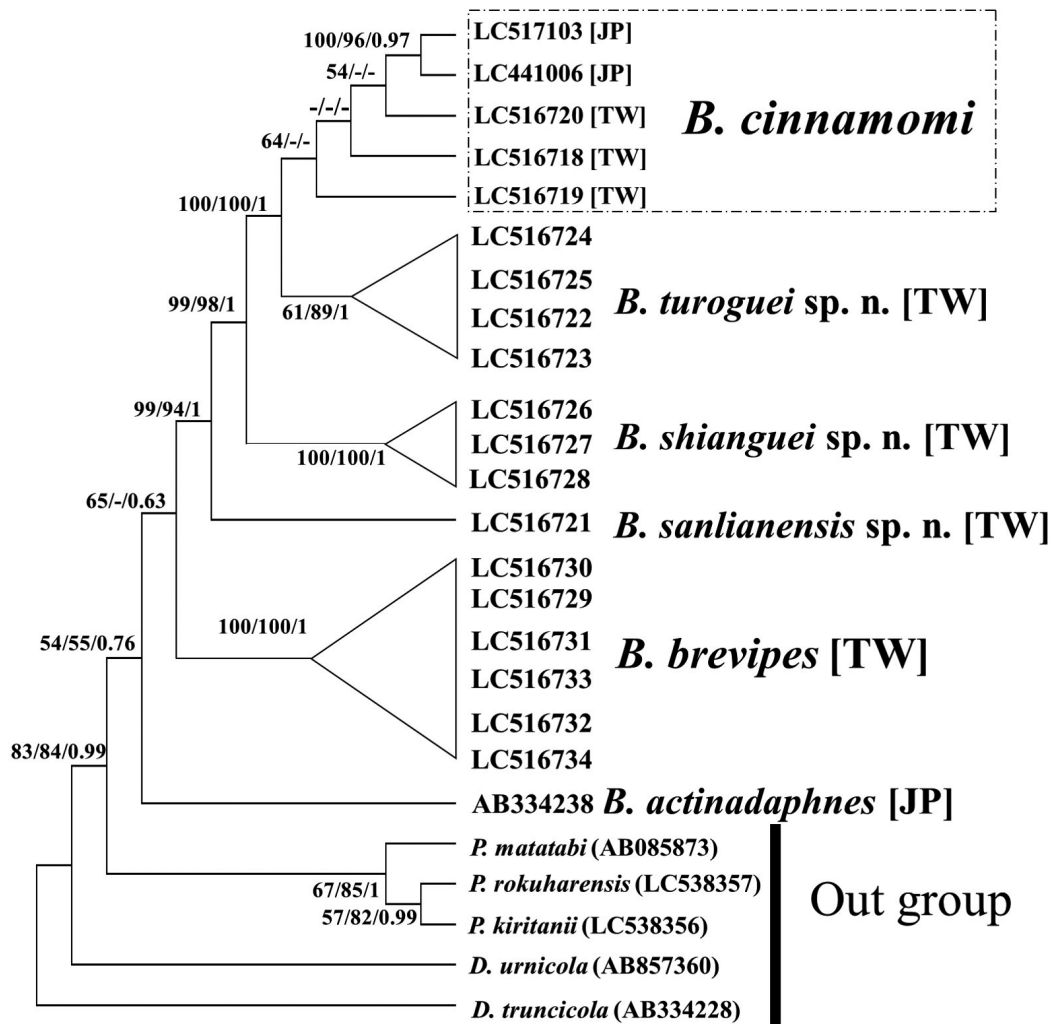
Fig. 8. Cladogram of East Asian *Bruggmanniella* based on the Neighbor-joining method based on the DNA *COI* region. Support values at the nodes are Neighbor-joining (left)/ Maximum-likelihood (media)/ Bayesian inference (right). "–" indicates support values less than 50% in ML and NJ, and 0.5 in BI inference.

cinnamomi was supported in both NJ and ML trees, but undetermined in the BI tree due to an unsolved relationship with *B. turoguei* sp. nov. In *B. cinnamomi*, Taiwanese taxa are monophyletic and sister to the Japanese taxa based on the ML method, but become a paraphyletic group with respect to Japanese taxa in the NJ tree (Fig. 8).

The genetic distances within species are 0.4%, 1.9%, 1.9% and 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*, the genetic distance of most species pairs do no exceed 7.0%. The lowest genetic distance is between *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 actually belong to the genus *Bruggmanniella*. In their scheme, *B. cinnamomi* and *B. actinadaphnes* are sister and members of *Pseudasphondylia*. In addition, *B. brevipes* was treated as a distinct genus (*Odontokeros*) and became a sister group of *Pseudasphondylia* + *Bruggmanniella*. However, our tree (Fig. 8) suggested that *Pseudasphondylia* and Asian *Bruggmanniella* are monophyletic and sister. In addition, our topology suggests that *B. brevipes* is sister to *Cinnamomum*-associated taxa with strong support, indicating that the species is more closely related to *Cinnamomum*-associated taxa than to *Pseudasphondylia*. That is, *B. cinnamomi* by no means belongs to genus *Pseudasphondylia* and *B. brevipes* should be treated as Asian *Bruggmanniella*. Characteristics of *Bruggmanniella* larvae and pupae have high heterogeneity in both Neotropcial and Asian groups. It is not suitable to erect a new genus or genera under the current taxonomic framework because it would lead to chaos in further taxonomic works. Presently, the phylogeny of *Bruggmanniella* is still inconclusive due to nodes with low support in the morphological-based cladogram (Garcia et al. 2020) and insufficient sampling of DNA fragments and taxa, especially DNA of the type species, *B. braziliensis*. Thus, the comprehensiveness of data—including taxonomic unit, species, morphological features and DNA information—on *Bruggmanniella* is essential for resolving this situation.

Molecular information is a useful tool for species identification, especially for gall midges, whose adult specimens can be very difficult to obtain; in addition, adults of this species share similar morphological features with genera such as *Asphondylia* (Yukawa et al. 2003; Uechi and Yukawa 2004; Elsayed et al. 2018). Three new *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. Ours is the first paper to address the molecular phylogeny of Asian *Bruggmanniella* and confirm the single origin of *Cinnamomum* association in the genus. Their pairwise genetic distances in a partial *COI* gene were large: for example, the maximum distance of the region was 7.0% within the species pair of *Cinnamomum*-associated *Bruggmanniella*, but it was 13.5–16.4% compared 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 the 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 early in the history.

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

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

*TW and JP refer to Taiwanese and Japanese.

Divergence among *Cinnamomum*-associated *Bruggmmanniella*

Diversification of gall inducers could be triggered by various biotic and/or abiotic factors. In gall inducing cecidomyiids, host plant and organ shifts are considered important mechanisms of divergence (Tokuda et al. 2004; Yukawa et al. 2005; Joy and Crespi 2007; 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 (2007) revealed that organ shifts are critical for divergence events in 14 *Asphondylia* species on their single host plant, the creosote bush *Larrea tridentata* (DC.) Coville.

In our study system of *Cinnamomum*-associated *Bruggmmanniella*, 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 confirm whether the divergence 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 they diverged around 0.35–0.4 Mya based on a 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 the Ryukyu Islands the last time they were connected to Taiwan (Kimura 1996; Osozawa et al. 2012), suggesting that *B. cinnamomi* expanded its range from Taiwan to the Ryukyu Islands and its host changed to *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 for understanding divergence pathways of cecidomyiids. *Bruggmanniella cinnamomi* utilizes *C. yabunikkei* on the Southwest Islands of Japan and *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 when the host is in a suitable development stage (Yukawa 2000). Alternatively, Taiwanese populations of *C. yabunikkei* may be resistant to *B. cinnamomi*. From a biogeographical point of view, *B. cinnamomi* has never been found in the Palearctic Region of Japan (*i.e.*, Kyushu or northern areas) or the Korean Peninsula (Yukawa and Masuda 1996; Paik et al. 2004; Tokuda and Yukawa 2006; Kim et al. 2015), even though its host plant is distributed in these areas. The mechanism inhibiting the distributional expansion of *B. cinnamomi* 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. *Bruggmanniella turoguei* sp. nov. and *B. sanlianensis* sp. nov. are associated with a 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. 2000). 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 the 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* are described from Taiwan, increasing the number of species in the genus to seven and 20 in Asia and the world, respectively. In this article, the molecular phylogeny of Asian *Bruggmanniella* species suggests its monophyletic and single origin of *Cinnamomum*-associated species, which is not consistent with the morphological-based phylogenetic concept in Garcia et al. (2020). Considering the genetic distance of the Asian species and morphological heterogeneity among *Bruggmanniella* species, Asian species should be divided into several genera. However, comprehensive data on *Bruggmanniella* species, including taxonomic unit, species, morphological features, and DNA information, are essential for resolving this situation.

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

Competing interests: All authors declare that they have no competing interest.

Availability of data and materials: DNA sequences generated in the study have been deposited into 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.

REFERENCES

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc Natl Acad Sci USA **91**:6491–6495. doi:10.1073/pnas.91.14.6491.

Cameron SL, Lambkin CL, Barker BC, Whiting MF. 2007. A mitochondrial genome phylogeny of Diptera: whole genome sequences resolve relationships over broad timescales with high precision. Syst Entomol **32**:40–59. doi:10.1111/j.1365-3113.2006.00355.x.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods **9**:772. doi:10.1038/nmeth.2109.

DeSalle R, Freeman T, Prager EM, Wilson AC. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. J Mol Evol **26**:157–164. doi:10.1007/BF02111289.

Dorchin N, Scott ER, Clarkin CE, Luongo MP, Jordan S, Abrahamson WG. 2009. Behavioural, ecological and genetic evidence confirm the occurrence of host-associated differentiation in goldenrod gall-midges. J Evol Biol **22**:729–739. doi:10.1111/j.1420-9101.2009.01696.x.

Elsayed AK, Matsuo K, Kim W, Uechi N, Yukawa J, Gyoutoku N, Tokuda M. 2018. A new *Asphondylia* species (Diptera: Cecidomyiidae) and a eulophid wasp (Hymenoptera) inducing similar galls on leaf buds of *Schoepfia jasminodora* (Schoepfiaceae), with reference to their ecological traits and a description of the new gall midge. Entomol Sci **21**:324–339. doi:10.1111/ens.12312.

Gagné RJ. 1994. The gall midges of the Neotropical region. Cornell University Press, Ithaca, NY, USA.

Gagné RJ, Jaschhof M. 2017. A catalog of the Cecidomyiidae (Diptera) of the world. 4th edn. https://www.ars.usda.gov/ARSUserFiles/80420580/Gagne_2017_World_Cat_4th_ed.pdf. Accessed 03 Apr. 2018.

Gagné RJ, Posada F, Gil ZN. 2004. A new species of *Bruggmanniella* (Diptera: Cecidomyiidae) aborting young fruit of avocado, *Persea americana* (Lauraceae), in Colombia and Costa Rica. Proc Entomol Soc Wash **106**:547–553.

Garcia CDA, Lamas CJE, Urso-Guimarães MV. 2020. Cladistic analysis of the genus *Bruggmanniella* Tavares (Diptera, Cecicomyiidae, Asphondyliini) with evolutionary inferences on the gall inducer-host plant association and description of a new Brazilian species. PLoS ONE **15**:e0227853. doi:10.1371/journal.pone.0227853.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser **41**:95–98.

Hassler M. 2019. World Plants: Synonymic Checklists of the Vascular Plants of the World. http://www.catalogueoflife.org/annual-checklist/2019. Accessed Nov. 2018.

Hebert PDN, Ratnasingham S, de Waard JR. 2003. Barcoding animal life: Cytochrome *c* oxidase subunit 1 divergences among closely related species. Proc Royal Soc B **270**:S96–S99. doi:10.1098/rsbl.2003.0025.

Huang JF, Li L, van der Werff H, Li HW, Rohwer JG, Crayn DM, Meng HH, van der Merwe M, Conran JG, Li J. 2016. Origins and evolution of cinnamon and camphor: A phylogenetic and historical biogeographical analysis of the *Cinnamomum* group (Lauraceae). Mol Phylgenet Evol **96**:33–44. doi:10.1016/j.ympev.2015.12.007.

Joy JB, Crespi BJ. 2007. Adaptive radiation of gall-inducing insects within a single host-plant species. Evolution **61**:784–795. doi:10.1111/j.1558-5646.2007.00069.x.

Kim W, Tokuda M, Yukawa J. 2015. Cecidomyiid galls found on Tsushima, a stepping stone island between the Korean Peninsula and Kyushu, Japan. Makunagi: Acta dipterologica **26**:21–38.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol **16**:111–120. doi:10.1007/BF01731581.

Kimura M. 1996. Quaternary paleogeography of the Ryukyu Arc. Geogr J **105**:259–285. doi:10.5026/jgeography.105.3_259. (in Japanese with English abstract)

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol **33**:1870–1874. doi:10.1093/molbev/msw054.

Lin SF, Tokuda M, Yang MM. 2019. The first record of genus *Bruggmanniella* (Diptera: Cecidomyiidae) from Taiwan with description of a new species inducing bud galls on *Neolitsea parvigemma* (Lauraceae). J Asia-Pac Entomol **22**:203–207. doi:10.1016/j.aspen.2018.12.012.

Lin SF, Tokuda M, Yang MM. 2020. Leaf gall polymorphism and molecular phylogeny of a new *Bruggmanniella* species (Diptera: Cecidomyiidae: Asphondyliini) associated with *Litsea*

acuminata (Laruaceae) in Taiwan, with ecological comparisons and a species description. Entomol Sci **22**:10–22. doi:10.1111/ens.12391.

Linnaeus C. 1758. Systema Naturae per Regna Tria Naturae. Edition 10, vol. I. - Stockholm, Sweden.

Lu FY, Ou CH, Chen YC, Chi YS, Lu KC. 2000. Trees of Taiwan. vol 1. – Department of Forestry, National Chung Hsing University, Taichung, Taiwan.

Maia VC, Fernandes GW, Oliveira LA. 2010. A new species of *Bruggmanniella* (Diptera, Cecidomyiidae, Asphondyliini) associated with *Doliocarpus dentatus* (Dilleniaceae) in Brazil. Rev Bras Entomol **54**:225–228. doi:10.1590/S0085-56262010000200004.

Mathur S, Cook MA, Sinclair BJ, Fitzpatrick SM. 2012. DNA barcodes suggest cryptic speciation in *Dasineura oxycoccana* (Diptera: Cecidomyiidae) on cranberry, *Vaccinium macrocarpon*, and blueberry, *V. corymbosum*. Fla Entomol **95**:387–394.

McAlpine JF. 1981. Morphology and terminology of-adults. *In*: Maclpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (ed) Manual of Nearctic Diptera, vol 1. Canadian Government Publishing Centre, Hull, pp. 9–63.

Möhn E. 1961a. Gallmücken (Diptera, Itonididae) aus El Salvador. 4. Zur Phylogenie der Asphondyliidi der neotropischen und holarktischen Region. Senckenbergiana biologica **42**:131–330.

Möhn E. 1961b. Neue Asphondyliini-Gattungen (Diptera, Itonididae). Stuttgarter Beiträge zur Naturkunde **49**:1–14.

Monzen K. 1955. Some Japanese gallmidges with the descriptions of known and new genera and species (II). Annu Rep Gakugei Fac Iwate Univ **9**:34–48.

Newman E. 1834. Attempted division of British insects into natural orders. Entomol Mag **2**:379–431.

Osozawa S, Shinjo R, Armid A, Watanabe Y, Horiguchi T, Wakabayashi J. 2012. Palaeogeographic reconstruction of the 1.55 Ma synchronous isolation of the Ryukyu Islands, Japan, and Taiwan and inflow of the Kuroshio warm current. Int Geol Rev **54**:1369–1388. doi:10.1080/00206814.2011.639954.

Paik JC, Yukawa J, Uechi N, Sato S, Ganaha T. 2004. Gall-inducing species of the family Cecidomyiidae (Diptera) recorded from the Korean Peninsula and surrounding islands, in comparison with the gall-midge fauna of Japan. Esakia **44**:57–66.

Pan LY, Chiang TC, Weng YC, Chen WN, Hsiao SC, Tokuda M, Tasi CL, Yang MM. 2015. Taxonomy and biology of a new ambrosia gall midge *Daphnephila urnicola* sp. nov. (Diptera: Cecidomyiidae) inducing urnshaped leaf galls on two species of *Machilus* (Lauraceae) in Taiwan. Zootaxa **3955**:371–388. doi:10.11646/zootaxa.3955.3.5.

Rodrigues AR, Carvalho-Fernandes Sheila P, Maia VC, Oliveira LA. 2020. Three new species of *Bruggmanniella* Tavares, 1909 (Diptera, Cecidomyiidae) from Brazil with a key to species. Rev Bras Entomol **64**:e201917. doi:10.1590/1806-9665-rbent-2019-17.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol **61**:539–542. doi:10.1093/sysbio/sys029.

Stireman III JO, Hilary D, Timothy GC, Abbot P. 2010. Evolutionary diversification of the gall midge genus *Asteromyia* (Cecidomyiidae) in a multitrophic ecological context. Mol Phylogenet Evol **54**:194–210. doi:10.1016/j.ympev.2009.09.010.

Tavares JS. 1909. Contributio prima ad cognitionem cecidologiae braziliae. Brotéria, Série Zoológica **8**:5–28.

Tavares JS. 1920. O gênero *Bruggmanniella* Tav. com a descripção de uma nova espécie e a clave dichotomica dos gêneros das Asphondyliariae. Brotéria, Série Zoológica **18**:33–42.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res **22**:4673–4680. doi:10.1093/nar/22.22.4673.

Tokuda M. 2004. *Illiciomyia* Tokuda, a new genus for *Illiciomyia yukawai* sp. n. (Diptera: Cecidomyiidae: Asphondyliini) inducing leaf galls on *Illicium anisatum* (Illiciaceae) in Japan. Esakia **44**:1–11.

Tokuda M. 2012. Biology of Asphondyliini (Diptera: Cecidomyiidae). Entomol Sci **15**:361–383. doi:10.1111/j.1479-8298.2012.00539.x.

Tokuda M. 2018. Arthropod galls found in northern parts of the Tokara Islands, Japan. Jap J Syst Entomol **24**:99–107.

Tokuda M, Tabuchi KY, Yukawa J, Amano H. 2004. Inter- and intraspecific comparisons between *Asteralobia* gall midges (Diptera: Cecidomyiidae) causing axillary bud galls on *Ilex* species (Aquifoliaceae): species identification, host range, and mode of speciation. Ann Entomol Soc Am **97**:957–970. doi:10.1603/0013-8746(2004)097[0957:IAICBA]2.0.CO;2.

Tokuda M, Yang MM, Yukawa J. 2008. Taxonomy and molecular phylogeny of *Daphnephila* gall midges (Diptera: Cecidomyiidae) inducing complex leaf galls on Lauraceae, with descriptions of five new species associated with *Machilus thunbergii* in Taiwan. Zool Sci **25**:533–545. doi:10.2108/zsj.25.533.

Tokuda M, Yukawa J. 2005. Two new and three known Japanese species of the genus *Pseudasphondylia* Monzen (Diptera: Cecidomyiidae) and their life history strategies. Ann Entomol Soc Am **98**:259–272. doi:10.1603/0013-8746(2005)098[0259:TNATKJ]2.0.CO;2.

Tokuda M, Yukawa J. 2006. First records of genus *Bruggmanniella* (Diptera: Cecidomyiidae: Asphondyliini) from Palaearctic and Oriental Regions, with descriptions of two new species that induce stem galls on Lauraceae in Japan. Ann Entomol Soc Am **99**:629–637. doi:10.1603/0013-8746(2006)99[629:FROGBD]2.0.CO;2.

Tokuda M, Yukawa J. 2007. Biogeography and evolution of gall midges (Diptera: Cecidomyiidae) inhabiting broadleaved evergreen forests in the Oriental and eastern Palaearctic Regions. Orient Insects **41**:121–139. doi:10.1080/00305316.2007.10417502.

Tung GS, Yang MM, Lin SF, Tang CT, Liao YC, Yeh HT, Wang CC, Huang MY. 2018. Let's gall Taiwan – A guidebook on insect galls. Forestry Bureau C. O. A, Taipei, Taiwan.

Uechi N, Yukawa J. 2004. Description of *Asphondylia itoi* sp. n. (Diptera: Cecidomyiidae) inducing fruit galls on *Distylium racemosum* (Hamameelidaceae) in Japan. Esakia **44**:27–43.

Yuasa H, Kumazawa T. 1938. A new gall midge from Nippon (Diptera, Cecidomyidae). J Pharmacol Soc Jpn **58**:204–206.

Yukawa J. 2000. Synchronization of gallers with host plant phenology. Popul Ecol **42**:105–113.

Yukawa J, Masuda H. 1996. Insect and mite galls of Japan in Colors. Zenkoku Nôson Kyôiku Kyôkai, Tokyo: 826 pp. (in Japanese with English explanation for color plates)

Yukawa J, Uechi N, Horikiri M, Tuda M. 2003. Description of the soybean pod gall midge, *Asphondylia yushimai* sp. n. (Diptera: Cecidomyiidae), a major pest of soybean and findings of host alternation. B Entomol Res **93**:73–86. doi:10.1079/BER2002218.

Yukawa J, Uechi N, Tokuda M, Sato S. 2005. Radiation of gall midges (Diptera: Cecidomyiidae) in Japan. Basic Appl Ecol **6**:453–461. doi:10.1016/j.baae.2005.07.004.