

# Biogeography and Ecological Differentiation of *Pseudasphondylia* gall midges (Diptera: Cecidomyiidae) Distributed in Taiwan and Japan, with Description of a New Species *P. kiwiphila* sp. nov. and the Southernmost Record of *P. elaeocarpi*

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*Pseudasphondylia* species (Diptera: Cecidomyiidae) are known to induce fruit galls on *Actinidia rufa* (Siebold & Zucc.) Planch. ex Miq. and finger-like leaf galls on *Elaeocarpus sylvestris* (Lour.) Poir. in Taiwan, but their taxonomic positions remain undetermined. Based on gall morphology and host plants, they were supposed to be the same or allied species of known Japanese congeners, i.e., *P. matatabi* Yuasa & Kumazawa inducing flower-bud galls on *Actinidia polygama* (Sieb. et Zucc.) Maxim and *P. elaeocarpi* Tokuda & Yukawa inducing finger-like leaf galls on *E. sylvestris*. Species identifications of these Taiwanese species provide us an opportunity to study biogeographical aspects and transition of ecological features in these *Pseudasphondylia* species distributed in East Asian Arc. Morphological comparisons and species delimitation by molecular analysis indicated that the cecidomyiid on the fruit of *A. rufa* is distinct from *P. matatabi* and thus it is described as a species new to science, *P. kiwiphila* sp. nov. Lin, Tokuda, & Yang. The leaf galler on *E. sylvestris* was identical to *P. elaeocarpi*, whose southernmost distribution range extended to Taiwan, a new record of its distribution. *COI*-based phylogenetic tree (Bayesian inference and IQ tree) of *Pseudasphondylia* suggested that leaf galling habitat and univoltine life history are ancestral, whereas fruit or flower-bud galling and multivoltine life history are derived. In addition, the monophyletic *Actinidia*-associated species lineage is sistered to the clade including the remaining Japanese fruit and flower-bud gallers, suggesting that *Pseudasphondylia* has colonized on the host genus *Actinidia* once and later speciated on different plant species of the host genus. As a biogeographical aspect of *P. elaeocarpi*, 2.7% of the *COI* distance between Japanese and Taiwanese individuals indicates that they have diverged around 1.2 mya, which corresponds to the last but second separation of Taiwan and Japan in the Pleistocene. As for *Actinidia*-associated *Pseudasphondylia* species, the two valid species are allopatric and have distinct areas of origin, suggesting they may have speciated allopatrically. Nevertheless, there is still the possibility of ecological speciation due to the following reasons: (1) Host species (and varieties)

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and unidentified congener of *Actinidia*-associated *Pseudasphondylia* are occurring China, revealing potential occurrence of these gall midges. (2) The divergence time (2.2–2.9 mya) of the two known species corresponds to the late Pliocene to Pleistocene, when China, Taiwan, and Japan were part of the East Asian continent. During this period, their host species were sympatric in southeast China. (3) The host of two named *Actinidia*-associated *Pseudasphondylia* species each belong to different plant groups with distinct fruit features. These presume that the speciation might have been caused via sympatric host shift.

**Key words:** *Actinidia*, Biogeography, *Elaeocarpus sylvestris*, Kiwi fruit gall, Speciation.

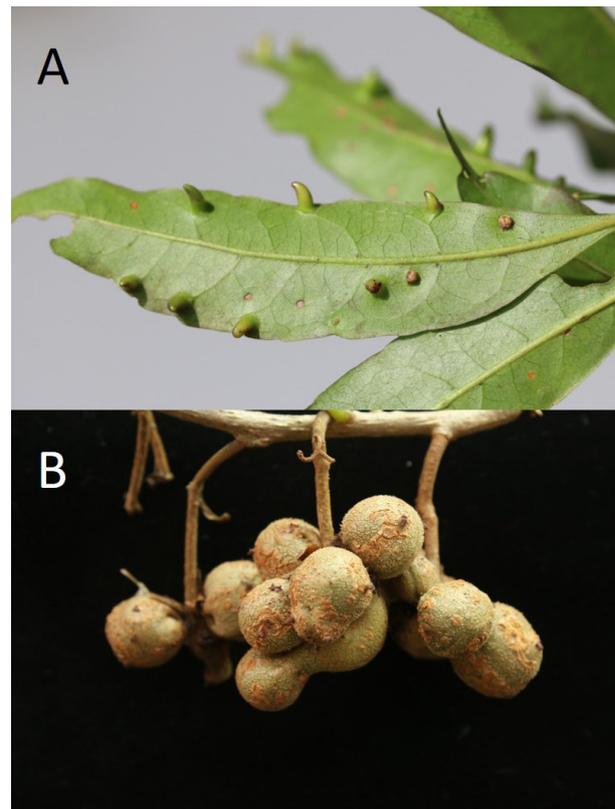
## BACKGROUND

The genus *Pseudasphondylia* Monzen (Diptera: Cecidomyiidae: Asphondyliini) is distributed in the Palearctic, Oriental, and Oceanian regions and comprises the following 12 gall-inducing species: *P. campanulata* (Mani, 1954), *P. diospyri* Mo & Xu, 1999, *P. elaeocarpi* Tokuda and Yukawa, 2005, *P. kiritanii* Tokuda & Yukawa, 2005, *P. matatabi* (Yuasa & Kumazawa, 1938), *P. neolitseae* Yukawa, 1974, *P. philadelphi* (Kovalev, 1964), *P. rauwolfiae* Coutin, 1980, *P. rokuharensis* Monzen, 1955, *P. saohimea* Matsuda, Elsayed & Tokuda, 2021, *P. tominagai* Elsayed & Tokuda, 2019, and *P. zanthoxyli* Mo, Bu & Li, 2007.

In Taiwan, Tung et al. (2018) assumed that three gall midge species belong to the genus *Pseudasphondylia*, all of which induce galls similar to those of Japanese congeners on the same or allied host plants. One species induces finger-like leaf galls on *Elaeocarpus sylvestris* (Elaeocarpaceae) (Fig. 1A), which resemble those induced by the Japanese *P. elaeocarpi* on the same host (Yukawa and Masuda 1996; Tokuda and Yukawa 2005); the second species induces ball-shaped leaf galls on five *Neolitsea* species (Lauraceae) (Liang et al. 1999; Tung et al. 2018) as Japanese *P. neolitseae* associated with *Neolitsea sericea* (Yukawa 1974; Tokuda and Yukawa 2005); the third species induces fruit galls on *Actinidia rufa* (Siebold & Zucc.) Planch. ex Miq. (Actinidiaceae) (Tung et al. 2018; Fig. 1B) and may be an allied species of the Japanese *P. matatabi* inducing a similar sort of gall on *Actinidia polygama* (Tokuda and Yukawa 2005). The fruit gall on *A. rufa* was also found in Japan, but the species remains unidentified (Nagai 2016). These findings suggest the high affinity of cecidomyiid fauna between Taiwan and Japan and provide an ideal system in biogeography to confirm divergence patterns and speciation processes of the insular species/populations.

Although Tung et al. (2018) treated the

abovementioned Taiwanese gall midges as the genus *Pseudasphondylia*, they were left unidentified. Therefore, in this study we determine the taxonomic positions of *Pseudasphondylia* gall midges associated with *A. rufa* and Taiwanese *E. sylvestris* by integration of morphological comparisons and molecular analyses. Then, we further explore their biogeographical aspects related to host distribution, genetic distances, ecological features, and their phylogenetic relationship within the genus *Pseudasphondylia*.



**Fig. 1.** Galls of Taiwanese *Pseudasphondylia* species. (A) Finger-shaped leaf galls on *Elaeocarpus sylvestris*. (B) Fruit gall on *Actinidia rufa*.

## MATERIALS AND METHODS

### Collecting and examination of specimens

Finger-like leaf galls on *E. sylvestris* in Taiwan and swollen fruit galls on *A. rufa* in Taiwan and Japan were surveyed and collected from 1999 to 2021. The collected galls were dissected under a microscope, and larval and pupal specimens of gall midges were preserved in plastic tubes with 95% ethanol for morphological and molecular studies. In addition, branches with galled leaves or fruit were inserted in a sealed water vial to keep moisture and maintained under laboratory conditions until adult emergence. Adult specimens were preserved in 70% ethanol for morphological examination. Specimens were mounted on slides following the procedures in Gagné (1994), and the morphological traits were examined according to Gagné (1994) and Tokuda (2004) using a microscope (Leica DM 750, Germany) and photographed with a digital camera (Canon 80D, Japan). We also examined the type specimens of *P. elaeocarpi* and *P. matatabi* that are kept in the collection of the Entomological Laboratory, Kyushu University, Fukuoka, Japan (ELKU). Taiwanese specimens examined in the present study were deposited into the Taiwan Forestry Research Institute, Taiwan (TFRI) and National Chung Hsing University, Taiwan (NCHU).

### DNA sequencing, genetic distance and species delimitation

Mitochondrial DNA sequences (partial region of the cytochrome oxidase subunit I; *COI* gene: 689 bp) of two cecidomyiid individuals inducing leaf

galls on *E. sylvestris* in Taiwan and five cecidomyiid individuals inducing fruit galls on *A. rufa* in Taiwan and Japan (Table 1) were obtained by the following process. Whole DNA was extracted from larval specimens adding 50 µl of QuickExtract Tissue Kit solution (Epicentre Biotechnologies, Madison, WI) following the manufacturer’s instructions. We used the following primers for six Taiwanese individuals and one Japanese individual: Diptera-49F (5'-AATCATAAAGATATTGGAAC-3') and Diptera-734R (5'-CAACATTTATTTTGGATTTTGG-3') (Pan et al. 2015), and LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer et al. 1994), respectively. The amplification and polymerase chain reaction condition followed Lin et al. (2020a). The purification of amplification products was carried out by QIAquick Gel Extraction Kit (Qiagen, Hilden, German) for direct sequencing. DNA sequences were checked via the software BioEdit (Hall 1999).

To compare genetic distances, two *COI* sequences of Japanese *P. elaeocarpi* (LC621306) and *P. matatabi* (AB085873) were downloaded from the National Center for Biotechnology Information and further compared with seven newly obtained *COI* sequences (Table 1) via *p*-distance using MEGA X (Kumar et al. 2018). For species delimitation, two downloaded and seven newly obtained *COI* sequences were aligned via ClustalW (Thompson et al. 1994) with BioEdit (Hall 1999) and further performed via Automatic Barcode Gap Discovery (ABGD) method (Puillandre et al. 2012; webpage: <http://www.wabi.snv.jussieu.fr/public/abgd>). Calculation mode and parameter as default setting: Jukes-Cantor (JC69) distance, X (relative gap width) = 1.5, Pmin = 0.001, Pmax = 0.1, Steps = 10, Number of

**Table 1.** DNA information on *Pseudasphondylia* species

Species name	Galling organ	Host/ Distribution	Accession No.*
<i>P. elaeocarpi</i>	Leaf	<i>Elaeocarpus sylvestris</i> / Yona, Japan	LC621306 <sup>a</sup>
<i>Pseudasphondylia</i> sp.	Leaf	<i>Elaeocarpus sylvestris</i> / Mt. Dahan, Taiwan	<b>LC710536-37</b>
<i>P. saohimeae</i>	Leaf	<i>Magnolia kobus</i> var. <i>borealis</i> / Tomakomai, Japan	LC621304 <sup>a</sup>
<i>P. matatabi</i>	Flower-bud	<i>Actinidia polygama</i> / Ino, Japan	AB085873 <sup>b</sup>
<i>Pseudasphondylia</i> sp.	Fruit	<i>Actinidia rufa</i> / Nobeoka, Japan	<b>LC710538</b>
<i>Pseudasphondylia</i> sp.	Flower bud/Fruit	<i>Actinidia rufa</i> / Mt. Yangming, Taiwan	<b>LC710532-35</b>
<i>P. kiritanii</i>	Flower-bud	<i>Cornus controversa</i> / Futo, Japan	LC538356 <sup>c</sup>
<i>P. tominagai</i>	Flower-bud	<i>Eleutherococcus spinosus</i> / Misugi, Japan	LC621302 <sup>a</sup>
<i>P. rokuharensis</i>	Fruit	<i>Viburnum dilatatum</i> / Hanayama, Japan	LC538357 <sup>c</sup>
Outgroups			
<i>Bruggmanniella litseae</i>	Leaf	<i>Litsea acuminata</i> / SanYi, Taiwan	LC475501 <sup>d</sup>
<i>Illiciomyia yukawai</i>	Leaf	<i>Illicium anisatum</i> / Mt. Hikosan, Japan	LC348719 <sup>e</sup>

\*Sequence information of “a”, “b”, “c”, “d”, and “e” refers to Matsuda et al. (2021), Yukawa et al. (2003), Lin et al. (2020b), Lin et al. (2020a), and Uechi et al. (2018), respectively; bold text refer to present study.

bins = 20.

### Phylogenetic placement and divergence time

Phylogenetic clustering was performed using the Maximum Likelihood (ML) method with IQTREE online version (Trifinopoulos et al. 2016; webpage: <http://iqtree.cibiv.univie.ac.at/>) and Bayesian inference (BI) via MrBayes 3.2 (Ronquist et al. 2012). Sequences of other four allied *Pseudasphondylia* species and one outgroup species *Illiciomyia yukawai* Tokuda (Table 1) were added to infer phylogenetic placement of Taiwanese species/taxa. For ML inference, the supported score caudated by 1000 ultrafast bootstrap (Minh et al. 2013) and 1000 bootstrap (Felsenstein 1985) replicates. As for BI, the best-fit substitution model GTR+G was determined via jModelTest 2.1.10 (Darriba et al. 2012) using the Bayesian Information Criterion (BIC). Markov chain Monte Carlo (MCMC) chain length was  $1 \times 10^6$  generations with sampling every 1000 generations with the first 25% of steps discarded as burn-in. Posterior probabilities (PP) were summarized on a maximum clade credibility tree. The phylogenetic tree was presented and edited by FigTree 1.4.3 (Rambaut 2009).

The divergence time of *Pseudasphondylia* on *E. sylvestris* and that on *Actinidia* were separately evaluated according to Brower (1994) as 2.3% of *COI* genetic distance per million years.

## RESULTS

### TAXONOMY

#### Order Diptera Linnaeus, 1758

#### Family Cecidomyiidae Newman, 1834

#### Tribe Asphondyliini Enderlein, 1914

#### Genus *Pseudasphondylia* Monzen, 1955

*Pseudasphondylia* Monzen 1955: 41. Type species: *P. rokuharensis* Monzen, 1955.

*Philadelphella* Kovalev 1964: 440. Type species. *P. philadelphia* Kovalev, 1964.

The genus is characterized as follows: the mediobasal lobes of the gonostyli is present and unsclerotized; two separate teeth of gonostylus on male terminalia; the presence of elongate spiracles on the pupal abdomen; and the absence of setae on the larval cervical papillae. General descriptions of the genus are shown in Tokuda and Yukawa (2005) and key to males of known species in Elsayed et al. (2019) and Matsuda

et al. (2021). In this paper, one new species is described and one known species is recorded for the first time in Taiwan.

#### *Pseudasphondylia kiwiphila* sp. nov. Lin, Tokuda & Yang

(Figs. 2–3; Table 2)

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*Type material: Holotype:* Male (on a slide, TFRI). TAIWAN: Taipei City, Erziping trail, 11.x.2014, leg. HL Lee. *Paratypes.* TAIWAN: 3 ♂, 4 ♀, 8 exuviae (on slides, TFRI), same data as holotype; 5 exuviae (in ethanol, NCHU), same data as holotype; 1 pupa (in ethanol, TFRI), 1 larva (on a slide, TFRI), Taipei City, Erziping trail, 1.ix.2017, leg. CT Hsu; 9 larvae (8 on slides, 1 in ethanol, TFRI) Taipei City, Mt. Yangming (GPS: N25.1717072, E121.5403027), 19.viii.2018, leg. J Liu.

*Distribution:* Taiwan and Japan.

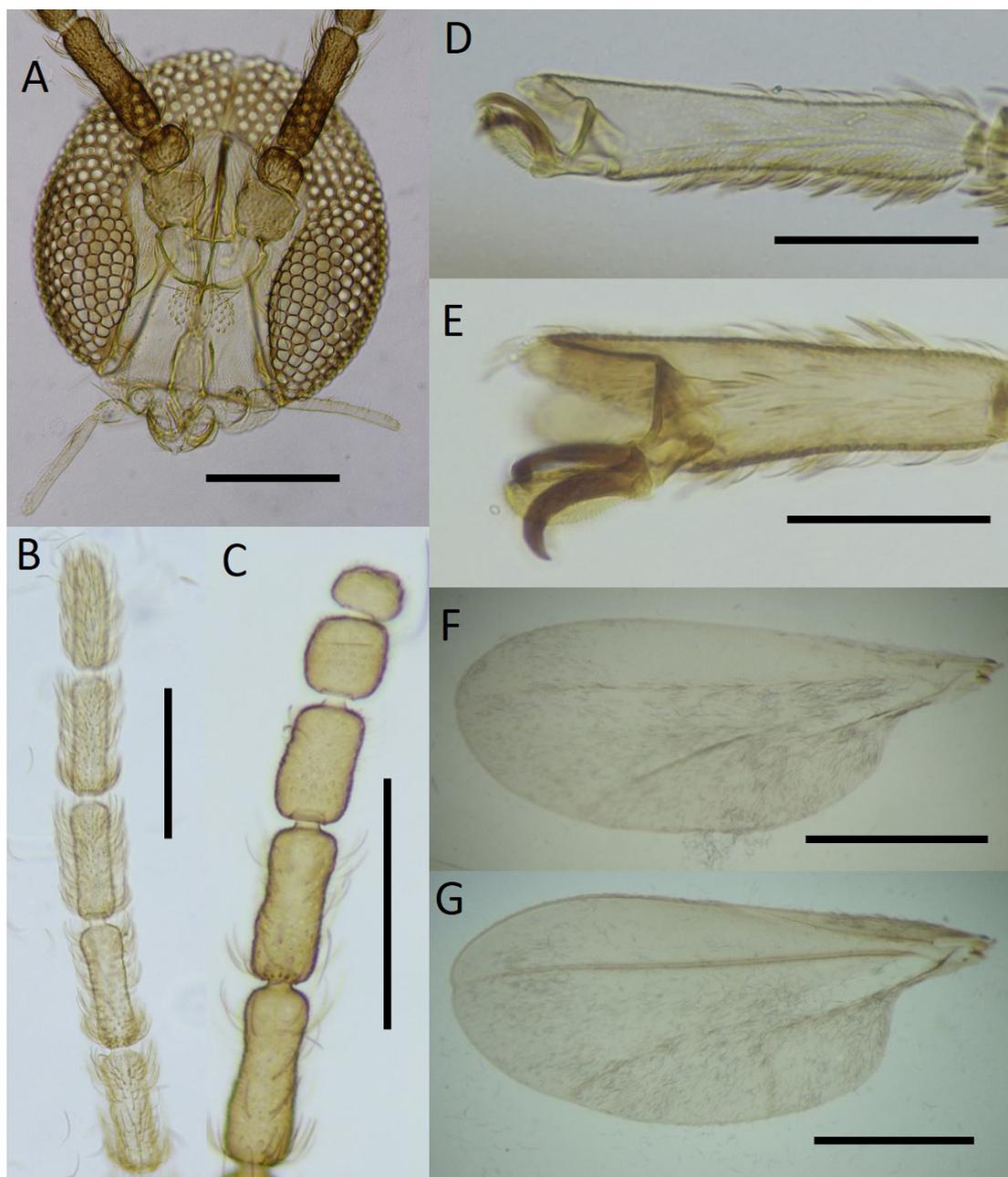
*Etymology:* The species name refers to its host which is derived from the common name of the fruit “kiwi” and favorite in Greek “phila”.

*Host:* *Actinidia rufa* (Siebold & Zucc.) Planch. ex Miq.

*Gall:* One or several chambers in each fruit gall, gall size 9–10 mm in diameter; smaller than normal fruit (Tung et al. 2018). Early stages of gall development occur at host flowering season in April to May. Galls continuously develop and mature in July or August. Galled fruit falls to the ground in October or November.

*Life history:* According to Tung et al. (2018), females lay their eggs on flowers of *A. rufa* during April and May, and larvae mature in the fruit galls in August and September. They pupate during September to October, and adults emerge from September to October. The life cycle of the new species is similar to the Japanese congener *P. matatabi*, which also has one generation on *Actinidia* from spring to autumn. The remaining parts of the life history are still unclear, but the gall midge possibly alternates host plant(s) to overwinter as has been suggested for *P. matatabi* and another congener *P. kiritanii* in Japan (Tokuda and Yukawa 2005). Some immature larvae of the new species, possibly diapausing, were found in fallen galls in winter.

*Adult: Head.* Eye bridge five to six facets long. Frontoclypeal setal counts as in table 2. Palpus three-segmented (Fig. 2A), first palpal segment 35–50  $\mu$ m long, same as width; second 1.3 $\times$  as long as first; third 3.7 $\times$  as long as first. Scape and pedicel with rather dense setae and scales; male first flagellomere ca. 200  $\mu$ m long, 4.0 $\times$  as long as wide and 1.1 $\times$  as long as second,



**Fig. 2.** *Pseudasphondylia kiwiphila* sp. nov. (A) Male head (ventral view) (B) Male antenna (8–12 segment). (C) Female antenna (8–12 segment). (D) Male 5th tarsomere. (E) Female 5th tarsomere. (F) Male wing. (G) Female wing. Scale bars: A–C = 0.02 mm; D–F = 0.1 mm; G–H = 1 mm.

**Table 2.** Frontoclypeal and thoracic setal of *Pseudasphondylia kiwiphila* sp. nov.

Specimens	Male			Female		
	<i>n</i>	Mean ± SD	Range	<i>n</i>	Mean ± SD	Range
Frontoclypeal setae	4	41.8 ± 13.0	27–55	4	36.0 ± 4.5	30–41
Anterior dorsolateral setae	4	75.3 ± 8.7	64–83	4	70.5 ± 7.0	66–81
Posterior dorsolateral setae	3	81.3 ± 21.2	57–96	3	80.0 ± 1.7	79–82
Mesopleural scales	4	44.8 ± 6.7	37–53	4	41.5 ± 8.9	30–49
Mesepimeral setae	4	56.3 ± 2.9	52–58	4	43.5 ± 8.4	35–55

1.6× as long as fifth, distal three segments similar length (Fig. 2B); female first flagellomere ca. 230  $\mu\text{m}$ , 1.4× as long as second and fifth, distal three flagellomeres gradually shortened and terminal one subglobular (Fig. 2C).

**Thorax:** Thoracic setal and scale counts as in table 2. All legs covered with many blackish scales, first tarsomeres of all legs with apical spur. Claws simple and strongly curved on all legs; pulvilli shorter than claws; empodia slightly longer than claw (Fig. 2D–E). Male wing 2.6–3.1 mm long, 2.2× as wide; female wing 3.0–3.5 mm long, 2.2× as long as wide; densely covered with dark grayish hairs; R5 joining costa a little beyond wing apex (Fig. 2F–G).

**Male Abdomen:** First through seventh tergites rectangular with three to six rows of posterior setae; elsewhere covered with scales on first through seventh tergites; eighth tergite without setae.

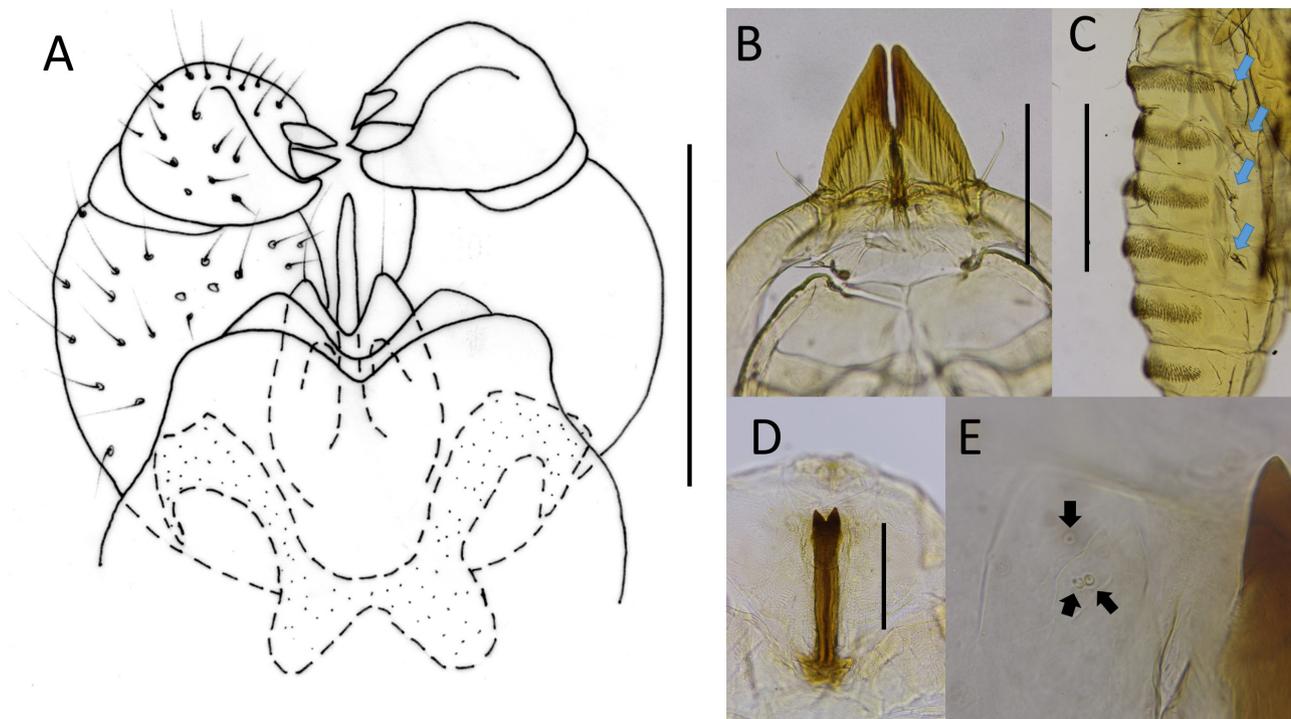
**Terminalia** (Fig. 3A): cerci incised deeply by U-shaped emargination, apex setose; hypoproct incised deeply by V-shaped emargination, each lobe with one apical seta; gonostylus suboval, situated posteriorly to gonocoxite; mediobasal lobe present, shorter than cerci and hypoproct; aedeagus laterally sclerotized, distally tapering.

**Female abdomen:** Ovipositor protractile, slender, aciculate, basally with a bilobed cerci-like structure; needle part 1.6 mm long, 2.8× as long as the length of

seventh sternite. Otherwise as in male.

**Pupa:** Body length ca. 3.2 mm, pupal skin not pigmented except for antennal horns. Antennal horn 320–420  $\mu\text{m}$  long, triangular, lateral smoothly (Fig. 3B); cephalic papilla with seta, 90–110  $\mu\text{m}$  long; frons without horns; a pair of lateral facial papillae on each side; prothoracic horn 380–530  $\mu\text{m}$  long; stigmatal tubercles present on second to fifth abdominal segments (Fig. 3C), 140–240  $\mu\text{m}$  long; second to seventh abdominal segments with 5 to 6 and eight abdominal segments with 3 to 5 transverse rows of spines, respectively; 6 dorsal papillae on first to seventh abdominal segments, outer and inner pairs, each with seta; 2 dorsal papillae on eighth abdominal segment, each with seta; each abdominal segment with pleural papilla, each with seta.

**Mature larva:** Body length ca. 2.9–3.5 mm, body color in life yellow; Second antennal segment short; cervical papillae without seta; sternal spatula 480–520  $\mu\text{m}$  long (Fig. 3D) anteriorly with two lobes; 2 sternal papillae present on all thoracic and first to seventh abdominal segments, each with seta; 3 lateral papillae (Fig. 3E) present on each side of all thoracic segments, inner two with seta; 2 pleural papillae present on each side of thoracic and abdominal segments, each with seta; 4 ventral papillae on first to seventh abdominal segments and 2 ventral papillae on eighth abdominal segment, each with seta; 4 dorsal papillae on



**Fig. 3.** *Pseudasphondylia kiwiphila* sp. nov. (A) Male genitalia. (B) Pupal antennal horn. (C) Pupal abdomen; arrow refer to spiracle tube. (D) Larval sternal spatula. (E) larval lateral papillae, arrow refers to lateral papillae. Scale bars: A = 0.1 mm; B and D = 0.3 mm; C = 1.0 mm.

each side of all thoracic and first to seventh abdominal segments, 2 dorsal papillae on eighth abdominal segment, each with seta; 8 terminal papillae present, two of them each with large seta, four of them each with minute seta, and remaining two without setae.

**Remarks:** The new species is morphologically similar to Japanese *P. matatabi*, but distinguishable by having the ovipositor (needle part: 1.6 mm) and palpal segments (35–50, 55–70, and 140–180  $\mu\text{m}$ ) that are longer than those of *P. matatabi* (needle part: 1.4 mm; palpal segments: 25, 45–62.5, and 76.5–130  $\mu\text{m}$ ) (Tokuda and Yukawa 2005). In addition, the mediobasal lobe of gonostyli in male is shorter than cerci in the new species but longer in *P. matatabi*.

***Pseudasphondylia elaeocarpi* Tokuda & Yukawa**  
(Fig. 4)

**Diagnosis:** Adult palpus three-segmented, first to sixth tergites with a single row of posterior setae, hypoproct basally rather wide and extremely narrowing at apex. Pupal antennal horn triangular, well developed (Fig. 4A). Pupal stigmatal tubercles present on second to fourth abdominal segments (Fig. 4B), reduced on fifth to eighth abdominal segments. Larval sternal spatula with four lobes and the inner pairs longer than the outer (Fig. 4C).

**Material examined:** *Type materials:* JAPAN: ♂ (on slide, Holotype, ELKU), Shiroyama, Kagoshima, Kyushu, galls collected 1.v.1976 and adult emerged on 2.v.1976., leg. J Yukawa; 10 ♂, 6 ♀, 7 Larvae (on

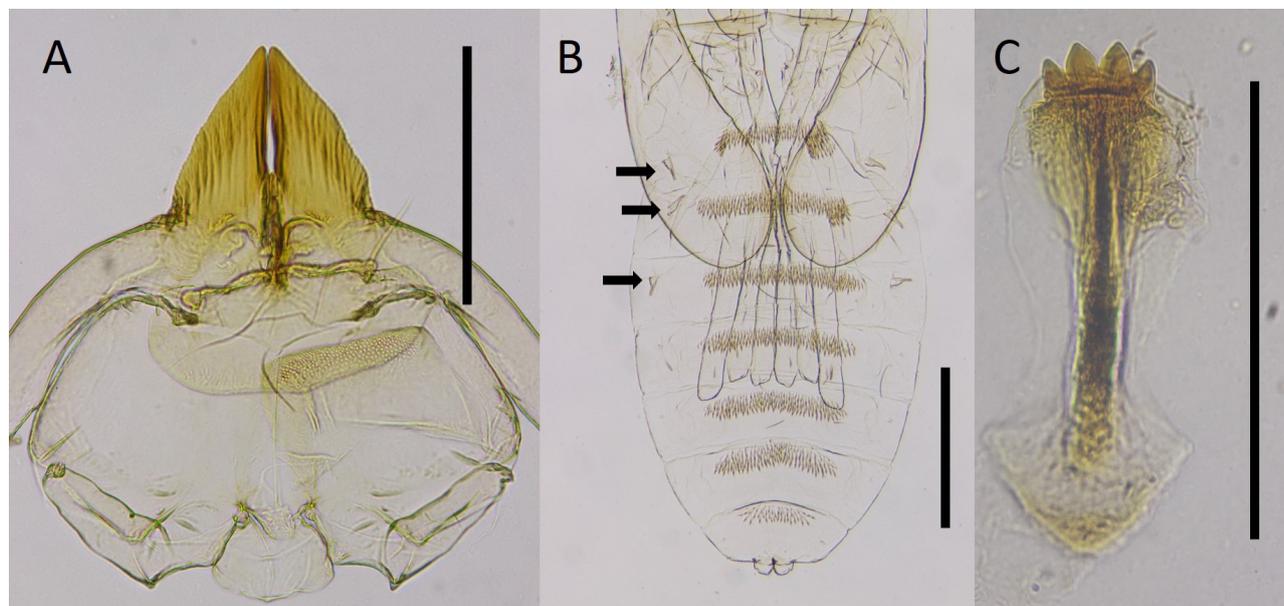
slides, Paratypes, ELKU), same data as holotype; 3 ♂ (on slides), Shiroyama, Kagoshima, Kyushu, 15.iv.1971, J Yukawa; 2 ♀ (on slides) Shiroyama, Kagoshima, Kyushu, galls collected on 22.iv.1974 and adults emerged 26.iv.1974, leg. K Takahashi; 2 ♂, 5 ♀, 2 Pupae (on slides) Kekura, Kagoshima, Kyushu, galls collected on 29.iv.1977 and adults emerged 4–11.v.1977, leg. T Sunose; 1 ♂, 1 ♀, 2 Pupae (on slides), Yona, Kunigami, Okinawa Island, 24.ii.2001, leg. M Tokuda and J Yukawa.

**Other materials examined:** TAIWAN: [Taichung City] 2 ♂, 2 ♀, 6 Pupae (on slides, NCHU), Mt. Tung-mao, galls collected on 25.iv.2017 and adults emerged on 3.v.2017, leg. SF Lin; 2 ♂, 7 ♀ (in EtOH, NCHU), Mt. Tung-mao, galls collected on 25.iv.2017 and adults emerged on 8–11.v.2017, SF Lin; [Pingtung Co.] 10 ♂, 7 ♀, (in EtOH, NCHU), Dahan forest rd., galls collected on 4.v.2017 and adults emerged on 8–25.v.2017, leg. YM Chao and SF Lin.

**Distribution:** JAPAN: Honshu, Kyushu, Tanegashima, Nakanoshima, Amamiyoshima, and Okinawajima (Tokuda and Yukawa 2005; Yukawa et al. 2013; Tokuda 2018); and TAIWAN (New distribution records, see Table 3).

**Genetic distance and species delimitation**

Sequences of five *P. kiwiphila* sp. nov. (one Japanese and four Taiwanese individuals) and two Taiwanese *P. elaeocarpi* individuals were obtained for a partial *COI* gene (658–689 bp). Species delimitation



**Fig. 4.** *Pseudasphondylia elaeocarpi* (A) Pupal face and antennal horns. (B) Pupal abdomen, arrows indicate stigmatal tubercles. (C) Larval sternal spatula. Scale bars: A and C = 0.3 mm; B = 0.5 mm.

of ABGD showed three presumptive species (Fig. 5) corresponding to our morphological examinations: (1) *P. matatabi*: one Japanese sequence; (2) *P. elaeocarpi*: one Japanese and two Taiwanese sequences; and (3) *P. kiwiphila* sp. nov.: one Japanese and four Taiwanese sequences.

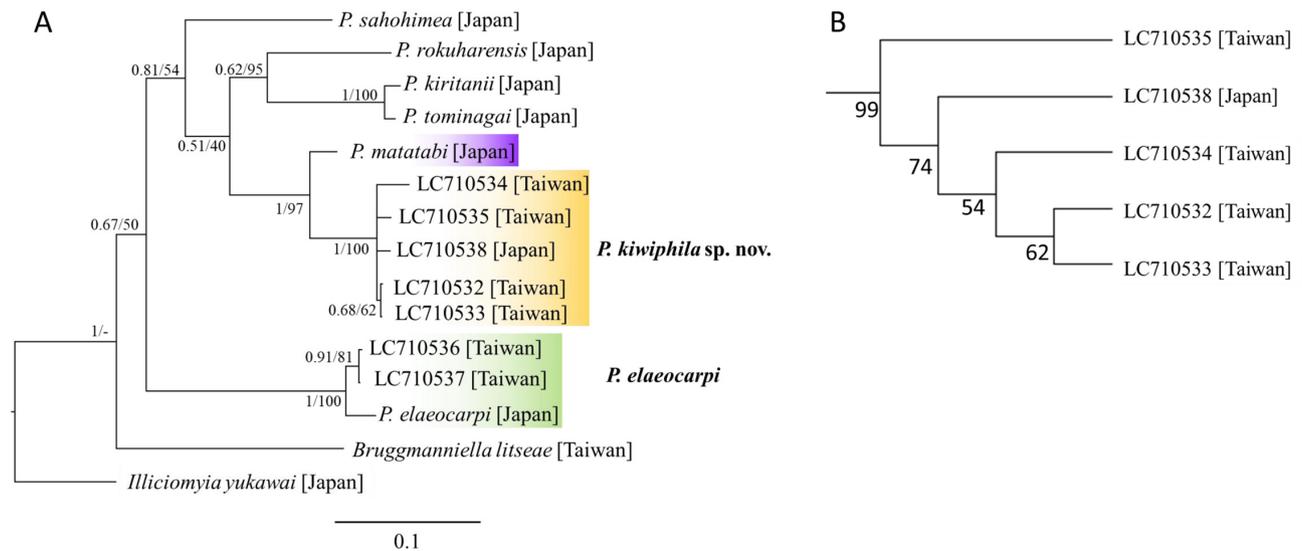
The genetic distance of *P. elaeocarpi* is 0.1% within Taiwanese individuals and 2.7% between Taiwanese and Japanese populations. As to *Actinidia*-associated species, the genetic distance is 0–2.6% among *P. kiwiphila* sp. nov. and 5.1–6.7% between *P. kiwiphila* sp. nov. and Japanese *P. matatabi*.

**Phylogeny and divergence time**

The phylogenetic trees constructed by Bayesian

inference (Fig. 5A) and by Maximum likelihood shared a similar topology except for the phylogenetic relationship among individuals of *P. kiwiphila* sp. nov., which was resolved in the IQ tree (Fig. 5B) but not the BI tree. Leaf galls of *Pseudasphondylia*, *P. elaeocarpi*, and *P. saohimea*, situated at the stem part of the tree and formed a paraphyletic group, whereas *Pseudasphondylia* species including all flower-bud and fruit galls formed a monophyletic clade (PP: 0.51 and ML: 40%). This clade is further divided into *Actinidia*-associated species, *P. kiwiphila* sp. nov. and *P. matatabi*, (PP: 1 and ML: 97%) and Japanese species on other hosts (PP: 0.62 and ML: 95%).

In *Actinidia*-associated species, the divergence time for the two species was estimated to be 2.2–2.9 mya (*COI* genetic distance: 5.1–6.7%). At the



**Fig. 5.** Phylogenetic tree of *Pseudasphondylia* species based on DNA partial *COI* region. (A) Bayesian tree of *Pseudasphondylia* species, supported values are shown beside nodes with the Bayesian posterior probability (left) and ultrafast bootstrap of the IQ tree (right). Taxa with different colors refer to distinct species based on summarized results of species delimitation (Automatic Barcode Gap Discovery analysis). (B) Cladogram of *Pseudasphondylia kiwiphila* sp. nov. lineage in IQ tree.

**Table 3.** Distribution records of *Pseudasphondylia elaeocarpi* in Taiwan

Locality	Gall shape	Collector	Collected date
North Taiwan			
Dalu forest Rd., Hsinchu County	Subconical	GS Tung, MM Yang	1996-II-9
Central Taiwan	Subconical		
Lianhuachi Research Center, Nantou County	Subconical	MM Yang	1999-IX-13
Zhonghe Vil., Taichung City	Subconical	MF Lou, MM Yang	2001-VI-1
Mt. Tung-mao, Taichung City	Subconical	SF Lin	2017-IV-5
South Taiwan	Subconical		
Shan-Ping Forest Ecological Garden, Kaohsiung City	Subconical	MF Lou	2005-VII-5
Dahan forest Rd., Kaohsiung City	Subconical	SF Lin	2014-XII-28
Dahan forest Rd., Kaohsiung City	Subconical	SF Lin	2016-III-8
Dahan forest Rd., Kaohsiung City	Subconical	YM Chao, SF Lin	2017-V-4

interspecific level, Taiwanese individuals of *P. kiwiphila* sp. nov. were paraphyletic to the Japanese individual. Taiwanese taxa of *P. elaeocarpi* was reconfirmed as a sister group of Japanese *P. elaeocarpi* (PP: 1 and ML: 100%), and their divergence time was estimated to be around 1.2 mya (*COI* genetic distance: 2.7%).

## DISCUSSION

*Pseudasphondylia* has a wide host spectrum even though the genus contains only 13 species (Gagné and Jaschhof 2021; Matsuda et al. 2021). Some species are univoltine and do not exhibit host alternation while others are multivoltine and are supposed to be host-alternating species (Tokuda and Yukawa 2005; Yukawa and Uechi 2021). The evolutionary scheme of host association is poorly known in *Pseudasphondylia*, although some taxonomic works have been performed recently (Elsayed et al. 2019; Lin et al. 2020b; Matsuda et al. 2021). In the phylogenetic analysis (Fig. 5), the leaf galls *P. elaeocarpi* and *P. saohimeae* were situated at more ancestral positions in the genus. This indicates that leaf galling is an ancestral habit, whereas fruit and flower-bud gallings are derived ones. Meanwhile, univoltine life history may also be ancestral in *Pseudasphondylia* in contrast to a multivoltine life history, because the leaf galls of *Pseudasphondylia* are univoltine whereas fruit and flower-bud gallings are multivoltine. From the viewpoint of speciation within *Pseudasphondylia*, it also provides a system to investigate how the gall midges have speciated accompanied by the transition of multiple ecological features, such as voltinism, galling organ, and host plants. Further ecological and molecular data of the remaining *Pseudasphondylia* species will facilitate further studies of the genus.

### **Actinidia-associated *Pseudasphondylia* species**

Host spectrum, ecology, and distribution of gall midges provide us valuable information to solve the biogeography and speciation processes of other species groups, such as *Ilex*-associated *Schizomyia* and *Cinnamomum*-associated *Bruggmanniella* (Tokuda et al. 2004; Lin et al. 2020b). In *Actinidia*-associated *Pseudasphondylia* species, *P. matatabi* occurs only in mainland Japan, whereas *P. kiwiphila* sp. nov. occurs in Taiwan and Kyushu. Both species have not yet been reported from the Ryukyu Islands (Tokuda and Yukawa 2005; Yukawa 2021), but host distribution and our findings provide a favorable opportunity to infer their biogeography.

Because *A. rufa* is also distributed on the Ryukyu

Islands between Taiwan and Kyushu, further intensive field surveys may find *P. kiwiphila* sp. nov. there. Taiwanese individuals became a paraphyletic group to the Japanese individual (Fig. 5) and the genetic distance was short between the two populations. These suggest that *P. kiwiphila* sp. nov. has a southern origin and its northward expansion through the Ryukyu Islands. In contrast, *P. matatabi* has been reported from Honshu, Shikoku, and Kyushu (Yuasa and Kumazawa 1938; Yukawa 1971; Tokuda and Yukawa 2005). However, its host *A. polygama* is not distributed on the Ryukyu Islands and Taiwan, suggesting that *P. matatabi* does not occur in southern parts of East Asia.

Morphological, phylogenetic, and ecological similarities between *P. kiwiphila* sp. nov. and Japanese *P. matatabi* demonstrated in the present study indicate that they are sibling species. Based on the phylogenetic analysis, *P. kiwiphila* sp. nov. has a southern origin while *P. matatabi* currently occurs only in mainland Japan. This supports the allopatric speciation scenario between the two species via geographical isolation. However, the two species still may have speciated sympatrically via ecological divergence, and then respectively dispersed along with their host species. The following ecological features will support the sympatric speciation scenario: the hosts of these two species belong to different groups of *Actinidia*, i.e., *A. polygama* belongs to the smooth-skinned fruit species group that occurs in relatively cool or high-altitudinal areas including northern China, Korea, and mainland Japan, whereas *A. rufa* belongs to hairy and/or spotted (lenticillate) fruit species groups that occurs in relatively warm and moist environments such as southern China and Taiwan (Huang 2014; Huang and Liu 2014; Liu et al. 2017). This implies that a host shift may have occurred in the common ancestor of *Actinidia*-associated *Pseudasphondylia*, leading to ecological speciation. To confirm the speciation process, further taxonomic and phylogenetic studies of *Actinidia*-associated *Pseudasphondylia* are needed, such as unidentified *Pseudasphondylia* species associated with fruit of *Actinidia valvata* Dun in China (Liu and Larsson 1996) and *Actinidia callosa* var. *discolor* and *A. chinensis* var. *setosa* in Taiwan (Tung et al. 2018).

### **Divergence and ecology of *P. elaeocarpi***

The discovery of *P. elaeocarpi* in Taiwan presents new information on the species distribution range extending beyond the previously reported place (Tokuda and Yukawa 2005), which also provides another study system in this region. The estimated divergence time (around 1.2 mya) between Taiwanese and Japanese populations corresponds to a later period of separation

between Taiwan and Japan in the late Pliocene (Kimura 1996). Further studies may also find *P. elaeocarpi* in mainland China and other areas because its host plant is commonly distributed in East Asia (Lu et al. 2000), and *P. elaeocarpi* represents a wide distribution range from the south (Taiwan) to north (Japan) in the East Asian Island Arc (Tokuda and Yukawa 2005, present study). In Japan, adults of *P. elaeocarpi* emerge in early May to mid-June and the larvae inside the galls pass through the summer, autumn, and winter as first instars. Then they reach third instars by early April and pupate by mid-April (Yukawa and Masuda 1996; Tokuda and Yukawa 2005). In Taiwanese populations of *P. elaeocarpi*, the larvae can be found until March and they pupate in March and April (Tung et al. 2018). Including the timing of adult emergence in Taiwan in late April to May, the whole life cycle of the Taiwanese population is similar to that of Japanese populations, but the development is slightly earlier than the latter. In addition, *P. elaeocarpi* induces dimorphic galls in Japan, one being subconical light green and the other hemispherical greenish-brown (Yukawa and Masuda 1996; Tokuda and Yukawa 2005). However, galls are usually subconical in Taiwan. Hemispherical galls are rarely found, and none have been found to contain live gall midge larvae. Gall dimorphism of *P. elaeocarpi* might not occur in Taiwan. Further intensive genetic and ecological studies will answer this question.

### Biogeography of Taiwanese Cecidomyiids

Insects distributed in the East Asian Island Arc are biogeographically interesting study subjects because the biodiversity in this region has been created by the complex geological history of repeated separations and connections within islands and between the Eurasian continent (Tojo et al. 2017). Generally, most of the insect species in the Ryukyu Islands diverged from Taiwanese or southern Chinese congeners (Tojo et al. 2017), such as *Repidolestis* spp. (Odonata: Megapodagrionidae) (Tojo and Itoh 2015) and *Polyura* butterfly species group (Lepidoptera: Nymphalidae) (Toussaint and Balke 2016). Nevertheless, the dispersal direction of insect species is not always from the south (Taiwan) to north (mainland Japan) through the Ryukyu Islands. Yukawa et al. (2012) indicated that the northern Taiwan and South-Ryukyu populations of *Pitydiplosis puerariae* Yukawa, Ikenaga & Sato (Diptera: Cecidomyiidae) are sister to the Central Taiwanese population and populations of North-Ryukyu, mainland China, the Korean Peninsula, and mainland Japan. In addition, a distributional gap of *P. puerariae* was detected in southern parts of Mid-Ryukyu. It suggests that *P. puerariae* has an alternative dispersal pathway

reaching mainland Japan through China and Korea rather than the Ryukyu Islands, as in the case of migratory locust *Locusta migratoria* L. (Orthoptera: Acrididae) (Tokuda et al. 2010).

Recently, Yukawa (2021) mentioned that 12 unidentified species of Taiwanese cecidomyiids shown in Tung et al. (2018) may be identical or closely related to Japanese species. In the present study, two of them were identified as *P. kiwiphila* sp. nov. and *P. elaeocarpi*. Our present findings together with previous studies highlight that Taiwanese cecidomyiids, such as the genera *Bruggmanniella*, *Daphnephila*, and *Pitydiplosis* (Tokuda and Yukawa 2007; Tokuda et al. 2008; Yukawa et al. 2012; Pan et al. 2015; Lin et al. 2020b; Yukawa 2021), have high affinities to Japanese species, and imply that their divergence may have occurred following the separation of mainland China and the East Asian Island Arc. To confirm this viewpoint, further intensive surveys are needed to clarify gall midge fauna in mainland China, where little is known about the gall midges belonging to these genera.

### CONCLUSIONS

In this study, the new species *P. kiwiphila* sp. nov. inducing fruit galls on *A. rufa* was described from Taiwan and Japan and one known species *P. elaeocarpi* was newly recorded from Taiwan. Species identities of the two Taiwanese *Pseudasphondylia* species were confirmed based on molecular (partial *COI* region) and morphological evidence. Based on the molecular phylogenetic analyses, the leaf-galling habit and univoltinism were regarded as ancestral states in *Pseudasphondylia*, whereas the fruit- or flower-bud-galling habitat and multivoltinism were derived ones. The biogeography and ecology of these two gall midge species highlight their possible divergence and dispersal pathways. The current distribution of *Actinidia*-associated *Pseudasphondylia* suggests their allopatric speciation, but based on genetic and host distribution data, there is still a possibility that they have sympatrically speciated via ecological isolation. As to *P. elaeocarpi*, we revealed that this species is widely distributed and Taiwan is currently located in its southmost distributional range. The presence of gall dimorphism and its fitness consequences in Taiwanese *P. elaeocarpi* is a future question to be solved.

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

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## 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.
- Coutin R. 1980. *Pseudasphondylia rauwolfiae*, nov. sp. Cécidomyie des fleurs de *Rauwolfia schumanniana* (Schl.) Boiteau, en Nouvelle Calédonie. *Annales de la Société Entomologique de France (N.S.)* **16**:501–508.
- 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.
- Elsayed AK, Yukawa J, Tokuda M. 2019. *Pseudasphondylia tominagai*, a new gall midge species (Diptera: Cecidomyiidae) inducing flower bud galls on *Eleutherococcus spinosus* (Araliaceae) in Japan. *Biodivers Data J* **7**:e35673. doi:10.3897/BDJ.7.e35673.
- Enderlein G. 1914. Ord. Diptera. Fliegen (Zweiflügler). In: Brohmer P (ed) *Fauna von Deutschland. Ein Bestimmungsbuch unserer heimischen Tierwelt*. Quelle & Meyer, Leipzig, pp. 272–334.
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**:783–791. doi:10.1111/j.1558-5646.1985.tb00420.x.
- Folmer O, Black M, Hoew H, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol Marine Biol Biotechnol* **3**:294–299.
- Gagné RJ. 1994. The gall midges of the Neotropical region. Cornell University Press, Ithaca, NY, USA.
- Gagné RJ, Jaschhof M. 2021. A catalog of the Cecidomyiidae (Diptera) of the world. 5th Edition. Digital, 813 pp. Available at: [https://www.ars.usda.gov/ARSUserFiles/80420580/Gagne\\_Jaschhof\\_2021\\_World\\_Cat\\_5th\\_Ed.pdf](https://www.ars.usda.gov/ARSUserFiles/80420580/Gagne_Jaschhof_2021_World_Cat_5th_Ed.pdf). Accessed 18 Apr. 2021.
- 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.
- Huang H. 2014. The genus *Actinidia*, a world monograph. Science Press, Beijing, China.
- Huang H, Liu Y. 2014. Natural hybridization, introgression breeding, and cultivar improvement in the genus *Actinidia*. *Tree Genet Genomes* **10**:1113–1122. doi:10.1007/s11295-014-0771-8.
- Kovalev OV. 1964. Review on gall-midges (Diptera, Itonididae) from the extreme south of far east. 1. The supertribe Asphondyliidi. *Entomol Obozr* **43**:418–446. English translation in *Entomol Rev* **43**:215–228.
- 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, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol* **35**:1547–1549. doi:10.1093/molbev/msy096.
- Liang LM, Yang SY, Yang JT, Chen MY. 1999. Development of the insect-induced galls of *Neolitsea aciculata* var. *variabilissima* and *Litsea acuminata* in Guandaushi forest ecosystem. *Quarterly Journal of Forest Research* **21**:75–89. (in Chinese with English abstract)
- Lin SF, Tokuda M, Yang MM. 2020a. Leaf gall polymorphism and molecular phylogeny of a new *Bruggmanniella* species (Diptera: Cecidomyiidae: Asphondyliini) associated with *Litsea acuminata* (Lauraceae) in Taiwan, with ecological comparisons and a species description. *Entomol Sci* **23**:10–22. doi:10.1111/ens.12391.
- Lin SF, Yang MM, Tokuda M. 2020b. 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. *Zool Stud* **59**:66. doi:10.6620/ZS.2020.59-66.
- Linnaeus C. 1758. *Systema Naturae per Regna Tria Naturae*. Edition 10, vol. I. Stockholm.
- Liu Y, Li D, Zhang Q, Song C, Zhong C, Zhang X, Wang Y, Yao X, Wang Z, Zeng S, Wang Y, Guo Y, Wang S, Li X, Li L, Liu C, McCann HC, He W, Niu Y, Chen M, Du L, Gong J, Datson PM, Hilario E, Huang H. 2017. Rapid radiations of both kiwifruit hybrid lineages and their parents shed light on a two-layer mode of species diversification. *New Phytol* **215**(2):877–890. doi:10.1111/nph.14607.
- Liu Z, Larsson S. 1996. The relationship between a flower gall midge *Pseudasphondylia* sp. (Diptera: Cecidomyiidae) and its host plant *Actinidia valvata*. *Entomol Sin (= Insect Sci)* **3**:271–282.
- 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.
- Mani MS. 1954. Entomological survey of the Himalayas. 1. Introduction, description of gall midges (Itonididae = Cecidomyiidae: Diptera) and plant galls from the western

- Himalayas. *Agra Univ J Res (Sci)* **3**:13–42.
- Matsuda H, Elsayed AK, Kim W, Yamauchi S, Libra M, Kamata N, Yukawa J, Tokuda M. 2021. A new species of *Pseudasphondylia* (Diptera: Cecidomyiidae) associated with *Magnolia kobus* DC. var. *borealis* Sarg. (Magnoliaceae) in Japan. *Biodivers Data J* **9**:e68016. doi:10.3897/BDJ.9.e68016.
- Minh BQ, Nguyen MAT, von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Mol Biol Evol* **30**:1188–1195. doi:10.1093/molbev/mst024.
- Mo T, Xu Z. 1999. A new record genus and a new species of Cecidomyiidae (Diptera) from China. *Entomotaxonomia* **21**:36–38. (in Chinese with English abstract)
- Mo T, Bu W, Li Q. 2007. A new species of the genus *Pseudasphondylia* from China (Diptera, Cecidomyiidae). *Acta Zootaxonomica Sinica* **32**:974–976. (in Chinese with English abstract)
- Monzen K. 1955. Some Japanese gallmidges with the descriptions of known and new genera and species (II). Annual report of the Gakugei Faculty of the Iwate University **9**:34–48.
- Nagai A. 2016. Insect galls of Miyazaki prefecture [VII]. *Creatures of Nishimoro* **9**:26–49.
- Newman E. 1834. Attempted division of British insects into natural orders. *Entomol Magazine* **2**:379–431.
- 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.
- Puillandre N, Lambert A, Brouillet S, Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol Ecol* **21**:1864–1877. doi:10.1111/j.1365-294X.2011.05239.x.
- Rambaut R. 2009. FigTree v1.4.3. Available at: <http://tree.bio.ed.ac.uk/software/figtree/>. (Accessed 16 Sept. 2021)
- 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.
- 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.
- Tojo K, Itoh T. 2015. The establishment patterns of the insect fauna of Japan, in comparison to its geological history. In: Oba Y, Osawa S (eds) *The Wonderful and Marvelous World of Insects from the Perspective of Genetic Analyses*. Tokyo: Yushokan, pp. 105–150.
- Tojo K, Sekiné K, Takenaka M, Isaka Y, Komaki S, Suzuki T, Schoville SD. 2017. Species diversity of insects in Japan: Their origins and diversification processes. *Entomol Sci* **20**:357–381. doi:10.1111/ens.12261.
- 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. 2018. Arthropod galls found in northern parts of the Tokara Islands, Japan. *Jpn 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, Tanaka S, Zhu DH. 2010. Multiple origins of *Locusta migratoria* (Orthoptera: Acrididae) in the Japanese Archipelago and the presence of two major clades in the world: evidence from a molecular approach. *Biol J Linn Soc* **99**:570–581. doi:10.1111/j.1095-8312.2010.01386.x.
- 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:TNAT KJ]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.
- Toussaint EFA, Balke M. 2016. Historical biogeography of *Polyura* butterflies in the oriental Palaeotropics: trans-archipelagic routes and South Pacific island hopping. *J Biogeogr* **43**:1560–1572. doi:10.1111/jbi.12741.
- Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucl Acids Res* **44**(W1):W232–W235. doi:10.1093/nar/gkw256.
- 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, 247 pp.
- Uechi N, Kim W, Tokuda M, Fujii T, Kikuchi H, Kakizaki M, Iwasaki A, Paik JC, Yukawa J. 2018. Genetic and ecological differences between *Asphondylia yushimai* and the ivy gall midge, *Asphondylia* sp. (Diptera: Cecidomyiidae), with a new distribution record of the former from Hokkaido and South Korea. *Appl Entomol Zool* **53**:363–371. doi:10.1007/s13355-018-0567-7.
- Yuasa H, Kumazawa T. 1938. A new gall midge from Nippon (Diptera, Cecidomyiidae). *J Pharmacol Soc Jpn* **58**:204–206.
- Yukawa J. 1971. A revision of the Japanese gall midges (Diptera: Cecidomyiidae). *Mem Fac Agric Kagoshima Univ* **8**:1–206.
- Yukawa J. 1974. Descriptions of new Japanese gall midges (Diptera, Cecidomyiidae, Asphondyliidi) causing leaf galls on Lauraceae. *Kontyû* **42**:293–304.
- Yukawa J. 2021. Diversity of gall-inducing cecidomyiid in Japan. In: Yukawa J, Tokuda M. editors. *Biology of Gall Midges*. Entomology Monographs. Singapore: Springer, pp. 61–79. doi:10.1007/978-981-33-6534-6\_4.
- Yukawa J, Ikenaga H, Sato S, Tokuda M, Ganaha-Kikumura T, Uechi N, Matsuo K, Mishima M, Tung GS, Paik JC, Ren BQ, Dong XY. 2012. Description and ecological traits of a new species of *Pitydiplosis* (Diptera: Cecidomyiidae) that induces leaf galls on *Pueraria* (Fabaceae) in East Asia, with a possible diversification scenario of intraspecific groups. *Entomol Sci* **15**:81–98. doi:10.1111/j.1479-8298.2011.00476.x.
- Yukawa J, Masuda H. 1996. *Insect and mite galls of Japan in Colors*. Zenkoku Nōson Kyōiku Kyōkai, Tokyo: 826 pp. (in Japanese with English explanation for color plates)
- Yukawa J, Ogata K, Kaburagi K, Tokuda M. 2013. Cecidomyiid galls found on Tanegashima Island. *Satsuma* **150**:48–61.
- Yukawa J, Uechi N. 2021. Life History Traits In: Yukawa J, Tokuda M (eds) *Biology of Gall Midges*. Entomology Monographs.

Singapore: Springer, pp. 119–149. doi:10.1007/978-981-33-6534-6\_6.  
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. Bull Entomol Res 93(1):73–86. doi:10.1079/BER2002218.