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Recent Divergence of *Neolitsea*-associated *Pseudasphondylia* Gall Midges (Diptera: Cecidomyiidae) with Description of a New Species from Taiwan

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A Taiwanese *Pseudasphondylia* species inducing spherical leaf galls on six *Neolitsea* hosts, viz, *N. acuminatissima*, *N. daibuensis*, *N. konishii*, *N. parvigemma*, *N. sericea* and *N. variabillima*, was regarded as a species allied to Japanese *P. neolitseae* Yukawa due to similarity of gall structure and host. The Taiwanese species is morphologically different from *P. neolitseae* in adult palpus segmental number, shapes of pupal antennal horn, pupal prothoracic spiracle, and larval sternal spatula, resulting in its description as a new species to science, *Pseudasphondylia hooki* sp. n. The species delimitation (Assemble Species by Automatic Partitioning method) supported that *P. hooki* sp. n. is distinct from *P. neolitseae*. The sistership of *P. hooki* sp. n. and *P. neolitseae* was also supported in the Maximum likelihood tree based on the 1st codon of *COI* region. The speciation event of them is assumed to be geographical isolation because the divergence corresponded to the separation of Taiwan and Japan in the Pleistocene. The patterns of ecological features (host species) on the phylogeny revealed that galling on *N. sericea* is primitive of *Neolitsea*-associated *Pseudasphondylia* lineage. Accordingly, the divergence pathway from north to south in Taiwan is suggested by the distributions of primitive (*N. sericea*) and most derived hosts (*N. daibuensis*).

Key words: Asphondyliina, Biogeography, East Asia, Phylogeny, Speciation

BACKGROUND

Gall midges (Diptera: Cecidomyiidae) are the largest gall-inducing insect group and use host of various plant families worldwide, although some species are saprophagous, mycophagous or predaceous (Gagné and Jaschhof 2021). Some galling cecidomyiids display unique phenotypes in their gall characteristics, such as shape, galling part/position, and host plant spectrum, which are useful cues to explore the species identity comparing with known cecidomyiid congeners (Tokuda et al. 2004; Lin et al. 2020b 2022). Besides, evolution of these ecological features assists us to know the eco-speciation and biogeography of cecidomyiids, in particular to similar gall sorts occurring on related plants or in adjacent localities, as has been done in *Schizomyia* species (= *Asteralobia* syn. in Elsayed et al. 2018) on *Ilex* (Aquifoliaceae) (Tokuda et al. 2004), *Bruggmannilla* species on *Cinnamomum* (Lauraceae) (Lin et al. 2020b), and *Pseudasphondylia* species on

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Actinidia (Actinidiaceae) (Lin et al. 2022) in the East Asian Arc.

Pseudasphondylia neolitseae Yukawa, 1974 induces spherical leaf galls on *Neolitsea sericea* var. *sericea* (Lauraceae) and widely occurs in Japan (Honshu, Shikoku, Kyushu, and Southwest Islands) and southern Korea (Jeju Island) (Paik et al. 2004; Tokuda and Yukawa 2005). In Taiwan, undescribed *Pseudasphondylia* species are assumed to have an allied relationship with *P. neolitseae* due to the similar gall features and host affinity. Namely, the spherical leaf galls induced by unclarified *Pseudasphondylia* species have been noted from six *Neolitsea* species consisting of two native species, *N. konishii* and *N. sericea* var. *sericea*, and four endemic species, *N. acuminatissima*, *N. daibuensis*, *N. parvigemma*, and *N. variabillima* (Liang et al. 1999; Yang et al. 1999; Tung et al. 2018; Fig. 1). These provide an opportunity to explore the ecological divergence and island biogeography of *Neolitseae*-associated *Pseudasphondylia* species.

In this article, the species identity of the Taiwanese *Pseudasphondylia* species on six *Neolitsea* host species was first clarified based on both morphological and molecular evidence. Then, we conducted phylogenetic analysis to address the following questions for *Neolitsea*-associated *Pseudasphondylia* species: (1) What is their possible divergence pathway in the East Asian Arc? (2) How did they diverse on *Neolitsea* plants? Clarifications of these two questions will contribute to the understanding of the divergence modes of cecidomyiids in this region.

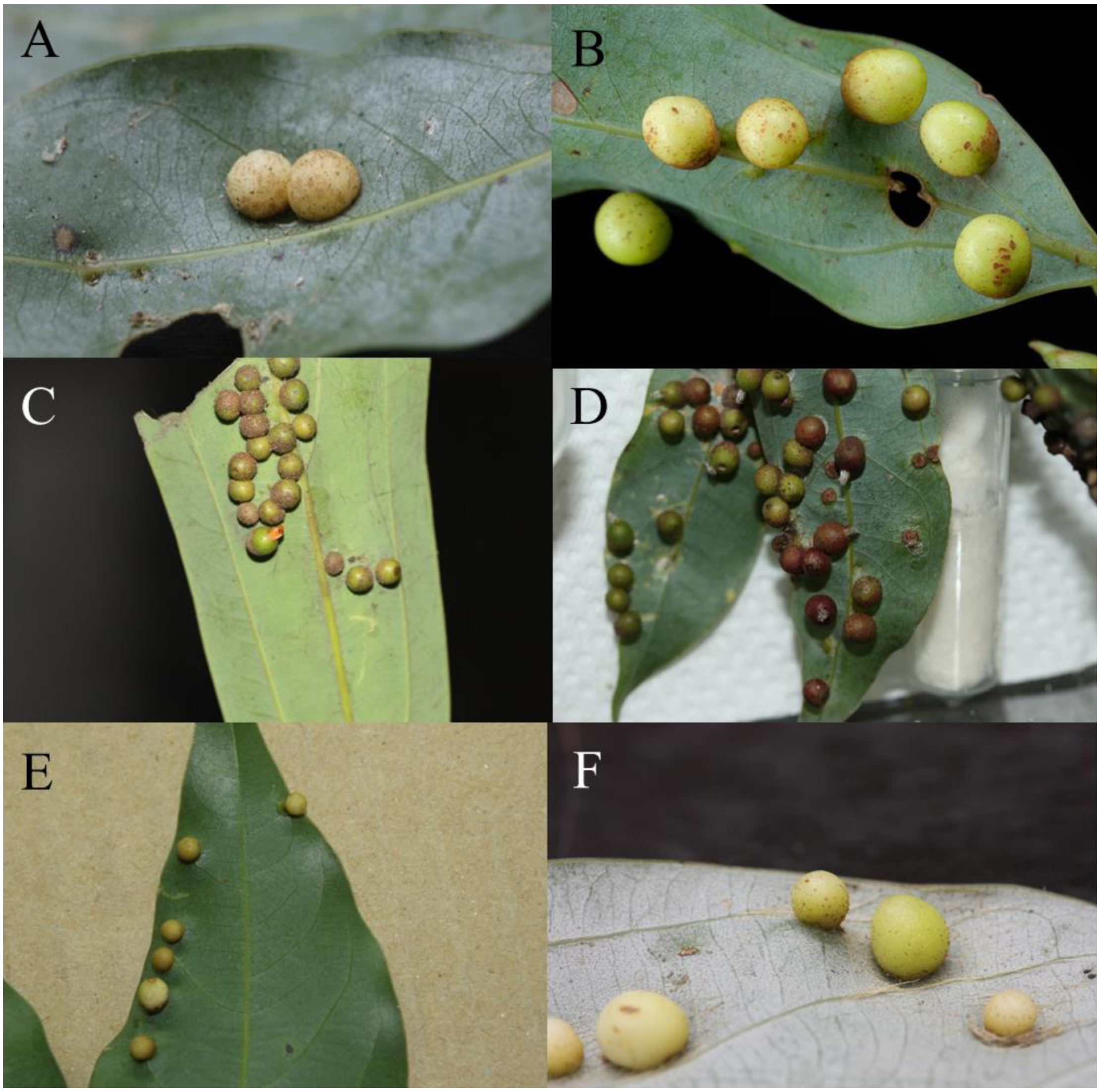

Fig. 1. Leaf galls of *Pseudasphondylia hooki* sp. n. on *Neolitseae* species (A) *N. acuminatissima*, (B) *N. daibuensis*, (C) *N. konishii*, (D) *N. sericea* (E) *N. parvigemma*, and (F) *N. variabillima*.

MATERIALS AND METHODS

Collecting and preparation of specimens

Leaf galls induced by *Neolitsea*-associated *Pseudasphondylia* species were collected from various localities in Taiwan and Japan between 2009 to 2024. Larval, pupal, and adult specimens were mostly preserved in 70% ethanol after dissecting the galls or adult emergence. A part of the fresh specimens was kept in 95% ethanol for molecular studies. Slide-mounted specimens were prepared following Gagné (1994). Specimens were observed using an optical microscope (Leica® DM 750, Germany) and deposited in National Chung Hsing University, Taiwan (NCHU), National Museum of Natural Science, Taiwan (NMNS), and Taiwan Forestry Research Institute, Taiwan (TFRI). The morphological terminology of adult followed Gagné (2018) and Tokuda (2004), and that of larva and pupa followed Gagné (1994).

Molecular analysis

Three individuals of *P. neolitseae* and 17 individuals of Taiwanese *Neolitsea*-associated *Pseudasphondylia* species were used in the present molecular work (Table 1). DNA extraction and PCR procedures followed Lin et al. (2019). The *COI* maker primers were referenced Cameron et al. (2007): Forward primer: Diptera-49F (5'-AATCATAAAGATATTGGAAC-3') and reverse primer: Diptera-734R (5'-CAACATTTATTTTGATTT TTTGG-3'). The 20 *COI* sequences were successfully analyzed and deposited in the DNA Data Bank of Japan (DDBJ) with accession numbers shown in table 1. The *COI* sequences of *Illiciomyia yukawai* (LC348719), *P. kiwiphila* (LC710532), and *P. rokuharensis* (LC538357) were used as outgroups. The sequences were aligned using ClustalW (Thompson et al. 1994) with BioEdit (Hall 1999) and the species delimitation methods using ASAP (Assemble Species by Automatic Partitioning) online service (Puillandre et al. 2021) with Jukes-Cantor model. The genetic distances among *Pseudasphondylia* species were calculated by *p*-distance with MEGA XI (Tamura et al. 2021) and the divergence time was assessed with the divergent rates of 2.0 and 2.3% pairwise sequence divergence per million years according to DeSalle et al. (1987) and Brower (1994).

For the phylogeny, we evaluate the saturation of codon substitutions in order to obtain greater phylogenetic signal. The analyzed *COI* dataset were evaluated for transition and transversion substitutions by DAMBE 7 (Xia 2018). As result, the first codon and first plus second codons dataset were analyzed. The best model (T93+G) was selected for phylogenetic analysis using MEGA XI (Tamura et al. 2021) with Maximum likelihood method. Bootstrap replication was set as 1000.

RESULTS

TAXONOMY

Order Diptera Linnaeus, 1758
Family Cecidomyiidae Newman, 1834
Tribe Asphondyliini Enderlein, 1914
Subtribe Asphondyliina Enderlein, 1914
Genus *Pseudasphondylia* Monzen, 1995

Pseudasphondylia Monzen 1955: 41. Type species: *Pseudasphondylia rokuharensis* Monzen, 1955.
Philadelphella Kovalev 1964: 440. Type species. *Philadelphella philadelphi* Kovalev, 1964.

The genus is known from China, Far-East Russia, India, Japan, New Caledonia, and Taiwan, and until now comprised 14 species (Gagné and Jaschhof 2021; Matsuda et al. 2021; Elsayed et al. 2023). A fifteenth

Table 1. *Pseudasphondylia* specimens used in the molecular phylogenetic analysis, with information on host plant, locality, and DNA accession numbers

Gall midge	Host plant	Locality*	Taxon No. [Accession No.]
P. neolitseae	*Neolitsea sericea*	Fukuoka, JP	A348-350 [PV446537-39]
P. hooki sp. n.	*N. acuminatissima*	Mt. Dahan, Pingtung, TW	A74,A77 [PV446529-30]
	N. daibuensis	Jinshuiying Trail 0.7K, Pingtung, TW	A354-356 [PV446540-42]
	N. konishii	Donggua Trial, Miaoli, TW	A180,A360 [PV446531,PV446543]
	N. parvigemma	Mt. Dahan forest Trial, Pingtung, TW	A257-258 [PV446532-33]
	N. sericea	Erziping trail, Taipei, TW	A247-249,A375-259 [PV446534-36,PV446544-46]
	N. variabillima	Mt. Beidongyan-Nantungyen 3.4K, Nantou, TW	A362-363 [PV446547-48]

*TW and JP indicate Taiwan and Japan, respectively.

species is described below from Taiwan on *Neolitseae* species. The description, DNA, and host spectrum of the new species were provided below.

Psuedasphondylia hooki **sp. n. Lin, Tokuda & Yang**

(Table 2; Figs. 3–4)
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Type materials: *Holotype*: ♂ (on slide, NCHU), TAIWAN: Taipei City, Mt. Yang-Ming National Park, gall collected on 17.ii.2011 and adult emerged on 23.ii.2011, ex. *Neolitsea sericea*, leg. SF Lin.

Paratypes: TAIWAN: Ex. ***Neolitsea acuminatissima*****: [Taipei City]** 4♀, 4 pupal exuviae (on slides, NCHU), gall collected on 22.i.2013 and adult emerged on 26.i.2013, Mt. Yang-Ming National Park, leg. SF Lin; **[Nantou Co.]** 4♂, 1♀, 10 pupal exuviae (on slides, NCHU), gall collected on 23.iii.2013 and adult emerged on 25–28.iii.2013, Mt. Liying, leg. SF Lin; **[Yilan Co.]** 2 pupae (on slides, NCHU), 12.ii.2009, Taiwan Beech National Trail, Mt. Taiping, leg. TC Tang. Ex. ***Neolitsea konishii*****: [Taipei City]** 4♂, 9♀, 3 pupae, 26 pupal exuviae (on slides, NCHU), gall collected on 17.ii.2011 and adult emerged on 24–26.ii.2011, Mt. Yang-Ming National Park, leg. SF Lin; 5♂, 8♀, 2 pupae, 6 pupal exuviae (on slide, NCHU), 11.iii.2011, Mt. Yang-Ming National Park, leg. SF Lin; 8♀, 12 pupal exuviae (on slides, NCHU), gall collected on 22.i.2013, adult emerged on 28.i.2013, Mt. Yang-Ming National Park, leg. SF Lin. Ex. ***Neolitsea sericea*** **[Taipei City]** 2♂, 6♀, 7 pupae, 50 pupal exuviae (on slides, NCHU), same data as holotype; 1♀, 2 pupae, 9 pupal exuviae (on slides, NCHU), gall collected on 18.iii.2011 and adult emerged on 20.iii.2011, Mt. Yang-Ming National Park, leg. SF Lin; 2 mature larvae (on slides, NCHU), 30.xii.2013, Mt. Yang-Ming National Park, leg. SF Lin; 5♂, 1 pupa (in EtOH, NCHU), 11.ii.2001, Mt. Yang-Ming National Park, leg. LH Liao, MM Yang; 10♂ (in EtOH, NMNS), gall collected on 29.i.2024 and adult emerged on 4~5.ii.2024, Mt. Yang-Ming National Park, leg. SF Lin. Ex. ***Neolitsea parvigemma*****: [Pingtung Co.]** 8 mature larvae (six in EtOH, two on slide, NCHU), 17.ii.2017, Dahan forest rd. leg. SF Lin. Ex. ***Neolitsea variabillima*****: [Nantou Co.]** 1 mature larva (in EtOH, TFRI), 18.i.2010, Mt. Peitungyen-Nantungyen 3.4K, leg. GS Tung.

Adult: Head. Eye bridge 3–4 facets long. Frontoclypeal setal count as in table 2. Labella hemispherical in lateral view, with setae. Palpus three-segmented (Fig. 2A), first segment globose, 25–30 µm long, with 2–4 setae; second 2.0 times as long as first, with 4–8 setae and third 2.2 times as long as first, with 7–10 setae. Twelve flagellomeres, the last three equal in length in male (Fig. 2B). Female distal flagellomeres gradually shortened, flagellomere 11 subglobular and terminal one globular (Fig. 2C).

Thorax: Thoracic setal and scale counts as in table 2. Legs with dense blackish scales; first tarsomeres of all legs with apicoventral spur; tarsal claws bent after midlength on all legs; pulvilli shorter than claws; empodia slightly shorter than claws on all legs (Fig. 2D–E). Wing 3.1–4.0 mm long, 2.0–2.4 times as long as wide in male; 3.2–3.4 mm, 2.3 times as long as wide in female. R_5 joining costa posterior to wing apex (Fig. 2F–G).

Male abdomen: Tergites I–VII rectangular, covered with scales on medium part, with a few lateral setae; tergites I–VI with 1–2 posterior row of setae; tergite VII with few setae on posterior part; sternites II–VII with anterior pair of lateral setae, cover with scattered setae and scales, sternites I–VI with three to four posterior row of setae; sternites VII with three to four posterior row of setae; tergite VIII covered with scattered setae and few scales.

Terminalia (Fig. 2H): cerci forming a pair of lobes, each lobe with a few apical setae; hypoproct bilobed, deeply separated, each lobe with two apical setae; gonostylus suboval, with two separate sclerotized teeth cover with setae; gonocoxite elongate, cover with setae dorsally and ventrally; mediobasal lobe of gonocoxite present; aedeagus laterally sclerotized in basal part, distally tapering.

Table 2. Frontoclypeal and thoracic setal counts of *Pseudasphondylia hooki* sp. n.

Specimens	Male			Female		
	n	Mean ± SD	Range	*n*	Mean ± SD	Range
Frontoclypeal setae	3	29.3 ± 4.7	24–33	2	41.5 ± 0.7	41–42
Anterior dorsolateral setae	4	66.5 ± 6.4	63–76	4	56.3 ± 5.7	49–63
Posterior dorsolateral setae	3	65.3 ± 7.0	58–72	4	64.3 ± 5.6	59–72
Mesopleural scales	5	14.6 ± 1.8	12–17	4	12.0 ± 1.8	10–14
Mesepimeral setae	6	46.5 ± 9.4	30–55	4	35.5 ± 4.1	32–40

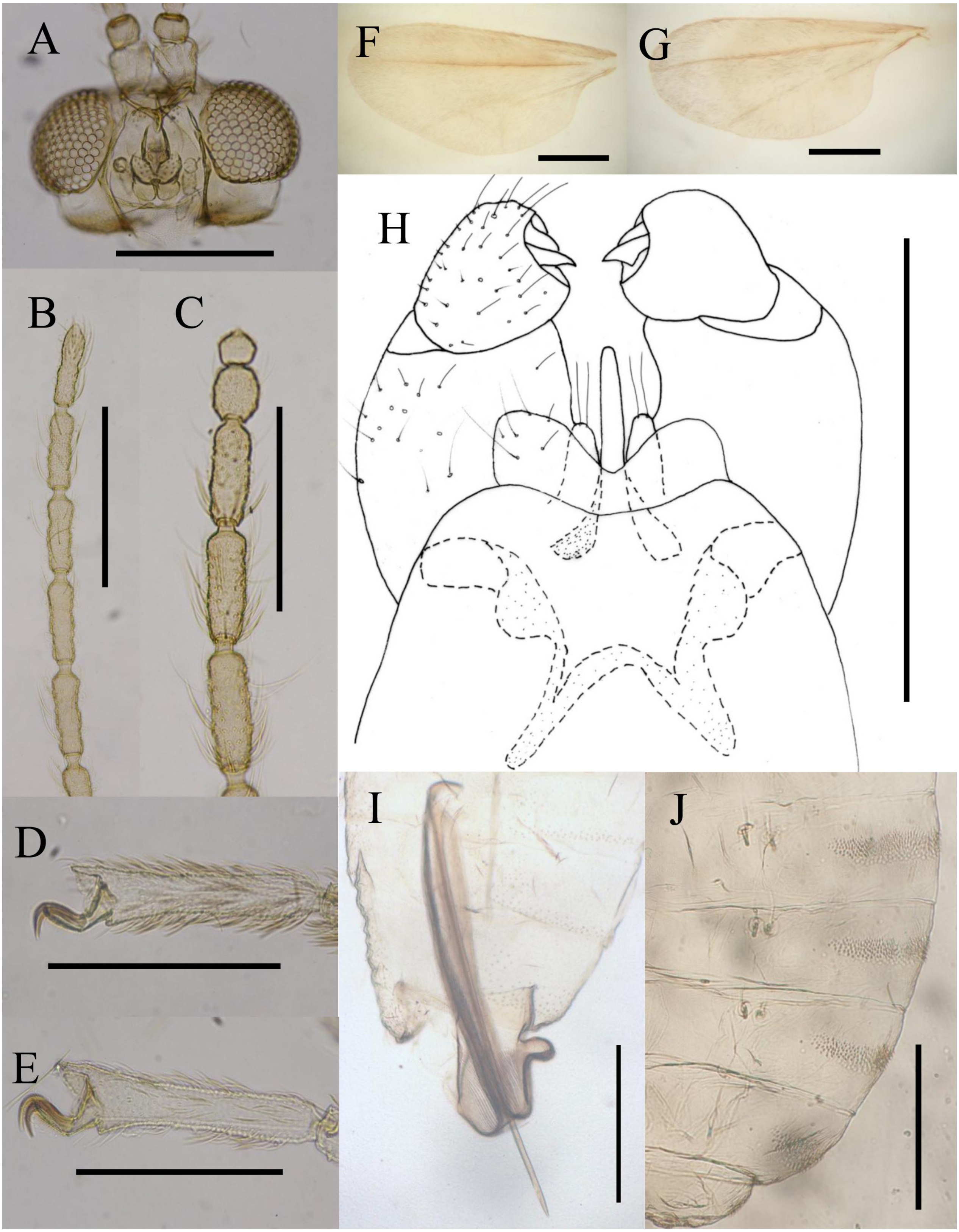

Fig. 2. *Pseudasphondylia hooki* sp. n. (A) Male head (ventral view) (B) Male antenna (8–12 segment). (C) Female antenna (8–12 fragellomeres). (D) Male 5th tarsomere. (E) Female 5th tarsomere. (F) Male wing. (G) Female wing. (H) Male genitalia. (I) Female abdomen. (J) Pupal abdomen. Scale bars: A–C = 0.03 mm; D–E = 0.2 mm; F–G = 1 mm; H = 0.2 mm; I–J = 0.5 mm.

Female abdomen: First through seventh tergites and second through sixth sternites as in male. Seventh sternite 0.5–0.6 mm long, protrusible part of ovipositor 1.3–1.7 mm long (Fig. 2I), 2.5–3.1 times as long as the length of seventh sternite.

Pupa: Body length 2.7–3.0 mm, pupal skin not pigmented except for antennal horns. Antennal horn 280–320 µm long, triangular, lateral margin without irregular serration (Fig. 3); cephalic papilla with seta, 40–65 µm long; frons without horns; two pairs of facial and three pairs lateral facial papillae present; prothoracic spiracle (Fig. 3) 180–210 µm long; second to sixth abdominal spiracles 50–70 µm long; second to seventh abdominal segments with 4 to 5 transverse rows of spines (Fig. 2J) ; eighth abdominal segments with 3 to 4 transverse rows of spines; eight dorsal papillae on first to the seventh abdominal segments, most outer and second inner pair papillae with seta; two dorsal papillae on eighth abdominal segment, each with seta; pleural papilla present on each side, with seta.

Mature Larva: Body color in life yellow, length 2.0–2.3 mm. Second antennal segment short, conical; cervical papillae without seta. Sternal spatula 330–370 µm long, anteriorly with two lobes (Fig. 3);

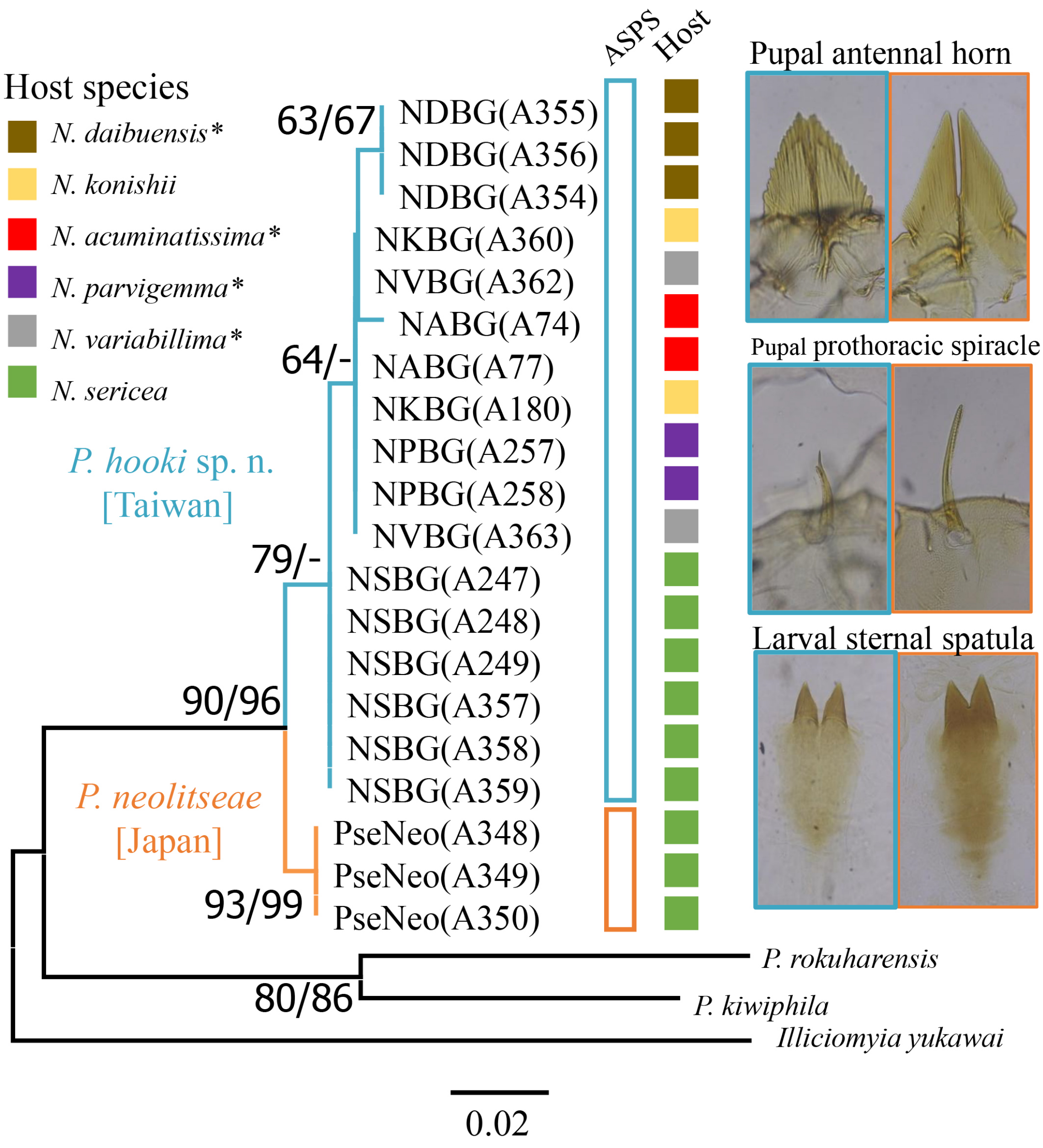

Fig. 3. Phylogenetic tree based on the 1st codon positions of *COI* (T93+G) and morphological differences of *Neolitsea*-associated *Pseudasphondylia* gall midge, *P. hooki* sp. n. (left) and *P. neolitseae* (right). The bootstraps (%) of Maximum-Likelihood inferences were shown beside nodes (1st codon/ 1st +2nd codons). Score less than 60 were hidden.

four lateral papillae and a sternal papilla present on each side of all thoracic segments, each with seta; four dorsal papillae on all thoracic and first to seventh abdominal segments, two dorsal papillae on eighth abdominal segment, each with seta; pleural papilla present on each side, with seta; 2 asetose terminal papillae present; anal papillae not apparent.

Distribution: Taiwan.

Gall and Host: Monothalamus, spherical gall on the leaves of six *Neolitsea* species, *N. acuminatissima*, *N. daibuensis*, *N. konishii*, *N. parvigemma*, *N. sericea*, and *N. variabillima*. Gall development and morphology are shown in Liang et al. (1999) and Tung et al. (2018). A total of 1059 galls of *P. hooki* were examined, including 225 galls on *N. acuminatissima*, 62 on *N. daibuensis*, 98 on *N. konishii*, 90 on *N. parvigemma*, and 500 on *N. variabillima*. All of them occurred on the abaxial side of the host leaf (Table 3).

Biological notes: This species is fundamentally univoltine. Adults emerge directly from galls in February and females lay eggs possibly into new buds as in *P. neolitseae*. Larvae soon hatch and remain inside the host tissue as the first instar until October. Liang et al. (1999) mentioned the gall started to grow from August and reached the maturation phase in October. Subsequently, larvae within the galls undergo maturation in January and pupate during middle mid-January and early February. While Takasu and Yukawa (1984) reported the occurrence of extended diapause and a semivoltine life cycle in Japanese *P. neolitseae*, the presence of such individuals in *P. hooki* remains unconfirmed

Remarks: Male terminalia and larval features, sternal spatula with two lobes and cervical and ventral papillae lacking seta, of the new species are similar to those of *P. neolitseae*. Nevertheless, the new species is distinguishable from *P. neolitseae* by the deeper insersion of larval sternal spatula (Fig. 3), serrate lateral margin of the antennal horns (Fig. 3) and hook-liked prothoracic spiracle (Fig. 3) in pupa, and the three-segmented palpus. In contrast to these features, *P. neolitseae* possesses sternal spatula with shallower insersion in larva, antennal horns with flat lateral margin and long prothoracic spiracle in pupa, and two-segmented palpus in adult.

Species delimitation and divergence

A total of 20 *COI* sequences (~658 bp) of *Neolitsea*-associated *Pseudasphondylia* species were obtained. Among them, 17 *COI* sequences of *P. hooki* were obtained from six host plants in Taiwan and three from *P. neolitseae* on *N. sericea* in Japan (Table 1). Excluding the three outgroups, the ASAP revealed that *P. neolitseae* formed one entity under all asap-score modes, whereas *P. hooki* formed one to 11 entities with different asap-score modes. The ASAP branch nodes of the two species did not support their distinction ($P > 0.1$) (Fig. 4). The *COI* genetic distance is 0.7% within *P. hooki* sp. n., 0% within *P. neolitseae*, while 2.7% (2.5–

Table 3. Galled position of *Pseudasphondylia hooki* sp. n. on *Neolitsea* leaves

Host	Locality	Abaxial side	Adaxial side
*N. acuminatissim*a	Mt. Beidongyan, Nantou	27	0
	Mt. Liying, Nantou	60	0
	Cika Cabin, Taichung	90	0
	Dasyueshan forest rd.	48	0
N. daibuensis	Jinshuiying Trail, Pingtung	62	0
N. konishii	Erziping Trail, New Taipei	48	0
	Sanyi, Maioli	4	0
	Huisun forest area, Nantou	16	0
	Mt. Dongmao, Taichung	25	0
	Dasyueshan forest rd., Taichung	5	0
N. parvigemma	Jinshuiying Trail, Pingtung	90	0
N. variabillima	Mt. Beidongyan, Nantou	23	0
	Mt. Baimao, Taichung	2	0
	Lianhuachi Research Center, Nantou	2	0
	Dasyueshan forest rd., Taichung	57	0
N. sericea	Erziping Trail, New Taipei	500	0

3.1%) between the two species. The divergence time between them was estimated at around 1.2–1.3 mya.

Molecular phylogeny

The comparison of the transition and transversion frequencies of our two dataset revealed that the 1st codon and 1st +2nd codons did not reach saturation. In contrast, saturation was observed in other datasets, including those containing the 2nd, 3rd, and all codon positions (Fig. 5). Therefore, a total of 202 positions from the 1st codon and 404 positions from the 1st + 2nd codons were used for further phylogenetic analysis. As a result, phylogenetic trees form both datasets shared similar topologies but some branches weakly supported in the tree reconstructed from the 1st + 2nd codons. In the present study, we showed the tree based on 1st codon dataset as it most clearly illustrated the phylogenetic relationship. The phylogenetic tree supported the monophyly of *Neolitsea*-associated *Pseudasphondylia* species (bootstrap value: 90; Fig. 3), as well as of *P. hooki* lineage (bootstrap value: 79) and *P. neolitseae* lineage (bootstrap value: 93). In the *P. hooki* clade, individuals associated with *N. sericea* situated at the basal part and formed a paraphyletic group with respective a clade of *P. hooki* individuals on the other hosts (bootstrap value: 64). Then, *P. hooki* individuals on *N. daibuensis* formed a subclade (bootstrap value: 63) and situated at the most terminal part.

DISCUSSION

The gall shapes together with host species of cecidomyiids are useful features for researchers to recognize cecidomyiid congeners in adjacent unexplored areas. In East Asia, *P. elaeocarpi* associated with *Elaeocarpus sylvestris* (Elaeocarpaceae) and inducing finger-shaped leaf galls in both Taiwan and Japan (Lin et al. 2022), and *Bruggmanniella cinnamomi* Tokuda & Yukawa inducing swollen stem galls on *Cinnamomum yabunikkei* (= *C. japonica*) in Japan and on *Cinnamomum insulari-montanum* in Taiwan (Lin et al. 2020b). However, similar ecological features may represent in distinct cecidomyiid species. For example, the shape of swollen stem galls of Taiwanese *Bruggmenniella turoguei* Lin, Yang, & Tokuda on *Cinnamomoum osmophloeum* is similar to Japanese *B. cinnamomi* on *C. yabunikkei* (Lin et al. 2020b). In the present study, spherical leaf galls on six Taiwanese *Neolitsea* species were all induced by *P. hooki*. This suggests the necessity of adopting the integrative taxonomic approach, in particular for galling cecidomyiid inducing similar galls and associated with related hosts in adjacent areas. Besides, gall morphospecies are used to evaluate the richness of galling insects in many studies (Yang et al. 2000; Tung et al. 2006; Pan et al. 2018). The case of *P. hooki* highlights the asymmetry of cecidomyiid species and gall morphospecies richness. In Taiwan, several Asphondyliina species are oligophagous and/or display

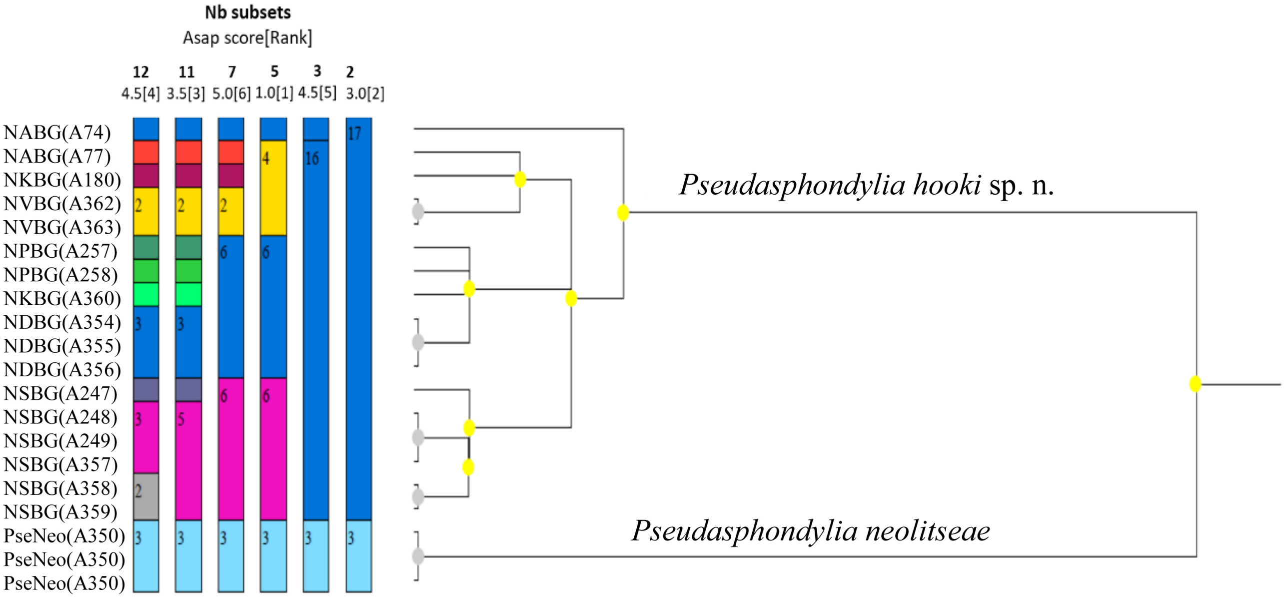

Fig. 4. Assemble Species by Automatic Partitioning (ASAP) analyses of *Neolitsea*-associated *Pseudasphondylia* gall midges based on mitochondrial partial *COI* region. Colors represent unique partitions. Gray and Yellow dots of branches refer to *P* value incalculable and greater than 0.1, respectively.

gall polymorphism (Pan et al. 2015; Lin et al. 2020a b; present study). These result in an overestimation of cecidomyiid richness based on host species and gall morphology. The further taxonomic efforts of Taiwanese Asphondyliina are needed to realize the actual cecidomyiid diversity. Additionally, these study systems are suitable materials to explore the divergence mode of galling cecidomyiids at the species level.

Species recognition and divergence

The mtDNA marker, the partial *COI* region, is a useful tool for the recognition of cecidomyiid species and the study of their evolutionary history. Tokuda et al. (2004) clarified the host spectrum and biogeography of *Ilex*-associated *S. sasakii* and *S. soyogo* (mentioned as *Asteralobia* species at that time) in Japan; Lin et al. (2020b) confirmed the species identify and possible evolutionary history of four *Cinnamomum*-associated

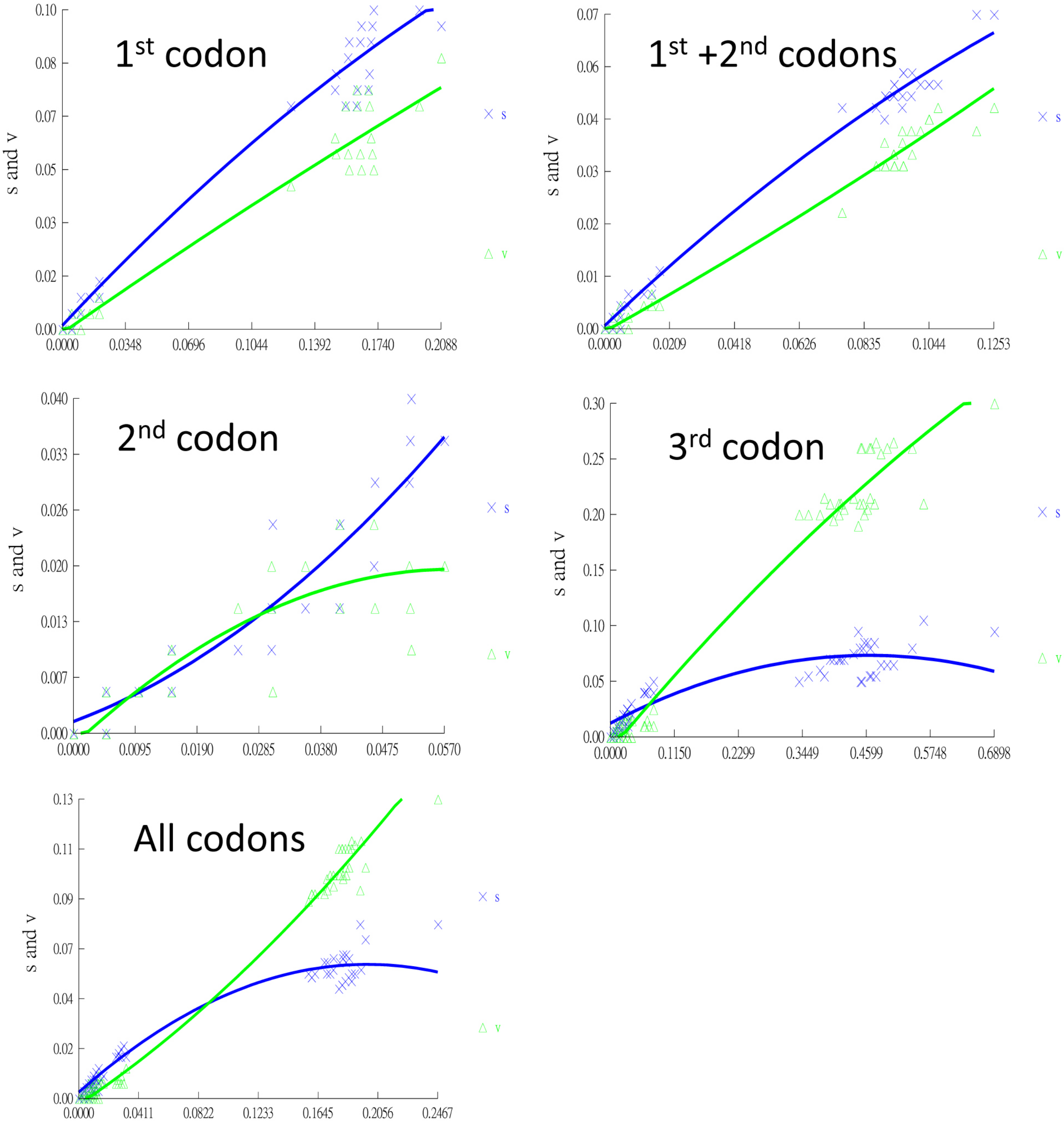

Fig. 5. Saturation plots of transition (blue line) and transversion (green line) rates against F84 distance.

Bruggmanniella species in the East Asian Arc. In the present study, the ASAP species delimitation (Fig. 4) showed the distinct relationship of *P. hooki* and *P. neolitseae*, which was also supported by morphological evidence and our ML tree (Fig. 3).

Our phylogenetic analysis of *Neolitsea*-associated *Pseudasphondylia* species highlighted the inter- and intraspecific divergence of cecidomyiids involving island biogeography and host expansion. In the biogeography of *Neolitsea*-associated *Pseudasphondylia* species, the divergence time (1.2–1.3 mya) of these two species corresponds to a later period of separation between Taiwan and Japan in the late Pliocene (Kimura 1996; Osozawa et al. 2012). This divergence timing is similar to that of Taiwanese and Japanese population of *P. elaeocarpi* (Lin et al. 2022). As to host expansion, *N. sericea* can be regarded as the primitive host of *Neolitsea*-associated *Pseudasphondylia* species based on the host distribution in the phylogeny (Fig. 3). The insular host expansion of *P. hooki* can be inferred together with the plant range and the dispersal route from north to south in Taiwan. The basal clade of *P. hooki* associated with *N. sericea*, which is mainly found in northern Taiwan and the terminal clade with *N. daibuensis*, which are endemic to southern Taiwan (Lu et al. 2000).

In *P. neolitseae*, galls were induced both on the abaxial and adaxial sides of leaf (mentioned as the lower and upper types in Mishima and Yukawa 2007) and these types were distributed parapatrically in Honshu (Kanto Region) and Kyushu (Sunose 1981; Mishima and Yukawa 2007). Host ploidy level is not related to the dimorphism (Mishima and Yukawa 2007), but, based on a transplanting experiment, gall midge genotype seems to be involved in it (Yukawa and Takahashi 2017). Unlike *P. neolitseae* representing gall dimorphism, all galls of *P. hooki* occur only on the abaxial side (Table 3), this type is assumed to be the primitive gall type in *Neolitsea*-associated *Pseudasphondylia* lineage relying on the parsimonic evolution in the gall habitat. More DNA data of *P. neolitseae* from Kyushu and Honshu (Kanto region) are needed to explore the evolutionary transition of gall type in *P. neolitseae*, as well their possible divergence route.

CONCLUSIONS

The Taiwanese *Pseudasphondylia* species associated with *Neolitsea* is morphologically distinct from Japanese congener *P. neolitseae*, and the result is supported by the species delimitation using mtDNA *COI* region and ASAP analysis. These resulted in the formal description of a new species *P. hooki*. The sistership of *P. hooki* and *P. neolitseae* may indicate their recent speciation via geographical isolation. Moreover, *N. sericea* is assumed to be the primitive host of *Neolitsea*-associated *Pseudasphondylia* species. Host expansion and insular dispersal route of *P. hooki* have been inferred, with evidence indicating a movement from north, associated with *N. sericea*, to south, associated with *N. daibuensis*, in Taiwan as supported by the phylogenetic relationship.

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