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Identification of silver vine, *Actinidia polygama* flower bud gall midge (Diptera: Cecidomyiidae) in Korea and its ecological traits

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The mature galled flower buds of the silver vine *Actinidia polygama* (Actinidiaceae) are used in traditional oriental medicine in Korea. In Japan, a similar gall maker of *A. polygama* is *Pseudasphondylia matatabi*. However, few taxonomic studies exist on gall makers in Korea. Comprehensive morphological and molecular analyses confirmed that the Korean specimens were identical to *P. matatabi* from Japan. Morphological characteristics, particularly the male genital structure and pupal horn morphology, matched the original description from Japan. Molecular phylogenetic analysis of the DNA barcode region revealed that the Korean and Japanese populations formed a monophyletic clade distinctly separated from other congeners, including the recently described *P. kiwiphila* from Taiwan, which induces galls in *A. rufa*. Life history observations showed that adult emergence occurred from September to October, when no suitable oviposition sites were available for the deciduous host plant, suggesting the existence of an unknown winter host plant.

Keywords: *Actinidia polygama*, gall midge, *Pseudasphondylia*

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Introduction

Galls are abnormally modified plant tissues or organs resulting from chemical and mechanical stimulation by gall-inducing organisms (Shorthouse and Rohfritsch, 1992). Several herbivorous insect groups include gall-inducing species (such as Hymenoptera, Lepidoptera, Diptera, and Hemiptera). Gall shape and host information represent an "extended phenotype" of the gall-inducing cecidomyiid (Yukawa, 2000). Cecidomyiid species can be easily distinguished based on gall morphology and host taxonomy. Certain cecidomyiid galls are used as herbal medicines in East Asia. The most prominent examples are the fruit galls of *Ampelopsis glandulosa* var. *heterophylla* (Vitaceae) and the flower bud gall of *Actinidia polygama* (Actinidiaceae). In Oriental Medicine, the gall of *A. polygama*, known as Mokcheonryoza (목천료자; 木天蓼子), has been used as a popular herbal medicine (Kim *et al*., 2023) (Fig. 1).

The gall inducer of flower buds on *A. polygama* is *Pseudasphondylia matatabi* in Japan, whereas in Taiwan, *Pseudasphondylia kiwiphila* is known to induce galls on *Actinidia rufa*, which belongs to the same genus as *A. polygama* (Yuasa and Kumazawa, 1938; Lin *et al*., 2022). A total of 14 described species of the genus *Pseudasphondylia* are known globally (Gagné and Jaschhof, 2021; Matsuda *et al*., 2021; Lin *et al*., 2022; Elsayed *et al*., 2023). Congeners and related genera exhibit alternating host behaviors (Tokuda and Yukawa, 2005; Uechi *et al*., 2004; Elsayed *et al*., 2019). *P. matatabi* is also a potential host-alternating species because its adult stage occurs from September to October. However, *A. polygama* is deciduous and leaves no oviposition sites on its host plants from autumn to winter. Winter host plant species are not yet identified in Japan (Tokuda and Yukawa, 2005). The similarity between the host plant and the gall shape suggests that the Korean species may be related to *P. matatabi*

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Fig. 1. Flower bud gall of *Actinidia polygama*; a: investigation site in Japan (Inumaki, Hisayama Town, Fukuoka), b: Early flower bud of *A. polygama*, c: Early stage of gall (12 June 2016), d: Bloomed flower bud gall.

(Hashim and Yukawa, 1992).

This study aimed to confirm whether the gall midge species on *A. polygama* in Korea are identical to *P. matatabi* in Japan and to provide basic ecological information on *P. matatabi* in Japan. This study contributes to a better understanding of this gall-inducing species and its relationship with *A. polygama* in East Asia.

Materials and Methods

Morphological studies, terminology, and depository of specimens

For morphological observation, pupal cases and adults stored in 80–85% ethanol were mounted on slides in Canada balsam, following Yukawa (1971) and Gagné (1989). Adult morphological terminology followed Cumming and Wood, 2017, except for the thoracic sclerites, which followed Yukawa and Ohsaki (1988). The pupal morphological terminology followed Gagné (1994). Specimens used in this study were deposited in the National Institute of Biological Resources, Korea.

Ecological surveys

In 2015 and 2016, from April to June, surveys were conducted at a colony of *Actinidia polygama* in Inumaki, Hisayama Town, Fukuoka, Japan (N33°40′33″, E130°32′46″), to confirm gall development and adult oviposition (Fig. 1a). The site was visited every two days; however, as the buds became more prominent, visits for daily observations increased. Sticky traps were placed near flowers to collect females for oviposition.

Molecular analysis

DNA samples were extracted from 80–85% ethanol-stored specimens using a DNeasy Blood and Tissue Kit (Qiagen, Tokyo, Japan) following the manufacturer's instructions. The 658 bp region of the COI gene used for the DNA barcoding project (Hebert *et al.*, 2003) was amplified, purified, and sequenced following the method described in Yukawa *et al.* (2003). The primers used for this analysis were: forward; LCO1490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G -3′ and reverse; HCO2198 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ (Folmer *et al.*, 1994). Sanger sequencing was performed

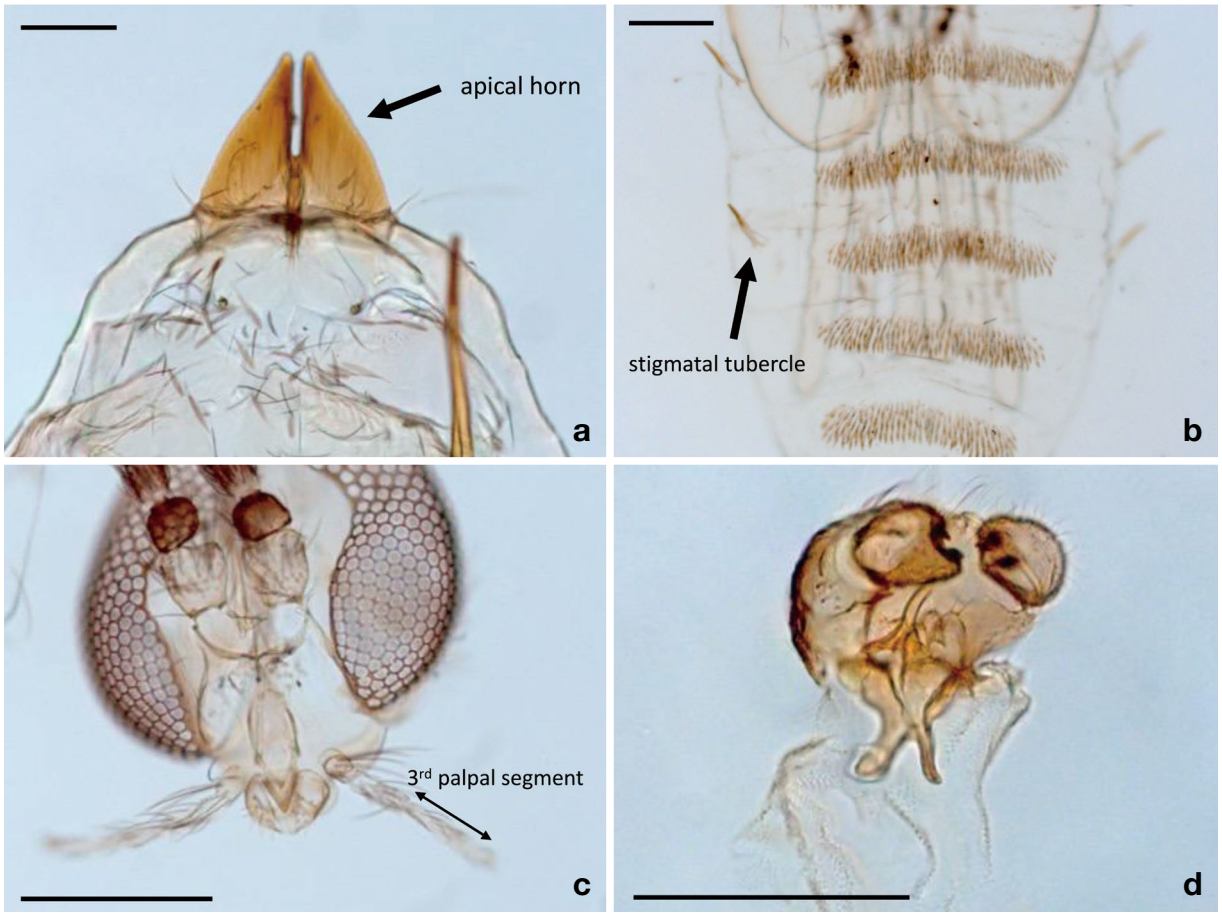

Fig. 2. Morphology of *Pseudasphondylia matatabi* in Korea; a: pupal head (ventral), b: pupal abdomen (dorsal), c: female head, d: male genitalia. Scale bar indicates 200 μm.

by Macrogen, Inc. (Seoul, Korea). Sequence data were aligned using MAFFT version 7 with the G-INS-I method (Golubchik *et al*., 2007) on the Geneious Prime program 2025.0.2 (https://www.geneious.com). Five species belonging to the same genus as *Pseudasphondylia* (*P. neolitseae*, *P. elaeocarpi*, *P. saohimea*, *P. tominagai*, and *P. kiwiphila*) were included and *Kiefferia pericarpiicola* was used as the outgroup taxon. Maximum likelihood trees were obtained using IQ-TREE (Trifinopoulos *et al*., 2016) with the TIM + F + G4 substitution model. The most suitable maximum likelihood model was chosen for the IQ-TREE using the Bayesian information criterion (BIC).

The nucleotide sequence data reported in this study were deposited in the NCBI GenBank database (https://www.ncbi.nlm.nih.gov).

Results

Taxonomic accounts
Family Cecidomyiidae
Subfamily Cecidomyiinae
Tribe Asphondyliini
Subtribe Asphondyiina
Genus *Pseudasphondylia* Monzen, 1955

Pseudasphondylia matatabi
(Yuasa and Kumazawa, 1938) (Fig. 2)
개다래꽃봉오리혹파리 (신칭)

Examined specimens. 2♂1♀ 3 pupa (NIBR, voucher number: 2019-IN-265-001−006), Chilbong-ro, Hojeo-myeon, Wonju-si, Gangwon-do, South Korea (N37°27′43″, E127°53′59″), 5 Oct. 2019, Coll. Junheon Kim (National Institute of Forest Science).

Diagnosis. Pupal apical horn 290−300 μm in length, acutely pointed, with finely denticulate outer margin; stigmatal tubercles long, 175−185 μm, present on second to fifth abdominal segments. Palpus three-segmented; third palpal segment about 2 times as long as second. Wing about 2 times as long as wide. Male genitalia cerci deeply emarginated with V-shaped incision, each lobe bearing numerous

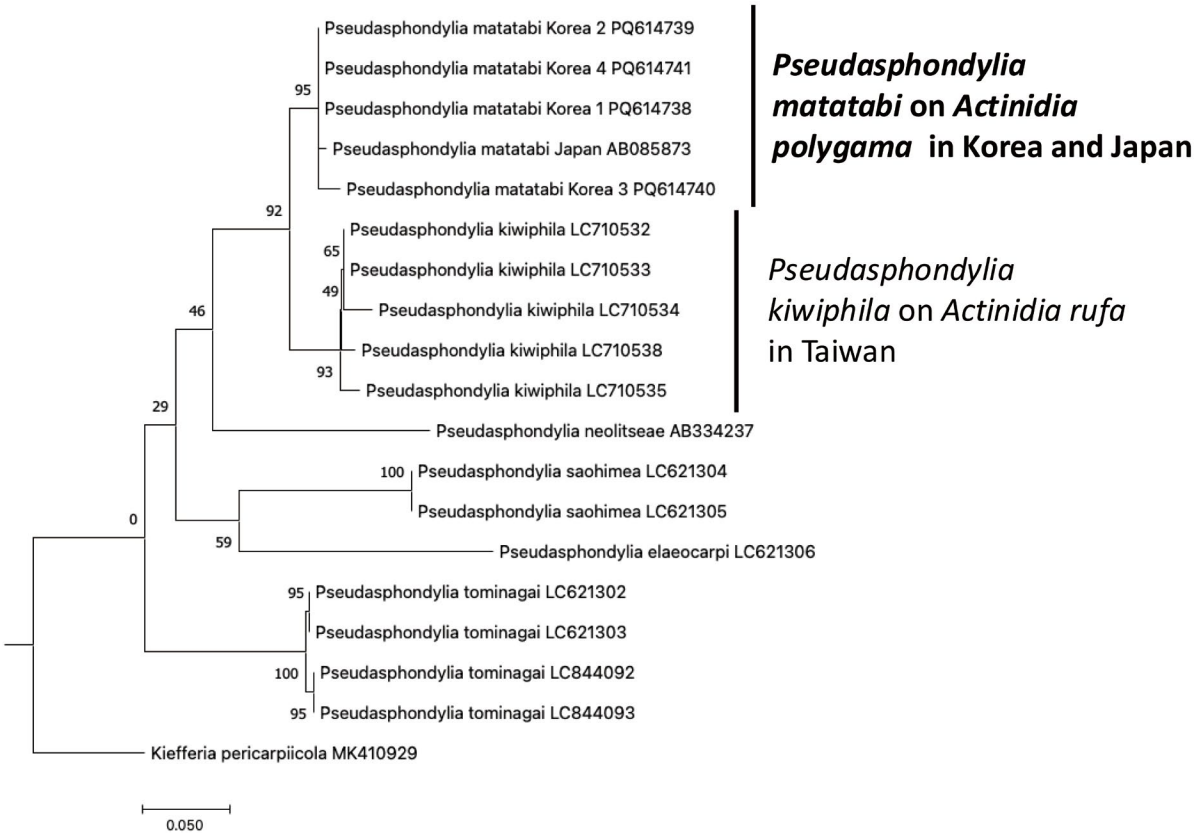

Fig. 3. Maximum likelihood tree of *Pseudasphondylia matatabi* and their congeners based on mtDNA COI sequences. Bootstrap values are indicated for nodes.

setae; gonostylus oval in shape, bearing two distinct sclerotized teeth at distal end; gonocoxite elongate in form, extending ventrally past gonostylar insertion point, with distinctive finger-like basal lobe; aedeagus narrow, gradually tapering toward apex. Needle part of ovipositor long about 1.5 mm (Fig. 2).

Gall. The ovary of the flower enlarged and swelled irregularly. Each gall contained multiple larval chambers with a maximum diameter of 15−38 mm and a height of 10−22 mm, which were larger than those of normal fruit (Yukawa and Masuda, 1996). Galls could form on flower buds before or during the flowering process. Considering their developmental characteristics, they should be classified as flower bud galls rather than fruit galls (Fig. 1c).

Distribution. Japan (Honshu, Shikoku, Kyushu) and Korea.

Life history. In Kyushu, Japan, the flower buds of *A. polygama* begin to develop during April and reach full bloom in June. The gall midge is assumed to lay eggs in early flower buds, although oviposition behavior was not observed during the daytime surveys (2015, 2016) (Fig. 1b). Galls developed from June to July, although the larvae remained in the first-instar stage until late August. Larval growth accelerates from September, with pupation and emergence occurring from late September to October (Yukawa and Masuda, 1996). However, from October onwards, no flower buds or fruits were available on *A. polygama* for oviposition, suggesting that the gall midge must move to a winter host; however, this process remains unknown.

Molecular analysis. Maximum likelihood tree analysis revealed that *P. matatabi* specimens from Korea and Japan formed a well-supported monophyletic clade with high bootstrap support (Fig. 3). The Korean population (NCBI accessions: PQ614738−PQ614741) clustered closely with the Japanese population (AB085873), indicating a close genetic relationship. This clade was distinctly separated from its congeners, including *P. kiwiphila* from Taiwan and other *Pseudasphondylia* species (*P. neolitseae*, *P. elaeocarpi*, *P. saohimea*, and *P. tominagai*). The tree topology strongly supported the genetic uniformity of *P. matatabi* populations across Korea and Japan.

Discussion

This study confirmed the identity of the flower bud gall midge of *A. polygama* in Korea as *P. matatabi* through comprehensive morphological and molecular analyses.

This identification was particularly crucial, given the recent discovery of *P. kiwiphila*, a closely related species inducing galls on *A. rufa* in Taiwan. The morphological characteristics of the Korean specimens aligned perfectly with those of the description of *P. matatabi* from Japan, particularly in terms of the male genitalia structure and pupal horn morphology (Yukawa, 1971). Molecular phylogenetic analysis strongly supported this identification.

Life history observations in Japan revealed synchronized development with host plant phenology, particularly during the flowering period. The absence of suitable oviposition sites on *A. polygama* during winter strongly suggests the existence of an alternative host plant, similar to that of the host-alternation behavior observed in other Asphondyliini species. Further research is required to identify the winter host plants of *P. matatabi*.

Conflicts of interest

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

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