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Reproductive biology and pollination ecology of two edible *Limnophila* species in Thailand

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Abstract. *Malaiphis R, Pakum W, Kaphang S, Chanokkhun T, Muangsan N, Warrit N, Traiyasut P, Barnes CH, Watthana S. 2026. Reproductive biology and pollination ecology of two edible* Limnophila *species in Thailand. Biodiversitas 27 (1): d270117. https://doi.org/10.13057/biodiv/d270117.* Culturally important native Thai herbs are declining due to habitat disturbance, overharvesting, and environmental stressors, including the use of herbicides. *Limnophila geoffrayi*, a native aquatic edible herb, has been especially affected by herbicide use and climate-driven rainfall anomalies. In contrast, the exotic *Limnophila aromatica*, introduced for edible herb usage, is widely vegetatively propagated. Both edible *Limnophila* species have poorly studied reproductive biology, which is important for native species conservation. The purpose of this study is to investigate the reproductive biology and pollination of the Thai native species *L. geoffrayi* to support conservation efforts, as well as to compare these traits to those of the introduced cultivated species, *L. aromatica*. Flowers of both species typically open for one day, with some *L. geoffrayi* flowers reopening on a second day. Diurnal opening and closing patterns corresponded with peak visitor activity. Solitary bees were the dominant pollinators, with 47.06% Sorensen's similarity index of visitors between the two plant species. Although both species exhibit floral traits conducive to self-pollination, *L. aromatica* failed to produce fruit under both natural and insect-excluded conditions, indicating self-incompatibility. In contrast, *L. geoffrayi* consistently set fruit in bagged treatments, demonstrating self-compatibility and autonomous self-pollination, which resulted from polinator scant. High fruit set under natural conditions suggests strong reproductive assurance in the native species. Field observations also confirmed that both species provided pollen resources for solitary bees, which may contribute to cross-pollination within populations. Due to self-incompatibility and clonal propagation, introducing diverse genotypes is necessary for the long-term cultivation of *L. aromatica*. As an autonomously self-pollinating species that produces many seeds, *L. geoffrayi* may serve as a potential adaptation or resilience mechanism in the face of habitat disturbance and herbicide use. Being an annual herbaceous plant with different color forms, seed banking is required to support its conservation and sustainable use.

Keywords: Breeding systems, floral phenology, flower visitors, *Limnophila*, pollination

INTRODUCTION

Native plant species with cultural and economic value are increasingly recognized as important resources for sustainable agriculture and biodiversity conservation (Goolmeer et al. 2022). In Thailand, many edible herbs traditionally used in local cuisine remain uncultivated and are harvested directly from natural habitats (Punchay et al. 2020), often without formal management or propagation strategies. Dependence on wild populations, in combination with habitat degradation and agrochemical exposure, has driven reductions in species abundance (Qi et al. 2020).

Effective conservation and cultivation planning for native plants require an understanding of reproductive biology and the ecological factors influencing reproductive success (Walsh et al. 2019; Far and Cursach 2022). Floral traits, pollinator resource collection strategies, and flowering phenology at the individual flower, entire plant, and population scales (Inouye et al. 2019) can influence pollination efficiency (Layek et al. 2022) and subsequent seed development and fruit production. Phenological investigations are crucial for understanding plant-pollinator interactions and population dynamics, providing essential knowledge for species management and conservation (Serna-González et al. 2022).

Plant reproduction systems and phenological occurrences range from complete self-incompatibility to full self-compatibility (Kuswantoro 2017). Small populations with low pollinator visitation and/or self-incompatible reproduction systems can be susceptible to extinction because they solely depend on cross-pollination from compatible mates to reproduce (Abrahamczyk et al. 2021). However, transfer of pollen into the stigma of self-compatible species potentially leads to inbreeding depression and diminished fitness in the resulting progeny (Cheptou 2024). Specifically, filament and style development are the primary mechanism allowing autogamous-selfing in plants (Ren et al. 2016). Understanding these reproductive strategies is crucial for evaluating extinction risks and population resilience under environmental change. This is particularly relevant for *Limnophila*, including *Limnophila aromatica* (Lam.) Merr. and *Limnophila geoffrayi* Bonati, which are widely used in Southeast Asian cuisine but remain poorly studied in terms of reproductive ecology in Thailand.

Limnophila (Plantaginaceae) including, *L. aromatica* and *L. geoffrayi*, which are used extensively in Southeast Asian cuisine. *L. aromatica* is native to Southeast Asia, except for Thailand, Myanmar, and the Malay Peninsula (Philcox 1970; POWO 2025). It has been introduced to Thailand as an edible crop, and vegetative propagation is the main method of cultivation. *L. geoffrayi* is a native aquatic herb in Thailand, Cambodia, Laos, and Vietnam (Philcox 1970; Yamazaki 1990; POWO 2025). It is typically collected from rice paddies and natural wetlands rather than being intentionally grown. Like other species, *L. geoffrayi* has been impacted by climate-driven rainfall anomalies (Geissler et al. 2023), and populations have also decreased in Thailand as a result of increased herbicide use. No prior reproductive ecology data exist for these species in Thailand, especially the native *L. geoffrayi*.

Sexual reproductive characteristics of *L. aromatica* and *L. geoffrayi* such as flower biology, breeding systems, and pollination methods are still mostly unknown, despite their cultural and gastronomic importance. These gaps restrict the ability to create well-informed conservation or cultivation strategies, especially for native species that might have reproductive assurance systems in pollinator-poor habitats. Therefore, pollination ecology research informs both agriculture and conservation, particularly for potential crop species such as *L. geoffrayi* that may have characteristics that guarantee reproduction even with limited pollinator visitation. Conservation priorities and sustainable agricultural use will be educated by knowledge of whether *Limnophila* species display autonomous pollination, obligate outcrossing, or mixed breeding systems.

This study provides the first comprehensive assessment of the reproductive biology of the Thai native *L. geoffrayi*, with comparative insights into the introduced cultivated *L. aromatica*. Our aims were to evaluate reproductive strategy and fruit set under different pollination treatments, describe floral morphology and flowering phenology, and identify insect pollinators. The results inform conservation and production planning for native edible plants with growing agroecological potential.

MATERIALS AND METHODS

Plant material and study area

The study was conducted in December 2024, during the peak flowering season of both species. *L. aromatica* was propagated from stem cuttings of a single genotype and cultivated in the Suranaree University of Technology Botanical Garden, Thailand. The cultivated plot was a wet, open area. Field research on *L. geoffrayi* was carried out in a repurposed abandoned rice field in Chum Phuang District, Nakhon Ratchasima Province, Thailand, in an open area that was wet from June to October and dry from November to May.

Floral morphology and anther dehiscence

We observed the floral development of *L. aromatica* and *L. geoffrayi* at three different stages: open flowers (corolla lobes expanded), late buds (completely formed but unopened corolla lobes), and early buds (corolla lobes remained closed but slightly extending from the calyx). The spatial arrangement of reproductive organs was revealed through extensive dissection. A Nikon SMZ645 stereomicroscope was used to take pictures of five flowers per developmental stage to record anther dehiscence, which had been previously kept in formalin acetic acid alcohol (FAA) solution.

Floral phenology

To monitor floral phenology, at least 40 late bud-stage flowers of *L. aromatica* and *L. geoffrayi* were randomly selected from a minimum of 15 individuals. Floral opening was observed from 06:00 to 18:00, with each bud checked at 1-hour intervals until withering. Elongation of the corolla tube and spreading of the corolla lobes were considered the first signs of flower opening. For both species, the average flower opening time was recorded. The humidity and average temperature at day time during the observation were 64% and 31°C, respectively.

Breeding system

Breeding systems of *L. aromatica* and *L. geoffrayi* were tested under two treatments, natural pollination and autogamy. Manual self- or cross-pollination and emasculation were not feasible due to small flower size and the tendency of anthers in floral buds to break easily. Fruit set was recorded under environmental conditions to evaluate natural pollination. Late buds were enclosed in nylon mesh bags to exclude insects and assess the capacity for autonomous self-pollination (autogamy). Each treatment involved ≥30 flowers from 10 individuals per species.

Floral visitors

Floral visitors were observed for 36 hours (06:00-18:00 over three consecutive days) on 30-40 flowers from three clumps per species at peak bloom. Visitors contacting stigma or anthers were photographed or collected with ethyl acetate-treated plastic bags (Animal License No. U1-11459-2566), preserved in 95% ethanol for identification.

Floral and insect morphometrics

We measured the apertures of 30 randomly chosen open flowers that insects could access to explore how they relate to flower size and insect morphology. We also assessed the lengths of their corolla tubes, measuring from the base of the calyx to the apex of the corolla tube. Lastly, we took measurements of the abdomens of the insects, noting their lengths and widths.

Data analysis

Flower longevity of both species was compared. Differences in first-day longevity between single- and double-opening flowers of *L. geoffrayi* were tested to evaluate energy conservation on the first day as a strategy for prolonging flower lifespan. Differences in the total opening period were also examined to assess the effect of opening patterns on overall duration. All statistical analyses were conducted using SPSS version 25. Longevity data were first assessed using the Shapiro-Wilk test, and then comparisons were conducted with the Mann-Whitney U test, due to non-normal data.

To test the matching of dimensions between flowers and insects, comparing the flower sizes of both *Limnophila* species with the body sizes of their insect visitors, we analyzed variance using the Kruskal-Wallis test. Pairwise comparison using Mann-Whitney U test with Bonferroni's adjustment at $p<0.05$ was adjusted to $p<0.005$. Similarity of visitor assemblages between the two species was estimated by Sorensen's similarity index, which is calculated by multiplying the number of species shared by both assemblages by 2, dividing the result by the total number of species recorded across both assemblages, and multiplying the quotient by 100 (Christopher 2020).

RESULTS AND DISCUSSION

Floral functional morphology

Color variation observed in *L. geoffrayi*, ranging from violet to purplish pink and white (Figure 1), has not been previously reported, especially in the population in Thailand (Yamazaki 1990). This study focused on the violet form of *L. geoffrayi*, as other forms were rare. *L. aromatica* and *L. geoffrayi* share floral structures favoring autogamy. Both species produced slightly bilaterally symmetrical tubular flowers, 1.0-1.3 cm long. Fully opened flowers displayed corollas with five unequal lobes: three upper and two lower (Figure 2). Trichomes were present on both the external and internal corolla surfaces. The outer surface was scattered with short hairs in both species, which was denser in *L. aromatica* (Figure 2.A, 2.B, 2.D, 2.E). Internally, dense trichomes developed from the base of the upper lobes, restricted to one side of the floral tube (Figure 2.C, 2.F). The summary of all the floral morphological appearances is shown in Table 1.

Stamen and stigma development were consistent across early bud, late bud, and open flower stages in both species. Each species bore four dorsifixed anthers in two unequal pairs, shorter stamens at the lower lobe and longer at the upper. All stamens were inserted in the corolla tube, aligned vertically near the lower lobes rather than the central hole (Figure 3.A, 3.C, 3.E, 3.G, 3.I, 3.K). In early buds, corolla lobes stayed closed but partly protruded from the calyx. Shorter stamens lay below the stigma, while longer stamens are aligned with it. Anthers were smooth and indehiscent, and the stigma lacked exudate, indicating non-receptivity. Longer stamens extended above the stigma, whereas shorter stamens remained below, and floral organs were more developed in late buds even though the corolla remained closed. Anthers remained indehiscent, and the stigma non-receptive. Corolla tubes were fully developed in open flowers, with shorter stamens staying basal and longer stamens extending over the stigma. The stigma displayed a sticky surface consistent with receptivity, and the anther walls were rough and split longitudinally, indicating dehiscence (Figures 3.E, 3.K). Both species showed rapid anther rupture immediately after flowers opened.

In both species, anther dehiscence and stigma receptivity at the open-flower stage ensure reproductive assurance through autonomous self-pollination, with stamen arrangement enabling direct pollen deposition onto receptive stigma. The pattern is similar to *Collinsia verna* (Plantaginaceae), in which the four anthers move towards the front of the flower and then dehisce sequentially (Kalisz et al. 1999). Stamen-stigma arrangements, together with a bell-shaped corolla and adaxial organ positioning, are linked to autonomous self-pollination in Scrophulariaceae and Plantaginaceae (Kampny 1995). Although stigma receptivity was not directly tested, visual cues and exudate indicated receptivity at open-flower stage, preventing premature fertilization and ensuring efficient self-pollen deposition through synchronized pollen release and stigma readiness (Zulkarnain et al. 2019). These mechanisms, like autonomous selfing in *Prunella vulgaris* (Lamiaceae), provide reproductive assurance and endure in environments with limited pollinators (Ling et al. 2017).

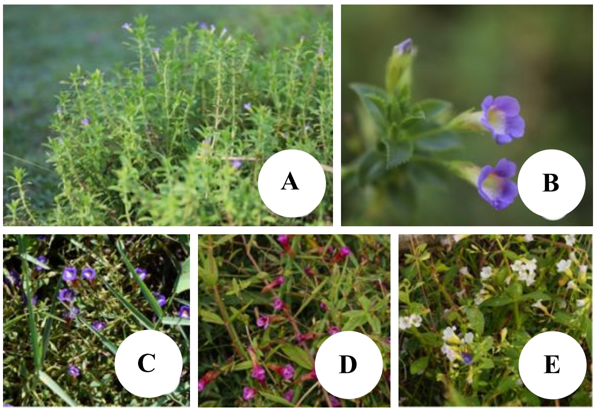


Figure 1. The variation of floral colors. A. *L. aromatica* B. *L. aromatica*, C. *L. geoffrayi*, D. *L. geoffrayi*, E. *L. geoffrayi*

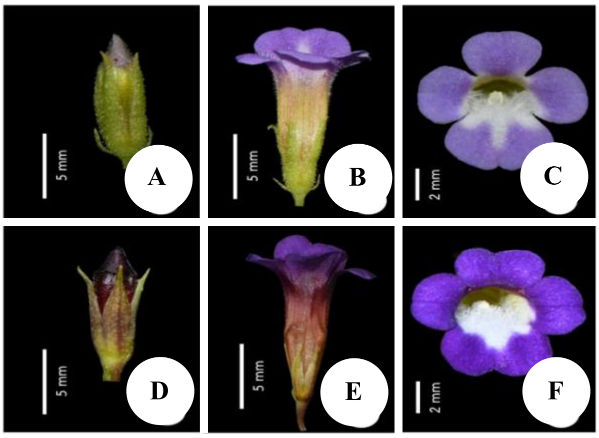


Figure 2. Floral buds and open flowers of *L. aromatica*: A. Floral bud, B. Open flowers, C. Top view of floral and *L. geoffrayi*: D. Floral bud, E. Open flowers, F. Top view of the flower. C and F are top view of the natural position

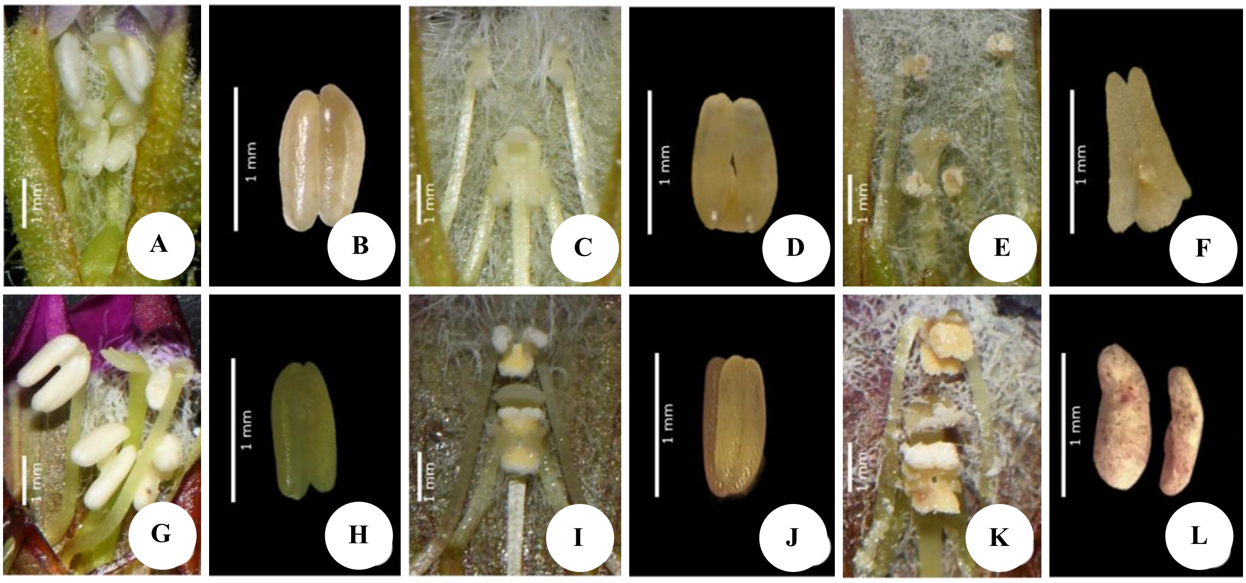


Figure 3. Position of *Limnophila aromatica* sex organs and anther dehiscence at different floral stages (A.-F): (A, B) Early bud (C, D) Late bud, and (E, F) Open floral stage. *Limnophila geoffrayi* (G-L): (G, H) Early bud, (I, J) Late bud, and (K, L) Open floral stage

Table 1. The comparative floral morphology of *L. aromatica* and *L. geoffrayi*

Characters	*Limnophila aromatica*	*Limnophila geoffrayi*
Inflorescence type	Terminal branches, usually solitary	Axillary or terminal racemes with 2-10 flowers
Flower size	1-1.3 cm long	1-1.3 cm long
Flower color	Violet	White, dark violet, or purplish pink
Flower shape	Campanulate-funel and slightly bilabiate	Campanulate-funel and slightly bilabiate
Corolla tube surfaces	Pubescens outside. White villous inside.	Pubescens outside. White villous inside.
Corolla lobes	3 upper and 2 lower. The middle upper lobe is rounded.	3 upper and 2 lower. The middle upper lobe is emarginate.

Floral longevity and opening patterns

Monitoring revealed diurnal flower opening in *L. aromatica* and *L. geoffrayi*, with slight interspecific differences. In *L. aromatica*, flowers opened at 10:00-11:00, reached full opening by 13:00-14:00, closed near sunset (17:00-18:00), and abscised the following day (Figure 4.A). Two patterns of flowering were observed in *L. geoffrayi*: single-opening (65%) and double-opening (35%). Single-opening flowers resembled *L. aromatica*, opening at 10:00-11:00, fully opening by 13:00-14:00, closing at 17:00-18:00, and abscising the next day. Double-opening flowers opened at 11:00-12:00, reached full opening by 13:00-14:00, closed at 17:00-18:00, reopened the next day at 10:00-11:00, again reached full opening by 13:00-14:00, closed at 17:00-18:00, and abscised the following day (Figure 4.B). Despite the relatively short lifespan of individual flowers, the overall flowering period for both species at the population level extended for approximately one month.

Flower longevity, measured as total opening period, averaged 6.31±0.66 h in *L. aromatica* and 6.42±2.45 h in *L. geoffrayi*, with no significant difference between species. (Mann-Whitney U=840.5, *p*=0.114) (Figure 5.A). In *L. geoffrayi*, first-day flower longevity did not differ significantly between single-opening (5.0 h) and double-opening (4.6 h) flowers (Mann-Whitney U=141.5, *p*=0.053) (Figure 5.B). However, the total opening period differed significantly between single-opening (5.0 h) and double-opening (9.0 h) flowers (Mann-Whitney U=22.0, *p*<0.001) (Figure 5.C).

The opening and closing of flowers are significant reproductive traits that regulate pollen removal. Different species display distinct patterns, such as single-day or multi-day opening, nocturnal, or diurnal (van Doorn and van Meeteren 2003). This study shows species-specific differences in *Limnophila* flower opening period and temporal patterns. *L. aromatica* flowers opened in a single day, although some *L. geoffrayi* flowers remain open for up to two days. This extended opening period may enhance opportunities for pollinator visitation and reproductive success (Jensen et al. 2019; Kehrberger and Holzschuh 2019). Additionally, the temporal separation promotes outcrossing and may prolong floral longevity relative to self-pollinating species (Pant et al. 2020), a pattern likely present in other Plantaginaceae species such as *Penstemon penlandii* (Tepedino et al. 1999). In contrast, self-fertilizing hermaphroditic plants often evolve shorter floral longevity due to reduced reliance on pollinator attraction and the advantage of conserving floral maintenance resources (Xu 2020). Although individual flowers in these species may remain open for only 1-2 days, the abundance of simultaneously open flowers within a population suggests an ecological trade-off, that they may maximize pollination success through temporal floral density.

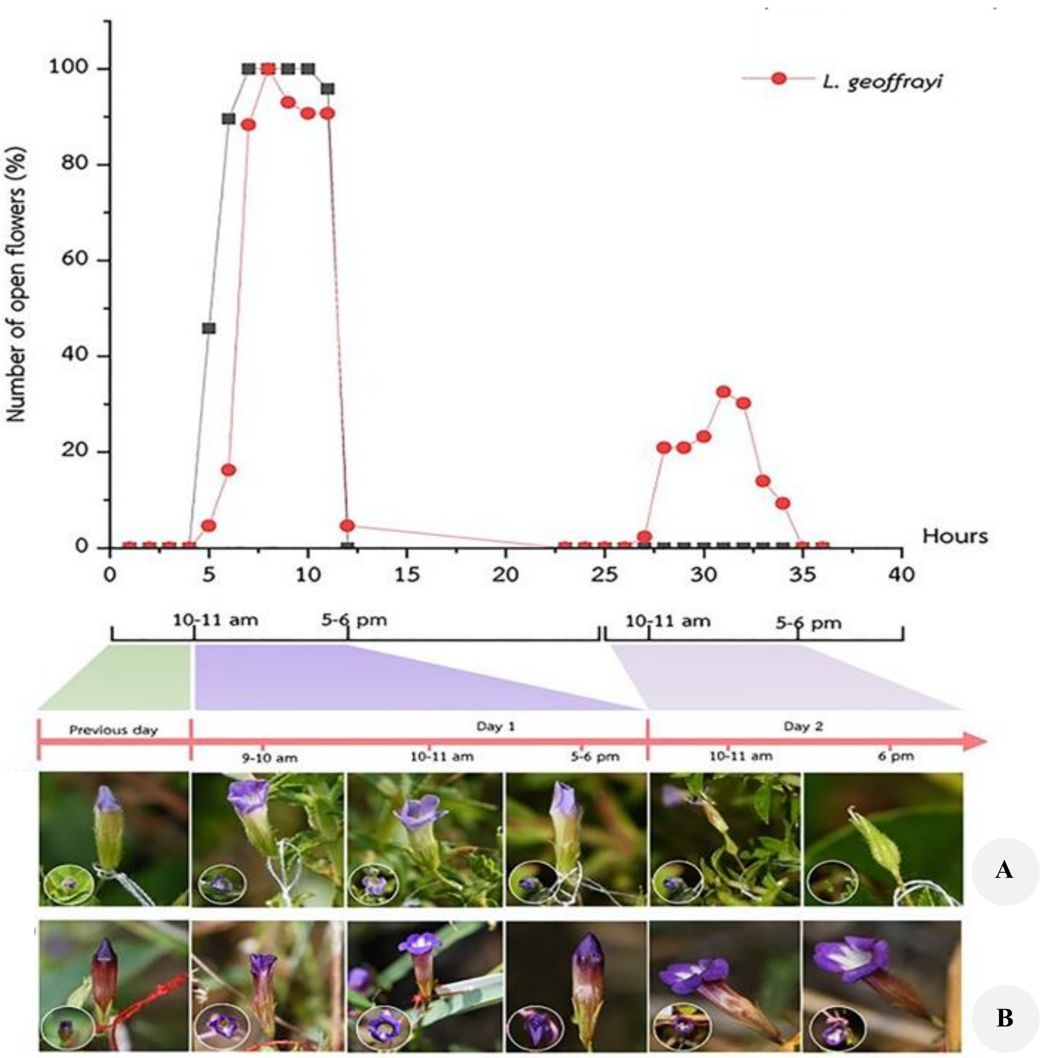
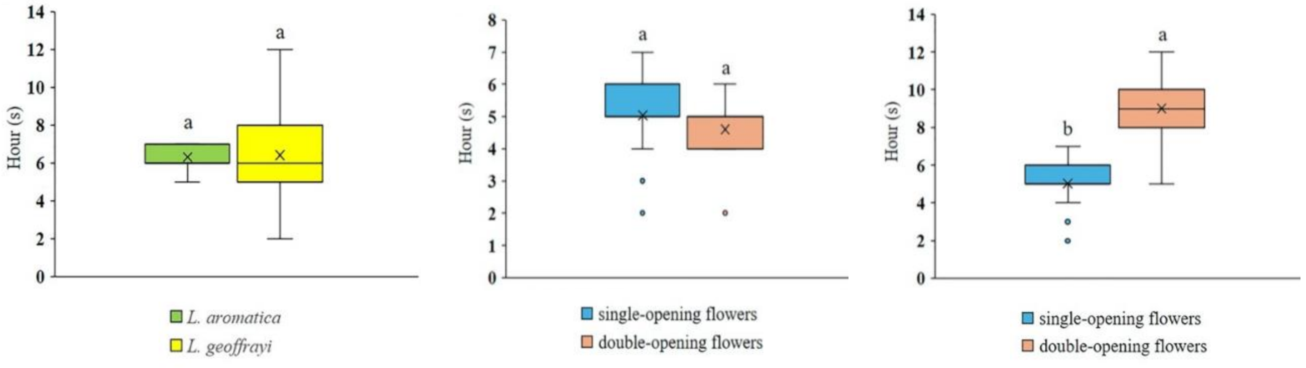


Figure 4. Comparative percentage of open flowers (top), A. opening patterns of *Limnophila aromatica* (middle), B. opening patterns of *L. geoffrayi* (bottom) in the floral opening period



A B C

Figure 5. A. Comparison of total flower longevity between *Limnophila aromatica* (n = 48) and *L. geoffrayi* (n = 43). B. First-day flower longevity and C. Total flower longevity of single-opening (n = 28) and double-opening (n = 15) flowers of *L. geoffrayi*. Box plots show the 25th-75th percentiles; dots represent outliers, the lines across the box indicate the median, and crosses indicate the mean. The same letters indicate non-significant differences based on the Mann-Whitney U test

Breeding system and fruit set

The responses of *L. aromatica* and *L. geoffrayi* to two pollination treatments, autogamy and natural pollination, are summarized in Table 2. *L. aromatica* did not produce fruit in either condition despite visible ovules, indicating a self-incompatible breeding mechanism. Since this plant is only found for cultivation and is not native to Thailand, fruit set observations were restricted to individuals grown from stem cuttings of a single genotype. These clones repeatedly failed to initiate fruit development, emphasizing the limited ability to reproduce sexually in the experimental setting and reinforcing self-incompatibility. Conversely, *L. geoffrayi* showed self-compatibility by exhibiting a consistent and complete fruit set (100%) under both autogamy and natural pollination.

To date, the breeding systems of *Limnophila* species remain largely undocumented. This study provides the first experimental evidence of contrasting reproductive strategies in *L. aromatica* and *L. geoffrayi*. Observations of anthers at the late flower-bud stage revealed no evidence of cleistogamous self-pollination, pollination occurring within unopened buds (Suetsugu et al. 2023). For *L. aromatica*, despite floral traits that promote self-pollination, no fruit set was observed under autogamy treatment including natural condition. These results strongly suggest a self-incompatible breeding system, in which seed production fails despite both male and female organs being functionally fertile. Such self-incompatibility may arise from pollen tube inhibition at the stigma surface or during style elongation, preventing successful fertilization (Li et al. 2023). Moreover, the absence of fruit production in clonal individuals supports the hypothesis of a narrow genetic base, which has been associated with poor sexual reproductive potential in other self-incompatible species (Wang et al. 2018). Reliance on a single genotype poses a significant risk to sustainable cultivation in Thailand, as self-incompatibility limits seed production and genetic resilience. Enhancing genetic variation through hybridization among genetically distinct clones could be a viable strategy to overcome this limitation and improve seed yield in cultivated populations (Guo et al. 2023).

The native Thai species, *L. geoffrayi*, on the other, hand demonstrated self-compatibility by consistently producing a high fruit set under both autogamy and natural pollination. This is consistent with other Plantaginaceae members that frequently display self-pollinating processes, such as *Digitalis*, *Ourisia*, *Parahebe* (syn. *Veronica*), *Antirrhinum*, and *Linaria*. Self-compatibility provides reproductive assurance, particularly in situations when pollinators are limited, although it tends to reduce genetic variation compared to outcrossing species (Ling et al. 2017; Zhang et al. 2024). To preserve genetic variety and advance the long-term sustainability of *L. geoffrayi*, seed banks with material from several populations must be established.

Floral visitors and pollination behavior

Solitary bees from the Hymenoptera order, which collect pollen and come into contact with the plant reproductive organs, were the main visitors to both *L. aromatica* and *L. geoffrayi* flowers. Other insect orders, Thysanoptera and Coleoptera, were rarely observed and did not engage in pollen collection. A total of 84 individual insects visited the flowers of *L. aromatica*, while 60 individual insects visited the flowers of *L. geoffrayi*. The frequency of visitors to *L. aromatica* flowers was 2.33 insects per hour, while *L. geoffrayi* was 1.67 insects per hour. Visitor assemblage similarity between the two species, measured by Sørensen's similarity index, was 47.06%. Shared visitors included *Lasioglossum vagans*, *Lasioglossum albescens, Thrips* sp., and *Braunsapis puangensis* (Table 3). The number and frequency of flower visits to *L. aromatica* in the cultivated plot were higher than *L. geoffrayi* in the natural habitat. The main visitors of both *Limnophila* species were pollen-collecting bees, which were also observed collecting pollen from other co-flowering species such as *Xyris* sp., *Ipomoea aquatica, Murdannia* sp., and *Lobelia thorelii* in the natural habitat of *L. geoffrayi* (Figure 6).

Table 2. *L. aromatica* and *L. geoffrayi* fruit set and treatments for pollination

Species	Pollination treatments	Number of flowers	Number of fruits	Fruit set (%)
L. aromatica	Autogamy	30	0	0
	Natural pollination	30	0	0
L. geoffrayi	Autogamy	30	30	100
	Natural pollination	30	30	100

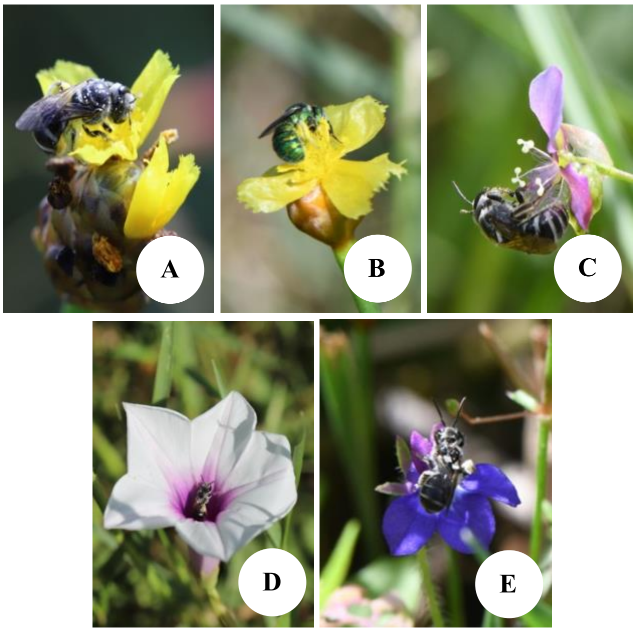


Figure 6. Some of the flowering plants in the study plots of *Limnophila geoffrayi* and solitary bees that forage in the flowers. A. *Lasioglossum albescens* on *Xyris* sp. flower, B. *Ceratina smaragdula* on *Xyris* sp. flower, C. *Lasioglossum* sp. on *Ipomoea aquatica* flower, D. *Lasioglossum albescens* on *Murdannia* sp. flower, E. *Lasioglossum* sp. on *Lobelia thorelii* flower

The activity period of the visitors spanned from 09:00 to 16:00, with peak visitation occurring between 11:00-12:00 (Figure 7), coinciding with the flower opening period of both *Limnophila* species and aligning with a previous study (Lim et al. 2025). The typical duration of a single visit was 3-12 sec (mean 10.76±11.36 s). The availability of alternative floral resources likely reduced the visitation frequency to *L. geoffrayi*. Specifically, insects visited *L. geoffrayi* much less frequently than *L. aromatica*. This low visiting rate is a key factor for self-pollination in *L. geoffrayi.* Single species cultivated plots appear to offer greater opportunities for insect visitation than natural habitats, where multiple species offer a variety of options.

Solitary bees were observed landing on the lower lobe of the corolla and clawing into the corolla tube, stepping on the anthers and stigma, with the lower pair of stamens facilitating deeper entry into the tube (Figures 8 and 9). Pollen-collecting bees frequently carried pollen grains in the scopa, and some pollen was observed attached to their legs, head, and other portions of their body prior to entering the corolla.

Limnophila, now placed in Plantaginaceae (tribe Gratioleae), was formerly included in Scrophulariaceae (Stevens 2025). Gratioleae flowers typically have open, bell-shaped corollas with stigma and anthers positioned adaxially, promoting mainly nototribic pollination (dorsal contact), although sternotribic pollination can occur when bees enter upside down (Kampny 1995). In contrast, our field observations of *L. aromatica* and *L. geoffrayi* consistently showed stamens and stigma on the abaxial side of the corolla tube, indicating a different configuration that may represent a derived condition within the tribe and warrants broader comparative study across Gratioleae. Solitary bees entering the corolla tube likely transfer pollen via ventral contact with reproductive organs (sternotribic pollination). Dense internal trichomes on one side of the tube appear to provide footholds for opportunistic visitors, facilitating general access rather than indicating specialized pollination adaptations (Kampny 1995).

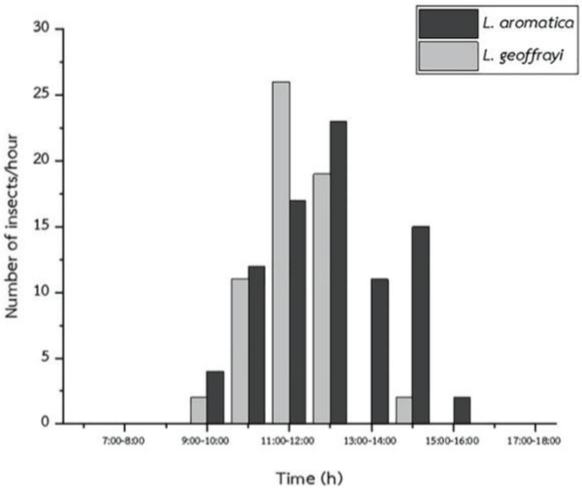


Figure 7. Number of insects visiting the flowers of *L. aromatica* and *L. geoffrayi* from 06:00 to 18:00, for 3 days (36 h) of the flowering season

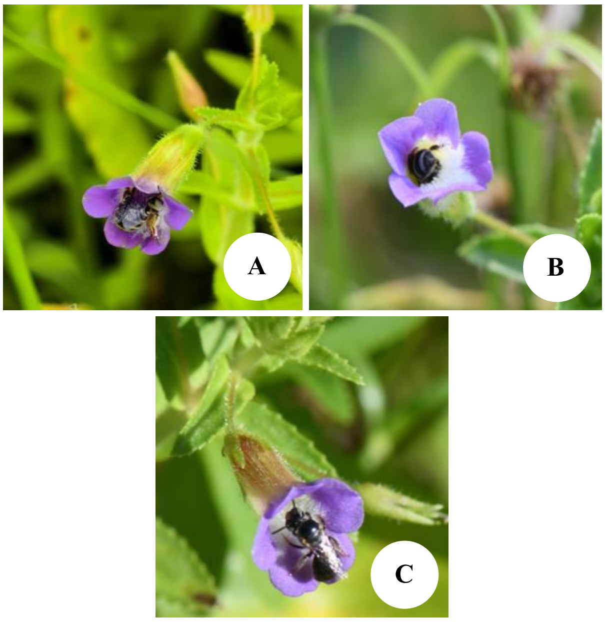


Figure 8. The foraging behaviors of solitary bees in the flowers of *Limnophila aromatica*, solitary bees insert their head into the flower and collect pollen. A. *Pseudapis siamensis*, B. *Lasioglossum* sp., C. *Braunsapis* sp.

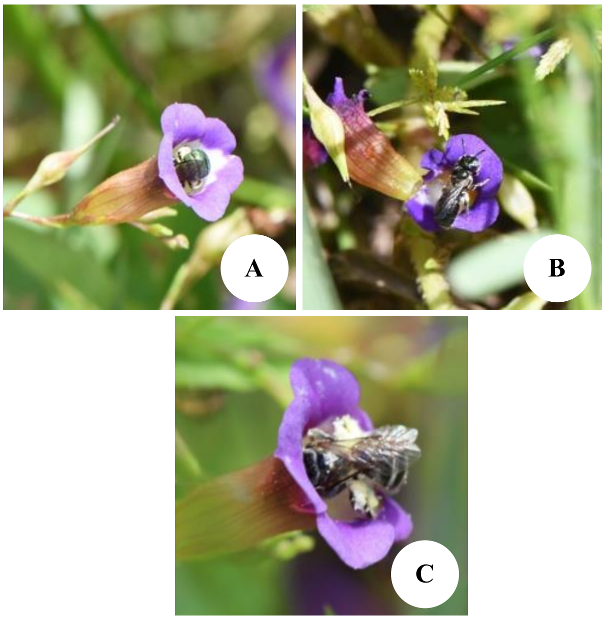
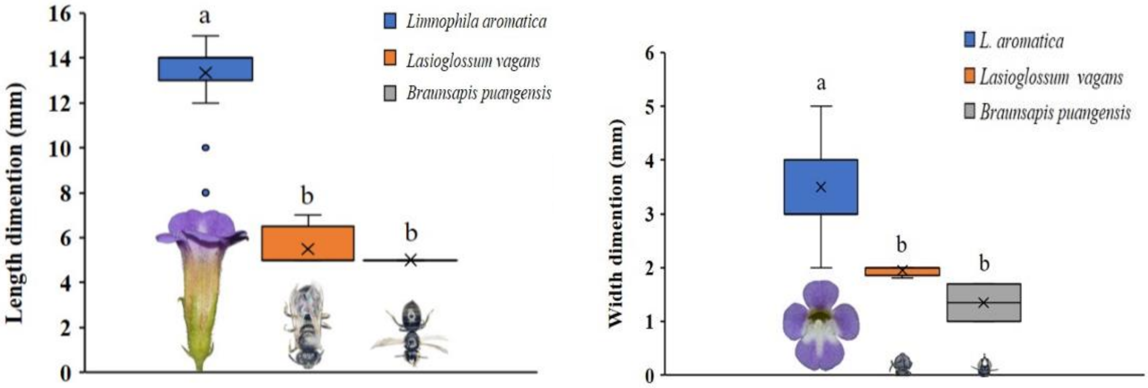


Figure 9. The foraging behaviors of solitary bees in the flowers of *Limnophila geoffrayi*, solitary bees insert the head into the flower, collect pollen. A. *Ceratina smaragdula*, B. *Braunsapis* sp., C. *Lasioglossum* sp.

Table 3. Insect visits of *Limnophila aromatica* (botanical garden) compared with *L. geoffrayi* (rice fields)

Orders	Families	Species	*L. aromatica*	*L. geoffrayi*
Coleoptera	Coccinellidae	*Micraspis* sp.		x
	Chrysomelidae	*Monolepta* sp.		x
Hymenoptera	Andrenidae	Unknown		x
	Apidae	*Ceratina dentipes* (Friese, 1914)		x
		Ceratina smaragdula (Fabricius, 1787)		x
		Braunsapis hewitti (Cameron, 1908)	x	
		Braunsapis malliki (Reyes, 1991)	x	
		Braunsapis puangensis (Cockerell, 1929)*	x	x
	Halictidae	*Ceylalictus* sp.		
		Lasioglossum albescens (Smith, 1853)*	x	x
		Lasioglossum vagans (Smith, 1857) gr.*	x	x
		Pseudapis siamensis (Cockerell, 1929)	x	
	Magachilidae	*Heriades* sp.		x
Thysanoptera	Thripidae	*Thrips* sp.*	x	x

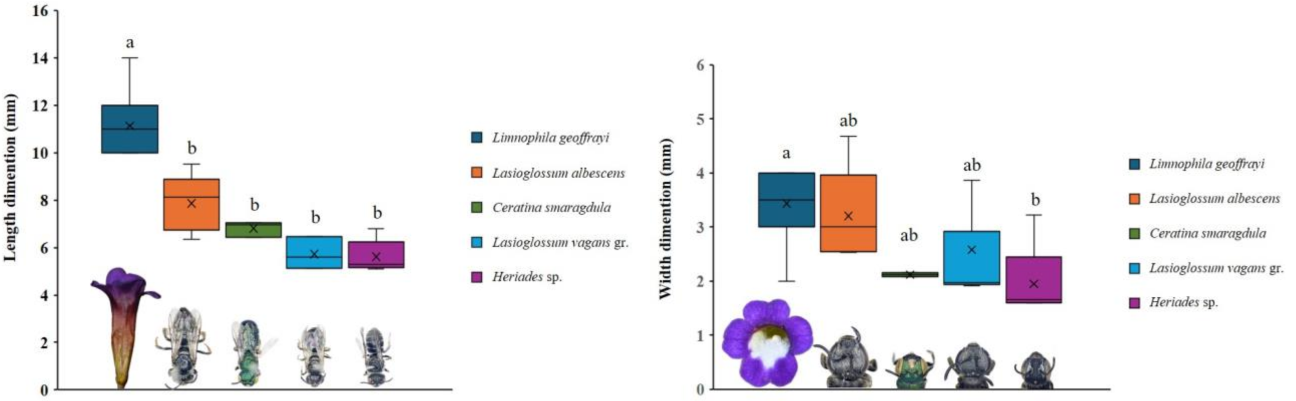
Note: * Means the species found in both *Limnophila* flowers



A

B

Figure 10. Comparison of pollination dimensions among plant species *Limnophila aromatica* (n = 30) and its visitors, *Lasioglossum vagans* (n = 4) and *Braunsapis puangensis* (n = 2). The dimensions measured were length (top) and width (bottom). Box plots display the 25th to 75th percentiles; dots represent outliers, the lines inside the boxes indicate the median, and crosses represent the mean. Kruskal-Wallis Test was used for analysis of variance. Identical letters denote non-significant differences based on the Mann-Whitney U test with Bonferroni correction, where the significance level was adjusted from $p<0.05$ to $p<0.0167$



A

B

Figure 11. Comparison of pollination dimensions among plant species *Limnophila geoffrayi* (n = 30) and its visitors, *Lasioglossum albescens* (n = 5), *Ceratina smaragdula* (n = 3), *Lasioglossum vagans* (n = 3) and *Heriades* sp. (n = 5). The dimensions measured were length (top) and width (bottom). Box plots display the 25th to 75th percentiles; dots represent outliers, the lines inside the boxes indicate the median, and crosses represent the mean. Kruskal-Wallis Test was used for analysis of variance. Identical letters denote non-significant differences based on the Mann-Whitney U test with Bonferroni correction, where the significance level was adjusted from $p<0.05$ to $p<0.005$

Floral and insect morphometrics

In *L. aromatica*, the floral chamber averaged 13.33±1.40 mm in length (n = 30) and 3.50±0.73 mm in width. Visitor body lengths were 5.50±1.00 mm for *L. vagans* and 5.00±0.00 mm for *B. puangensis*, with no significant difference between them. The chamber was significantly longer than both visitors, while its width (3.43±0.63 mm) exceeded their body widths (*L. vagans* 1.95±0.10 mm, *B. puangensis* 1.35±0.49 mm), which also did not differ significantly (Figure 10).

In *L. geoffrayi*, the average floral chamber length was 11.13±1.11 mm (n = 30). The main floral visitors of *L. geoffrayi* were *L. albescens*, *Ceratina smaragdula*, *L. vagans* gr., and *Heriades* sp., with body lengths of 7.87±1.21, 6.81±0.34, 5.73±0.68, and 5.61±0.70 mm, respectively. The floral chamber was longer than all visitor body lengths, with no significant differences among the insects. The chamber width averaged 3.43±0.63 mm (n = 30). The body widths of *L. albescens*, *C. smaragdula*, *L. vagans*, and *Heriades* sp. were 3.20±0.88, 2.12±0.04, 2.58±1.11, and 1.95±0.71 mm, respectively. Only *Heriades* sp. showed a smaller significant mismatch in width compared to the floral chamber (Figure 11). According to morphometric comparisons, *L. geoffrayi* shows closer size matching with its bee visitors except *Heriades* sp., which is consistent with effective pollination and high fruit set, while *L. aromatica* displays mismatches between floral chamber dimensions and visitor body size, suggesting limited pollination efficiency and possible pollen theft. Comparing the size matching between flowers and visitors allows us to understand which bee species are likely to function as pollinators or as pollen thieves, in the ecosystem (Solís-Montero and Vallejo-Marín 2017; Ornai and Keasar 2020; Opedal 2023). This study was constrained by the limited number of insect samples, resulting from the low frequency of insect visitation during the experiment. Consequently, the data provided only preliminary insights into insect and flower size.

In conclusion, this study provides the first insights into the reproductive biology of two edible *Limnophila* species, *L. aromatica* and *L. geoffrayi*. Despite similar floral morphologies that appear to favor self-pollination, their breeding systems differ. *L. aromatica* is self-incompatible, producing no fruit or seed after selfing, while *L. geoffrayi* is self-compatible and capable of autogamous self-pollination, resulting in substantial seed set. Both species are mainly visited by solitary bees that collect pollen. *L. geoffrayi* may function as a potential adaptation or resilience mechanism in the face of habitat disruption and herbicide use because it is an autonomously self-pollinating species that produces a large number of seeds. Seed banking is necessary to assist the conservation and sustainable usage of this annual herbaceous plant, which comes in a variety of colors. Meanwhile, overcoming self-incompatibility in *L. aromatica* may increase its cultivation potential. Future studies should focus on genetic diversity evaluations of *L. geoffrayi* populations, as well as experimental ways to improve seed production in *L. aromatica*, in order to support conservation initiatives and the long-term usage of these culturally and commercially vital herbs.

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REFERENCES

Abrahamczyk S, Thielen M, Weigend M. 2021. Frequency of flower visitors and achene production increase with rising population size in the self-incompatible herb *Centaurea scabiosa* (Asteraceae). Plant Ecol 222: 613-623. https://doi.org/10.1007/s11258-021-01129-1.

Cheptou PO. 2024. The evolutionary ecology of inbreeding depression in wild plant populations and its impact on plant mating systems. Front Plant Sci 15: 1359037. https://doi.org/10.3389/fpls.2024.1359037.

Christopher AO. 2020. Comparative analyses of diversity and similarity indices of west bank forest and block A forest of the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State, Nigeria. Intl J For Res 2020: 4865845. https://doi.org/10.1155/2020/4865845.

Far AJ, Cursach J. 2022. Reproductive biology insights for the conservation of *Euphorbia fontqueriana*, a rare endangered plant species from the western Mediterranean Basin. Plant Biosyst 157 (1): 129-139. https://doi.org/10.1080/11263504.2022.2089767.

Geissler C, Davidson A, Niesenbaum RA. 2023. The influence of climate warming on flowering phenology in relation to historical annual and seasonal temperatures and plant functional traits. PeerJ 11: e15188. https://doi.org/10.7717/peerj.15188.

Goolmeer T, Skroblin A, Grant C, van Leeuwen S, Archer R, Gore-Birch C, Wintle BA. 2022. Recognizing culturally significant species and Indigenous-led management is key to meeting international biodiversity obligations. Conserv Lett 15 (6): e12899. https://doi.org/10.1111/conl.12899.

Guo S, Ji P, Wang J, He Y, Zhang Y, Zhang F, Yun Y, Zhang G. 2023. Estimation of genetic diversity between and within biparental clones and full-sib families of the Chinese pine using SSR markers. Horticulturae 9 (11): 1205. https://doi.org/10.3390/horticulturae9111205.

Inouye BD, Ehrlén J, Underwood N. 2019. Phenology as a process rather than an event: From individual reaction norms to community metrics. Ecol Monogr 89 (2): e01352. https://doi.org/10.1002/ecm.1352.

Jensen AM, Schamp BS, Belleau A. 2019. Evidence of temporal niche separation via low flowering time overlap in an old-field plant community. Oecologia 189 (4): 1071-1082. https://doi.org/10.1007/s00442-019-04386-0.

Kalisz S, Vogler D, Fails B, Finer M, Shepard E, Herman T, Gonzales R. 1999. The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). Am J Bot 86 (9): 1239-1247. https://doi.org/10.2307/2656771.

Kampny CM. 1995. Pollination and flower diversity in Scrophulariaceae. Bot Rev 61: 350-366. https://doi.org/10.1007/BF02912622.

Kato M, Kosaka Y, Kawakita A, Okuyama Y, Kobayashi C, Phimminith T, Thongphan D. 2008. Plant-pollinator interactions in tropical monsoon forests in Southeast Asia. Am J Bot 95 (11): 1375-1394. https://doi.org/10.3732/ajb.0800114.

Kehrberger S, Holzschuh A. 2019. How does timing of flowering affect competition for pollinators, flower visitation and seed set in an early spring grassland plant? Sci Rep 9 (1): 15593. https://doi.org/10.1038/s41598-019-51916-0.

Kline O, Joshi NK. 2020. Mitigating the effects of habitat loss on solitary bees in agricultural ecosystems. Agriculture 10 (4): 115. https://doi.org/10.3390/agriculture10040115.

Kuswantoro F. 2017. Flower-insect visitor interaction: Case study on *Rhododendron inundatum* Sleumer in Bali Botanic Garden. J Trop Biodiv Biotech 2: 35-38. https://doi.org/10.22146/jtbb.25443.

Laha S, Chatterjee S, Das A, Smith BM, Basu P. 2020. Non-crop floral traits as determinants of bee visitation in a tropical agricultural landscape. Proc Zool Soc 73 (4): 441-445. https://doi.org/10.1007/s12595-019-00302-9.

Layek U, Das U, Karmakar P. 2022. The pollination efficiency of a pollinator depends on its foraging strategy, flowering phenology, and the flower characteristics of a plant species. J Asia-Pac Entomol 25 (2): 101882. https://doi.org/10.1016/j.aspen.2022.101882.

Li Y, Mamonova E, Köhler N, van Kleunen M, Stift M. 2023. Breakdown of self-incompatibility due to genetic interaction between a specific *S*-allele and an unlinked modifier. Nat Commun 14: 3420. https://doi.org/10.1038/s41467-023-38802-0.

Lim Z, Lord J, Johnson S. 2025. Understanding foraging and nesting behaviour of ground nesting bees in Dunedin, New Zealand. N Z J Ecol 49 (1): 3579. https://doi.org/10.20417/nzjecol.49.3579.

Ling TC, Wang LL, Zhang ZQ, Dafni A, Duan YW, Yang YP. 2017. High autonomous selfing capacity and low flower visitation rates in a subalpine population of *Prunella vulgaris* (Lamiaceae). Pl Ecol Evol 150 (1): 59-66. https://doi.org/10.5091/plecevo.2017.1274.

Opedal Øystein H. 2023. A functional view of flower-pollinator trait matching. A commentary on 'mismatching explained: Constricted corolla tubes in Roscoea elevate the nectar'. Ann Bot 132 (6): 5-7. https://doi.org/10.1093/aob/mcad162.

Ornai A, Keasar T. 2020. Floral complexity traits as predictors of plant-bee interactions in a Mediterranean pollination web. Plants (Basel) 9 (11): 1432. https://doi.org/10.3390/plants9111432.

Pant P, Singh VP, Panwar R, Jat R. 2020. Dichogamy and its relevance in fruit crops: An overview. Intl J Curr Microbiol App Sci 9 (8): 698-708. https://doi.org/10.20546/ijcmas.2020.908.076.

Philcox D. 1970. A taxonomic revision of the genus *Limnophila* R. Br. (Scrophulariaceae). Kew Bull 24 (1): 101-170. https://doi.org/10.2307/4103255.

POWO. 2025. Plants of the World Online. Royal Botanic Gardens, Kew. https://powo.science.kew.org/.

Punchay K, Inta A, Tiansawat P, Balslev H, Wangpakapattanawong P. 2020. Traditional knowledge of wild food plants of Thai Karen and Lawa (Thailand). Genet Resour Crop Evol 67: 1277-1299. https://doi.org/10.1007/s10722-020-00910-x.

Qi Y, Li J, Guan X, Yan B, Fu G, He J, Du L, Zhao C, Zhang D. 2020. Effects of herbicides on non-target plant species diversity and the community composition of fallow fields in northern China. Sci Rep 10: 9967. https://doi.org/10.1038/s41598-020-67025-2.

Ren YQ, Xu YP, Zhang T, Ma YP, Marczewski T. 2016. Growth discrepancy between filament and style facilitates self-fertilization in *Brandisia hancei* (Paulowniaceae). Plant Species Biol 31 (2): 153-158. https://doi.org/10.1111/1442-1984.12099.

Schlessman MA. 1986. Floral protogyny, self-competibility, and the pollination of *Ourisia macrocarpa* (Scrophulariaceae). N Z J Bot 24 (4): 651-656. https://doi.org/10.1080/0028825X.1986.10409948.

Schmid R. 1976. Fly pollination of *Penstemon davidsonii* and *P. procerus* (Scrophulariaceae). Madroño 23 (7): 400-402. https://doi.org/jstor.org/stable/41424070.

Serna-González M, Urrego-Giraldo LE, Santa-Ceballos JP, Suzuki-Azuma H. 2022. Flowering, floral visitors and climatic drivers of reproductive phenology of two endangered magnolias from neo-tropical Andean forests. Plant Species Biol 37 (1): 20-37. https://doi.org/10.1111/1442-1984.12351.

Solís-Montero L, Vallejo-Marín M. 2017. Does the morphological fit between flowers and pollinators affect pollen deposition? an experimental test in a buzz-pollinated species with anther dimorphism. Ecol Evol 7 (8): 2706-2715. https://doi.org/10.1002/ece3.2897.

Stevens PF. 2025. Angiosperm Phylogeny. University of Missouri, St Louis, and Missouri Botanical Garden http://www.mobot.org/MOBOT/research/APweb/.

Suetsugu K, Fukushima K, Makino T, Ikematsu S, Sakamoto T, Kimura S. 2023. Transcriptomic heterochrony and completely cleistogamous flower development in the mycoheterotrophic orchid *Gastrodia*. New Phytol 237 (1): 323-338. https://doi.org/10.1111/nph.18495.

Tepedino VJ, Sipes SD, Griswold TL. 1999. The reproductive biology and effective pollinators of the endangered beardtongue *Penstemon penlandii* (Scrophulariaceae). Pl Syst Evol 219: 39-54. https://doi.org/10.1007/BF01090298.

van Doorn WG, Van Meeteren U. 2003. Flower opening and closure: A review. J Exp Bot 54 (389): 1801-1812. https://doi.org/10.1093/jxb/erg213.

Walsh SK, Pender RJ, Junker RR, Daehler CC, Morden CW, Lorence DH. 2019. Pollination biology reveals challenges to restoring populations of *Brighamia insignis* (Campanulaceae), a critically endangered plant species from Hawai'i. Flora 259: 151448. https://doi.org/10.1016/j.flora.2019.151448.

Wang Z, Xie L, Prather CM, Guo H, Han G, Ma C. 2018. What drives the shift between sexual and clonal reproduction of *Caragana stenophylla* along a climatic aridity gradient? BMC Plant Biol 18 (1): 91. https://doi.org/10.1186/s12870-018-1313-6.

Xu K. 2020. The coevolution of flower longevity and self-fertilization in hermaphroditic plants. Evolution 75 (8): 2114-2123. https://doi.org/10.1111/evo.14303.

Yamazaki T. 1990. Scrophulariaceae. In: Smitinand T, Larsen K (eds). Flora of Thailand. The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand.

Zhang D, Li YY, Zhao X, Zhang C, Liu DK, Lan S, Yin W, Liu ZJ. 2024. Molecular insights into self-incompatibility systems: from evolution to breeding. Plant Commun 5 (2): 100719. https://doi.org/10.1016/j.xplc.2023.100719.

Zulkarnain Z, Eliyanti E, Swari EI. 2019. Pollen viability and stigma receptivity in *Swainsona formosa* (G.Don) J.Thompson (Fabaceae), an ornamental legume native to Australia. Ornam Hortic 25 (2): 158-167. https://doi.org/10.14295/oh.v25i2.2011.