Variation in floral morphology, histochemistry, and floral visitors of three sympatric morning glory species

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Abstract

Three morning glory species in the genus *Argyreia* Lour., *A. lycioides* (Choisy) Traiperm & Rattanakrajang, *A. mekongensis* Gagnep & Courchet, and *A. versicolor* (Kerr) Staples & Traiperm, were found co-occurring and co-flowering. ~~Two of these species (~~*A. mekongensis* and *A. versicolor*) are rare and endemic to Thailand, while ~~the third (~~*A. lycioides*) has not yet been evaluated. We investigate key floral characters (floral morphology and phenology, as well as the micromorphology of the floral nectary disc and staminal trichomes) and screened for important chemical compounds hypothesized to contribute to pollinator attraction. Our results found some overlap among the three study species in terms of floral visitors. However, pollinator composition appears to be influenced by floral shape and size; morning glory species with wider corolla tubes were pollinated by larger bees. The morphology of the floral nectary disc was similar in all species while variation in staminal trichomes was observed across species. Glandular trichomes were found in all three species, while non-glandular trichomes were found only in *A. versicolor*. Histochemical results revealed different compounds in the floral nectary and staminal trichomes of each species, which contribute to both floral attraction and defense. These findings demonstrate some segregation

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Comentado [JG2]: I understand those flower characteristics will improve pollinator attraction, but I think it will be better to mention first some results about the flower characters evaluated, and Later you will talk about visitors' composition

of floral visitors among sympatric co-flowering morning glory species, which appears to be influenced by the macro- and micromorphology of flowers and their chemical compounds. Moreover, understanding the floral morphology and chemical attractants of these sympatric co-flowering *Argyreia* spp. may help to maintain their common pollinators in order to conserve these rare and endangered species, especially *A. versicolor*.

Keywords: *Argyreia*, Biodiversity, Convolvulaceae, Histochemistry, Plant Conservation, Pollinator, Trichome, *Xylocopa*

Introduction

Much of floral evolution is driven by pollinator attraction (Fenster et al., 2004) and pollination efficiency (Stewart et al., 2022). To attract pollinators, floral morphology is one prominent and important feature (Bobisud & Neuhaus, 1975; Schemske, 1981), including flower size, color, scent, and phenology (Rathcke, 1983; Waser & Price, 1983; Spaethe, Tautz & Chittka, 2001; Waser & Ollerton, 2006; Willmer, 2011; Hassa, Traiperm & Stewart, 2020, 2024). For example, large flowers tend to be favored and selected for by insects since they are more visible (Chittka & Raine, 2006; Naug & Arathi, 2007; Benitez-Vieyra et al., 2010) and reduce search time during pollinator foraging (Spaethe, Tautz & Chittka, 2001). Similarly, Thompson (2001) demonstrated the importance of floral size or display on variation in visitation patterns between different insect types; hawkmoths and butterflies both preferred larger displays. Floral color is another important trait for pollinator attraction since different species have different spectral receptor cells; for example, hummingbirds tend to select red flowers because of high chromatic contrast to the background (Herrera et al., 2008), whereas bees are uncommon visitors to red flowers given their lower sensitivity to red wavelengths (Bergamo et al., 2016). Flowering time and floral phenology also influence

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Comentado [JG4]: Large floral displays? Or large flowers? please review

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pollination and reproductive success (Evans, Smith & Gendron, 1989; Elzinga et al., 2007). Phenology has been shown to affect pollinator variation and effectiveness, which can impact fitness, as has been demonstrated through reduced seed mass throughout the flowering season (Gallagher & Campbell, 2020).

While floral morphology tends to be highly prominent, there are other floral traits that are less visible, yet are also important in plant-pollinator interactions. For example, the floral nectary is an important floral organ as it provides the primary reward for many pollinators~~,~~ ~~nectar~~ (Irwin et al., 2010; Pacini & Nicolson, 2007). The floral nectary is important not only in terms of providing food resources for pollinators (Simpson & Neff, 1981; Proctor, Yeo & Lack, 1996; Neiland & Willcock, 1998; Nicolson, 2007) but also for manipulating pollinator behavior (Bailey et al., 2007). However, nectar not only attracts ~~beneficial pollinators,~~ ~~but~~pollinators but can also lead to visits by less beneficial species at the same time. Consequently, many plant species have evolved features that only allow visitation by specific pollinators (e.g., pollination syndromes; Fenster et al., 2004); nectar production is one trait that has an important role in confining the range of visitors to species that benefit the plants (Irwin, Adler & Agrawal, 2004). While floral morphology is an important component to predicting pollination syndromes, other characters should also be considered, such as flowering phenology, anthesis start time and duration, and chemical compounds in nectar that are certainly associated with pollination and pollinator activities (Southwick, Loper & Sadwick, 1981; Baker & Baker, 1983; Pleasants, 1983; Waser et al., 1996; Galetto & Bernardello, 2005; Ollerton et al., 2009; Bobrowiec & Oliveira, 2012). Additionally, macroevolutionary studies can reveal associations between nectar traits and pollinator types, for example, finding similar nectar properties in plants that are visited by the same pollinators (Faegri & van der Pijl, 1979; Proctor, Yeo & Lack, 1996). Floral chemistry can mediate

Comentado [JG6]: Could you please explain, if these less beneficial visitors are secondary pollinators, antagonistic, or what kind of behavior have these insects?
Also, include reference

Comentado [JG7]: Please review redaction interactions with pollinators, pathogens, and/or herbivores, and are therefore influence plant fitness (Strauss & Whittall, 2006; Irwin et al., 2010; Good et al., 2014).

Another plant organ important in mediating plant-animal interactions is the trichome. Trichomes initially originate from expansions or appendages of the epidermis (Evert, 2006), and have diverse biological functions, such as in herbivore defense, pollinator attraction, or tissue protection and maintenance (Nihoul, 1993; Van Dam & Hare, 1998; Kennedy, 2003; Moyano, Cocucci & Sersic, 2003; Simmons & Gurr, 2005; Liu et al., 2006; Horgan et al., 2007; Gonzalez et al., 2008; Romero Souza & Vasconcellos-Neto, 2008; Nonomura et al., 2009; Kang et al., 2010; Karabourniotis et al., 2020), which may be due to the synthesis and storage of biologically active metabolites (Alonso et al., 1992; Antonious, 2001; Iijima et al., 2004; Siebert, 2004; Deschamps et al., 2006; Nagel et al., 2008; Wang et al., 2008; Biswas, Foster & Aung, 2009; Sallaud et al., 2009; Luo et al., 2010). Trichomes are typically found on vegetative and reproductive organs, such as leaves, stems, petals, petioles, peduncles, and seeds (Wagner, Wang & Shepherd, 2004). Trichomes can also be found on staminal filaments, although they have been less studied and their function in many cases is still unclear. Staminal trichomes have been reported in five species in the genus *Teucrium* L. (Lamiaceae) (Bini Maleci & Servettaz, 1991) and in some species of *Argyreia* Lour. (van Ooststroom, 1943, 1945, 1950, 1952; Hoogland, 1952; van Ooststroom & Hoogland, 1953; Chitchak et al., 2018; Chitchak, 2019) and *Rivea* Choisy (Chitchak, Stewart & Traiperm, 2022). Staminal trichomes have been proposed to contribute to pollinator attraction (Jirabanjongjit et al., 2021; Chitchak, Stewart & Traiperm, 2022).

Floral traits such as color, size, shape, and the chemical composition of the nectary and staminal trichomes can all influence floral visitors. Therefore, the objectives of this study were to study and compare how these traits influence floral visitor composition in three sympatric species of *Argyreia* ~~Lour.~~: *A. lycioides* ~~(Choisy) Traiperm & Rattanakrajang~~, *A.*

Comentado [JGGF8]: please do not repeat species authors, I suggest just mentioning them in Methods *mekongensis* Gagnep & Courchet, and *A. versicolor* (Kerr) Staples & Traiperm. Two of these species, *A. mekongensis* and *A. versicolor*, are rare and endemic to Thailand, especially the latter, which is extremely rare and near extinction (Staples et al. 2021; Jirabanjongjit et al. 2024). All three species are naturally found in the Watthana Nakhorn district of Sa Kaeo province, Thailand, and they have nearly identical flowering periods. The study area is found in eastern Thailand, has a tropical climate, and is classified as a lowland watershed with undulating plains at around 74 m above sea level. Human settlements are interspersed with natural habitat, and natural areas are primarily covered with deciduous dipterocarp forest that occasionally experiences fire forests.

Materials & Methods

Study species and sample collection

Three species of sympatric *Argyreia* Lour. were collected in this study. *Argyreia mekongensis* Gagnep & Courchet and *A. versicolor* (Kerr) Staples & Traiperm are woody perennial twiner (Staples & Traiperm, 2010), while *A. lycioides* (Choisy) Traiperm & Rattanakrajang is woody shrub (Staples, 2010; Rattanakrajang, Traiperm & Staples, 2018; Rattanakrajang et al., 2022). The nectaries of all three species surround the ovary at the base of the corolla. In general, five stamens circle the outer edge of the nectary. The bases of the filaments are covered by dense trichomes (i.e., staminal trichomes). Fresh mature flowers (five per species) were collected and kept in 4ºC for histochemical examination.

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Floral characters and flowering phenology

Floral characters were observed and recorded during field work following terminology from Kew glossary (Beentje, 2010) and the Flora of Thailand (Convolvulaceae) (Staples, 2010), as well as from recent studies of the three species (Staples & Traiperm, 2017; Rattanakrajang et

Comentado [JGGF11]: Probably I am lost, pero how long was the fieldwork?

al., 2022). The floral phenology of *A. lycioides* was assessed from field work, herbarium specimens, and relevant literature (Staples, 2010; Rattanakrajang, Traiperm & Staples, 2018; Rattanakrajang et al., 2022). The floral phenologies of *A. mekongensis* and *A. versicolor* were recently observed and published in our earlier study (Jirabanjongjit, et al. 2024).

Floral visitor observation

The floral visitors of *Argyreia mekongensis* and *A. versicolor* have recently been reported (Jirabanjongjit et al., 2024), but no records of the floral visitors of *A. lycioides* were found. We used action cameras (Xiaomi YI Z15, Xiaomi, Beijing, China) placed in front of mature *A. lycioides* flowers to capture animal visits. We did not collect floral visitors to avoid disturbing subsequent animal visits and to avoid damaging flowers with sweep nets. All footage was reviewed and floral visitors were identified to the lowest taxonomic level possible with help from a local entomologist (see Acknowledgments). Permission to work with animals was granted by MUSC-IACUC (Faculty of Science, Mahidol University-Institutional Animal Care and Use Committee) (Protocol numbers MUSC60-037-387 and MUSC63-031-539).

Histochemical examination

Histochemical techniques were used to detect the presence of chemical compounds of interest in the floral nectary discs and staminal trichomes. Nectary discs were free hand-sectioned both transversally and longitudinally. Staminal trichomes were removed from the filament base. Staminal trichome morphology was described based on Chitchak (2019). All sample specimens were treated with the following histochemical assays: NADI reagent to test for terpenes (David & Carde, 1964; Olaranont et al., 2018), Sudan Black B and Sudan III to test for lipids (Brundrett, Kendrick & Peterson, 1991), and Naturstoff to test for flavonoids

(Olaranont et al., 2018; Tattini et al., 2000). Samples stained with NADI reagent, Sudan Black B, and Sudan III were examined under a light microscope (Olympus CX21 equipped with a Sony 6400 digital camera, Tokyo, Japan) and samples stained with Naturstoff were examined under a fluorescent microscope (Olympus BX53 with a DP73 camera set, Waltham, MA, USA) with a 436-nm exciter filter.

Results

Floral characters and flowering phenology

All three sympatric species have a campanulate floral shape but differ in size; flowers of *A. versicolor* and *A. mekongensis* are similar in size and significantly larger than *A. lycioides.* Moreover, both *A. versicolor* (Figure 1A, D) and *A. mekongensis* (Figure 1B, E) produce several flowers per inflorescence and flowers exhibit a corolla limb, while *A. lycioides* (Figure 1C, F) produces axillary solitary flowers that typically lack a corolla limb but are occasionally found with a very small corolla limb. In terms of floral color, *A. versicolor* has a whitish corolla tube with a purple corolla limb, *A. mekongensis* has a pure white corolla tube and limb with small brownish dots scattered across the flower, and *A. lycioides* has a waxy greenish-white tube with a dense concentration of dark purple dots inside the corolla (Figure 1). Additionally, *A. versicolor* and *A. mekongensis* are lianas, while *A. lycioides* is a shrub. Our previous work found that the flowers of *A. versicolor* start to open around 5:00 h, are fully open around 7:00 h, and flowers last until sunset; similarly, the flowers of *A. mekongensis* start to open around 5:00 h, are fully open around 7:00-8:00 h, but flowers last until the evening of the following day (Jirabanjongjit et al., 2024). The flowering periods of both *A. versicolor* and *A. mekongensis* occur from August to December and fruits are mature approximately 12-15 weeks later (Jirabanjongjit et al., 2024). From the observations of this study, flowers of *A. lycioides* generally open around 5.00 h, are fully open around 8.00-9.00

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Comentado [JG14]: This information is different from those in the paper of 2024

Comentado [JGGF15]: I am not sure if this published information can be included in the result section, because they were not obtained in the present study.

Probably is better just to mention the results of the present study, and make a table comparing the data of the three species

Comentado [JG16]: The paper mentioned 10-12 weeks, please review h, and last until the evening of the following day. The flowering period of *A. lycioides* lasts from early September until late October, and fruits are mature approximately 10-12 weeks later.

Comentado [JGGF17]: I understand that anthesis was daily observed from a specific flower number, however, authors do have to show how they made the field observations. Review

Floral visitor observation

The flowers of *A. versicolor* and *A. mekongensis* have the same pollinators: *Xylocopa latipes* and *X. aestuans* (Jirabanjongjit et al., 2024) (Figures 2A-D). The campanulate form of their flowers allows large carpenter bees to enter while foraging on nectar, and the thorax of both *Xylocopa* species was observed to contact stigmas and stamens; pollen was also observed on the thorax of both bee species (Figures 2A-B). A number of other diurnal floral visitors were observed at *A. mekongensis* flowers, including *Amegilla* blue-banded bees (Figure 2E), *Mylabris phalerata* blister beetles (Figure 2F), *Cinnyris jugularis* sunbirds (Figure 2G), Hesperiidae skipper butterflies, and wasps; visits by other animal taxa to *A. versicolor* flowers were extremely uncommon. Several diurnal animal taxa were observed visiting *A. lycioides* flowers, including wasps (Vespidae; Figure 2I), an unknown bee (possibly *Lipotriches* sp.; Figure 2H), several ants (Formicidae), *Cinnyris jugularis* sunbirds (Figure 2J), cockroaches (Blattodea), and skipper butterflies (Hesperiidae). No nocturnal visitors were observed. Vespid wasps were the most frequent visitors. They entered the corolla to forage on nectar, during which their thorax was observed to touch the anthers and stigmas; pollen was observed on their thorax (Figure 2I). The unknown bee species, possibly in the genus *Lipotriches*, was also observed contacting floral reproductive structures, but was only rarely recorded visiting flowers. The cockroaches and skipper butterflies appear not to pollinate flowers, as they simply crawled along the outside of the corolla. Ants were occasionally observed walking on the inside of the corolla, in addition to the outside, but were never observed contacting anthers or stigmas. Sunbirds were observed robbing nectar;

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they would perch on branches near flowers and use their beaks to pierce the corolla base (Figure 2J).

Histochemistry of floral nectary discs

The sectioned nectaries of the three study species revealed some similarities and some differences among the tested compounds (Table 1). NADI reagent tested positive in all three species, revealing that terpenes are produced and/or accumulated in the epidermis, around the nectary ducts, and in the parenchyma cells of *A. versicolor* (Figure 3A) and *A. mekongensis* (Figure 4A), while terpenes were strongly detected throughout the entirety of the nectary disc of *A. lycioides* (Figure 5A). Similarly, flavonoids were detected in all species throughout the nectary disc (Figure 3B, Figure 4D, Figure 5B). In contrast, lipids were found only in *A. mekongensis* and appear to accumulate in the epidermis layer and nectary ducts (Figure 4B-C), while the other two study species tested negative for lipids using both Sudan Black B and Sudan III.

Morphology and histochemistry of staminal trichomes

Argyreia versicolor has two types of trichomes (Table 1), glandular trichomes (Figure 3D, E) and non-glandular trichomes (Figure 3F). The trichomes were dispersed across the lower part of the filaments, and the highest density of trichomes and the longest trichomes were found at the center of the distribution. Glandular trichomes were shorter than non-glandular trichomes and were fewer in number and shorter in length towards the margins of their distribution. Each glandular trichome consisted of a head cell (apical cell), stalk, and basal cell; the head cells are unicellular apical glands which are either rounded cylindrical, globose (Figure 3D), or obovoid (Figure 3E)~~, or globose (Figure 3D)~~. Stalks were observed to have different lengths; long stalks (Figure 3E) were mostly found at the very base of the filaments while short stalks (Figure 3D) were densely scattered around the middle of filaments and sparsely

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Comentado [JG20]: How the degree of this chemical was measured?

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scattered near the tops of filaments. We also observed simple non-glandular staminal trichomes (Figure 3F), which were present only in this species and only at the center of the filament base. The non-glandular trichomes consisted of a basal cell and a long slender apical cell. The glandular staminal trichomes tested positive for terpenes (Figure 3C) while the simple non-glandular staminal trichomes did not (Table 1). Both glandular and non-glandular trichomes tested negative for lipids (Table 1). Flavonoids were detected in the apical cells of non-glandular trichomes, especially where the apical cell connects to the base (Figure 3F), but not in glandular trichomes (Table 1).

Only glandular trichomes were observed for *A. mekongensis*, and they were distributed across the base of filaments, densely at the center of their distribution and more sparsely towards the margins of their distribution. These glandular trichomes also consisted of a head cell (apical cell), stalk, and basal cell. The head cells are unicellular apical glands and five gland shapes were observed; rounded conical (Figure 4F), bell-shaped (Figure 4F), rounded cylindrical (Figure 4G), convex (Figure 4G), and globose (Figure 4H). Stalks were longer at the center of their distribution and shorter towards the margins of their distribution. Histochemical analysis revealed the presence of terpenes (Figure 4E) and lipids (Figure 4F-H), both of which appear to accumulate in the glands (Table 1). However, flavonoids were not detected (Table 1).

We also only observed glandular trichomes in *A. lycioides*, which were distributed across the base of the filaments, densely at the center of their distribution and more sparsely towards the margins of their distribution. Similar to the other two study species, these glandular trichomes consisted of a head cell (apical cell), stalk, and basal cell. The apical gland cells were observed to have four shape types: rounded cylindrical (Figure 5C), globose (Figure 5C), obovoid (Figure 5D), and pyriform (Figure 5D). In contrast to the other two study species, the glandular trichomes of *A. lycioides* have very short unicellular stalks

(Figure 5D). Histochemical analysis revealed the presence of terpenes (Figure 5C), while lipids and flavonoids were not detected (Table 1).

Discussion

Floral visitors in relation to floral characters

We observed high overlap in floral visitor composition between *A. versicolor* and *A. mekongensis*, and some overlap between *A. mekongensis* and *A. lycioides*, but no overlap in the taxa visiting *A. versicolor* and *A. lycioides*. *Argyreia versicolor* and *A. mekongensis* were both almost exclusively visited by *Xylocopa* carpenter bees (*X. aestuans* and *X. latipes*). These species are very similar in shape and size, both of which allow their large bee pollinators to enter the flower and contact floral reproductive structures with their thorax. Moreover, these species likely attract their pollinators with their relatively showy floral displays: bright colors, a large corolla limb, and numerous flowers per inflorescence (5-9 flowers). While these two species do differ in color (*A. versicolor* is purple and white, while *A. mekongensis* is pure white), *Xylocopa* bees have been reported to favor both purplish-white and creamy white flowers (Raju & Rao, 2006).

We also observed some overlap in the animal taxa that visited *A. mekongensis* and *A. lycioides*, namely, wasps, small bees, skipper butterflies, and sunbirds. However, most of these taxa were uncommon visitors and unlikely pollinators. For example, for both *Argyreia* species, skipper butterflies (Hesperiidae) were only ever observed on the outside of the corolla, and sunbirds (*Cinnyris jugularis*) were only ever observed robbing nectar. The one exception is wasps in the family Vespidae, which were frequent visitors to and likely pollinators of *A. lycioides* (see paragraph below). The similarities in floral visitor composition between *A. mekongensis* and *A. lycioides* may be due to their similar coloration and floral heights. Both species have pale-colored flowers and were found about 1.5 m above ground,

Comentado [JGGF22]: I would like to see a table indicating flower visitors and their frequency

Comentado [JGGF23]: How you are sure that these characters attract flower visitors? Did you make an experiment by changing some of the colors or cutting the limb? The authors mention that visitor frequencies were very low. If the characteristics were as attractive visitors were frequent, as occurring in some orchid species.

Comentado [JG24]: Authors do not show the frequency of the flower visitors whereas twining *A. versicolor* was usually found climbing tall trees and its flowers were typically around 5 m above ground.

Argyreia lycioides is the most different of the three study species in terms of shape and size, and it also had different pollinators than the other two. While *A. versicolor* and *A. mekongensis* were pollinated by *Xylocopa* carpenter bees (Jirabanjongjit et al., 2024), *A. lycioides* appears to be pollinated primarily by wasps in the family Vespidae. Key floral features that distinguish *A. lycioides* from the other two species are its floral arrangement (axillary solitary instead of inflorescence), size (about three-fourths the size of the other two), and reduced corolla limb (minimal to absent). The flowers of *A. lycioides* are too small for carpenter bees, but are appropriately sized for wasps or smaller bees, as were observed in this study. Wasps appear to be the main pollinators of *A. lycioides* given the frequency of their visits and their consistent contact with stigmas and anthers. Wasps can be found as pollinators of both generalist and specialized flowers (Heithaus, 1979; Nilsson, 1981; Kephart, 1983; Vieira & Shepherd, 1999; Ollerton et al., 2003; Johnson, 2005; Shuttleworth & Johnson, 2006, 2008, 2009a, b, c, d; Johnson, Ellis & Dötterl, 2007). While Faegri & Van der Pijl (1979) did not specifically describe a wasp pollination syndrome, evidence suggests that wasps often pollinate easily approachable flowers that have dull or cryptic coloration, a strong or unusual scent, and concentrated nectar (Heithaus, 1979; Proctor, Yeo & Lack, 1996; Ollerton & Watts, 2000; Johnson, Ellis & Dötterl, 2007; Shuttleworth & Johnson, 2009a; Shuttleworth & Johnson, 2009d). According to Kingston and McQuillan (2000), flowers that are visited by wasps mainly have pale colors, followed by yellow and some purple flowers. These results correspond with our findings, as *A. lycioides* has a greenish white corolla with a dense concentration of dark purple dots on the inside the corolla, and the wide corolla entrance and tube make nectar easily accessible. However, given that our study only opportunistically examined a single population, additional research is needed to assess the

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Comentado [JGGF26]: The authors mention superficially about how flowers display, but they do not present any data about quantitative measures of flower size visitation frequency and effectiveness of animal taxa visiting *A. lycioides* to determine its main pollinators.

Comentado [JGGF27]: I agree with the authors. They do not have information (or at least they did not present it) for consider pollinators some insect species, even in the other two plant species.

Histochemistry of the floral nectary discs

In all three study species, the floral nectary surrounds the base of the ovary; this nectary disc is a conserved character within the Convolvulaceae (Govil, 1972; Deroin, 1992, 2002; Galetto & Bernardello, 2004; Wright, Welsh & Costea, 2011). However, investigation of terpenes, flavonoids, and lipids in the floral nectaries revealed some differences between the three sympatric *Argyreia* species.

Terpenes were detected in the floral nectaries of all three study species, and have also been reported in several other plant species (Giuliani, Bini & Lippi, 2012; Machado & Souza, 2016; Wiese et al., 2018; Farinnaso et al., 2021; Jirabanjongjit et al., 2021; Chitchak, Stewart & Traiperm, 2022). Terpenes are important secondary metabolites in plants that contribute to pollinator attraction by providing scent compounds (Knudsen & Gershenzon, 2006) that are recognized to attract bees (Bergström, Dobson & Groth, 1995; Robertson et al., 1995). Moreover, terpenes can contribute to plant-insect interactions for bees that forage for biologically active plant products (Harrewijn, Minks & Mollema, 1994; Stevenson, Nicolson & Wright, 2017). Therefore, apart from the food resources provided by nectar, the floral nectary can also produce other important chemical substances to attract and reward pollinators.

Flavonoids were also detected in the floral nectaries of all three study species, are prevalent throughout plants and their tissues especially in higher plants (Wollenweber & Dietz, 1981; Harborne, 1988; Taylor & Grotewold, 2005), and have previously been reported in several plant taxa (Ferreres et al., 1996; Truchado et al., 2008; Machado & Souza, 2016; Jirabanjongjit et al., 2021; Chitchak, Stewart & Traiperm, 2022). Several functions of flavonoids are well-known, such as their role in plant reproduction, namely, as a color attractant that advertises flowers to pollinators and fruits to seed dispersers (Dakora, 1995). Flavonoids can also absorb UV wavelengths, providing visual cues that guide bees or other insects to floral nectar (Thorp et al., 1975; Harborne, 1979; Agati & Tattini, 2010). Additionally, flavonoids can protect nectar from pathogens or microbes, preserving it for pollinators, which can also benefit plant reproduction (Treutter, 2005).

In contrast to terpenes and flavonoids, lipids were only found in one of the three study species, *A. mekongensis*. Lipids have been reported in the floral nectaries of many plant taxa, such as Anacardiaceae, Bignoniaceae, Convolvulaceae, and Orchidaceae (Figueiredo & Pais, 1992; Stpiczynska, 1997; Stpiczynska & Matusiewicz, 2001; Stpiczynska & Davies, 2006; Kowalkowska et al., 2015; Machado & Souza, 2016; Tolke et al., 2018; Phukela, Adit & Tandon, 2021; Jirabanjongjit et al., 2021; Chitchak, Stewart & Traiperm, 2022). Lipids are frequently found in the nectar of vertebrate-pollinated species due to their importance in the diets of vertebrates (Varassin, Trigo & Sazima, 2001; Gumede & Downs, 2020), however, their potential role in plant-insect interactions has not been widely studied. Previous research suggests that the positive detection of lipids in the floral nectary could indicate the presence of laticifers (Martin et al., 2012) or may provide nutrition for nectar-feeding insects, in addition to polysaccharides (Bernardello, 2007). From the results of this study, terpenes and flavonoids appear to be important substances that potentially attract pollinators, while lipids (which were only detected in *A. mekongensis*) may serve as additional nutrition for pollinators.

Comentado [JGGF28]: However, the authors mentioned that carpenter bees are the main visitors and pollinators, even in the other species A. versicolor. Were is the difference? One presents lipids and the other none, what happens then?

Histochemistry of the staminal trichomes

Argyreia versicolor is the only species examined in this study that has both non-glandular and glandular trichomes on the staminal filaments. Non-glandular staminal trichomes appear to be very uncommon, as Chitchak (2019) observed the staminal trichomes of 31 taxa and only observed non-glandular trichomes in *A. versicolor*. Traditionally, non-glandular trichomes have been considered unimportant in the storage, production, and secretion of biologically active compounds (Werker, 2000). However, non-glandular trichomes have been found to store phenolic compounds, despite not having secretion abilities, and such phenolics are important in the protection against and regulation of biotic and abiotic stresses (Koudounas et al., 2015; Karabourniotis et al., 2020). Among secondary metabolic compounds, flavonoids, a type of phenolic compound, has been shown to substantially accumulate in non-glandular trichomes (Skaltsa et al., 1994; Valkama et al., 2004; Tattini et al., 2007; Koudounas et al., 2015), as we also observed in *A. versicolor*. Non-glandular trichomes are typically considered to provide physical plant defenses against biotic or abiotic stresses, such as protection against insect oviposition or herbivory (Levin, 1973; Baur, Binder & Benz, 1991), or as protection against drought (Ichie et al., 2016), low or high humidity, high solar radiation, or high light intensity (Werker, 2000; Ichie et al., 2016).Thus, it is possible that the non-glandular staminal trichomes found in *A. versicolor* may help protect the ovary from herbivory, as the ovary is located directly below the staminal trichomes.

Glandular trichomes were observed in all three study species, and had similar distribution patterns, but varied in some of their morphological features. *Argyreia lycioides* had shorter stalks than the other two species, which may be due to phenotypic integration of floral traits such as corolla size, as was reported in Chitchak (2019). The function of staminal trichomes is still unclear and they have received less attention than other types of trichomes. However, a recent study by Dieringer and Cabrera (2022) suggested one ecological advantage of staminal trichomes in *Agalinis auriculata*, in which they appear to facilitate the grasping of flowers during buzz pollination; filament trichomes had a positive effect on pollen removal in sternotribic pollination but a negative effect in nototribic pollination. Riviere et al. (2013) reported a different function of filament trichomes in the genus *Cuscuta*, where glandular trichomes appear to play a role in the protection of the nectar or ovary (ovules).

Glandular trichomes usually secrete and accumulate specific secondary metabolites such as terpenes and other essential oils (Metcalf & Kogan, 1987). In the species that we examined, there were some differences in the histochemical results of glandular staminal trichomes, but terpenes were found in all species. In general, terpenes are reserved in specialized structures within plant tissues, such as secretory cavities, resin canals, latex canals, and glandular trichomes (Holopainen et al., 2013). Previous studies examining terpenes have mostly reported their presence in the glandular trichomes of leaves, and they are generally recognized as being defensive substances against microbes, fungi and/or herbivores (Harborne, 1993; Kelsey Reynolds & Rodriguez, 1984; Olaranont et al., 2018). However, more recent work has started to improve our understanding of how insects respond to terpenes and their function in attracting pollinators (Raguso & Light, 1998; Dudareva et al., 2006; Knudsen & Gershenzon, 2006). In contrast to the ubiquity of terpenes, lipids were only detected in the glandular staminal trichomes of *A. mekongensis* while flavonoids are not detected in the glandular staminal trichomes of any species. Previous work examining other morning glory species, such as *Rivea ornata* (Chitchak, Stewart & Traiperm, 2022) and *Argyreia siamensis* (Jirabanjongjit et al., 2021), have hypothesized that staminal trichomes are important for pollinator attraction since glandular staminal trichomes contain chemical substance such terpenes and flavonoids. The results of this study indicate that glandular staminal trichomes, with their accumulation of terpenes, may help protect the nectar and ovary from microbes, fungi, and herbivores, and/or may help attract pollinators and guide them to the nectar.

Conclusion

The findings of this study reveal that the three sympatric, co-flowering *Argyreia* species exhibit some similarities and some differences in terms of floral visitors, floral characters, and histochemical compounds. When comparing pollinators, the two twining species, *A. versicolor* and *A. mekongensis*, shared carpenter bee pollinators due to their similar floral sizes and shapes. In contrast, *A. lycioides* is a perennial shrub with a smaller corolla tube, and appears to be pollinated by wasps (Vespidae) and possibly small- to medium-sized bees. All species exhibited trichomes at the base of staminal filaments; glandular trichomes were observed in all species but non-glandular trichomes were found only in *A. versicolor*. Histochemical investigation of the floral nectary and staminal trichomes revealed that all three species contain chemical substances that may be related to pollinator attraction and floral defense, although there were slight differences among species. Terpenes and flavonoids in the floral nectar may contribute to pollinator attraction while lipids may provide additional nutrition for pollinators. In terms of staminal trichomes, glandular trichomes may secrete chemical substances for pollinator attraction or plant defense, while non-glandular trichomes may help protect the nectary and ovary from herbivory. The knowledge gained from this study regarding flower morphology and chemical compounds, in combination with what we know about the breeding system of the rare species, *A. versicolor* (Jirabanjongjit et al. 2024), are necessary for plant conservation in terms of preserving their overlapping pollinators.

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References

Agati G, Tattini M. 2010. Multiple functional roles of flavonoids in photoprotection. *New Phytologist* 186(4):786-793.

Alonso WR, Rajaonarivony JI, Gershenzon J, Croteau R. 1992. Purification of 4S-limonene synthase, a monoterpene cyclase from the glandular trichomes of peppermint (*Mentha x piperita*) and spearmint (*Mentha spicata*). *Journal of Biological Chemistry* 267(11):7582-7587.

Antoniou GF. 2001. Production and quantification of methyl ketones in wild tomato accessions. *Journal of Environmental Science and Health, Part B* 36(6):835-48.

Bailey SF, Hargreaves AL, Hechtenthal SD, Laird RA, Latty TM, Reid TG, Teucher AC, Tindall JR. 2007. Empty flowers as a pollination-enhancement strategy. *Evolutionary Ecology Research* 9(8):1245-1262.

Baker H, Baker I. 1983. Floral nectar sugar constituents in relation to pollinator type. In: Jones CE, Little RJ, eds. *Handbooks of experimental pollination biology*. New York: Van Nostrand Reinhold, 131-41.

Baur R, Binder S, Benz G. 1991. Nonglandular leaf trichomes as short-term inducible defense of the grey alder, *Alnus incana* (L.), against the chrysomelid beetle, *Agelastica alni* L. *Oecologia* 87(2):219-226.

Beentje HJ. 2010. *The Kew plant glossary: an illustrated dictionary of plant terms*. UK: Royal Botanic Gardens.

Benitez-Vieyra S, Ordano M, Fornoni J, Boege K, Domínguez CA. 2010. Selection on signal-reward correlation: limits and opportunities to the evolution of deceit in *Turnera ulmifolia* L. *Journal of Evolutionary Biology* 23(12):2760-7.

Bergamo PJ, Rech AR, Brito VL, Sazima M. 2016. Flower colour and visitation rates of *Costus arabicus* support the 'bee avoidance' hypothesis for red-reflecting hummingbird-pollinated flowers. *Functional Ecology* 30(5):710-720.

Bergström G, Dobson HE, Groth I. 1995. Spatial fragrance patterns within the flowers of *Ranunculus acris* (Ranunculaceae). *Plant Systematics and Evolution* 195:221-242.

Bernardello G. 2007. A systematic survey of floral nectaries. In: Nicolson SW, Nepi M, Pacini E, eds. *Nectaries and nectar*. Springer: Dordrecht, 19-128.

Bini Maleci L, Servettaz O. 1991. Morphology and distribution of trichomes in Italian species of *Teucrium* sect. *Chamaedrys* (Labiatae)-a taxonomical evaluation. *Plant Systematics and Evolution* 174:83-91.

Biswas KK, Foster AJ, Aung T, Mahmoud SS. 2009. Essential oil production: relationship with abundance of glandular trichomes in aerial surface of plants. *Acta Physiologiae Plantarum* 31:13-19.

Bobisud L, Neuhaus R. 1975. Pollinator constancy and survival of rare species. *Oecologia* 21:263-272.

Bobrowiec PED, Oliveira PE. 2012. Removal effects on nectar production in bat-pollinated flowers of the Brazilian Cerrado. *Biotropica* 44(1):1-5.

Brundrett MC, Kendrick B, Peterson CA. 1991. Efficient lipid staining in plant material with sudan red 7B or fluoral yellow 088 in polyethylene glycol-glycerol. *Biotechnic & Histochemistry* 66(3):111-116.

Chitchak N, Traiperm P, Staples G, Rattanakrajang P, Sumanon P. 2018. Species delimitation of some *Argyreia* (Convolvulaceae) using phenetic analyses: insights from leaf anatomical data reveal a new species~~.~~. *Botany* 96(4):217-233.

Chitchak N, Stewart AB, Traiperm P. 2022. Functional ecology of external secretory structures in *Rivea ornata* (Roxb.) Choisy (Convolvulaceae~~).~~). *Plants* 11(15):2068.

Chitchak N. 2019. Staminal trichome morphology of genus *Argyreia* Lour. (Convolvulaceae) and its taxonomic implications. MSc thesis of Plant Sciences, Mahidol University.

Chittka L, Raine NE. 2006. Recognition of flowers by pollinators. *Current Opinion in Plant Biology* 9(4):428-435.

Dakora FD. 1995. Plant flavonoids: biological molecules for useful exploitation. *Functional Plant Biology* 22(1):87-99.

David R, Carde JP. 1964. Histochimie-coloration differentielle des inclusions lipidiques et terpeniques des pseudophylles du pin maritime au moyen du reactif NADI. *Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences* 258(4):1338.

Deroin T. 1992. Anatomie florale de Humbertia madagascariensis Lam. Contribution à la morphologie comparée de la fleur et du fruit des Convolvulaceae~~.~~. Bull Musé Natl d'hist Nat Sec B. *Adansonia* 14(2):235-255.

Deschamps C, Gang D, Dudareva N, Simon JE. 2006. Developmental regulation of phenylpropanoid biosynthesis in leaves and glandular trichomes of basil (*Ocimum basilicum* L~~.). I~~ *nternational Journal of Plant Sciences* 167(3):447-454.

Dieringer G, Cabrera RL. 2022. Stamen dimorphism, bee visitation, and pollen removal in three species of *Agalinis* (Orobanchaceae~~).~~). *Botany* 100(4):377-386.

Dudareva N, Negre F, Nagegowda DA, Orlova I. 2006. Plant volatiles: recent advances and future perspectives~~.~~. *Critical Reviews in Plant Sciences* 25(5):417-440.

Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G. 2007. Time after time: flowering phenology and biotic interactions.. *Trends in Ecology & Evolution* 22(8):432-439.

Evans EW, Smith CC, Gendron RP. 1989. Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds.. *Oecologia* 78:220-230.

Evert RF. 2006. *Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development*. John Wiley & Sons.

Faegri K, van der Pijl L. 1979. *The principles of pollination ecology*. Oxford: Pergamon Press.

Farinasso HC, Consolaro H, Gomes SM, Aguiar AJ. 2021. From generalization to pollination syndromes: filtering and dependency on functional-group of pollinators in two cassava wild relatives.. *Arthropod-Plant Interactions* 15(2):235-247.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization.. *Annual Review of Ecology, Evolution and Systematics* 35:375-403.

Ferreres F, Andrade P, Gil MI, Tomás-Barberán FA. 1996. Floral nectar phenolics as biochemical markers for the botanical origin of heather honey. *Zeitschrift für Lebensmittel-Untersuchung und Forschung* 202:40-44.

Figueiredo ACS, Pais MS. 1992. Ultrastructural aspects of the nectary spur of *Limodorum abortivum* (L) Sw. (Orchidaceae).). *Annals of Botany* 70(4):325-331.

Galetto L, Bernardello G. 2004. Floral nectaries, nectar production dynamics and chemical composition in six *Ipomoea* species (Convolvulaceae) in relation to pollinators.. *Annals of Botany* 94(2):269-280.

Galetto L, Bernardello G. 2005. Rewards in flowers: Nectar.. In: Dafni A, Kevan PG, Husbands BC, eds. *Practical pollination biology*, *enviroquest*. 261-313.

Gallagher MK, Campbell DR. 2020. Pollinator visitation rate and effectiveness vary with flowering phenology. *American Journal of Botany* 107(3):445-455.

Giuliani C, Bini LM, Lippi MM. 2012. Two structures and functions in the nectary of Frankincense tree (*Boswellia sacra* Flueck.). *Flora* 207(1):74-79.

Gonzales WL, Negritto MA, Suarez LH, Gianoli E. 2008. Induction of glandular and non-glandular trichomes by damage in leaves of *Madia sativa* under contrasting water regimes. *Acta Oecologica* 33(1):128-132.

Good AP, Gauthier MPL, Vannette RL, Fukami T. 2014. Honey bees avoid nectar colonized by three bacterial species, but not by a yeast species, isolated from the bee gut. *PloS ONE* 9(1):e86494.

Govil CM. 1972. Morphological studies in the family convolvulaceae: IV. *Vascular anatomy of the flower*. In Proceedings/Indian Academy of Sciences 75(6). New Delhi: Springer India, 271-282.

Gumede ST, Downs CT. 2020. Preliminary observations suggest Common Myna prefer lipid over protein and carbohydrate foods in a pairwise choice experiment. *Ostrich* 91(1):95-100.

Harborne JB. 1979. Correlations between flavonoid chemistry, anatomy and geography in the Restionaceae. *Phytochemistry* 18(8):1323-1327.

Harborne JB. 1988. *The flavonoids: Advances in research since*. New York, NY: Springer.

Harborne JB. 1993. *Introduction to ecological biochemistry*. 4th edn. London: Academic Press.

Harrewijn P, Minks AK, Mollema C. 1994. Evolution of plant volatile production in insect-plant relationships. *Chemoecology* 5:55-73.

Hassa P, Traiperm P, Stewart AB. 2020. Pollinator visitation and female reproductive success in two floral color morphs of *Ipomoea aquatica* (Convolvulaceae). *Plant Systematics and Evolution* 306:88.

Hassa P Traiperm P, Stewart AB. 2023. Compatibility systems and pollinator dependency in morning glory species (Convolvulaceae). *BMC Plant Biology* 23:432.

Heithaus ER. 1979. Flower visitation records and resource overlap of bees and wasps in northwest Costa Rica. *Brenesia* 16:9-52.

Herrera G, Zagal JC, Diaz M, Fernández MJ, Vielma A, Cure M, Martinez J, Bozinovic F, Palacios AG. 2008. Spectral sensitivities of photoreceptors and their role in colour discrimination in the green-backed firecrown hummingbird (*Sephanoides sephaniodes*). *Journal of Comparative Physiology A* 194:785-794.

Holopainen JK, Himanen SJ, Yuan JS, Chen F, Stewart CN. 2013. Ecological functions of terpenoids in changing climates. *Journal of Natural Products* 2913-2940.

Hoogland KD. 1952. The Convolvulaceae of Malaysia, VIII. *Blumea* 7(1):179-192.

Horgan FG, Quiring DT, Lagnaoui A, Pelletier Y. 2007. Variable responses of tuber moth to the leaf trichomes of wild potatoes. *Entomologia Experimentalis et Applicata* 125(1):1-12.

Ichie T, Inoue Y, Takahashi N, Kamiya K, Kenzo T. 2016. Ecological distribution of leaf stomata and trichomes among tree species in a Malaysian lowland tropical rain forest. *Journal of Plant Research* 129:625-635.

Iijima Y, Gang DR, Fridman E, Lewinsohn E, Pichersky E. 2004. Characterization of geraniol synthase from the peltate glands of sweet basil. *Plant Physiology* 134(1):370-379.

Irwin RE, Adler LS, 2004. Agrawal AA. Community and evolutionary ecology of nectar. *Ecology* 85(6):1477-1478.

Irwin RE, Bronstein JL, Manson JS, Richardson L. 2010. Nectar robbing: ecological and evolutionary perspectives. *Annual Review of Ecology, Evolution and Systematics* 41:271-292.

Jirabanjongjit A, Traiperm P, Sando T, Stewart, AB. 2021. Pollination and floral biology of a rare morning glory species endemic to Thailand, *Argyreia siamensis*. *Plants* 10(11):2402.

Jirabanjongjit A, Traiperm P, Rattanamanee C, Stewart AB. 2024. Near extinct *Argyreia versicolor* and rare *Argyreia mekongensis* are dependent on carpenter bee pollinators. *AoB Plants* 16:(1-10).

Johnson SD. 2005. Specialized pollination by spider-hunting wasps in the African orchid *Disa sankeyi*. *Plant Systematics and Evolution* 251:153-160.

Johnson SD, Ellis A, Dötterl S. 2007. Specialization for pollination by beetles and wasps: the role of lollipop hairs and fragrance in *Satyrium microrrhynchum* (Orchidaceae). *American Journal of Botany* 94(1):47-55.

Kang JH, Shi F, Jones AD, Marks MD, Howe GA. 2010. Distortion of trichome morphology by the hairless mutation of tomato affects leaf surface chemistry. *Journal of Experimental Botany 61*(4):1053-1064.

Karabourniotis G, Liakopoulos G, Nikolopoulos D, Bresta P. 2020. Protective and defensive roles of non-glandular trichomes against multiple stresses: structure-function coordination. *Journal of Forestry Research* 31(1):1-12.

Kelsey RG, Reynolds GW, Rodriguez E. 1984. Chemistry of biologically active constitutents secreted and stored in plant glandular trichomes. *Biology and chemistry of plant trichomes*. New York: Plenum Press.

Kennedy GG. 2003. Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. *Annual Review of Entomology* 48(1):51-72.

Kephart SR. 1983. The partitioning of pollinators among three species of *Asclepias*. *Ecology*. 64(1):120-33.

Kingston AB, Mc Quillan PB. 2000. Are pollination syndromes useful predictors of floral visitors in Tasmania?. *Austral Ecology* 25(6):600-609.

Knudsen JT, Gershenzon J. 2006. The chemical diversity of floral scent. In: Dudareva N, Pichersky E, eds. *Biology of floral scent*. USA: CRC Press, 27-52.

Koudounas K, Manioudaki ME, Kourti A, Banilas G, Hatzopoulos P. 2015. Transcriptional profiling unravels potential metabolic activities of the olive leaf non-glandular trichome. *Frontiers in Plant Science* 6:633.

Kowalkowska AK, Kostelecka J, Bohdanowicz J, Kapusta M, Rojek J. 2015. Studies on floral nectary, tepals' structure, and gynostemium morphology of *Epipactis palustris* (L.) Crantz (Orchidaceae). *Protoplasma*. 252(1):321-333.

Levin Donald A. 1973. The role of trichomes in plant defense. *The Quarterly Review of Biology* 48(1):3-15.

Liu J, Xia KF, Zhu JC, Deng YG, Huang XL, Hu BL, Xu X, Xu ZF. 2006. The nightshade proteinase inhibitor IIb gene is constitutively expressed in glandular trichomes. *Plant and Cell Physiology* 47(9):1274-1284.

Luo SH, Luo Q, Niu XM, Xie MJ, Zhao X, Schneider B, Gershenzon J, Li SH. 2010. Glandular trichomes of *Leucosceptrum canum* harbor defensive sesterterpenoids. *Angewandte Chemie* 122(26):4573-4577.

Machado SR, Souza CVD. 2016. A reduced, yet functional, nectary disk integrates a complex system of floral nectar secretion in the genus *Zeyheria* (Bignoniaceae). *Acta Botanica Brasilica* 31:344-357.

Martins FM, Lima JF, Mascarenhas AAS, Macedo TP. 2012. Secretory structures of *Ipomoea asarifolia*: anatomy and histochemistry. *Revista Brasileira de Farmacognosia* 22: 13-20.

Metcalf RL, Kogan M. 1987. Plant volatiles as insect attractants. *Critical Reviews in Plant Sciences* 5(3):251-301.

Moyano F, Cocucci A, Sersic A. 2003. Accessory pollen adhesive from glandular trichomes on the anthers of *Leonurus sibiricus* L. (Lamiaceae). *Plant Biology* 5(04):411-418.

Nagel J, Culley LK, Lu Y, Liu E, Matthews PD, Stevens JF, Page JE. 2008. EST analysis of hop glandular trichomes identifies an O-methyltransferase that catalyzes the biosynthesis of xanthohumol. *Plant Cell* 20(1):186-200.

Naug D, Arathi HS. 2007. Receiver bias for exaggerated signals in honeybees and its implications for the evolution of floral displays. *Biology Letters* 3(6):635-657.

Neiland MRM, Wilcock CC. 1998. Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany* 85(12):1657-1671.

Nicolson SW. 2007. Nectar consumers. In: Nicolson SW, Nepi M, Panini E, eds. *Nectaries and nectar*. Heidelberg: Springer-Verlag GmbH, 289-342.

Nihoul P. 1993. Do light intensity, temperature and photoperiod affect the entrapment of mites on glandular hairs of cultivated tomatoes?. *Experimental and Applied Acarology* 17(9):709-718.

Nilsson LA. 1981. The pollination ecology of *Listera ovata* (Orchidaceae). *Nordic Journal of Botany* 1(4):461-480.

Nonomura T, Xu L, Wada M, Kawamura S, Miyajima T, Nishitomi A, Kakutani K, Takikawa Y, Matsuda Y, Toyoda H. 2009. Trichome exudates of *Lycopersicon pennellii* form a chemical barrier to suppress leaf-surface germination of *Oidium neolycopersici* conidia. *Plant Science* 176(1):31-37.

Olaranont Y, Stauffer F, Traiperm P, Staples GW. 2018. Investigation of the black dots on leaves of *Stictocardia* species (Convolvulaceae) using anatomical and histochemical analyses. *Flora* 249: 133-142.

Ollerton J, Watts S. 2000. Phenotype space and floral typology: towards an objective assessment of pollination syndromes. *Det Norske Videnskaps-Akademi. I. Matematisk-Naturvidenskapelige Klasse, Skrifter, Ny Serie* 39:149-159.

Ollerton J, Johnson SD, Cranmer L, Kellie SAM. 2003. The pollination ecology of an assemblage of grassland asclepiads in South Africa. *Annals of Botany* 92(6):807-834.

Ollerton J, Alarcón R, Waser NM, Price MV, Watts S, Cranmer L, Hingston A, Peter CI, Rotenberry J. 2009. A global test of the pollination syndrome hypothesis. *Annals of Botany* 103(9):1471-1480.

Pacini E, Nicolson SW. 2007. Introduction. In: Nicolson SW, Nepi M, Panini E, eds. *Nectaries and nectar*. Heidelberg: Springer-Verlag GmbH, 1-18.

Phukela B, Adit A, 2021. Tandon R. Evolutionary trends and diversity of major floral nectary types across Solanaceae. *Planta* 254(3):1-20.

Pleasants JM. 1983. Nectar production patterns in *Ipomopsis aggregata* (Polemoniaceae). *American Journal of Botany* 70(10):1468-1475.

Proctor M, Yeo P, Lack A. 1996. *The natural history of pollination.* Portland, Oregon: Timber press.

Raguso RA, Light DM. 1998. Electroantennogram responses of male *Sphinx perelegans* hawkmoths to floral and 'green-leaf volatiles'. *Entomologia Experimentalis et Applicata* 86(3):287-293.

Raju AS, Rao SP. 2006. Nesting habits, floral resources and foraging ecology of large carpenter bees (*Xylocopa latipes* and *Xylocopa pubescens*) in India. *Current Science* 1210-1217.

Rathcke B. 1983. Competition and facilitation among plants for pollination. In: Real L, editor. *Pollination biology*. London: Academic press, 305-329.

Rattanakrajang P, Sumanon P, Traiperm P, Staples GW, Utteridge T. 2022. Reduction of *Blinkworthia* (Convolvulaceae) based on multilocus phylogenetic reconstruction and resurrection of a species from synonymy revealed by phenetic analyses*. Kew Bulletin* 77(4):859-883.

Rattanakrajang P, Traiperm P, Staples GW. 2018. Re-evaluation of generic characters for *Blinkworthia* (Convolvulaceae) based on morphology and reproductive organ development. *Plant Systematics and Evolution* 304:415-429.

Riviere S, Clayson C, Dockstader K, Wright MA, Costea M. 2013. To attract or to repel? diversity, evolution and role of the "most peculiar organ" in the *Cuscuta* flower (dodder, Convolvulaceae)-the infrastaminal scales. *Plant Systematics and Evolution* 299(3):529-552.

Robertson GW, Griffiths DW, Woodford JAT, Birch ANE. 1995. Changes in the chemical composition of volatiles released by the flowers and fruits of the red raspberry (*Rubus idaeus*) cultivar glen prosen. *Phytochemistry* 38(5):1175-1179.

Romero GQ, Souza JC, Vasconcellos-Neto J. 2008. Anti-herbivore protection by mutualistic spiders and the role of plant glandular trichomes. *Ecology* 89(11):3105-3115.

Sallaud C, Rontein D, Onillon S, Jabes F, Duffé P, Giacalone C, Thoraval S, Escoffier C, Herbette G, Leonhardt N, Causse M. 2009. A novel pathway for sesquiterpene biosynthesis from Z, Z-farnesyl pyrophosphate in the wild tomato *Solanum habrochaites*. *Plant Cell* 21(1):301-317.

Schemske DW. 1981. Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. *Ecology* 62(4):946-954.

Shuttleworth A, Johnson SD. 2006. Specialized pollination by large spider-hunting wasps and self-incompatibility in the African milkweed *Pachycarpus asperifolius. International Journal of Plant Sciences* 167(6):1177-1186.

Shuttleworth A, Johnson SD. 2008. Bimodal pollination by wasps and beetles in the African milkweed *Xysmalobium undulatum*. *Biotropica* 40(5):568-574.

Shuttleworth A, Johnson SD. 2009a. The importance of scent and nectar filters in a specialized wasp-pollination system. *Functional Ecology* 23(5):931-940.

Shuttleworth A, Johnson SD. 2009b. Specialized pollination in the African milkweed *Xysmalobium orbiculare*: a key role for floral scent in the attraction of spider-hunting wasps. *Plant Systematics and Evolution* 280:37-44.

Shuttleworth A, Johnson SD. 2009c. Palp-faction: an African milkweed dismembers its wasp pollinators. Environmental Entomology 38(3):741-747.

Shuttleworth A, Johnson SD. 2009d. A key role for floral scent in a wasp-pollination system in *Eucomis* (Hyacinthaceae). *Annals of Botany* 103(5):715-725.

Siebert DJ. 2004. Localization of salvinorin A and related compounds in glandular trichomes of the psychoactive sage, *Salvia divinorum*. *Annals of Botany* 93(6):763-771.

Simmons AT, Gurr GM. 2005. Trichomes of Lycopersicon species and their hybrids: effects on pests and natural enemies. *Agricultural and Forest Entomology* 7(4):265-276.

Simpson BB, Neff JL. 1981. Floral rewards: alternatives to pollen and nectar. *Annals of the Missouri Botanical Garden* 68:301-322.

Skaltsa H, Verykokidou E, Harvala C, Karabourniotis G, Manetasi Y. 1994. UV-B protective potential and flavonoid content of leaf hairs of *Quercus ilex*. *Phytochemistry* 37(4): 987-990.

Southwick EE, Loper GM, Sadwick SE. 1981. Nectar production, composition, energetics and pollinator attractiveness in spring flowers of western New York. *American Journal of Botany* 68(7):994-1002.

Spaethe J, Tautz J, Chittka, L. 2001. Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. *Proceedings of the National Academy of Sciences* 98(7):3898-3903.

Staples G. 2010. Convolvulaceae. In: Santisuk T, Larsen K, eds. *Flora of Thailand* 10(3). Bangkok: Prachachon, 330-468.

Staples GW, Chitchak N, Kochaiphat P, Rattamanee C, Rattanakrajang P, Traiperm P. 2021. Convolvulaceae in the Flora of Thailand: Addenda, Corrigenda and Emendanda, I. *Thai Forest Bulletin (Botany)* 49(1):88-101.

Staples G, Traiperm P. 2010. *Argyreia*. In: Santisuk T, Larsen K, eds. *Flora of Thailand* 10(3). Bangkok: Prachachon, 337-371.

Staples G, Traiperm P. 2017. A nomenclatural review of *Argyreia* (Convolvulaceae). *Taxon* 66(2):445-477.

Stevenson PC, Nicolson SW, Wright GA. 2017. Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. *Functional Ecology* 31(1):65-75.

Stewart AB, Diller C, Dudash MR, Fenster CB. 2022. Pollination-precision hypothesis: support from native honey bees and nectar bats. *New Phytologist* 235(4):1629-1640.

Stpiczyńska M. 1997. The structure of nectary of *Platanthera bifolia* L. Orchidaceae. *Acta Societatis Botanicorum Poloniae* 66(1):5-11.

Stpiczynska M, Davies KL. 2006. Nectary structure in *Symphyglossum sanguineum* (Rchb. f.) Schltr. (Orchidaceae). *Acta Agrobotanica* 59(1):7-16.

Stpiczyńska M, Matusiewicz J. 2001. Anatomy and ultrastructure of spur nectary of *Gymnadenia conopsea* (L.) Orchidaceae. *Acta Societatis Botanicorum Poloniae* 70(4):267-272.

Strauss SY, Whittall JB. 2006. Non-pollinator agents of selection on floral traits. In: Harder LD, Barrett SCH, eds*. Ecology and evolution of flowers*. Oxford: Oxford University press, 120-138.

Tattini M, Gravano E, Pinelli P, Mulinacci N, Romani A. 2000. Flavonoids accumulate in leaves and glandular trichomes of *Phillyrea latifolia* exposed to excess solar radiation. *New Phytologist* 148(1):69-77.

Tattini M, Matteini P, Saracini E, Traversi ML, Giordano C, Agati G. 2007. Morphology and biochemistry of non-glandular trichomes in *Cistus salvifolius* L. leaves growing in extreme habitats of the Mediterranean basin. *Plant Biology* 9(03):411-419.

Taylor LP, Grotewold E. 2005. Flavonoids as developmental regulators. *Current Opinion in Plant Biology* 8(3):317-323.

Thompson J. How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system?. Oecologia. 2001;126(3):386-94.

Thorp RW, Briggs DL, Estes JR, Erickson EH. 1975. Nectar fluorescence under ultraviolet irradiation. *Science* 189(4201):476-478.

Tölke ED, Bachelier JB, Lima EA, Galetto L, Demarco D, Carmello-Guerreiro SM. 2018. Diversity of floral nectary secretions and structure, and implications for their evolution in Anacardiaceae. *Botanical Journal of the Linnean Society* 187(2):209-231.

Treutter D. 2005. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biology* 7(06):581-591.

Truchado P, Ferreres F, Bortolotti L, Sabatini AG, Tomás-Barberán FA. 2008. Nectar flavonol rhamnosides are floral markers of acacia (*Robinia pseudacacia*) honey. *Journal of Agricultural and Food Chemistry* 56(19):8815-8824.

Valkama E, Salminen JP, Koricheva J, Pihlaja K. 2004. Changes in leaf trichomes and epicuticular flavonoids during leaf development in three birch taxa. *Annals of Botany* 94(2):233-42.

Van Dam NM, Hare JD. 1998. Biological activity of *Datura wrightii* glandular trichome exudate against *Manduca sexta* larvae. Journal of Chemical Ecology 24:1529-1549.

van Ooststroom SJ. 1943. The Convolvulaceae of Malaysia, IV. *Blumea* 5(2):339-411.

van Ooststroom SJ. 1945. A new species of *Argyreia* from Sumatra (Convolvulaceae) *Blumea* 5:686-8.

van Ooststroom SJ. 1950. The Convolvulaceae of Malaysia, VI. *Blumea* 6(2):337-348.

van Ooststroom SJ. 1952. The Convolvulaceae of Malaysia, VII. *Blumea* 7(1):171-178.

van Ooststroom SJ, Hoogland RD. 1953. *Convolvulaceae*. In: Steenis CGGJ, ed. Flora Malesiana, ser. I (4), 388-512.

Varassin IG, Trigo JR, Sazima M. 2001. The role of nectar production, flower pigments and odour in the pollination of four species of *Passiflora* (Passifloraceae) in south-eastern Brazil. *Botanical Journal of the Linnean Society* 136(2):139-152.

Vieira MF, Shepherd GJ. 1999. Pollinators of *Oxypetalum* (Asclepiadaceae) in southeastern Brazil. *Revista Brasileira de Biologia* 59:693-704.

Wagner GJ, Wang E, Shepherd R. 2004. New approaches for studying and exploiting an old protuberance, the plant trichome. *Annals of Botany* 93(1):3.

Wang G, Tian L, Aziz N, Broun P, Dai X, He J, King A, Zhao PX, Dixon RA. 2008. Terpene biosynthesis in glandular trichomes of hop. *Plant Physiology* 148(3):1254-1266.

Waser NM, Price MV. 1983. Optimal and actual outcrossing in plants, and the nature of plant-pollinator interaction. In: Jones CE, Little RJ, eds. *Handbook of experimental pollination biology*. New York: Van Nostrand Reinhold, 341-359.

Waser NM, Chittka L, Price MV, Williams NM, Ollerton J. 1996. Generalization in pollination systems, and why it matters. *Ecology* 77(4):1043-1060.

Waser NM, Ollerton J. 2006. *Plant-pollinator interactions: from specialization to generalization*. Chicago: University of Chicago Press.

Werker E. 2000. Trichome diversity and development. *Advances in Botanical Research* 31:1-35.

Wiese N, Fischer J, Heidler J, Lewkowski O, Degenhardt J, Erler S. 2018. The terpenes of leaves, pollen, and nectar of thyme (*Thymus vulgaris*) inhibit growth of bee disease-associated microbes. *Scientific Reports* 8(1):1-12.

Willmer P. 2011. *Pollination and floral ecology*. Princeton University press.

Wollenweber E, Dietz VH. 1981. Occurrence and distribution of free flavonoid aglycones in plants. *Phytochemistry* 20(5):869-932.

Wright MA, Welsh M, Costea M. 2011. Diversity and evolution of the gynoecium in *Cuscuta* (dodders, Convolvulaceae) in relation to their reproductive biology: two styles are better than one. *Plant Systematics and Evolution* 296:51-76.

Figure captions

Figure 1. Photos showing the floral characters of three sympatric *Argyreia* species. (A, D) *Argyreia versicolor*; (B, E) *Argyreia mekongensis*; and (C, F) *Argyreia lycioides*. Photo A, D and F were taken by Yotsawate Sirichamorn, and B, C and E were taken by Tripatchara Atiratana.

Figure 2. Animal visitors of three sympatric species of *Argyreia*. (A) *Xylocopa latipes* visiting *A. versicolor.* (B) *X. aestuans* visiting *A. versicolor.* (C) *X. latipes* visiting *A. mekongensis.* (D) *X. aestuans* visiting *A. mekongensis.* (E) *Amegilla* sp. visiting *A. mekongensis.* (F) *Mylabris phalerata* beetle consuming the corolla of *A. mekongensis.* (G) *Cinnyris jugularis* sunbird robbing nectar from *A. mekongensis*. (H) Unknown bee species visiting *A. lycioides.* (I) Wasp (Vespidae) visiting *A. lycioides.* (J) *Cinnyris jugularis* sunbird robbing nectar from *A. lycioides*.

Figure 3. Results of histochemical analysis conducted in *Argyreia versicolor.* (A) Transversal section of the floral nectary showing the presence of terpenes; positive staining shown at the blue arrow pointing to a nectary duct (ND) and red arrows pointing to the nectary epidermis. (B) Longitudinal section of the floral nectary showing the presence of flavonoids under a fluorescence microscope; positive staining shown by the white arrow pointing to the nectary disc. (C) Staminal trichomes showing the presence of terpenes; positive staining shown by the red arrow pointing to the apical gland cell. (D) Unstained short glandular trichomes at the middle of staminal filaments; red arrow pointing to an apical gland cell that is globose shaped (GLO). (E) Unstained long glandular trichomes at the base of staminal filaments; red arrows pointing to rounded cylindrical (RCY) and obovoid (OBO) apical gland cells. (F) Staminal trichomes stained with Naturstoff reagent and viewed under fluorescent microscope reveal the presence of flavonoids; strong staining shown at the blue arrow, white arrow pointing to an apical cell of a non-glandular trichome.

Figure 4. Results of histochemical analysis conducted in *Argyreia mekongensis.* (A) Transversal section of the floral nectary showing the presence of terpenes; positive staining shown at the red arrows pointing to the nectary duct (ND) and epidermis (EP). (B)

Transversal section of the floral nectary stained with Sudan Black B showing the presence of lipids; positive staining shown at the red arrow pointing to the nectary duct (ND). (C) Transversal section of the floral nectary stained with Sudan III showing the presence of lipids in black. (D) Transversal section of the floral nectary stained with Naturstoff reagent and viewed under a fluorescent microscope showing the presence of flavonoids. (E) Staminal trichomes tested positive for terpenes; red arrows point to apical gland cells showing terpenes inside of the glands. (F) Staminal trichomes stained with Sudan Black B tested positive for lipids; red arrows point to apical gland cells containing lipids, and demonstrate the different types of gland cells: BE=bell-shaped, RCO=rounded conical. (G-H) Staminal trichomes stained with Sudan III tested positive for lipids; red arrows point to lipids inside of the apical gland cells, while green arrows demonstrate the different types of gland cells: RCY=rounded cylindrical, CON=convex, GLO=globose. Other abbreviations: N=nectary, OV=ovary, EP=epidermis.

Figure 5. Results of histochemical analysis conducted in *Argyreia lycioides*. (A) Longitudinal section of the floral nectary showing the presence of terpenes. (B) Longitudinal section of the floral nectary stained with Naturstoff reagent and viewed under a fluorescent microscope showing the presence of flavonoids. (C) Staminal trichomes tested positive for terpenes; positive staining shown at the red arrows, which also demonstrate some of the different types of apical gland cells: GLO=globose, RCY=rounded cylindrical. (D) Unstained glandular trichomes; red arrows demonstrate some of the different types of apical gland cells: PY=pyriform, OBO=obovoid. Other abbreviations: N=nectary, OV=ovary, BS=basal cells, S=stalk.

Table caption

Table 1: Results of histochemical analysis testing for the presence of terpenes, lipids, and flavonoids in the floral nectary and staminal trichomes of three sympatric *Argyreia* species.