

The lignin fluorescence at the base of the petals guiding the pollinators to the pollination chamber in *Artabotrys hainanensis*

Bingxin Li

South China Botanical Garden

Xiu Liu

South China Botanical Garden

Yanli Lin

South China Botanical Garden

Fengxia Xu

`xfx@scbg.ac.cn`

South China Botanical Garden

Research Article

Keywords: Annonaceae *Artabotrys hainanensis* Fluorescence Lignin Pollination chamber

Posted Date: December 10th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8198406/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Plant Biology on April 23rd, 2026.
See the published version at <https://doi.org/10.1186/s12870-026-08769-3>.

Abstract

In *Artabotrys hainanensis*, the pollination chamber aperture of the flowers at the blooming stage is narrow, and only exposed in the pistillate phase. It is worth studying how the pollinating insects enter the pollination chambers accurately through the narrow aperture. During flowering, the dynamics of the opening and closing of the pollination chambers ensure that sufficient pollen was carried by pollinating beetles (Carpophilinae) and facilitating successful pollination. *A. hainanensis* exhibits fluorescence at the base of the inner and outer whorls of petals, which marks the entrance to the pollination chamber, acting a role in pollinator attraction. The lignin content gradually accumulated as the epidermal hairs in pollination chamber part of petals increase in density and volume during flower development and the lignin fluorescent signals at the pollination chamber part reach their brightest at the blooming stage. The green fluorescence emitted by lignin under visible light could attract beetles, which have visual receptors that are sensitive to green light. Therefore, the lignin fluorescence produced by epidermal hairs that located at the aperture of the pollination chamber serves as a visual signal that guides the pollinating insects through the narrow pollination aperture and into the flower pollination chamber at anthesis. It is reasonable to speculate that lignin fluorescent guidance may exist in more other Annonaceae species, which provide visual cues for pollinators precisely locate the pollination chambers.

Background

Fluorescence as a common phenomenon in the flowers, leaves, stalks and roots of plant organism has some functions, such as biocommunication roles, ultraviolet defense, etc. The most common fluorescent substances in plants are lignin and chlorophyll. Additionally, there are numerous other fluorescent molecules in plant, including pigments, tannins, flavonoids, phenolic, etc. [1, 2]. Since flowers are important for plant reproduction, the fluorescence phenomenon in flowers has received much attention. The fluorescence appears in different structures of flowers, such as the nectary [3], stigma [4], ovary [4], pollen [5, 6], and petal [7, 8]. It plays an important role in the biocommunication between flowering plants and pollinators [1, 7]. Up to date, reports on fluorescent compounds that attract pollinators in flowers are very few. The blue fluorescence emitted by the hydroxycinnamoyl derivatives in pollen and anthers serve as a visual cue to attract honeybees under sunlight [9]. According to Gandía-Herrero et al. [7, 8], the betaxanthins in the petals of *Portulaca grandiflora* and *Mirabilis jalapa* produce a green fluorescence that may serve as a visual signal to attract pollinators. Also, the ferulic acid in petal, stamen and pollen emitted blue fluorescence in grasses play a potential role in insect attraction [6]. However, only two fluorescent compounds, ferulic acid and betaxanthins, have been reported in petals, which may serve as a visual cue to attract pollinators [6–8].

Lignin, a complex polyphenolic polymer synthesized in plants, is an important component of the secondary cell wall and widely distributed in plant stems and roots with physiological functions in structural support, water transport and disease defense [10]. As a natural fluorescent substance, lignin contains diverse fluorophores with a wide range of emission spectra, emitting blue, green and red fluorescence under UV and visible light excitation [11, 12]. The fluorescent properties of lignin have been

extensively used for cellular imaging and studying the structural characteristics of plant tissues, including investigate the cellular structure and cell type of xylem and vascular bundles, as well as the structure of wood-based products [10, 12–14]. As for the distribution of lignin in the flower structure, the reports in this area are very limited. As far as now know, the function of lignin fluorescence in flowers seems have never been recognized.

Annonaceae are a diverse family of flowering trees and climbers widespread in wet lowland and lower montane forests throughout the tropics. Annonaceae flowers possess whorls of distinct sepals and petals. The two petal whorls are furthermore generally morphologically distinct, with the inner petals often convergent or connivent to form a partially enclosed pollination chamber [15]. Pollination chamber provides a place for insects to feed and mate [16–18]. While in some sense, it plays as a trap [19, 20]. *Artabotrys hainanensis*, a species from Annonaceae, exhibits a tightly enclosed pollination chamber with an elaborate rim between the inner petal blade and claw. The pollination chamber aperture of the flowers at the blooming stage is narrow and its opening time is very short only about 8 hours in *A. hainanensis*. Aimed to explore how insects accurately locate and enter the pollination chamber in *A. hainanensis*, we observed the floral phenology and pollinator circadian rhythms, monitored the fluorescence of petal under blue light, compared the morphology and structure of epidermal hairs in petal, and the lignin content of the petals during flower development stages.

Materials and Methods

Artabotrys hainanensis R. E. Fries is a climbing shrub that inhabits subtropical forests in southeastern China. *Artabotrys hainanensis* was identified by Prof. Xueliang Hou. Voucher specimens have been deposited in the publicly available herbarium of the South China Botanical Garden, Chinese Academy of Sciences (IBSC:0077396). Field observations of *A. hainanensis* were conducted in the South China National Botanical Garden, Guangdong Province, China (113.373°N, 23.188°E). The petal of buds and mature flowers from differentiation up to anthesis were collected during the period of September 2021 to November 2022, and separately immediately fixed in prepared 2.5% glutaraldehyde (4°C) or in formalin acetic alcohol (FAA (v/v): 70% alcohol, formaldehyde, and glacial acetic acid at a ratio of 18:1:1) for more than 24 hours to preserve.

Floral Phenology

Phenological studies were conducted over two successive flowering seasons (2021–2022) to identify the timing and duration of floral anthesis. A total of 50 flower buds were tagged and monitored every day and hourly for bud development and anthesis, respectively. The onset and duration of stigmatic receptivity, presence or absence of stigmatic exudate and fragrance, and color changes in the petal and stigmas were recorded throughout the pistillate phase. The presence of stigmatic exudate was observed to be correlated with stigmatic receptivity and was accordingly used as supporting evidence for determining the onset of the pistillate phase [21]. Stigmatic receptivity was confirmed by immersing the stigmas in 3% hydrogen peroxide (H₂O₂) solution and observing bubble formation, which is the result of

peroxidase enzyme activity [22]. The staminate phase was recognized by anther dehiscence, associated color changes recorded throughout the staminate phase. In addition, the presence of an interim phase of asexual functioning between the end of the pistillate phase and the beginning of the staminate phase was recorded.

Petal Fluorescence Survey

The whole flower and inner and outer petal of *A. hainanensis* at different developmental stages were observed and photographed under white light and blue light in a fluorescence stereomicroscope (LEICA 205FA), respectively, with the excitation wavelength of blue light at 450–490 nm. For each developmental stage, at least five biological replicates were analyzed. The average fluorescence intensity in the pollination chamber part of the petals were measured using ImageJ software, and the resulting data were analyzed and plotted.

Epidermal Hair Morphological Observation

The fresh petal materials of *A. hainanensis* at different developmental stages that fixed in FAA, followed by dehydration and transparency for paraffin-embedded. Serial sections of 8 µm thick were prepared using automatic rotary microtome (Thermo Scientific HM355S) and stained with Hematoxylin-Eosin. The other part of the paraffin section without staining was directly placed under fluorescence stereomicroscope and observed by blue light irradiation.

The fresh petal materials of *A. hainanensis* at different developmental stage and in different parts of the petal that fixed in 2.5% glutaraldehyde, followed by dehydration in a graded water-ethanol series, and dried with a CO₂ critical-point dryer. After being sputter-coated with gold, the dried petals were examined with a JSM-6360LV (JEOL, Tokyo, Japan) scanning electron microscope operated at 25kV.

Fluorescent Compound Identification

Fresh petal segments (1 cm × 1 cm) from both the pollination chamber and non-pollination chamber regions were dissected and immersed in a dissociative solution (30% hydrogen peroxide: acetic acid (v/v) = 1:1), and incubated in a thermostatic water bath at 60°C for 2 h. After the petal tissues and epidermal cells were separated, the dissociated materials were removed, and the broken tissues and cells were gently swept away with a brush and rinsed in water. The upper epidermis of different parts of the petals were placed on slides dripping with water, sealed with glycerol. Another dissociated petal epidermis was placed on a slide and a drop of 2% phloroglucinol ethanol solution (2 g of phloroglucinol dissolved in 100 mL of 95% ethanol) on the sample stained for 2 min, and then an equal amount of 25% HCl was dripped in to visualize the coloration for 2 min, and then the excess of the staining solution was sucked off, covered with a coverslip. All the above sections were observed and photographed using a light microscope (LEICA DM5500 B).

Fresh petal epidermal materials of *A. hainanensis* flowers at different developmental stages were collected and assayed for lignin content using the acetyl bromide method, with reference to Fang et al.

[23]. The lignin content was measured in the pollination chamber parts of inner and outer whorl petals as well as in the non-pollination chamber parts, respectively. A standard curve was generated using purified lignin, with glacial acetic acid serving as a blank control. The UV absorption values of the samples were measured at 280 nm, with three independent biological replicates performed.

Floral Visitors and Visiting Behavior Analysis

We observed floral visitors to 50 flowers of *A. hainanensis* at the South China National Botanical Garden in 2021–2022. Flowers were observed throughout anthesis at 1h intervals between 9:00 a.m. and 9:00 p.m. each day to record the arrival and departure patterns of the floral visitors. The following criteria were used to determine whether floral visitors were effective pollinators: (1) relative visitation rates; (2) the coincidence of visits and the duration of pistillate or staminate phases in the flowers; (3) the attachment of *A. hainanensis* pollen grains to the floral visitors, determined using LM and/or SEM; and (4) evidence of movement of floral visitors between flowers of different anthesis phases [24]. The floral visitor was captured into plastic centrifuge tube and after checking for attached pollen grains, preserved in 70% alcohol for further identification.

The flowers of *A. hainanensis* in the pistillate phase were collected, and captured pollinators hidden in the pollination chambers of flowers in the interim phase and staminate phase in the morning and brought them back to the laboratory every day. The collected flowers in the pistillate phase were divided into two groups, A and B, in which the flowers of group A were painted black at the pollination chamber part to block the fluorescent of the petals, and the flowers of group B were without any treatment, and three flowers of each group were placed in two glass vials, and four pollinators were carefully put into each glass vial, the caps of the vials were then sealed to prevent the beetles from flying away. The behavior of insects visiting flowers was observed and recorded under both natural light and blue light. Each observation session lasted 4–5 hours, and the experiment was replicated five times, encompassing a total of 30 flowers.

Results

Floral Phenology

The flowers of *A. hainanensis* have two whorl petals, with three petals per whorl, the base of each petal is concave and converges to form a closed pollination chamber that surrounds the reproductive organs (Fig. 1), which are hermaphroditic and protogynous, with anthesis lasting over two days (Fig. 2). Duration from the early bud stage to the end of flowering lasts about 32 days (Fig. 1), and six different stages of floral development are apparent as follows:

Stage I: early bud (ca. 15d). Flower buds are green, petal length approximate 0.5cm, with initially the larger sepals enclosing the petals, which beginning to separate after 2 or 3 days, exposing the outer whorl of petals, with the apical portion of the inner whorl of petals are visible (Fig. 1A).

Stage II: petal elongation (7-8d). Both whorls of petals remain green, petal length approximate 1.5cm. At this stage the petals elongate rapidly, and as the petals progress the base of inner whorl petals begin to concave and tightly compress against each other forming the prototype of the pollination chamber, while this curvature is not obvious on the outer petals. The flowers gradually become pendulous with increasing weight (Fig. 1B).

Stage III: pollination chamber formation (ca. 8d). The petals of the inner and outer whorls continue to enlarge and gradually turn yellow, petal length approximate 3-4cm, with the bases of the inner and outer whorl petals become constricted and inwardly curved and the close-type pollination chamber fully developed. The pollination chambers have three apertures located between the basal edges of adjacent inner whorl petals and are sealed by the outer whorl petals (Fig. 1C).

Stage IV: pistillate phase (ca. 8h). The pistillate stage commenced at approximately 9:00 a.m. and persisted until 5:00 p.m., on the first day of flowering (Fig. 2: A-B). Flowers are off-white, petal length approximate 4 cm, and the outer whorl of petals gradually opens to a position that is nearly horizontal, thereby exposing the apertures of the pollination chamber. The apertures are approximately 5 mm in length and 3 mm in width (Fig. 1D, E). Additionally, a fruity-sweet fragrance can be perceived. The stigma is tender and succulent, with a thin layer of secretion appearing on the surface. The yellow stamens were tightly packed together and the anthers did not dehisce (Fig. 1. F). Toward the end of the stage, the outer whorl of petals converged inward, the pollination aperture closed, and the floral scent fades and difficult to smell.

Stage V: interim phase (ca. 16h). The interim phase commences at approximately 5:00 p.m. on the first day of flowering and terminates at 9:00 a.m. on the second day of flowering (Fig. 2: B-C). Flowers are off-white and at this stage the outer whorl of petals pendulous, the pollination chamber is closed (Fig. 1G, H). The stigma is soft and easily dislodged, the stigma surface no longer produces secretion, and there is no perceptible scent ((Fig. 1I).

Stage VI: staminate phase (ca. 2h). The staminate stage commences at 9:00 a.m. on the second day of flowering and persists until approximately 11:00 a.m. (Fig. 2: C-D). Flowers are off-white, pollination chambers remain closed, petal bases become deepen and petal tips soften, gaps between stamens widen begin to abscise, and the thecae dehisce pollen disperses inside pollination chambers (Fig. 1J, K). Stigma tip turns dark brown to black and abscise from ovary. At the end of this stage, the stamens and pollen are gradually fall from the receptacle along with the petals, within a very brief period (Fig. 1L).

Petals Fluorescence of *A. hainanensis*

The petal pollination chamber part was observed emitting green fluorescence under blue light illumination, which gradually increases with the petal development (Fig. 3, 4). The fluorescence intensity was very faint with approximately 3.1 as measured by the ImageJ software (Fig. 4), but was almost indistinguishable by naked eye (Fig. 3A-F) at S1. The fluorescence intensity could be observed with an intensity of 11.6 (Fig. 3G-L) when the petal developed to S2 and that increased to 20.9 after the

pollination chamber formed (S3), makes a clearer distinction in the pollination chamber of the petals in comparison with the non-pollination chamber part of the petals (Fig. 3M-R). The fluorescence intensity of the pollination chamber site was 24.3 at S4 (Fig. 4), and the outer whorl of petals opens to expose the pollination chamber apertures, which shown as three bright circles around the center of the flower (Fig. 3S-X). In addition, the fluorescence of petals non-pollination chamber part was always inconspicuous, and the fluorescence intensity of the pollination chamber portion of the inner whorl petals was consistently brighter than that of the outer whorl petals.

The Epidermal Hairs Morphology

The mature peals transverse sections of paraffin sections at the pollination chamber area and non-pollination chamber parts of the two whorls petals show that except the epidermal hairs exhibit various morphology, no significant differences were observed in histological structures between the inner and outer whorl petals (Fig. 5). The epidermal hairs were curly and dense at the petal pollination chamber site (Fig. 5A, B, D, E, G, H, J, K), whereas they were acicular erect and sparse at the non-pollination chamber part of petal (Fig. 5C, F, I, L).

The morphology of petal epidermal hairs was observed using a scanning electron microscope, the pollination chamber part of S1 petals were smooth and free of epidermal hairs (Fig. 6: A, C), whereas the non-pollination chamber parts of petals were densely covered with erect acicular epidermal hairs (Fig. 6: B, D). The densely curled and twisted epidermal hairs were observed in the pollination chamber part of S2 petal (Fig. 6: E, G), and the density of epidermal hairs in non-pollination part of petals relatively reduced and exhibiting an erect acicular morphology resemble S1 (Fig. 6: F, H). Epidermal hairs in the pollination chamber further curled and twisted and increased in length as the petals grow to S3 and S4 (Fig. 6: I, K, M, O), and as the petal elongated, the distribution of epidermal hairs in non-pollination chamber parts became more sparser (Fig. 6: J, L, N, P). The averages length of epidermal hairs in the pollination chamber part of mature petal was 50 μm , and the density of epidermal hairs per unit area was about 2000 hairs/ mm^2 , while the average length of epidermal hairs in the non-pollination chamber part was 700 μm , and the density of epidermal hairs per unit area was about 35 hairs/ mm^2 .

Fluorescent Compound Lignin and Content

Unstained transverse paraffin sections of petals emitted bright green fluorescence of epidermal hairs and vascular bundles under the blue light of a fluorescent stereomicroscope (Fig. 7A-D). The fluorescent properties of epidermal hairs are like those of the vascular bundles, and the latter can fluoresce due to the presence of the fluorescent compound lignin, so it is assumed that the petals fluorescence of *A. hainanensis* flowers may be derived from the lignin within the epidermal hairs. To prove the above hypothesis, the epidermal hair together with the epidermis cell layers of the mature petals were separated from petal tissue (Fig. 7E, F). Then stained with phloroglucinol, epidermal hairs of both morphologies were stained red, while the epidermal cell layer was not colored, indicating that both epidermal hairs contained lignin and the epidermal cells lacked lignin (Fig. 7G, H).

The lignin content of the petals gradually increased as flowers developed from S1 to S4 (Fig. 8). The lignin content of the non-pollination chamber part of the petals was higher than that of the pollination chamber part of the petals only at the early bud stage (S1), and thereafter, the lignin in the pollination chamber part gradually accumulated, and its lignin content was significantly higher than that of the non-pollination chamber part (S2-S4). Moreover, the lignin content of the pollination chamber part of the inner whorl petals was always higher than that of the outer whorl petals. Corresponding to the lignin content, the fluorescence intensity of the petal pollination chamber part also gradually increased with flower development, the fluorescence of the pollination chamber area was more obvious than that of the non-pollination chamber part, and the fluorescence of the pollination chamber part of the inner whorl petals was brighter than that of the outer whorl petals. That is, the lignin contained within the epidermal hairs determines the fluorescence intensity emitted by the petals of *A. hainanensis* flower, while the number and density of the epidermal hairs affects the lignin content in the petal.

Floral Visitors and Pollinator Flower Visiting Behavior

The insect observed in the field identified three kinds visiting *A. hainanensis* flowers during the anthesis period, including small beetles (Fig. 9A), cockroach larvae (Fig. 9D), and ants (Fig. 9E). The observation of cockroach larvae entering the pollination chambers was noted to occur during the pistillate phase, with the larvae remaining inside for approximately 30 minutes before climbing out, when the pollination chamber was opened by hand, some petals and stamens were blackened by eating (Fig. 9D). Only one visit of cockroach larvae to flowers was observed and they did not move from flower to flower, which assumed that they only feed on petals and stamens and therefore cockroach is not considered an effective pollinator, but an opportunist. The ants mainly crawled on the petals and moved around the base of the petals (Fig. 9E), were not observed entering the pollination chamber, and the limited mobility of the ants led to consider that the ants were not effective pollinators of *A. hainanensis*.

The small beetles were observed to fly into the petals of flowers in the pistillate phase, subsequently crawling towards the basal part of the flower and entering the pollination chamber through the apertures. Gently open the petals of a flower that had small beetles entered, it was found that the small beetles mostly stayed near the stigma and touching the stamens as they moved in the flower (Fig. 9B). After entering the pollination chamber, most of the small beetles stayed inside, and at night when the apertures of the pollination chamber closed the beetles were trapped inside until the end of the staminate phase when the petals fell off and the beetles were released (Fig. 9C). In field observations, beetles were leaving a flower at the end of the staminate phase and flying to visit another *A. hainanensis* flower at pistillate phase. The small beetles (ca. 3mm long, abdomen ca. 2mm wide) were captured back to the laboratory and identified as belonging to the subfamily Carpophilinae (Coleoptera: Nitidulidae) via stereomicroscope and scanning electron microscopic. The beetles carried pollen from *A. hainanensis* flowers (Fig. 9F-I), combined with the observation that the timing of the beetles' activities corresponded to the flower anthesis, suggests that the small beetles of the subfamily Carpophilinae are effective pollinators.

Untreated flowers in vials at the laboratory of group B, the beetles entered the vials and flew to the flowers for about 6 minutes in average, with a frequency of about 3 visits/hour and a residence time of 2 hour (Fig. 9J, K; Table. 1). Artificially blackened petal pollination chamber in vials of group A, the beetles entered the vials and flew to the flowers for an average time of about 15 minutes, with a visit frequency of about 1 visit/hour and a residence time of 15 minutes (Fig. 9L; Table. 1). Comparison of the results revealed that after blocking the pollination chamber of the flower petals which could emit fluorescence, the beetles needed to spend longer time to find the flower, and the frequency of visits and the time they stayed on the flowers were also significantly reduced. Besides, beetles in glass vials of group A visited flowers are disoriented, crawling in all directions of the petals, indicating that pollinators were unable to accurately locate the flower when blocking the fluorescence of petals.

Table 1
Records of laboratory insect visits to flowers.

	Autofluorescence uncovered	Autofluorescence covered
Mean time from bottle entry to flower alighting for a beetle	6 minutes	15 minutes
Average visiting frequency (times/hour)	3	1
Average handling time	2 hours	15 minutes

Discussion

The Pollination Chambers as Pollinator Traps Increase Pollination Efficiency

The *A. hainanensis* flower exhibits hermaphroditic and protogyny characteristics, and possesses a close-type pollination chamber, which was considered as the pollinators trap, a structure that prevents a flower visitor from leaving before pollination has been completed [19]. Upon entering the pistillate phase at 9:00 a.m., on the first day of blooming, the pollination chamber apertures were opened, and remained opening throughout the pistillate phase, allowing pollinators access to the chamber. The pistillate phase concludes at 5:00 p.m. on the first day of flowering, at which point the pollinator chamber apertures were closed, blocking the pollinators leaving from the chamber, and trapping the potential pollinators into the chamber. The pollinator chamber apertures were maintained in a closed position throughout the interim phase (from 5:00 p.m. on the first day to 9:00 a.m. on the second day of anthesis) and the staminate phase (from 9:00 a.m. to 11:00 p.m. on the second day of anthesis). During the latter, due to the closed pollination chamber, the pollinators can only move around the pollen-filled pollination chamber, carrying enough pollen on their body. The pollination chamber structure was destroyed when the staminate phase was concluded (at 11:00 a.m.), as the petals had fallen off the receptacle, and the pollinators were able to fly out of the chamber. In field observations, the end of the staminate phase of one flower of *A. hainanensis* was found to coincide with the peak of stigma receptivity of another flower at pistillate phase, and the pollen-laden pollinators exited the flowers at the end of the staminate phase and were

attracted by flowers in the pistillate phase, subsequently entering their pollination chamber and transferring pollen to the stigma. Alternatively, they may feed on the nectar-rich secretions within the flower and subsequently fly to another flower, also in the pistillate phase, with the potential to pollinate several flowers.

In most species of Annonaceae, such as *Goniothalamus tapisoides* [18], *Dasymaschalon trichophorum*, and, *Friesodielsia borneensis* [19], the pollination chamber acts as a pollinator trap, and exhibits resemble dynamic changes in anthesis as *A. hainanensis*, although the duration of the phases in these species may be different, but the anthesis phenology of these species was found to cooperate with the activity patterns of pollinating insects to ensure efficient pollination. Lau et al. [19] posit that such pollinator traps, when acted upon by pollinator chambers, possess distinct selective advantages. These include the expansion of the range of potential pollinators based on their activity patterns, the prolongation of the time during which stamen pollen is deposited on the bodies of pollinators, and the enhancement of inter-flower movement of pollinators. The ultimate purpose of this process was to successfully pollination and promote fruiting. Although the structure of pollination chambers and the operational mechanisms of pollinator traps may be different between species, this is a common feature in Annonaceae, and is likely to be a key evolutionary innovation of considerable functional importance.

Flower fluorescence attracts pollinators to visit flowers

The flower fluorescence was widespread in plants and has been proposed as a potential visual signal to attract pollinators [2, 4, 9]. Pollen and anthers of many plants usually emit bright blue fluorescence under UV light [5, 25], which may protect the genes in pollen from harmful UV energy through a transduction of absorbed UV to the fluorescence [26, 27], and may be the attraction of pollinators by highlighting pollen as food as well [9]. The pollen of *A. hainanensis* also exhibits pronounced fluorescence, yet it could not be detected by the pollinators outside, even when pollen was dispersed during the staminate phase, since the close-type pollination confines the pollen effectively. Olfactory, as another cues during the flowering period, often attracts pollinators over long distances. Previous research indicated that the visual signals are more effective than olfactory signals when insects approach flowers [28–30], since the diffuse nature of these olfactory signals makes it difficult for pollinators to precisely locate the flower using their sense of smell alone [31, 32], especially in some species with pollination chambers, such as Annonaceae, Myristicaceae, Araceae and Dipterocarpaceae [33–35]. In *A. hainanensis*, flowers exhibit an obvious scent during the pistillate phase, while the pollination chamber aperture was narrow (approximately 5 mm in length and 3 mm in width, comparing the petals of mature flowers are approximately 4.5cm in length and 7mm in width), and its opening time was only about 8 hours (constraining to the pistillate phase, from 9:00 a.m. to 5:00 p.m. on the first day of anthesis). Had no assistance, it would be with great effort for the pollinating insects to enter the pollination chamber through the narrow aperture in a relatively short time. Fortunately, the fluorescence exhibited by the two whorls petals provided an orientation signal for the position of the pollination chamber aperture. The effective pollinator of the *A. hainanensis* flower was the small beetles (ca. 3mm long, abdomen ca. 2mm wide) belonging to the subfamily Carpophilinae (Coleoptera: Nitidulidae), and their eyes could perceive a

broad spectrum of light, ranging from 340 nm (ultraviolet, UV) to 540 nm (green light) [36–38]. We suggested that the green fluorescence around the aperture of the pollination chamber could guide the pollinators speed through the apertures and enter the chamber with precision. It was terrified when the fluorescence around the aperture of the pollination chamber was artificially obscured in the lab. We observed that the frequency of the beetles visiting the flowers was reduced. Even the beetles landed on the flowers, they became disoriented and crawled randomly on the petals, unable to locate accurately the aperture of the pollination chamber and enter it. It indicated that the pollinators can be attracted to visit the flowers by the olfactory, but they cannot accurately localize the pollination chamber apertures without the fluorescence around the aperture. In *A. hainanensis*, the challenge of a closed pollination chamber is overcome by strong autofluorescence at the base of the petals, which provides visual guidance that directs beetles—initially attracted by long-range scent—from long-range olfactory attraction to pinpointing the chamber opening via short-range fluorescent cues, thus ensuring successful pollination.

Lignin Acts as a Fluorescent Component in the Flower

Most flowers typically contain a diverse array of pigment compounds that can exhibit fluorescence in response to varying light irradiation. The compounds that make petals fluoresce of the flowers were pigment molecules, including betaxanthin (*Portulaca grandiflora* and *Mirabilis jalapa*) [7, 8], aurones (*Antirrhinum majus*) [39], and flavonoids (*Ornithogalum thyrsoides*) [4]. However, the substance that made the petals of *A. hainanensis* fluoresce was lignin, which is unique and noteworthy. The current research on lignin fluorescence characteristics has been no reports of lignin present in floral structures that have demonstrated its fluorescent properties with specific functions, but was primarily concerned with utilizing it for the cell structure and cell type of xylem and vascular bundles [10, 12–14].

In *A. hainanensis*, at the early stage of flower development (S1 stage), when the epidermal hairs of the petal initiation appeared, weak fluorescence could be observed by fluorescence stereomicroscopy, and the lignin began to accumulate in epidermal hairs. The fluorescence intensity of the petals is dependent on the lignin content within the epidermal hairs, that exhibited both an erect morphology and a curly one, with the former sparsely distributed at the non-pollination chamber part, and the later densely distributed at the pollination chamber part of the petal, with higher lignin content and relative to the fluorescence of the non-pollination aperture. During the flower development from S2 to S4, lignin accumulates and the epidermal hairs in pollination chamber part of petals undergo a significant increase in density and volume, as observed by scanning electron microscopy. Correspondingly, the lignin content of the petal pollination chamber part obviously increased, and the fluorescence enhanced at the pollination chamber part of petals. The fluorescent signals at the pollination chamber part of *A. hainanensis* reach their brightest when the flowers are in blooming. Lignin has a broad range of autofluorescence emission and can be excited with both UV and visible light, emitting blue, green and red fluorescence [12, 40–42]. The green fluorescence emitted by lignin under visible light could attract beetles (Nitidulidae), which have visual receptors that are sensitive to green light. The investigation of this present study revealed that lignin fluorescence can serve as an optical signal in the context of plant-animal interactions, particularly

between insects and plants, analogous to other flower fluorochrome molecules. As a widespread component of plant organisms, the distribution of lignin in floral structures is a topic worthy of further investigation and the function of lignin fluorescence phenomena in flowers deserves attention.

Conclusion

During flowering, the dynamics of the opening and closing of the pollination chambers and the pollinators were able to fly out of the chamber until the petals fallen off ensures that sufficient pollen was carried by pollinating beetles and facilitating successful pollination. The pollinators entered the pollination chamber through the narrow aperture guided by the lignin fluorescence from the base of the petal. The epidermal hairs on the petals contained lignin. With the increase of density and volume of the hairs, the fluorescence signals enhanced, and reached their brightest when the flowers were in blooming.

Abbreviations

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication*

Not applicable.

Data availability

All the data are contained within the article.

Competing Interests

The authors declare that this study does not involve any conflicts of interest. All authors have read and agreed to the published version of the manuscript.

Funding

This work was financially supported by the National Natural Science Foundation of China (32370406; 32300193; 31970188), the Natural Science Foundation of Fujian Province of China (2022J011187), and the Start-up Foundation for Advanced Talents in Sanming University (RD21002P).

Authors' contributions

Bingxin Li: Conceptualization, Funding acquisition, Data curation, Formal analysis, Methodology, Investigation, Visualization, Software, Writing – original draft. Xiu Liu: Conceptualization, Data curation, Formal analysis. Yanli Lin: Investigation, Methodology, Formal analysis, Visualization. Fengxia Xu: Writing – review & editing, Project administration, Funding acquisition, Conceptualization, Supervision, Validation.

Acknowledgements

Not applicable.

References

1. Lagorio MG, Cordon GB, Iriel A. Reviewing the relevance of fluorescence in biological systems. *Photoch Photobio Sci*. 2015;14:1538–59.
2. Donaldson L. Autofluorescence in Plants. *Molecules*. 2020;25:2393.
3. Davies KL, Stpiczynska M, Gregg A. Nectar-secreting floral stomata in *Maxillaria anceps* Ames. *Ann Bot*. 2005;96:217–27. C. Schweinf. (Orchidaceae).
4. Iriel A, Lagorio MG. Is the flower fluorescence relevant in biocommunication? *Naturwissenschaften*. 2010;97:915.
5. Roshchina VV. Vital Autofluorescence: Application to the study of plant living cells. *J Spectro SC*. 2012;124672:14.
6. Baby S, Johnson AJ, Govindan B, Lukose S, Gopakumar B, Koshy KC. UV induced visual cues in grasses. *Sci Rep*. 2013;3:2738.
7. Gandía-Herrero F, Escribano J, García-Carmona F. Betaxanthins as pigments responsible for visible fluorescence in flowers. *Planta*. 2005a;222:586–93.
8. Gandía-Herrero F, Garcia-Carmona F, Escribano J. Floral fluorescence effect. *Nature*. 2005b;437:334–334.
9. Mori S, Fukui H, Oishi M, Sakuma M, Kawakami M, Tsukioka J, Goto K, Hirai N. Biocommunication between plants and pollinating insects through fluorescence of pollen and anthers. *J Chem Ecol*. 2018;44:591–600.
10. Kitin P, Nakaba S, Hunt C, Lim S, Funada R. Direct fluorescence imaging of lignocellulosic and suberized cell walls in roots and stems. *Aob Plants*. 2020;12:plaa032.
11. Hafren J. Excitation wavelength-specific changes in lignocellulosic autofluorescence. *J Wood Sci*. 2007;53:358–60.
12. Donaldson L. Softwood and hardwood lignin fluorescence spectra of wood cell walls in different mounting media. *Iawa J*. 2013;34:3–19.
13. Castellan A, Ruggiero R, Frollini E, Ramos LA, Chirat C. Studies on fluorescence of cellulose. *Holzforschung*. 2007;61:504–8.

14. Donaldson LA, Kroese HW, Hill SJ, Franich RA. Detection of wood cell wall porosity using small carbohydrate molecules and confocal fluorescence microscopy. *J Microsc.* 2015;259:228–36.
15. Saunders RMK. The evolution of key functional floral traits in the early divergent angiosperm family Annonaceae. *J Syst Evol.* 2020;58:369–92.
16. Thien LB, Azuma H, Kawano S. New perspectives on the pollination biology of basal angiosperms. *Int J Plant Sci.* 2000;161:225–35.
17. Seymour RS, White CR, Gibernau M. Heat reward for insect pollinators. *Nature.* 2003;426:243–4.
18. Lau JYY, Pang CC, Ramsden L, Saunders RMK. Reproductive resource partitioning in two sympatric *Goniothalamus* species (Annonaceae) from Borneo: floral biology, pollinator trapping and plant breeding system. *Sci Rep.* 2016;6:35674.
19. Lau JYY, Guo X, Pang CC, Tang CC, Thomas DC, Saunders RMK. Time dependent trapping of pollinators driven by the alignment of floral phenology with insect circadian rhythms. *Front Plant Sci.* 2017;8:11–9.
20. Chen J, Liu MF, Saunders RMK. Contrasting floral biology of *Artabotrys* species (Annonaceae): implications for the evolution of pollinator trapping. *Plant Species Biol.* 2020;1–14.
21. Dafni A. Pollination ecology: a practical approach. Oxford: Oxford University Press; 1992.
22. Galen C, Plowright RC. Testing the accuracy of using peroxidase activity to indicate stigma receptivity. *Can J Bot.* 2011;65:107–11.
23. Fang L, Ishikawa T, Rennie EA, Murawska GM, Lao JM, Yan JW, Tsai AYL, Baidoo EK, Xu J, Keasling JD, Demura T, Kawai-Yamada M, Scheller HV, Mortimer JC. Loss of inositol phosphoryl ceramide sphingolipid mannosylation induces plant immune responses and reduces cellulose content in *Arabidopsis*. *Plant Cell.* 2016;28:2991–3004.
24. Pang CC, Saunders RMK. Floral biology and pollination ecology of *Desmos chinensis* (Annonaceae): assessing the efficacy of floral synchrony for promoting xenogamy. *Int J plant sci.* 2015;176:333–45.
25. Fukui H, Hirai N, Mori S, Goto K, Toyoda J, Tsukioka J. Floral fluorescence database. The Garden of Medicinal Plants, Kyoto Pharmaceutical University. 2017. <http://labo.kyoto-phu.ac.jp/mpgkpu/ffd.html>
26. Hoque E, Remus G. Natural UV-screening mechanisms of Norway spruce (*Picea abies* [L.] Karst.) needles. *Photochem Photobiol.* 1999;69:177–92.
27. Rozema J, Broekmana RA, Blokkerb P, Meijkampa BB, de Bakker N, van de Staaij J, van Beem A, Ariese F, Kars SM. UV-B absorbance and UV-B absorbing compounds (para-coumaric acid) in pollen and sporopollenin: the perspective to track historic UV-B levels. *J Photochem Photobiol B Biol.* 2001;62:108–17.
28. Dafni A, Bernhardt P, Shmida A, et al. Red bowl-shaped flowers: convergence for beetle pollination in the Mediterranean region. *Isr J Bot.* 1990;39:81–92.

29. Giurfa M, Vorobyev M, Kevan P, Menzel R. Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. *J Comp Physiol A*. 1996;178:699–709.
30. Dötterl S, Vereecken NJ. The chemical ecology and evolution of bee-flower interactions: a review and perspectives. *Can J Zool*. 2010;88:668–97.
31. Gong YB, Huang SQ. On methodology of foraging behavior of pollinating insects. *Biodivers Sci*. 2017;15:576–83.
32. Reeves JL. Vision should not be overlooked as an important sensory modality for finding host plants. *Environ Entomol*. 2011;40:855–63.
33. Armstrong JE, Drummond BA. Floral biology of *Myristica fragrans* Houtt. (Myristicaceae), the nutmeg of commerce. *Biotropica*. 1986;18:32–8.
34. Young HJ. Beetle pollination of *Dieffenbachia longispatha* (Araceae). *Am J Bot*. 1986;73:931–44.
35. Momose K, Nagamitsu T, Inoue T. Thrips cross-pollination of *Popowia pisocarpa* (Annonaceae) in a lowland dipterocarp forest in Sarawak. *Biotropica*. 1998;30:444–8.
36. Briscoe AD, Chittka L. The evolution of color vision in insects. *Annu Rev Entomol*. 2001;46:471–510.
37. Döring TF, Skellern M, Watts N, Cook SM. Colour choice behaviour in the pollen beetle *Meligethes aeneus* (Coleoptera: Nitidulidae). *Physiol Entomol*. 2012;37:360–78.
38. van der Kooi CJ, Vallejo-Marín M, Leonhardt SD. Mutualisms and (A)symmetry in Plant-Pollinator Interactions. *Curr Biol*. 2021;31:91–9.
39. Ono E, Fukuchi-Mizutani M, Nakamura N, Fukui Y, Yonekura-Sakakibara K, Yamaguchi M, Nakayama T, Tanaka T, Kusumi T, Tanaka Y. Yellow flowers generated by expression of the aurone biosynthetic pathway. *PNAS*. 2006;103:11075–80.
40. Radotić K, Kalauzi A, Dikanović D, Jeremić M, Leblanc RM, Cerović ZG. Component analysis of the fluorescence spectra of a lignin model compound. *J Photochem Photobiol B Biol*. 2006;83:1–10.
41. Djikanović D, Kalauzi A, Radotić K, Jeremić M. Deconvolution of lignin fluorescent spectra: A contribution to the comparative structural studies of lignins. *Russ J Phys Chem A*. 2007;81:1425–8.
42. Donaldson LA, Knox JP. Localisation of cell wall polysaccharides in normal and compression wood of radiata pine – Relationships with lignification and microfibril orientation. *Plant Physiol*. 2012;158:642–53.

Figures



Figure 1

Flowers of *Artabotrys hainanensis* at different phenological stages. A: Early bud stage; B: Petal elongation stage; C: Pollination chamber formation stage; D-F: Pistillate phase, outer petals extend outward, exposing the pollination chamber holes (arrows); G-I: Interim phase, outer wheel petals pendulous, arrows indicate pollinating hole closed; J-L: Staminate phase, outer petals extend outward,

exposing the pollination chamber holes (arrows); L: End of staminate phase, petals and stamens detached from receptacle.

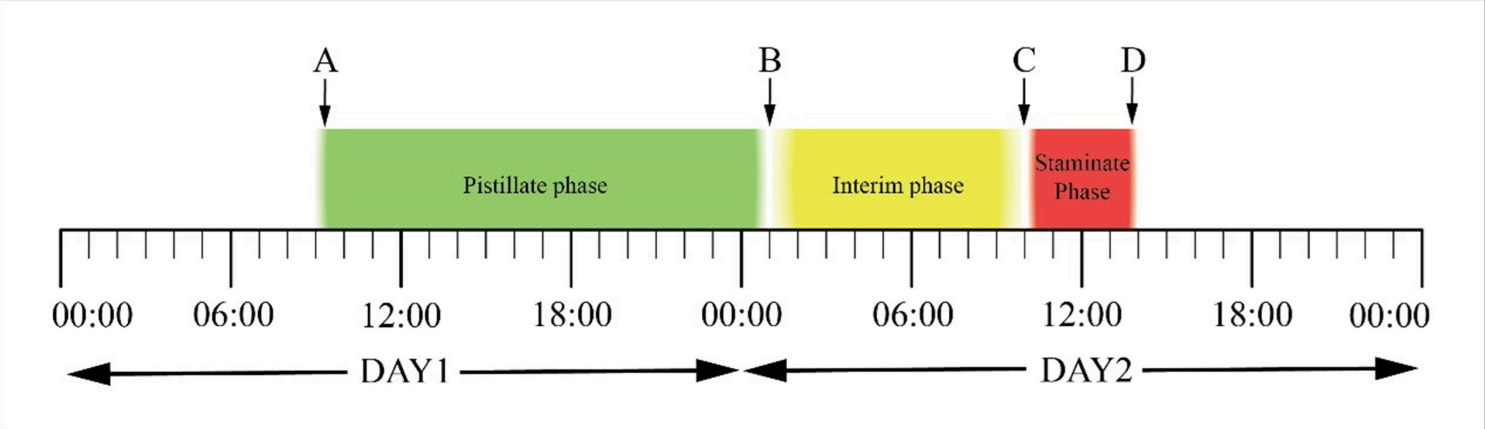


Figure 2

Flowers of *Artabotrys hainanensis* at different phenological stages. A: Initiation of pistillate phase, rise of outer petals and exposing the basal apertures; B: Initiation of interim phase, close of outer petals and blocking the basal apertures; C: Initiation of staminate stage; D: End of staminate stage, abscission of petals and stamens, and departure of pollinators.

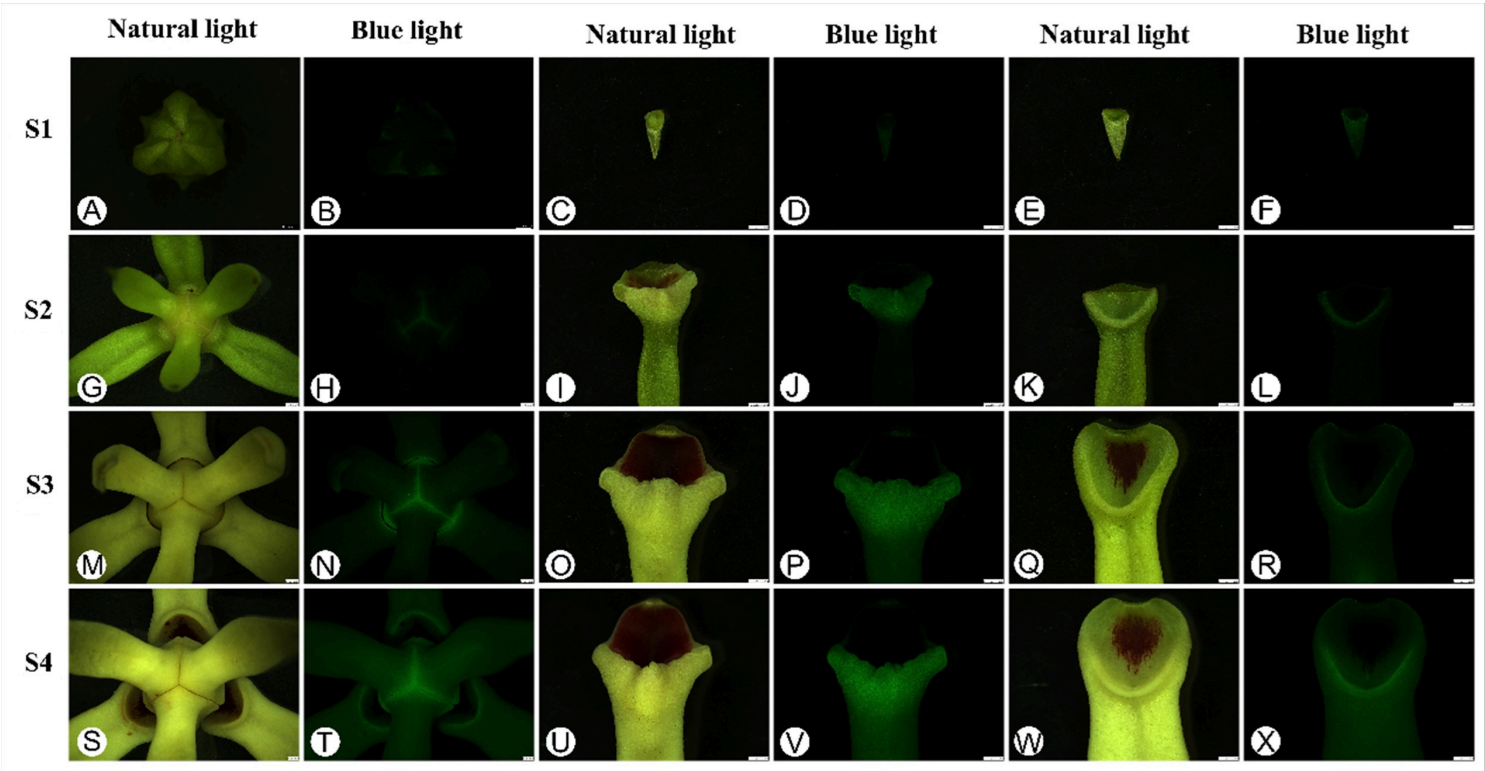


Figure 3

Autofluorescence phenomenon of *Artabotrys hainanensis*. A-B: Image of flower in stage 1 under bright field and blue light; C-F: Image of inner petal in stage 1 under bright field and blue light; G-H: Image of flower in stage 2 under bright field and blue light; I-L: Image of inner petal and outer petals in stage 2

under bright field and blue light; M-N: Image of flower in stage 3 under bright field and blue light; O-R: Image of inner petal and outer petals in stage 3 under bright field and blue light; S-T: Image of flower in stage 4 under bright field and blue light; U-X: Image of inner petal and outer petals in stage 4 under bright field and blue light. Scale bars:5 mm.

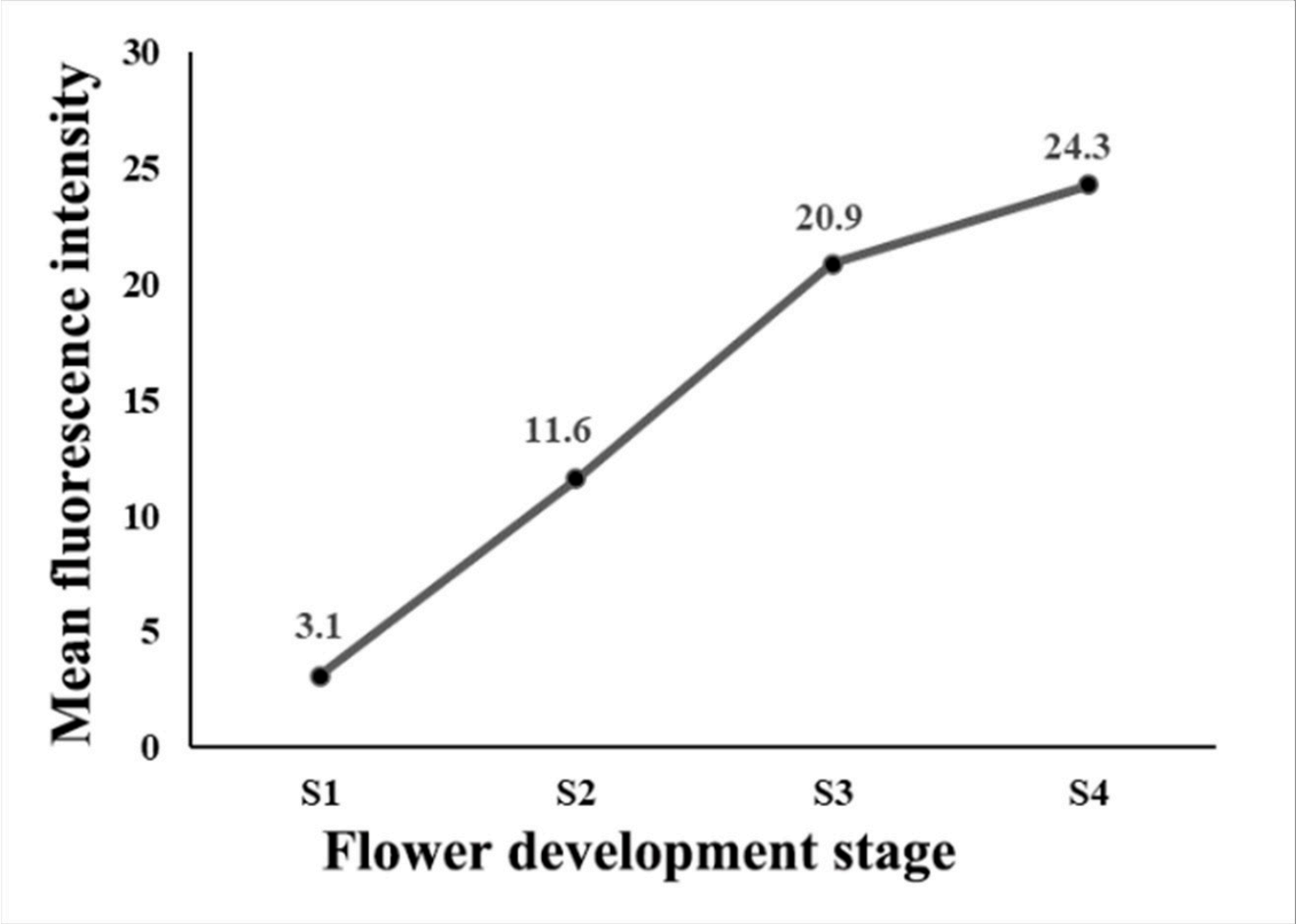


Figure 4

Average fluorescence intensity of *Artabotrys hainanensis*

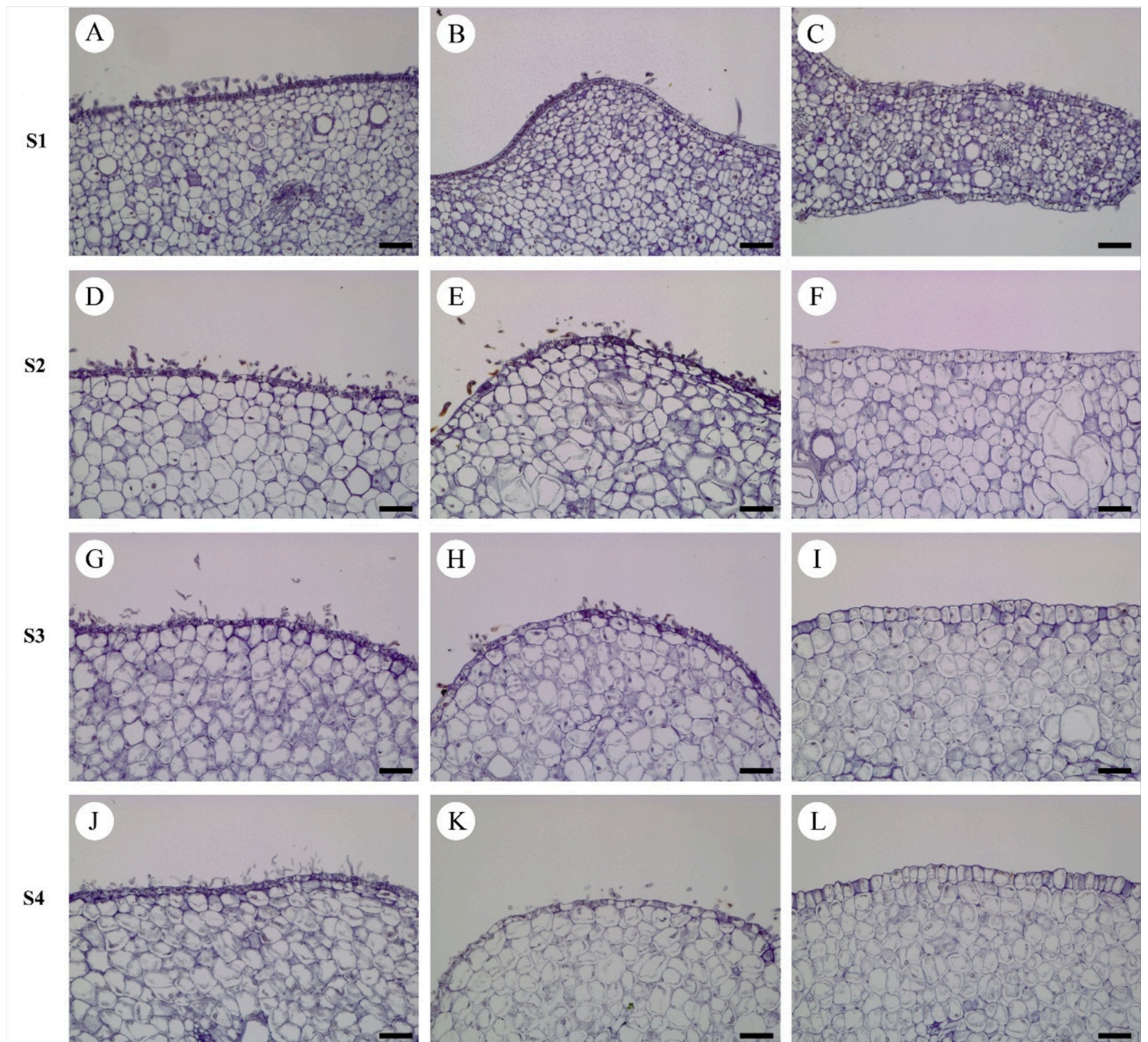


Figure 5

The structure of epidermal hair at different developing stages in *Artabotrys hainanensis*. A-C: The chamber area of the inner petals, outer petals and non-chamber region of stage 1; D-F: The chamber area of the inner petals, outer petals and non-chamber region of stage 2; G-I: The chamber area of the inner petals, outer petals and non-chamber region of stage 3; J-L: The chamber area of the inner petals, outer petals and non-chamber region of stage 4. Scale bars: 50 μm.

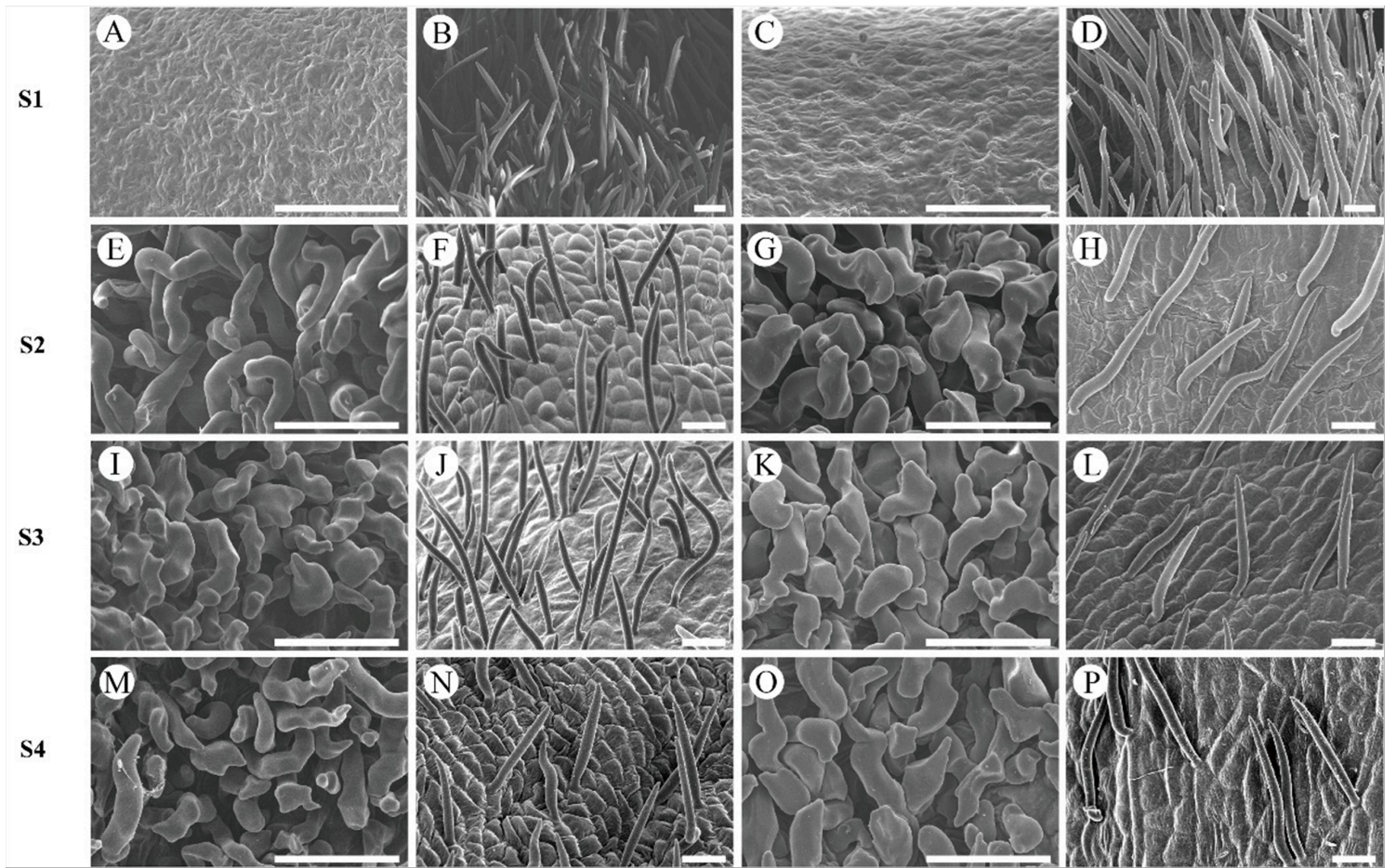


Figure 6

The morphology of epidermal hair at different developing stages in *Artabotrys hainanensis*. A-B: Epidermal hairs at the chamber area and non-chamber region of the inner petals at stage 1; C-D: Epidermal hairs at the chamber area and non-chamber region of the outer petals at stage 1; E-F: Epidermal hairs at the chamber area and non-chamber region of the inner petals at stage 2; G-H: Epidermal hairs at the chamber area and non-chamber region of the outer petals at stage 2; I-J: Epidermal hairs at the chamber area and non-chamber region of the inner petals at stage 3; K-L: Epidermal hairs at the chamber area and non-chamber region of the outer petals at stage 3; M-N: Epidermal hairs at the chamber area and non-chamber region of the inner petals at stage 4; O-P: Epidermal hairs at the chamber area and non-chamber region of the outer petals at stage 4. Scale bars: 50 μm .

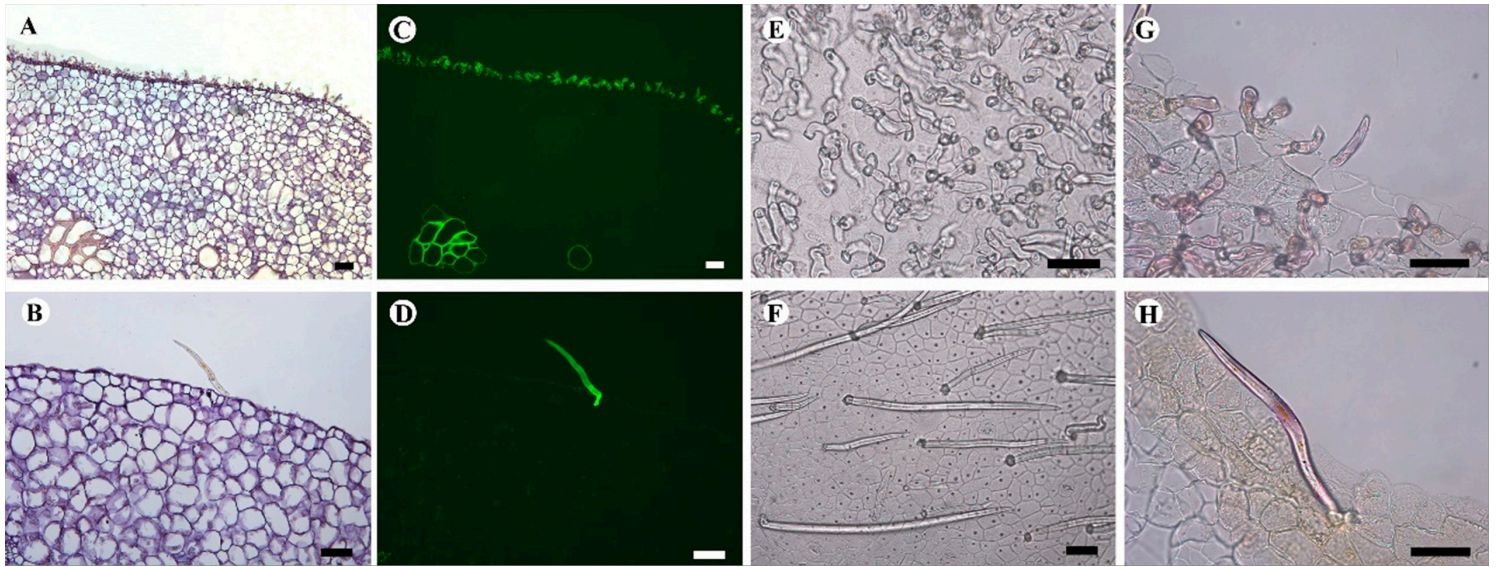


Figure 7

Structure and composition of epidermal hairs in *Artabotrys hainanensis*. A: Paraffin section of the raised part of the pollination chamber; B: Paraffin section of non-pollinating chamber; C: Epidermal hairs of the raised part of the pollination chamber emit green fluorescence when excited by blue light; D: Epidermal hairs of non-pollination chamber emit green fluorescence when excited by blue light; E: Epidermis of the pollination chamber area; F: Epidermis of non-pollination chamber region; G: The pollination chamber area after phloroglucinol staining; H: The non-pollination chamber region after phloroglucinol staining. Scale bars: 50 μm .

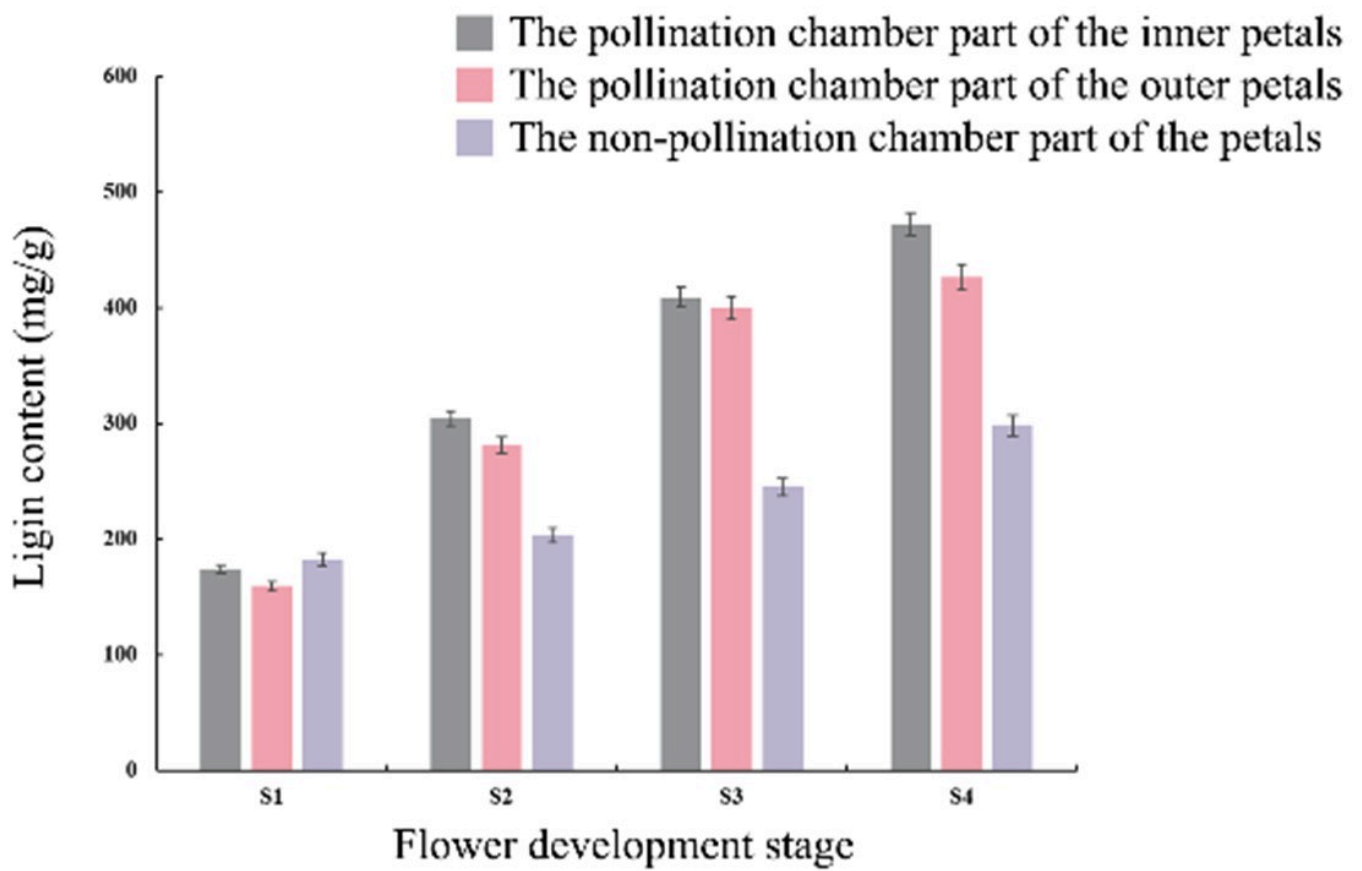


Figure 8

The lignin content at different developmental stages.

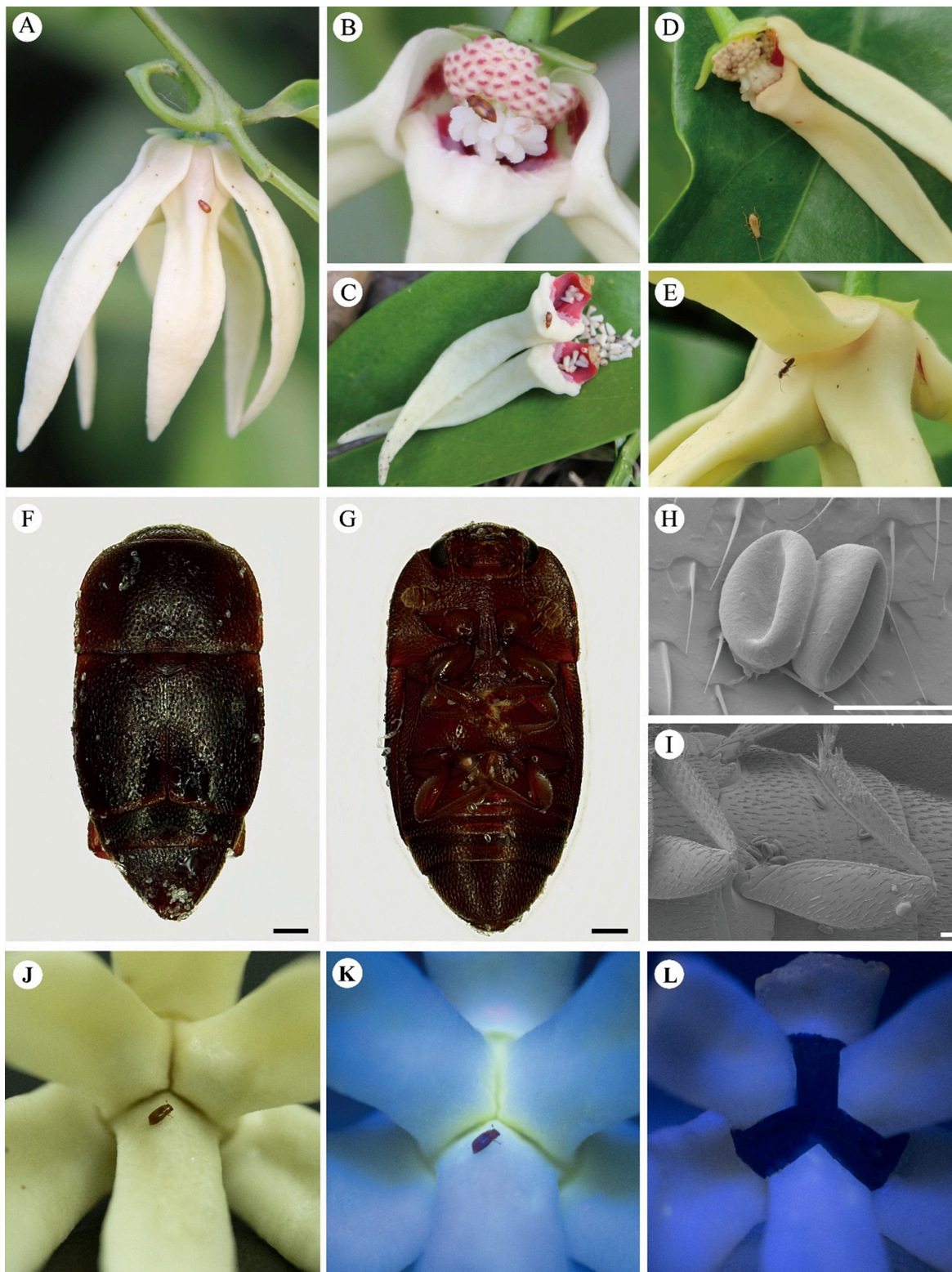


Figure 9

A survey of pollinators of *Artabotrys hainanensis*. A: Pistillate phase beetle began to visit flower; B: Beetle move in chamber; C: Beetle release at the end of the staminate phase; D: The larvae of Blattaria visit flower; E: Ant visit flower; F, G: Carpophilinae beetle; H: Pollen of *Artabotrys hainanensis*; I: Carpophilinae beetles with pollen on their bodies; J: Beetles visit flowers in natural light; K: Under blue light, the

autofluorescence of the base of the pollination chamber attract beetle to visit flowers; L: Under blue light, the base of the pollination chamber is covered with black paint. Scale bars: F, G=250 μm ; H, I=40 μm .