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Morphological variations of gynostemium and floral biology of *Thottea ponmudiana* Sivar., an endemic species of the Western Ghats, India

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Abstract

Thottea ponmudiana is an endemic species restricted to the Ponmudi hills, a part of Agasthyamala Biosphere Reserve, Western Ghats, India. The gynostemium morphology is quite interesting in *T. ponmudiana* as this is a partial fusion product of the male and female reproductive parts. The present study deals primarily with the micromorphological analysis of gynostemium with particular emphasis on its appendages and lobes and the floral biology of *T. ponmudiana*. The morphological variations in the gynostemium are discussed from an evolutionary point of view.

Keywords

floral biology; gynostemium; morphology; *Thottea ponmudiana*

Introduction

Thottea is a medicinal undershrub belonging to the birthwort family Aristolochiaceae. The genus is reported to have 45 species in Asia (1). In India, it is represented by 14 species, out of which ten are endemic to Peninsular India (2). The plants have petiolate leaves and the inflorescence pattern is a raceme, racemose corymb or cyme. The flowers are radially symmetrical with 3 valvately aestivated perianth lobes. There are 6–42 stamens with large degree of variations in their pattern and arrangement. The ovary is tetralocular and the fruits are capsules with pendulous seeds (1, 3, 4).

The flower of *Thottea* is particular owing to the presence of a synorganized structure called the gynostemium. Gynostemium is reported as a characteristic feature of families like Aristolochiaceae, Orchidaceae, Hypoxidaceae, Corsiaceae and Stylidiaceae (5-8). Synorganization is the congenital or post-genital fusion of organs and may pave the way for floral evolution in angiosperms (9). However, in *Thottea*, the gynostemium shows only a partial fusion between the reproductive organs and there are different views on the morphological characterization of the structure with confusion about the 'stylar lobes'. Some morphologists treat them as the structures for pollen capture (10), some consider them as the actual stylar lobes (4) and some others are of the view that they are the stigmatic lobes (2, 11, 12). Even though there are different perspectives regarding the morphology of the gynostemium and its associated structures, they are vastly used in the taxonomic literature to identify *Thottea* spp. It is unnatural to use these as taxonomic markers even without identifying the true reproductive potential of the gynostemium and its associated structures. The present study was designed to explore the morphological and functional parameters of gynostemium concerning the reproductive mechanism in the genus by focusing on an endemic species of *Thottea*- *T. ponmudiana*. The gynostemium with its associated structures is very prominent in *T. ponmudiana*, an endemic species located at the Ponmudi hills of the Western Ghats. The flower of *T. ponmudiana* is trimerous with yellow perianth lobes and a dark purple eye-shaped mark on the adaxial surface of each lobe (4). This plant is an ingredient in some traditional medicinal preparations and has antimicrobial properties (13).

Materials and Methods

Periodic field visits were conducted to the Ponmudi hills of the Western Ghats and the flowers of *Thottea ponmudiana* Sivar. (Fig. 1A) were collected from the plant populations. The voucher specimen was deposited in the Herbarium of JNTBGRI, Palode, Thiruvananthapuram (Voucher no. TBGT 95928, GPS coordinates: 8.74ºN & 77.12ºE).

Floral phenology

The flowering period of *T. ponmudiana* was determined by frequent field visits. Since the number of plants was not

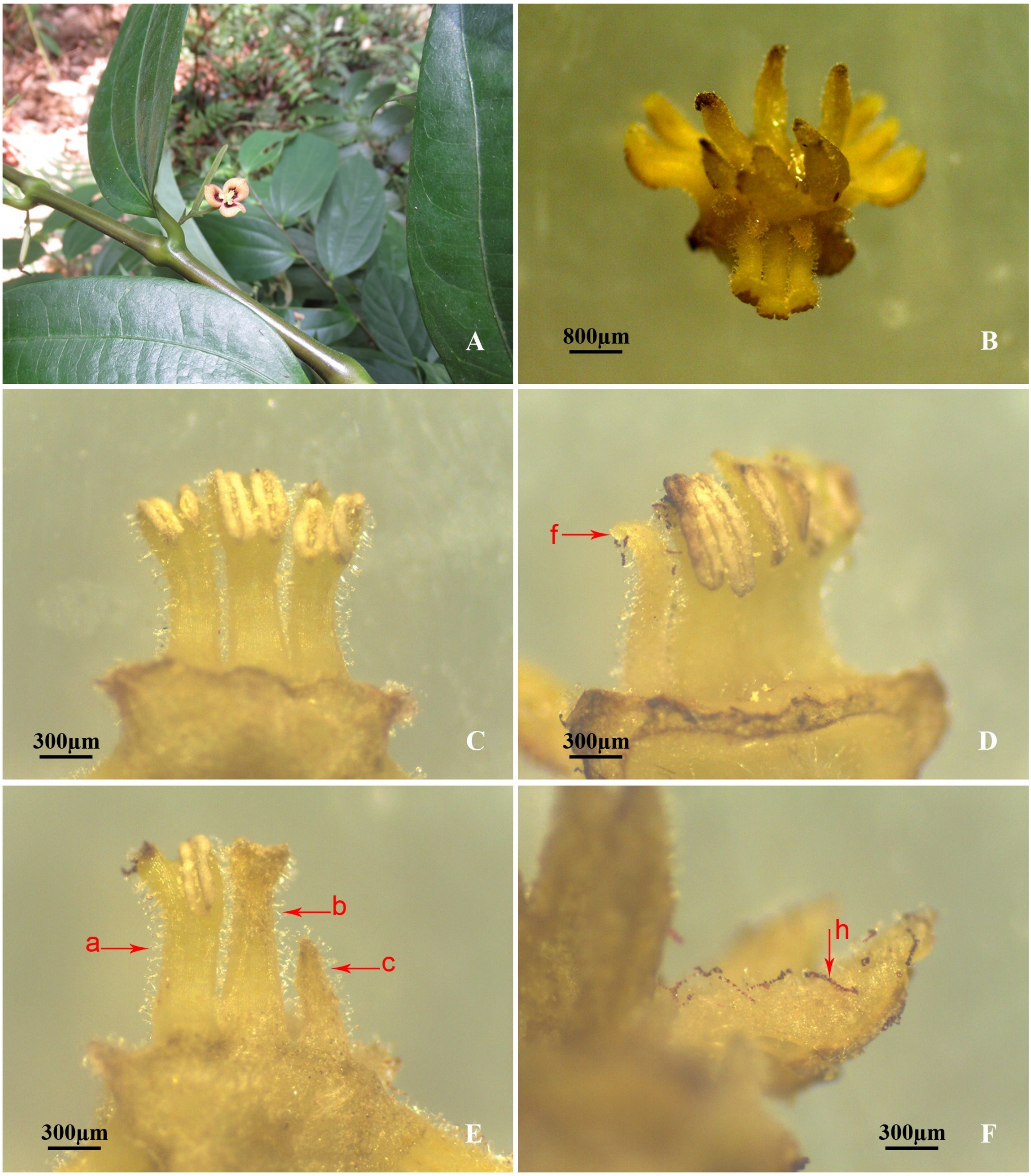


Fig. 1. Habit and variations in the morphology of stamens in *T. ponmudiana* (**A**) Habit; (**B**) Gynostemium; (**C**) Stamen bundle; (**D**) Presence of a filament (f) along with the stamens in the stamen bundle; (**E**) Stamen bundle showing stamen with a single anther lobe (a) Stamen without anther lobe (b) and stamen reduced to an appendage (c); (**F**) Stamen with reddish brown filamentous hairs (h) on adaxial surface.

very large, all the plants within each population were labelled for observation. Frequent field observations were carried out monthly for three consecutive years to identify the peak flowering period. For floral phenological studies, a sufficient number (30) of flower buds that would open the next day were selected from different plants and then tagged. Temporal floral phenological details from initiation of anthesis to completion were recorded every day. This procedure was repeated several times a year, with each observation session conducted on seven successive days. The flowers were observed continuously from 5:00 am to 7:00 pm and each flower was observed at a time interval of 30 min. The longevity of the flowers was determined to be the time up to which they remained fresh, with their parts remaining turgid and functional. The functionality of the flowers was considered to be lost when the floral parts became flaccid and the stylar lobes and stamens became dry and senesced (14). The floral phenological parameters recorded include the temporal details of anthesis, spreading and wetting of stylar lobes, closing of stylar lobes, anther dehiscence, closing of tepals and flower shedding.

Floral Biology

Morphology of gynostemium

Gynostemium morphology was analyzed with the help of a stereo zoom microscope (LEICA EZ4 HD, Switzerland) and Scanning Electron Microscopy (ZEISS EVO 18 Research microscope, Germany). For SEM studies, the gynostemia of the flowers was fixed in FAA. The samples were dehydrated through an ethanol series with increasing concentration (50-100 %). Finally, the samples were air-dried, mounted on metallic stubs and coated with a gold film in a sputter chamber.

Pollen viability and fertility

To assess the pollen viability, the FDA (Fluorescein Diacetate) test (also called the FCR test) was conducted (15). Brewbaker and Kwack's medium supplemented with varying sucrose concentrations (0.03–50 %) were used for *in vitro* pollen germination. The pollen grains collected at different time intervals on the day of anther dehiscence were incubated in the medium taken in a humidity chamber and observed after 4 h to determine the percentage of pollen germination (14, 16). The percentage of pollen fertility was determined using the Acetocarmine staining method (14). All the preparations were analyzed and documented using a trinocular fluorescent research microscope (ZEISS Axio Observer.A1, Germany, for the FDA test and Nikon Eclipse Ci epifluorescence microscope, Japan, for *in vitro* pollen germination and pollen fertility studies).

Stigma receptivity

To check the stigma receptivity, a hydrogen peroxidase test was conducted at different time intervals by dipping the pistils in 4 % aqueous solution of hydrogen peroxide (17). Cytochemical localization of stigma-surface esterases was performed to locate the stigmatic area (receptive area) on the gynostemium, which appeared red after the test (18). Artificial pollination was used as an *in vivo* pollen germination technique to check stigma receptivity. For xenogamy and geitonogamy, the flower buds were emasculated one day before anthesis, bagged and labelled. Artificial pollination was carried out the next morning (on the day of anthesis) and the pollinated flowers were then bagged again and tagged (19). Autogamy was also conducted artificially by bagging single flowers without emasculating them. The flowers were collected 24 h after pollination. The gynostemia of the collected flowers was fixed in Carnoy's fluid for one day and then stored in ethanol (70 %). The pistils were softened using 4 N NaOH. After rinsing, the pistils were mounted in a 1:1 mixture of Aniline blue and 10 % glycerine (20). The preparations were observed under a trinocular fluorescent research microscope (ZEISS Axio Observer.A1, Germany).

Results

Floral phenology

T. ponmudiana shows year-round flowering. However, the intensity of flowering was the highest during August to October. Flowers opened between 6:00 am and 7:30 am. The time of anthesis was extended up to 9:00 am at more shady areas. Spreading of the stylar lobes was observed between 7:00 am and 9:00 am, exhibiting the receptive stage. Spreading of the stylar lobes was at its peak between 8:00 am and 1:00 pm. In most flowers, the stylar lobes became wet along with anthesis. The stylar lobes remained adequately wet up to 3:00 pm. After 3:00 pm, they started to wilt and close partially. The stylar lobes started to close entirely on the second day of anthesis, between 12:00 pm and 5:00 pm. The stamens began to expand along with flower opening. They showed extrorse dehiscence between 7:30 am and 8:30 am on the second day of anthesis. This indicated the protogynous nature of the flowers. Closing of tepals started from the 3rd day of anthesis. Floral longevity was calculated as three days on average. However, the floral organs stay attached to the capsules until they mature. Unfertilized flowers shed within one week from the 3rd day of anthesis.

Floral Biology

Morphology of gynostemium

T. ponmudiana has a pale yellow-coloured gynostemium. It is formed by the incomplete fusion of the basal, proximal part of style and filaments (Fig. 1B). The androecium consists of 3 groups of 3 extrorse stamens (3+3+3). But, flowers with 2+2+3, 2+3+3, 3+3+4 and 3+4+4 stamen arrangements were also observed. A large number of multicellular uncinate hairs were observed on the filaments. Generally, physical fusion is absent between the adjacent stamens within a group so that the stamens within a group remain unbound from their top to base (Fig. 1C).

Some exciting variations were noticed in the stamen architecture of certain flowers. In some flowers, filament alone was present along with the stamens in the bundle (Fig. 1D). The presence of rudimentary stamen (without anther lobes), stamen with a single anther lobe and stamen reduced to an appendage with a pointed apex, which reaches half the length of normal stamen were also observed in some flowers (Fig. 1E). In still other flowers, a few numbers of reddish-brown filamentous hairs were observed on the adaxial surface of the stamens (Fig. 1F).

Some solitary appendages are seen on the outer side of the stamen bundles, alternating with the tepals and stamen bundles (Fig. 2A). One, two or three such solitary appendages can be observed in a flower. These appendages are dome-like and entirely covered by uncinate hairs. During the receptive stage, these appendages do not release any exudates, whereas the stylar lobes release some exudates during the receptive stage. Interestingly, in some flowers, instead of this solitary appendage, a normal stamen was found to be developed in its position (Fig. 2B). The percentage of the fertility of such stamens was observed to be 95.5 ± 0.41 % as per Acetocarmine staining technique, and their pollen viability was observed to be 94.76 ± 0.25 %.

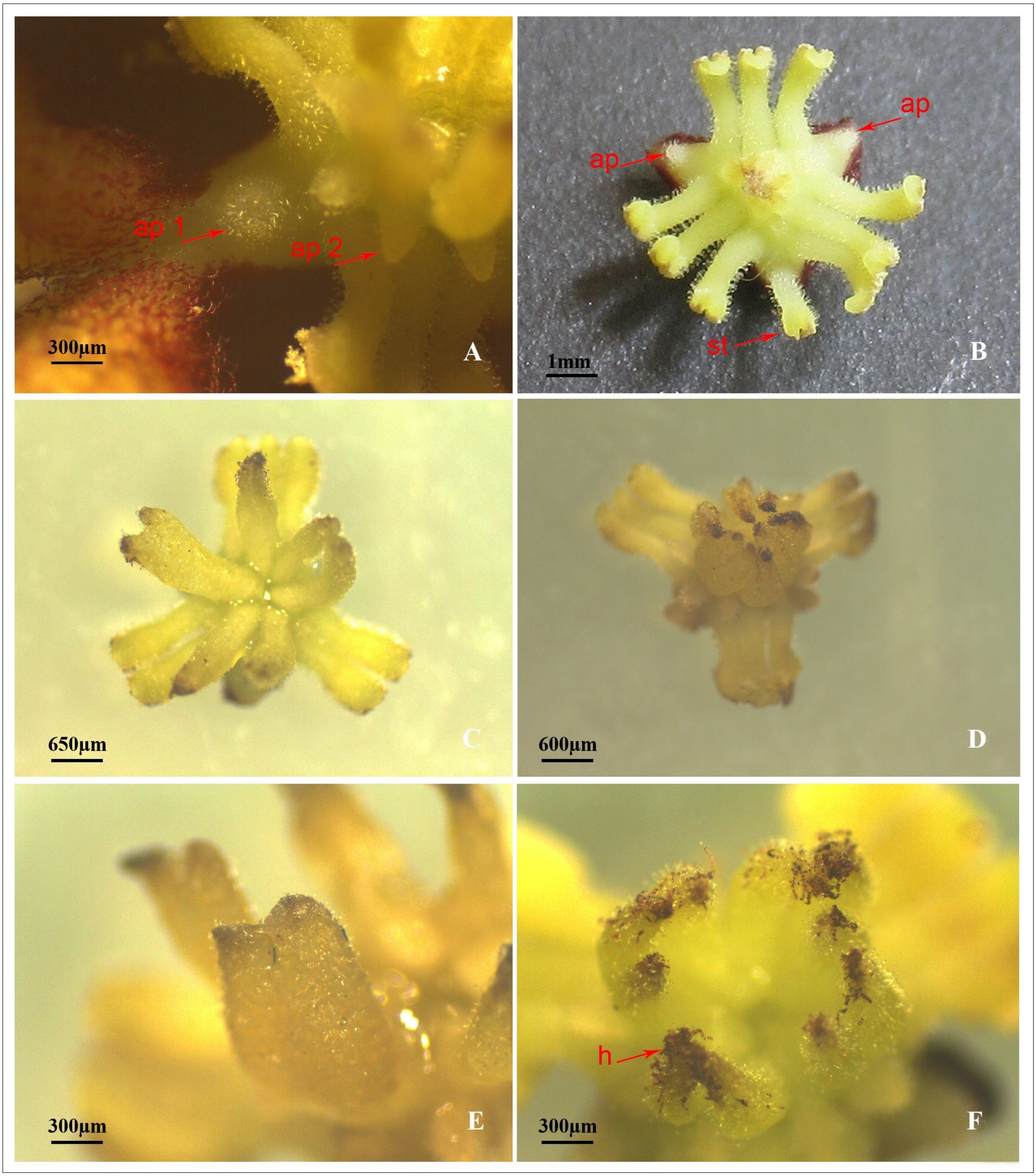


Fig. 2. Gynostemium morphology and its variations in *T. ponmudiana* (**A**) Part of a flower showing appendage alternating with the stamen bundles and tepals (ap1) and whorl of appendages just below the stylar lobes (ap2); (**B**) Development of a stamen (st) in the position of appendage alternating with the stamen bundles; ap: appendage; (**C**) Gynostemium with open stylar lobes (receptive stage); (**D**) Gynostemium with closed stylar lobes (non-receptive stage); (**E**) Bifurcate stylar lobe; (**F**) Stylar lobes with elongated reddish brown hairs (h) on their terminal regions.

Six to 10 stylar lobes are present just above the stylar column. They are arranged as a ring (Fig. 1B). During the receptive stage of the flower, these stylar lobes remain fleshy, wet and spreading (Fig. 2C). During the non-receptive stage, they become dry and closed (Fig. 2D). The stylar lobes have tapering ends. In some flowers, bifurcate stylar lobes were also noticed (Fig. 2E). Large number of uncinate hairs cover the stylar lobes either entirely or mainly towards the terminal region, starting halfway from the base of the stylar lobe. The development of a few reddish-brown elongated hairs on the terminal parts of the stylar lobes was observed in some flowers (Fig. 2F). Such hairs were also observed on the stamen bundles. These hairs were filamentous, uniseriate, multicellular and consisting of elongated cells (Fig. 3A). Hairs similar to these are present on the adaxial side of the tepals also (Fig. 3B). Just below the stylar lobes, a whorl of pointed appendages is present (6 to 14 in number). These appendages are also covered with a lot of uncinate hairs. These appendages do not produce any visually identifiable exudates, even

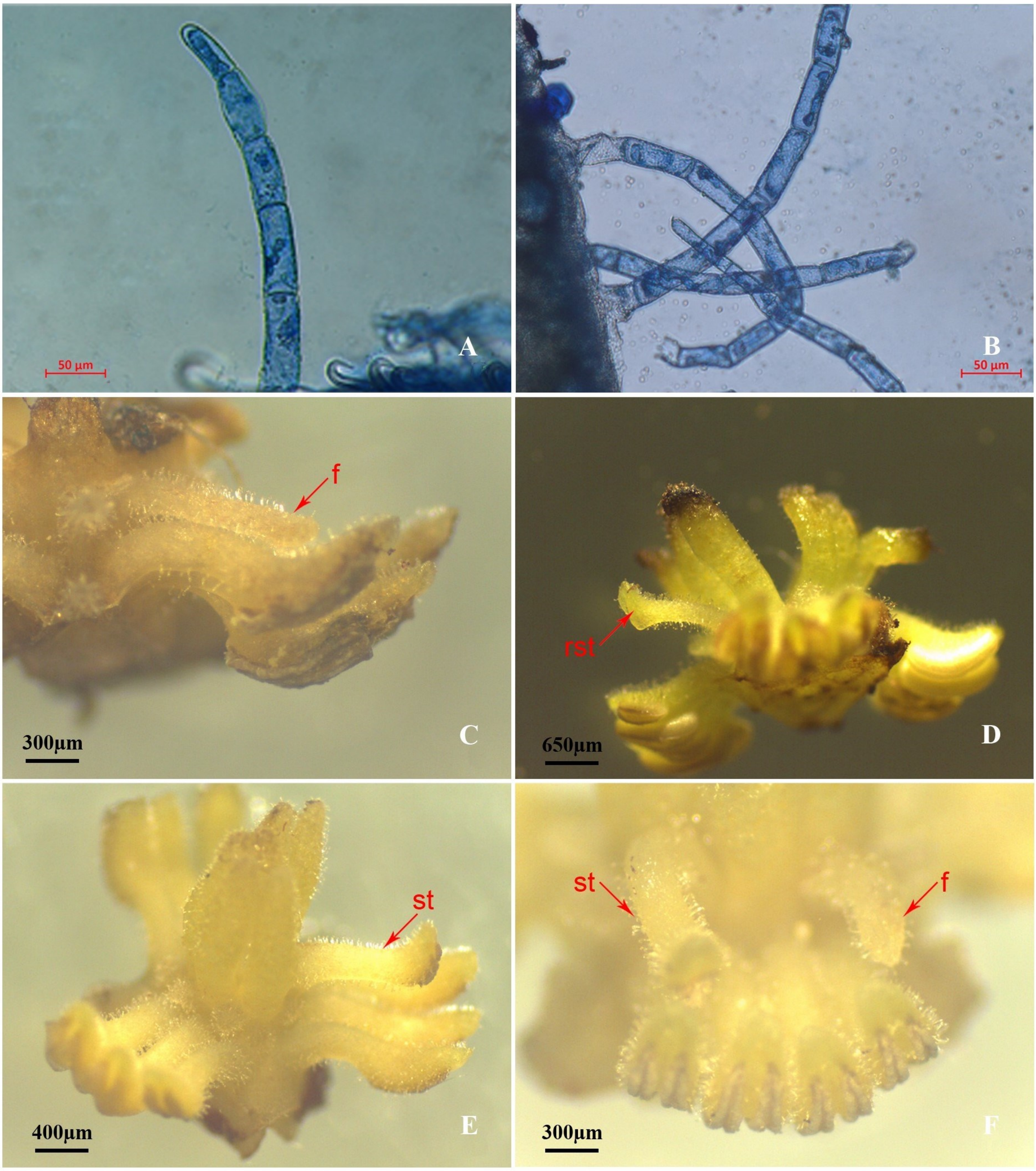


Fig. 3. Morphological variations in the gynostemium of *T. ponmudiana* (**A**) Light microscopic view of the filamentous hairs on the apex of the stylar lobes; (**B**) Light microscopic view of the filamentous hairs on the adaxial surface of the tepal; (**C**) Development of a filament (f) from the whorl of appendages below the stylar lobes; (**D**) Development of rudimentary stamen (rst) from the whorl of appendages below the stylar lobe; (**E**) Presence of a complete stamen (st) developed from the whorl of appendages below the stylar lobes; (**F**) Presence of stamen (st) and filament (f) developed from the whorl of appendages within the same flower.

during the receptive stage of the flower.

There were some odd specimens in which filaments of stamens (i.e., without anther) had developed by the elongation of some appendages of the whorl below the stylar lobes (Fig. 3C). In some other flowers, rudimentary stamens were produced from this whorl of appendages and they contained poorly developed pollen grains (Fig. 3D). In still others, complete stamens were also seen developed from some of these appendages (Fig. 3E). The percentage of pollen fertility in such stamens was 94.85 ± 0.51 % (Acetocarmine staining method). Their pollen viability was 94.03 ± 0.18 % (FDA staining technique). Such stamens dehisced and released their pollen grains along with the anthers of the stamen bundles. Very rarely, the above kind of development of filament and stamen was observed within the same flower (Fig. 3F).

The SEM analysis of the gynostemium shows that the stylar lobes have a waxy-coated, irregular surface with ridges and furrows. However, prominent stigmatic structures such as stigmatic papillae or stigmatic protuberanc-

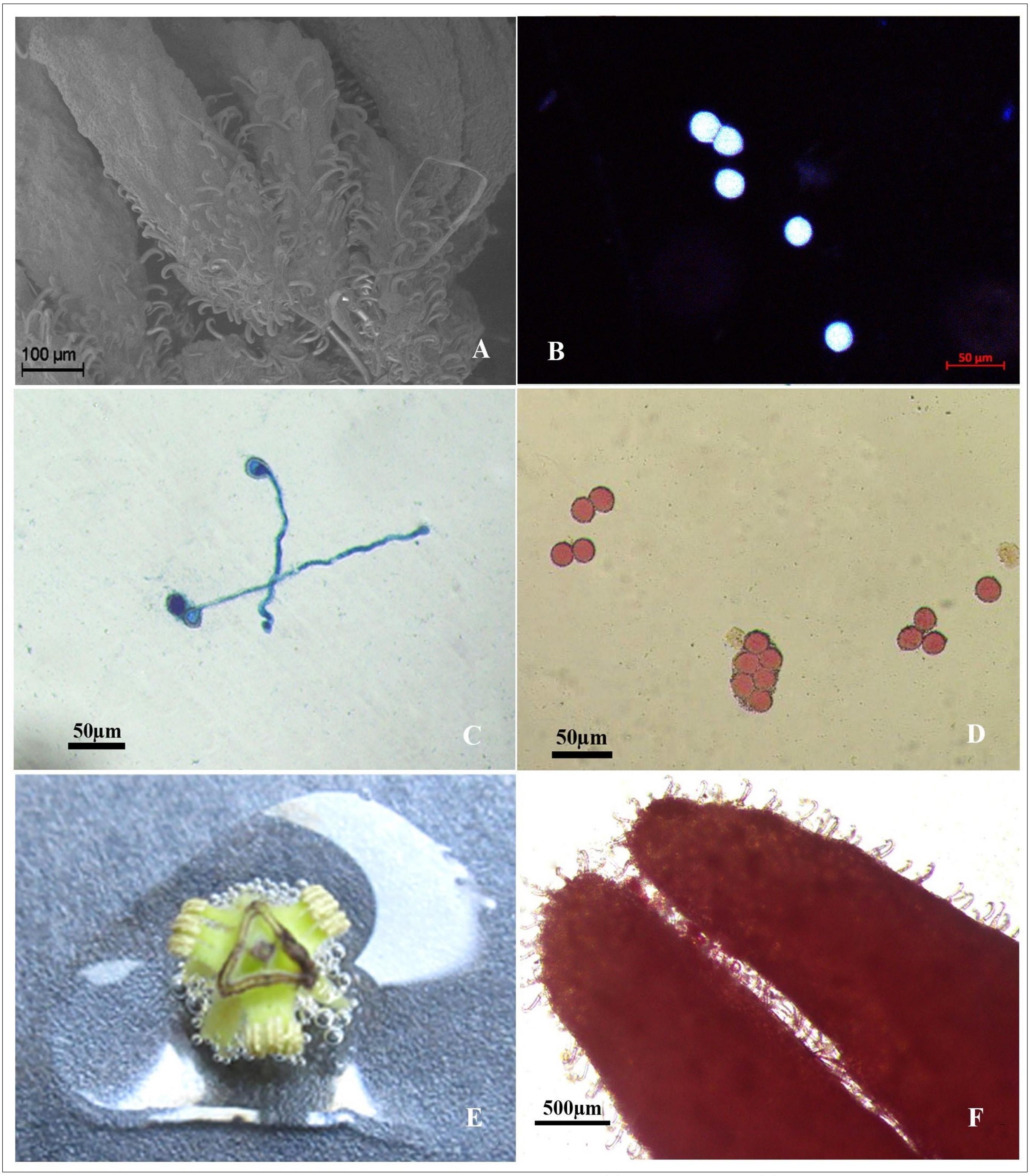
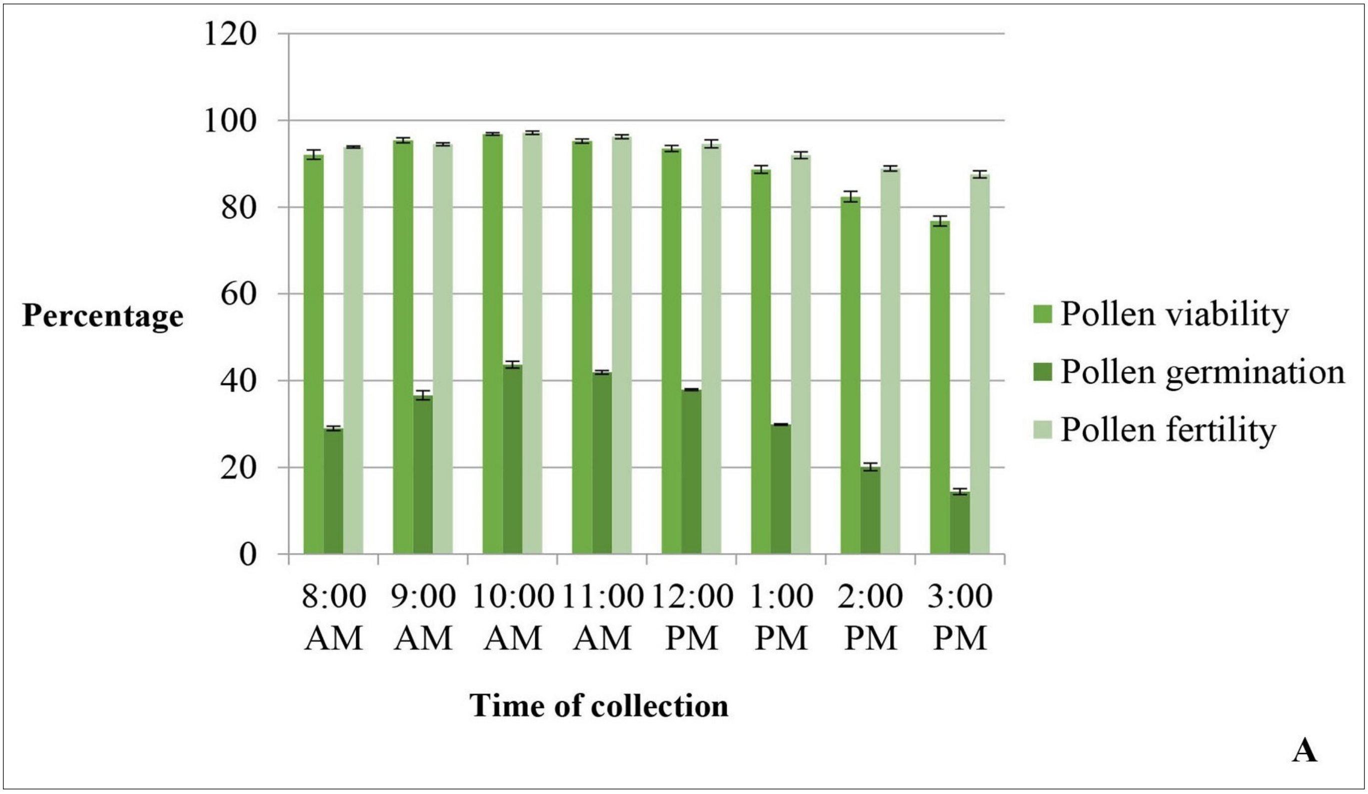


Fig. 4. Morphology of gynostemium and reproductive biological studies in *T. ponmudiana* (**A**) SEM of stylar lobes; (**B**) Viable pollen grains showing fluorescence by FDA staining; (**C**) *in vitro* pollen germination; (**D**) Fertile pollen grains after acetocarmine staining; (**E**) Release of oxygen bubbles from receptive area (Peroxidase activity test); (**F**) Reddish appearance of receptive area on stylar lobe (cytochemical localization of stigma specific esterases).

es are absent on the stylar lobes. The stylar lobes have many uncinate hairs on their surface (Fig. 4A).

Pollen viability and Pollen fertility

Pollen viability, germination and fertility studies were carried out on the second day of anthesis because the flowers were protogynous and anther dehiscence occurred on the second day of anthesis. The highest rate of pollen viability was observed in the pollen grains collected between 9:00 am and 11:00 am on the second day of anthesis. It was 96.86 ± 0.28 % (Fig. 4B and Fig. 5A). The highest percentage of pollen germination was observed in the pollen grains collected between 9:00 am and 11:00 am on the second day of anthesis. It was 43.67 ± 0.79 % (Fig. 4C and Fig. 5A). The pollen grains showed maximum response when using Brewbaker and Kwack's medium for pollen germination supplemented with 7 % sucrose. Pollen fertility was the highest for the pollen grains collected between



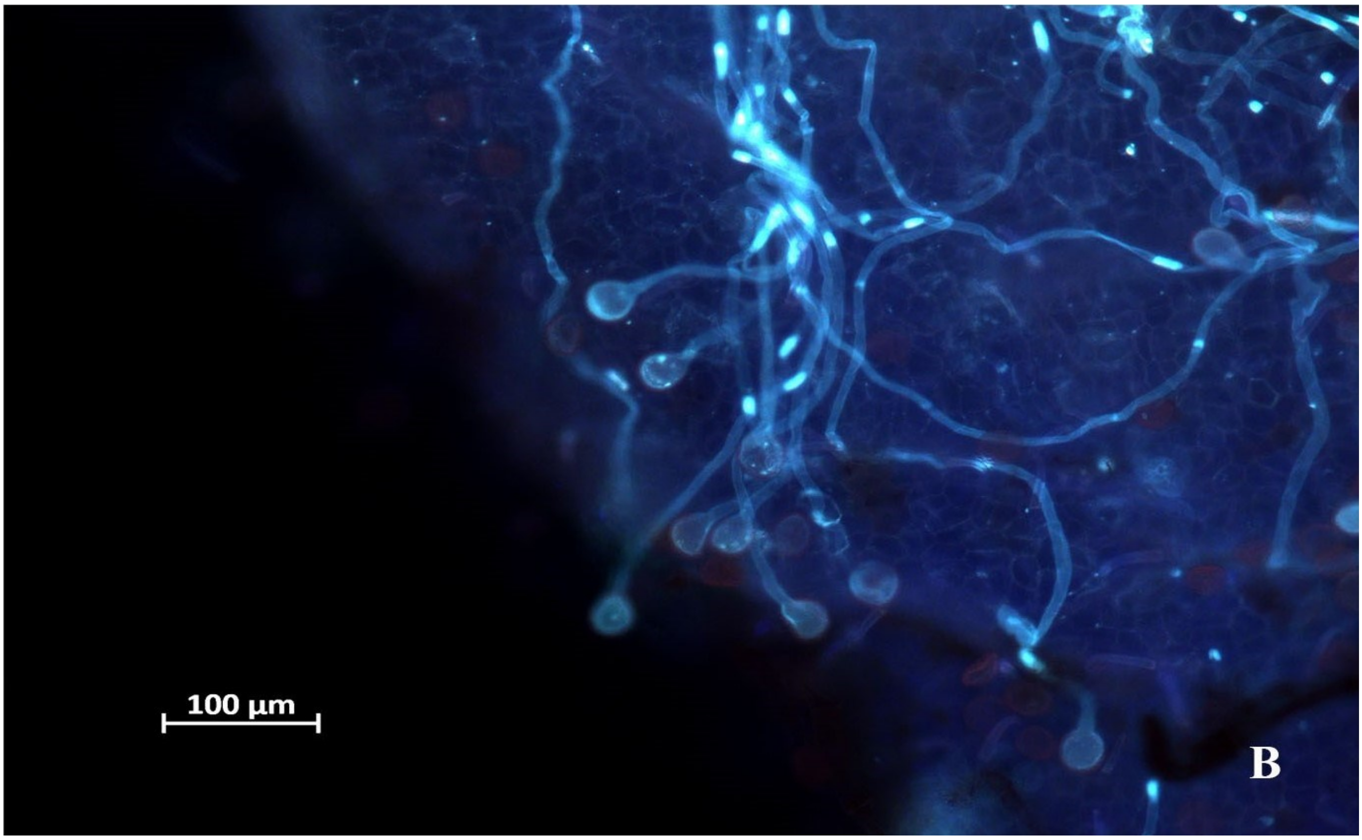


Fig. 5. Reproductive biological studies in *T. ponmudiana* (**A**) Pollen viability, *in vitro* pollen germination and pollen fertility studies; (**B**) Germinating pollen grains on the surface of the stylar lobe (Aniline blue fluorescence microscopic method).

9:00 am and 11:00 am on the second day of anthesis. The highest percentage of pollen fertility was 97.14 ± 0.38 % (Fig. 4D and Fig. 5A).

Stigma receptivity

Stigma receptivity was found to be maximum between 9:00 am and 12:00 pm on the day of anthesis (Fig. 4E). After 2:30 pm, a sharp decrease in stigma receptivity was observed. However, a persisting lower rate of stigma receptivity was noticed on the second day of anthesis, resulting in fewer oxygen bubble emissions. Cytochemical localization of stigma-surface esterases depicted the area of stigma receptivity. The whole stylar lobe appeared red after the test, irrespective of the occupancy of the uncinate hairs. Hence, the entire stylar lobe is receptive (Fig. 4F). The appendages showed no positive response to the test. Aniline blue fluorescence microscopic method indicated that pollen grains were germinated on the entire stylar lobes irrespective of the presence or absence of uncinate hairs on them (Fig. 5B). Pollen grains germinated successfully on both self-pollinated and cross-pollinated stigmas. However, pollen germination was absent on the appendages of the gynostemium.

Discussion

Flowers are the organs meant for sexual reproduction in angiosperms (14). The uniqueness of angiosperms that distinguish them from the rest of the land plant forms is the presence of flowers (21). The most interesting character in the flowers of *Thottea* is the gynostemium, which is unique. Studies on the floral morphological features in 8 species of *Thottea* (*T. idukkiana, T. duchartrei, T. siliquosa, T. ponmudiana, T. sivarajanii, T. dinghoui, T. abrahamii* and *T. barberi*) led to the conclusion that out of the 8 species, 5 species (*T. idukkiana, T. duchartrei, T. siliquosa, T. ponmudiana* and *T. sivarajanii*) have a distinct gynostemium (4). In the present study, it was observed that an imperfect and proximal fusion between the stamen bundles and the stylar column formed the gynostemium. Flexibilities in the number and arrangement of stamens were noticed in *T. ponmudiana* and similar cases were reported earlier in *T. idukkiana, T. duchartrei* and *T. sivarajanii* (3, 4, 22).

The present study revealed some interesting morphological variations relating to the stamen architecture in *T. ponmudiana*. The presence of incompletely developed stamen, stamen with a single anther lobe, filament alone and an appendage formed due to stamen reduction in the stamen bundles, was very unusual. All these structures denote floral evolution in *T. ponmudiana, which* involves the formation of staminode from a wholly developed stamen. In plants, vestigial staminodes can exist as a sterile stamen whorl with tiny anthers (rarely fertile) on the apices of the stamens. These filaments lack anthers, small remnants or tiny organs visible only during floral development (23). A noticeable percentage of stamen diversity exists in the genus *Thottea*. For example, single whorl of stamens is present in *T. sumatrana*, 2 in *T. tapanuliensis*, 3 in *T. triserialis*, 4 in *T. parviflora* etc. (24-26). Such discrepancies indicate that the staminode whorls in *T. pon-mudiana* may be formed by reducing the number of stamen whorls. According to phylogenetic studies, stamen reduction by evolution is the reason for the origin of staminodes. Plants of at least a single species of 32.5 % Angiosperm families have staminodes in their flowers (27). Angiosperms are found to adopt an economic principle in creating reproductive parts and androecium evolution by stamen reduction can be regarded as its general example (28).

Functional stamen with high pollen fertility and pollen viability percentages developed in the position of the solitary appendages alternating with stamens and tepals in *T. ponmudiana* indicates that these solitary appendages are staminodes. Striking variations, such as the development of a filament, rudimentary stamen and functional stamen, arising from the whorl of appendages just below the stylar lobes, indicate the identity of these appendages in the whorl as staminodes. In clades containing actinomorphic flowers, when staminodes replace stamens, the stamen whorls become entirely and irreversibly replaced (27). But, in the present study, the results contrast with the above report, so stamen to staminode evolution seems reversible. The morphological modifications of stamens to appendages and *vice versa* help conclude that both progressive and retrogressive evolution coincide in the flowers of *T. ponmudiana*. Observations similar to the above situation, i.e., both the appearance and disappearance of stamens were reported in the genus *Mentzelia* also(29).

The stylar lobes of the gynostemium in *T. ponmudiana* exhibit disparities in their number. A previous study reported no interdependence between the number of stylar lobes and the number of carpels, except that the number of stylar lobes is never lesser than that of the number of carpels (4). The reddish-brown filamentous hairs on the stylar lobes and stamen bundles of *T. ponmudiana* are similar to those on its perianth. The presence of such filamentous hairs on the perianth was reported earlier while studying the trichomes of Indian *Thottea* (30). The presence of such hairs on the stylar lobes and stamen bundles in the present study may be a natural occurrence or the filamentous hairs on the perianth might be incorporated into the tissue of stamens and stylar lobes at the bud stage or during some other stages of flower development and grown along with them. The SEM studies could not pinpoint any specific stigmatic structures or protuberances on the stylar lobes other than the presence of ridges, grooves and waxy coating on their surface.

The reproductive events in flowering plants follow a seasonal order and the term plant phenology involves observing and analyzing the timely events occurring in the life span of a plant (14, 31). The term floral phenology is more specific as it involves the study of temporal changes of a flower from its anthesis until it loses its functionality (14). In *Thottea ponmudiana,* the flowers remain fresh for three days from the day of anthesis and exhibit protogyny. Protogyny is known as a mechanism to prevent autogamy (32), and it is a kind of dichogamy. Dichogamy has some influence on the floral biological aspects, including the interference between the male and female reproductive organs of the same plant (33). In *T. ponmudiana*, incomplete protogyny was observed in most flowers as the stigmas were receptive even at the time of anther dehiscence. Incomplete protogyny allows the flowers to bring about delayed self-pollination, which is a privileged system in some species and helps tackle pollination failure that sometimes occurs (34, 35).

Fertility, viability and germinability of pollen grains and stigma receptivity are the key factors regulating adequate pollination, fertilization and seed formation (36-40). The pollen grains of *T. ponmudiana* express high pollen viability and fertility and medium pollen germination rates. However, poor pollen viability and germination rates were reported in *T. barberi* (41). *T. abrahamii, T. dinghoui, T. duchartrei, T. idukkiana, T. ponmudiana, T. siliquosa T . sivarajanii* and *T. barberi* were reported to exhibit higher pollen viability rates in a study conducted earlier (42). In the present study, stigma-surface esterases were localized on the entire stylar lobe of the gynostemium and pollen germination was also observed on the whole stylar lobe, indicating that the entire stylar lobe of *T. ponmudiana* is receptive. Even though the flower exhibits protogyny, longer stigma receptivity period overlaps with the initial stage of anther dehiscence and allows pollen germination on stigmas pollinated by both autogamy and geitonogamy. In *Aristolochia*, stigma receptivity is lost at or before anther dehiscence, promoting cross-pollination (43-45). However, pollination studies in *Aristolochia tagala* have proved that the plant allows geitonogamous pollination also (46).

Conclusion

The gynostemium of *T. ponmudiana* is formed by the partial fusion of androecium and gynoecium. There is a lot of disparity in the number and arrangement of stamens, and the number of stylar lobes. Analysis of variations in the morphological architecture of stamens and floral biological studies indicate that the appendages present just below the stylar lobes and those alternating with the tepals are staminodes formed by the evolutionary reduction of stamens. The plant shows the tendency of both stamen reduction and reversion of stamen reduction, which are eye-catching occurrences for the researchers of plant evolution. Studies on the morphology and reproductive biology of the gynostemium of *T. ponmudiana* helped to unfold the fact that the whole surface of each stylar lobe is receptive. Hence, the entire stylar lobe forms the stigmatic tissue irrespective of the distribution of uncinate hairs on its surface. Therefore, it is evident that all the stylar lobes on the gynostemium together form the entire stigmatic tissue of the flower.

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Authors' contributions

PN, AK and KS conceived the idea and planned the experiments. PN and KS collected the plant samples. KS performed the experiments under the supervision of PN and AK. All authors analyzed the research data and drafted the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Yao TL. Aristolochiaceae. In: Kiew R, Chung RCK, Saw LG, Soepadmo E, editors. Flora of Peninsular Malaysia, Series II: seed plants, vol 5. Kepong: Forest Research Institute Malaysia; 2015. p. 5-46.
2. Kumar ES, Mathew SP, Jabbar MA, Krishnan SG, Murugesan, K. Rediscovery of *Thottea dalzellii* (Hook.f.) Karthik and Moorthy (Aristolochiaceae) from the Western Ghats. Int J Advanced Res. 2015;3(4):1-4.
3. Nazarudeen A, Sabu T. Staminal instability in *Thottea duchartrei.* Indian J Forest. 2002;25(2):194-95.
4. Shaiju PN, Omanakumari N. Floral morphology and systematics of the genus *Thottea* Rottb. (Aristolochiaceae) from Western Ghats, India. Pl Syst Evol. 2010;288:213-25. https://doi.org/10.1007/s00606-010-0326-x
5. Gonzalez F, Stevenson DW. Gynostemium development in *Aristolochia* (Aristolochiaceae). Bot Jahrb Syst. 2000;122(2):249-91.
6. Rudall PJ, Bateman RM. Roles of synorganisation, zygomorphy and heterotopy in floral evolution: the gynostemium and labellum of orchids and other lilioid monocots. Biol Rev Cambridge Philos Soc. 2002;77(3):403-41. https://doi.org/10.1017/S1464793102005936
7. Wagstaff SJ, Wege J. Patterns of diversification in New Zealand Stylidiaceae. Amer J Bot. 2002;89(5):865-74. https://doi.org/10.3732/ajb.89.5.865
8. Peréz-Mesa P, Ortíz-Ramírez CI, González F, Ferrándiz C, Pabón-Mora N. Expression of gynoecium patterning transcription factors in *Aristolochia fimbriata* (Aristolochiaceae) and their contribution to gynostemium development. EvoDevo. 2020;11(4):2-16. https://doi.org/10.1186/s13227-020-00149-8
9. Endress PK. Synorganisation without organ fusion in the flowers of *Geranium robertianum* (Geraniaceae) and its not so trivial obdiplostemony. Ann Bot. 2010;106(5):687-95. https://doi.org/10.1093/aob/mcq171
10. Leins P, Erbar C, Van Heel WA. Note on the floral development of *Thottea* (Aristolochiaceae). Blumea. 1988;33(2):357-70.
11. Renuka C, Swarupanandan K. Morphology of the flower in *Thottea siliquosa* and the existence of staminodes in Aristolochia-

ceae. Blumea. 1986;31(2):313-18.

12. Sunil CN, Kumar VVN. *Thottea adichilthottiana* (Aristolochiaceae), a new species from Ernakulam, Western Ghats, India. Webbia. 2014;69(2):239-42. https://doi.org/10.1080/00837792.2014.951205
13. Moorthy K, Punitha T, Vinodhini R, Mickymaray S, Shonga A, Tomass Z, et al. Efficacy of different solvent extracts of *Aristolochia krisagathra* and *Thottea ponmudiana* for potential antimicrobial activity. J Pharm Res. 2015;9(1):35-40.
14. Shivanna KR, Tandon R. Reproductive ecology of flowering plants: A manual. New Delhi: Springer. 2014;2. https://doi.org/10.1007/978-81-322-2003-9
15. Heslop-Harrison J, Heslop-Harrison Y. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. Stain Technol. 1970;45(3):115-20. https://doi.org/10.3109/10520297009085351
16. Brewbaker JL, Kwack BH. The essential role of calcium ion in pollen germination and pollen tube growth. Amer J Bot. 1963;50 (9):859-65. https://doi.org/10.1002/j.1537-2197.1963.tb06564.x
17. Kearns CA, Inouye DW. Techniques for the pollination biologists. Niwot: University press of Colorado; 1993.
18. Mattsson O, Knox RB, Heslop-Harrison J, Heslop-Harrison Y. Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions. Nature. 1974;247:298-300. https://doi.org/10.1038/247298a0
19. Sharma MV, Kuriakose G, Shivanna KR. Reproductive strategies of *Strobilanthes kunthianus*, an endemic, *Semelparous* species in southern Western Ghats, India Bot J Linn Soc. 2008;157(1):155 -63. https://doi.org/10.1111/j.1095-8339.2008.00786.x
20. Shivanna KR, Rangaswamy NS. Pollen biology: a laboratory manual. Berlin: Springer; 1992. https://doi.org/10.1007/978-3-642-77306-8
21. Alvarez-Buylla ER, Benítez M, Corvera-Poiré A, Cador CÁ, de Folter S, de Buen AG, et al. Flower development. The *Arabidopsis* Book. 2010;8:1-57. https://doi.org/10.1199/tab.0127
22. Tennakoon TMSG, Borosova R, Suraweera C, Herath S, De Silva T, Padumadasa C, et al. First record of *Thottea duchartrei* Sivar., A. Babu and Balach. (Aristolochiaceae) in Sri Lanka. J *Natn Sci Foundation Sri Lanka.* 2022;50(2):441-52. https://doi.org/10.4038/jnsfsr.v50i2.10546
23. Decraene LPR, Smets EF. Merosity in flowers: definition, origin and taxonomic significance. Pl Syst Evol. 1994;191:83-104. https://doi.org/10.1007/bf02858099
24. Hou D. Florae malesianae praecursores LXII on the genus *Thottea* (Aristolochiaceae). Blumea. 1981;27(2):301-32.
25. Yao TL. Nine new species of *Thottea* (*Aristolochiaceae*) in Peninsular Malaysia and Singapore, with two taxa in Peninsular Malaysia redefined and a taxon lectotypified. Blumea. 2013;58 (3):245-62. https://doi.org/10.3767/000651913X675791
26. Mustaqim WA, Putra HF. *Thottea tapanuliensis* (Aristolochiaceae): A new species from Sumatra, Indonesia. Telopea. 2020;23:163-68. https://doi.org/10.7751/telopea14535
27. Walker-Larsen J, Harder LD. The evolution of staminodes in angiosperms: patterns of stamen reduction, loss and functional re-invention. Amer J Bot. 2000;87(10):1367-84. https://doi.org/10.2307/2656866
28. Rao RR. Trends in the evolution of the angiosperm flowers. Palaeobotanist. 1992;41:167-75. https://doi.org/10.54991/jop.1992.1118
29. Botnaru L, Schenk JJ. Staminode evolution in *Mentzelia* section *Bartonia* (Loasaceae) and its impact on insect visitation rates. Bot J Linn Soc. 2019;190:151-64. https://doi.org/10.1093/botlinnean/boz012
30. Kumar ESS, Chitra CR, Khan AES. Studies on the trichomes of Indian *Thottea* Rottb. (Aristolochiaceae). J Econ Taxon Bot Addit Ser. 2001;19:237-40.
31. Gu L, Post WM, Baldocchi D, Black TA, Verma SB, Vesala T, et al. Phenology of vegetation photosynthesis. In: Schwartz MD, editor. Phenology: An Integrative Environmental Science. Tasks for Vegetation Science, vol 39. Springer, Dordrecht; 2003;467-85.https://doi.org/10.1007/978-94-007-0632-3_29
32. Stace HM. Protogyny, self-incompatibility and pollination in *Anthocercis gracilis* (Solanaceae). Aust J Botany. 1995;43(5):451-59. https://doi.org/10.1071/BT9950451
33. Lloyd DG, Webb CJ. The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy,N.Z.J.Bot.1986;24(1):135-62. https://doi.org/10.1080/0028825X.1986.10409725
34. Goodwillie C, Weber JJ. The best of both worlds? A review of delayed selfing in flowering plants. Am J Bot. 2018;105(4):641-55. https://doi.org/10.1002/ajb2.1045
35. Lemos AL, Moreira MM, Benevides CR, Miranda AS, Rodarte ATA, Lima HA. Reproductive biology of *Prepusa hookeriana* (Gentianaceae): an endangered species of high-altitude grasslands in Brazil. Braz J Bot. 2020;43:379-87. https://doi.org/10.1007/s40415-020-00611-w
36. Yang WQ, Lai Y, Li MN, Xu WY, Xue YB. A novel C2-domain phospholipid-binding protein, OsPBP1, is required for pollen fertility in rice. Mol Plant. 2008;1(5):770-85. https://doi.org/10.1093/mp/ssn035
37. Prasad PVV, Boote KJ, Allen Jr LH. Longevity and temperature response of pollen as affected by elevated growth temperature and carbon dioxide in peanut and grain sorghum. Environ Exp Bot. 2011;70(1):51-57. https://doi.org/10.1016/j.envexpbot.2010.08.004
38. Bhattacharya A. Does canopy height determine the pollen viability and stigma receptivity? A Cross-population Observation on *Shorea robusta* Gaertn. f. Our Nature. 2011;9(1):41-48. https://doi.org/10.3126/on.v9i1.5732
39. Makwana MA, Akarsh P. Stigma receptivity test in diverse species of tomato. Int J Agric Sci. 2017;7(5):1-8. https://doi.org/10.24247/ijasroct20171
40. Hine A, Rojas A, Suarez L, Murillo O, Espinoza M. Optimization of pollen germination in *Tectona grandis* (Teak) for breeding programs. Forests. 2019;10(10):908. https://doi.org/10.3390/f10100908
41. Femy KH, Radhamany PM, Gangaprasad A. Reproductive biology of *Thottea barberi* (Gamble) Ding Hou. (Aristolochaceae) - an endemic taxon of southern Western Ghat, Kerala, India. Int J Plant Rep Biol. 2014;6(1):99-104.
42. Shaiju PN, Omanakumari N. Chromosomal evolution in the genus *Thottea* Rottb. (Aristolochiaceae) from the Western Ghats, India. Nucleus. 2013;56(3):179-82. https://doi.org/10.1007/s13237-013-0095-3
43. Hipólito J, Viana BF, Selbach-Schnadelbach A, Galetto L, Kevan PG. Pollination biology and genetic variability of a giant perfumed flower (*Aristolochia gigantea* Mart. and Zucc., Aristolochiaceae) visited mainly by small Diptera. Botany. 2012;90(9):815-29. https://doi.org/10.1139/b2012-047
44. Çetinbaş A, Ünal M. An overview of dichogamy in angiosperms. Res Plant Biol. 2014;4(5):9-27.
45. Nakonechnaya OV, Koren OG, Sidorenko VS, Shabalin SA, Markova TO, Kalachev AV. Poor fruit set due to lack of pollinators in *Aristolochia manshuriensis* (Aristolochiaceae). Plant Ecol Evol. 2021;154(1):39-48. https://doi.org/10.5091/plecevo.2021.1747
46. Murugan R, Shivanna KR, Rao RR. Pollination biology of *Aristolochia tagala*, a rare species of medicinal importance. Curr Sci India. 2006;91(6):795-98.