



Anatomy, vascular pattern and development of gynostemium in *Thottea ponmudiana* Sivar. (Aristolochiaceae), an endemic species of the Western Ghats

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Abstract

Thottea ponmudiana Sivar. is a species endemic to the Ponmudi hills of the Western Ghats, India. In terms of morphology and reproductive biology, the species is significant due to the very special reproductive part-the gynostemium, which is formed as a result of partial fusion between the stamen and the pistil. The present study addresses the anatomical and developmental features of the gynostemium, with special emphasis on its vascular pattern and appendages and the results indicate that the appendages in the gynostemium are underdeveloped and exist morphologically as staminodes, not as sterile stigmatic lobes. The irregular distribution of appendages, both in the solitary and whorled pattern and their developmental sequences and vascular pattern point out an evolutionary instability.

Keywords: development; gynostemium; staminode; *Thottea ponmudiana*; vascular pattern; Western Ghats

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1. Introduction

Thottea is a medicinal genus in the family Aristolochiaceae, which comprises 46 species worldwide and 14 species in India (1, 2). These include *T. duchartrei*, *T. ponmudiana*, *T. siliquosa*, *T. idukkiana*, *T. sasidharaniana*, *T. adichilthottiana*, *T. abrahamii*, *T. barberi*, *T. dalzellii*, *T. dinghoui*, *T. sivarajanii*, *T. paucifida*, *T. celebica* and *T. tomentosa*. Among them, nine species (*T. ponmudiana*, *T. idukkiana*, *T. sasidharaniana*, *T. adichilthottiana*, *T. abrahamii*, *T. barberi*, *T. dalzellii*, *T. dinghoui* and *T. sivarajanii*) are endemic to peninsular India and three (*T. paucifida*, *T. celebica* and *T. tomentosa*) are endemic to the Andaman and Nicobar Islands. *Thottea siliquosa* and *T. duchartrei* are distributed in India and Sri Lanka (3–9). The floral morphological characters, especially those of the gynostemium are very significant in the taxonomy of the genus *Thottea* (4–7). Gynostemium is a specialised reproductive structure in some angiosperms, formed by the congenital or post-genital union of stamen and pistil by a developmental phenomenon called synorganisation and it sometimes leads to certain evolutionary modifications in plants (10). In the genus *Thottea*, the gynostemium is formed by the joining of the basal part of

the style and filaments of the stamen (3). Among the *Thottea* species present in peninsular India, *T. siliquosa* and *T. sasidharaniana* have a simple gynostemium without any appendages, whereas the others possess a complex gynostemium with appendages (3, 6). Among them, *T. ponmudiana* is of unique significance due to its endemism in the Ponmudi hills of the Western Ghats and has a very distinct and complex gynostemium with morphological fluctuations. Here, the gynostemium has a well-developed stylar column and at its terminal region, it divides into a number of stigmatic lobes (3). There is a whorl of appendages just below the lobes of the gynostemium. There are three stamen bundles; alternating with these, one to three solitary appendages are observed. All these appendages are considered for species identification and characterisation, even though their actual identity is not revealed on the basis of any scientific data. The appendages are variously considered as either staminodes or an additional whorl of stigmas by several researchers (5, 7, 11). The present study aims to reveal the anatomical and developmental details of the flower of *T. ponmudiana*, with special emphasis on the vascular pattern of the gynostemium and the characterisation of its appendages.

2. Materials and Methods

Flowers of *T. ponmudiana* (Fig. 1A and 1B) from the floral bud to anthesis were collected from the Ponmudi hills of Thiruvananthapuram district, a region of the Agasthyamala Biosphere Reserve, Western Ghats, South Kerala. The voucher specimens were then submitted to the herbarium of JNTBGRI, Palode (Voucher No. 95928).

Anatomy and vascular pattern of gynostemium

For the anatomical studies, both hand sections and permanent serial sections were used. For hand sections, both cross-sections (CS) and longitudinal sections (LS) of the gynostemium were taken using a razor blade. The sections were then stained using safranin, mounted with glycerine and analysed and photographed using a trinocular microscope (ZEISS Axio Observer.A1, Germany). The flower materials were fixed for 24 hr in formalin-aceto-alcohol (FAA) prepared using 5 % formaldehyde, 60 % alcohol, 10 % acetic acid and 25 % water. These specimens were then used for taking permanent serial sections. They were first dehydrated using the tertiary-butanol (TBA) series and then moved to paraffin oil. Later, the specimens were transferred to melted paraffin wax (62 °C), kept overnight and then replaced twice with fresh melted wax (Table 1). The materials were finally embedded in paraffin wax by pouring the melted wax containing the specimens into a paper boat rubbed with glycerin. The specimens were oriented using a hot needle and the boat was placed in cold water. Later, the boat was removed and trapezoid blocks were cut so that each block contains a single specimen. Then each block was attached to a metallic block holder using melted wax. A rotary microtome (Spencer type, AMBA instruments, India) was used to take serial sections (both CS and LS) at a thickness of 10 µm. The sections were then dewaxed using xylol-alcohol mixture, followed by xylol and then treated with a graded, decreasing alcohol series. The sections were then stained with alcoholic safranin for 1 hr and incubated in an ascending alcohol series, followed by xylol-alcohol mixture, then xylol and finally mounted in dibutylphthalate polystyrene xylene (DPX) (12). The sections were then analysed using a trinocular microscope (ZEISS Axio Observer.A1, Germany). Particular attention was given to the vascular traces to find out the similarities and dissimilarities in their path and direction to check and clarify if the appendages are staminodes or not.

Development of gynostemium

The developmental stages were analysed using permanent serial sections and scanning electron microscopic (SEM) studies. The initial flower buds were subjected to permanent serial sectioning and the sections were analysed using a trinocular microscope (ZEISS Axio Observer.A1, Germany). For SEM studies, flower buds at different stages of development were collected, the perianth was removed when necessary, fixed in FAA and stored in ethanol. They were air-dried, then mounted on metallic stubs to coat with gold film in

a sputter chamber. Later, the materials were observed and the developmental stages were photographed using a SEM (ZEISS EVO 18 research microscope).

Table 1. Block preparation using the paraffin method/ tertiary-butanol (TBA) method

Step No.	TBA (mL)	Water (mL)	95 % alcohol (mL)	100 % alcohol (mL)	Paraffin oil (mL)	Safranin O
1	10	40	50	-	-	-
2	20	30	50	-	-	-
3	35	15	50	-	-	-
4	50	-	50	-	-	-
5	75	-	-	25	-	0.1 % (0.1 gm/ 100 mL)
6	75	-	-	25	-	0.1 %
7	100	-	-	-	-	-
8	100	-	-	-	-	-
9	67	-	-	-	33	-
10	50	-	-	-	50	-
11	-	-	-	-	100	-
12	Overnight treatment with liquid paraffin wax at 62 °C in a hot air oven					

TBA: tertiary-butanol.

3. Results

Longitudinal section of gynostemium

The LS of the gynostemium reveals a mass of thin-walled, irregular parenchyma cells bordered by an epidermis bearing several multicellular uncinat hairs. (Fig. 1C and 1D). There are some starch grains within the tissue (Fig. 1E). The parenchyma cells are more closely packed and smaller towards the outer region than in the inner region. The vascular tissue is present in the central region, which divides and forms narrow vascular traces. These vascular traces then contribute to the perianth lobes, the stamen bundles and the stylar lobes. The vascular traces are not present in the solitary appendages located between the stamen bundles and the whorl of appendages present below the stylar lobes (Fig. 1F and 1G). Notably, in the LS of one specimen, an appendage from the whorl below the stylar lobes had developed into a rudimentary stamen. The vascular supply was also present within this structure (Fig. 2A).

Longitudinal section of stylar lobe

The LS of the stylar lobe consists of thin-walled and irregular parenchyma cells, which are bordered by a single epidermis. The epidermis bears several multicellular uncinat hairs towards the apex of the stylar lobe, starting at the halfway mark from its basal region (Fig. 2B). The parenchyma cells are larger towards the centre and smaller and compactly packed towards the peripheral regions. The peripheral or bordering regions sometimes have a nebulous appearance as the cells present there are stained more. The vascular traces are placed longitudinally along the centre of the stylar lobes.



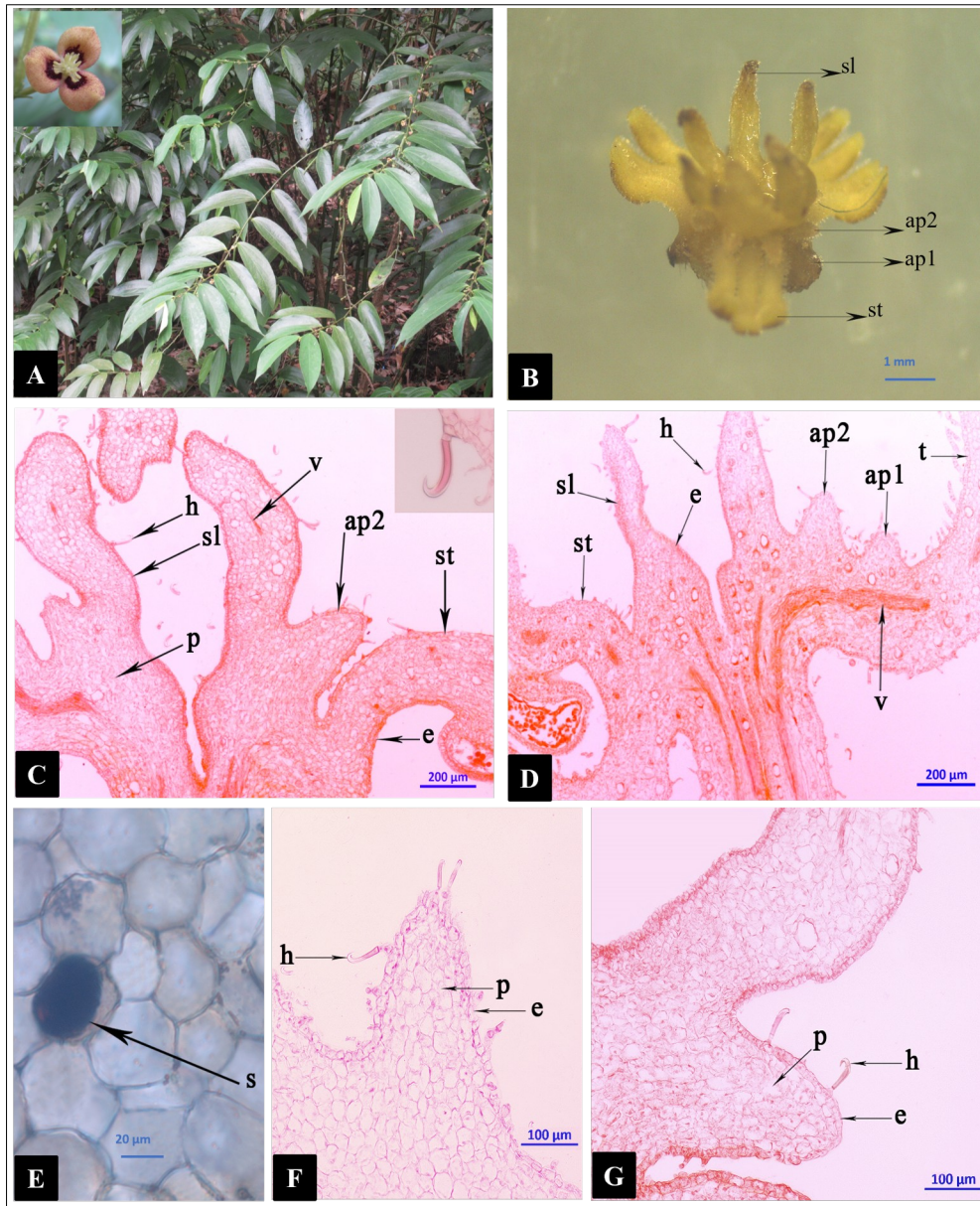


Fig. 1. Habit and gynostemium anatomy of *T. ponmudiana* (A) Habit; inset: flower (B) Gynostemium; ap1: solitary appendage, ap2: whorled appendage, st: stamen bundle, sl: stylar lobe; (C) and (D) Longitudinal section (LS) of gynostemium; t: tepal; st: stamen, ap1: solitary appendage between the stamen bundles, ap2: appendage in the whorl below stylar lobes, sl: stylar lobe, e: epidermis, p: parenchyma, v: vascular trace, h: uncinete hair, inset: uncinete hair; (E) Starch grain (s) stained with IKI solution; (F) The LS of solitary appendage between the stamen bundles; e: epidermis, p: parenchyma, h: uncinete hair; (G) The LS of appendage in the whorl below stylar lobes, e: epidermis, p: parenchyma, h: uncinete hair.

Cross-section of stylar column

In the CS of the stylar column, there is a mass of irregular, thin-walled parenchyma cells which surround a central cruciform hollow space (Fig. 2C). This tissue mass is bordered by a single-layered epidermis which is covered by multicellular uncinete hairs. There are 6–10 patches of vascular traces (that is, equal in number to the stylar lobes) surrounding the central hollow region, arranged in a circular manner. This ring of patches is surrounded by another ring of vascular traces, with a number equal to that of the stamens in the stamen bundles. They are generally arranged in groups of 3–5, corresponding to the stamen bundles. The parenchyma cells of the stylar column are very small, compactly packed and densely stained when approaching both the

inner and outer border regions, whereas the area in between these two is occupied by comparatively larger parenchyma cells. Some starch grains are also seen deposited within the tissue.

Cross-sections of stylar lobe

The CS of the stylar lobe has an irregularly circular to oval shape (Fig. 2D). Its boundary is formed by a single-layered epidermis formed of irregular parenchyma cells. The epidermis is surrounded by multicellular uncinete hairs. Within the epidermis, the major tissue of the stylar lobe is formed by irregularly shaped, thin-walled parenchyma cells. Some starch grains are randomly distributed within the tissue. The cells are smaller and tightly packed towards both the central and peripheral regions. The peripheral regions have a hazy appearance

since the cells take up more stain in those areas. At the centre of the CS, a vascular trace is present.

Cross-section of anther

The CS of the anther consists of irregular and thin-walled parenchyma cells (Fig. 2E). It is surrounded by irregular epidermal cells, which remain narrower towards the outer region of the pollen sacs. Inner to the epidermis, a layer of endothecium is present. Each anther has two thecae within it, each theca with two pollen sacs. The pollen sacs are surrounded by 1–3 middle layers. Inside the pollen sacs, numerous pollen grains can be seen. The two thecae of the anther are connected by a mass of connective tissue, which

contains a central vascular strand that later forms three branches.

Cross-section of appendages

The CS of the appendages on the gynostemium (both solitary appendages and appendages in the whorl below stylar lobes) are irregularly oval to irregularly circular in outline (Fig. 2F). These are formed of thin-walled, irregular parenchyma cells. The CS is bordered by a single layer of irregular parenchyma cells. Several multicellular uncinata hairs are seen arising from the epidermis. The appendages are devoid of any vascular tissues.

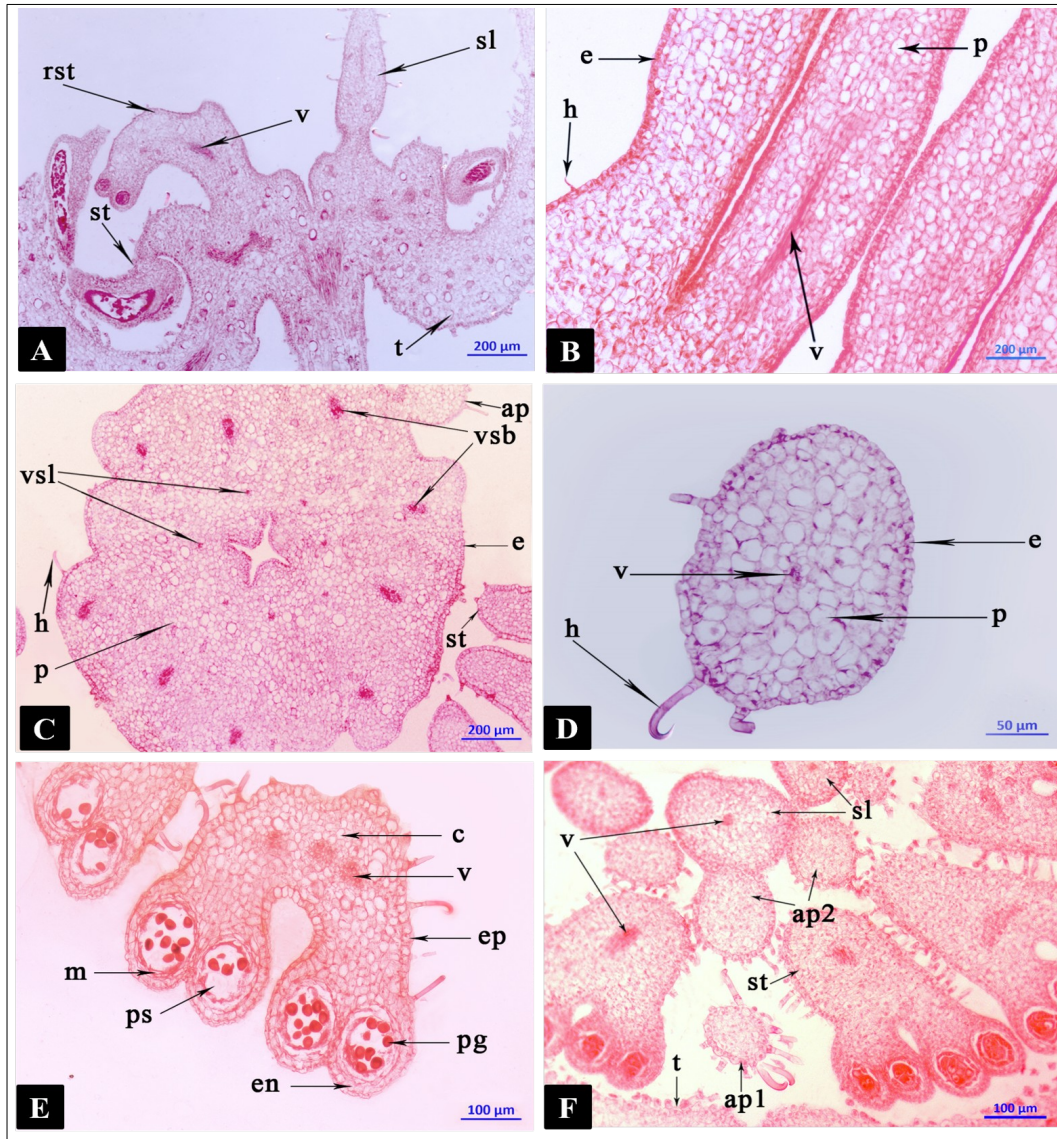


Fig. 2. Anatomy of gynostemium in *T. ponmudiana* (A) Longitudinal section (LS) of gynostemium; rst: rudimentary stamen developed in the position of a whorled appendage, t: tepal, st: normal stamen, sl: stylar lobe, v: vascular trace; (B) The LS of stylar lobes; e: epidermis, p: parenchyma, v: vascular trace, h: uncinata hair; (C) Cross section (CS) of stylar column; e: epidermis, p: parenchyma, h: uncinata hair; vsb: vascular trace towards stamen bundle, vsl: vascular trace towards stylar lobe, ap: solitary appendage, st: stamen; (D) CS of stylar lobe; e: epidermis, p: parenchyma, v: vascular trace, h: uncinata hair; (E) The CS of anther; ep: epidermis, en: endothecium, m: middle layer, ps: pollen sac, pg: pollen grain, c: connective tissue, v: vascular trace; (F) CS of gynostemium; t: tepal; st: stamen, ap1: solitary appendage between the stamen bundles, ap2: appendage in the whorl below stylar lobes, sl: stylar lobe, v: vascular trace.

Vascular pattern of gynostemium

Thottea ponmudiana has a tetracarpellary tetralocular ovary. In the CS of the ovary, there are eight conjoint collateral vascular bundles (Fig. 3A), with four dorsal vascular bundles and four placental vascular bundles. The dorsal vascular bundles lie outside and opposite to the ovary locules, whereas the placental vascular bundles are seen inside and opposite to the septal region between the adjacent locules. The dorsal vascular bundles show endarch xylem vessels. In these bundles, phloem lies towards the exterior and xylem towards the interior, but the placental vascular bundles have an inversely oriented xylem and phloem. The ovules are supplied by the placental bundles (Fig. 3B). Both the dorsal vascular strands and the placental vascular strands run towards the upper region of the ovary, forming two concentric rings, each with 4 vascular bundles (Fig. 3C). Beyond this region, the vascular bundles of both the rings divide forming 6–8 vascular traces (Fig. 3D). Each of the vascular traces of the outer ring divide to form two horizontal secondary

branches, such that one of them lies above the other (Fig. 3E). Out of these, the lower horizontal branches split up to form three groups of vascular traces, each group corresponding to a tepal and thus each group finally contributes to each tepal (Fig. 3F). The upper horizontal branches of vascular traces undergo further divisions to form three groups of vascular traces. The number of vascular traces in each of these groups is equal to the number of stamens in the stamen bundle into which that particular group of vascular traces finally enter. Thus, each stamen in the stamen bundle gets supplied by a single vascular trace (Fig. 4A). The vascular traces of the inner ring traverse upwards and undergo further divisions so that the resulting number of vascular traces is equal to the number of stylar lobes (Fig. 4B). Thus, each stylar lobe is eventually contributed by a single vascular trace (Fig. 4C–4E and Fig. 5). It was noticed that both the solitary appendages alternating with the stamen bundles and the whorl of appendages just below the stylar lobes were devoid of any vascular tissues.

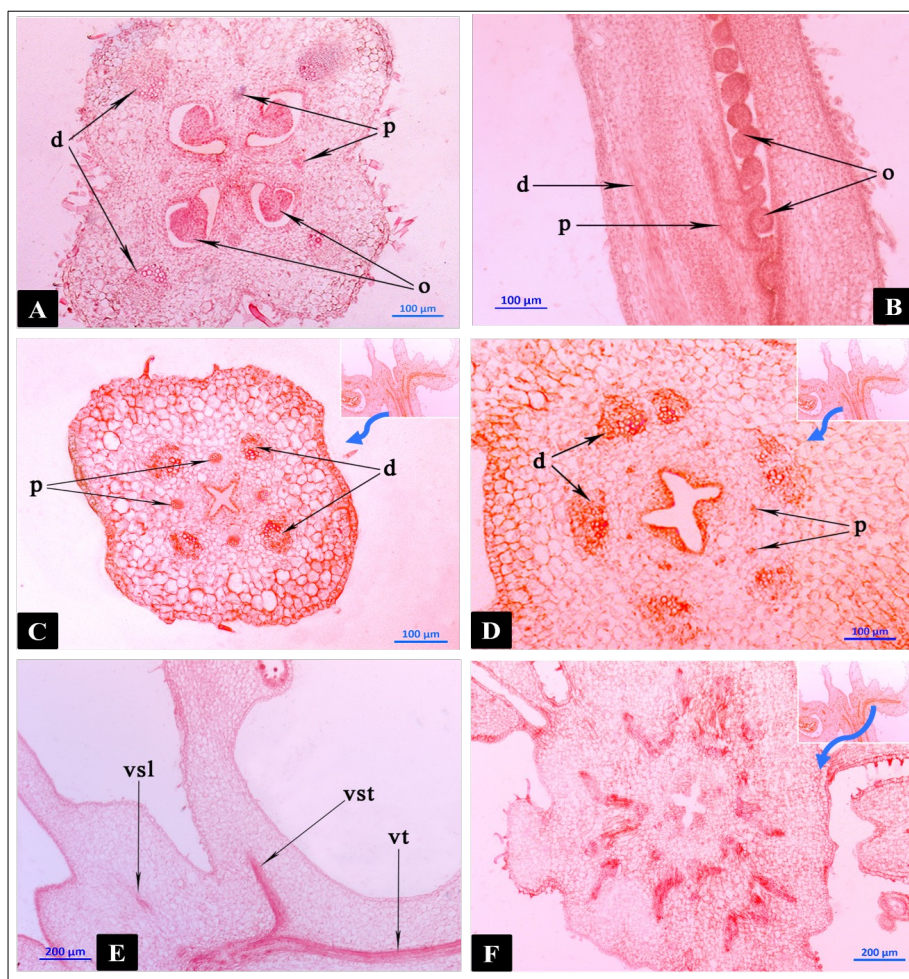


Fig. 3. Vascular pattern of gynostemium in *T. ponmudiana* (A) Cross section (CS) of ovary showing vascular bundles; d: dorsal vascular bundle, p: placental vascular bundle, o: ovule; (B) Longitudinal section (LS) of ovary; d: dorsal vascular strand, p: placental vascular strand, o: ovule; (C) The CS through the top of ovary showing vascular bundles; d: dorsal vascular bundle, p: placental vascular bundle, inset: LS of gynostemium; (D) The CS through the region just below perianth showing the division of vascular bundles; d: dorsal vascular bundle, p: placental vascular bundle, inset: LS of gynostemium; (E) The LS of gynostemium-branching of vascular tissue; vt: vascular trace towards tepal, vst: vascular trace towards stamen, vsl: vascular trace towards stylar lobe; (F) CS of flower through the base of perianth - branching of vascular traces; inset: LS of gynostemium.

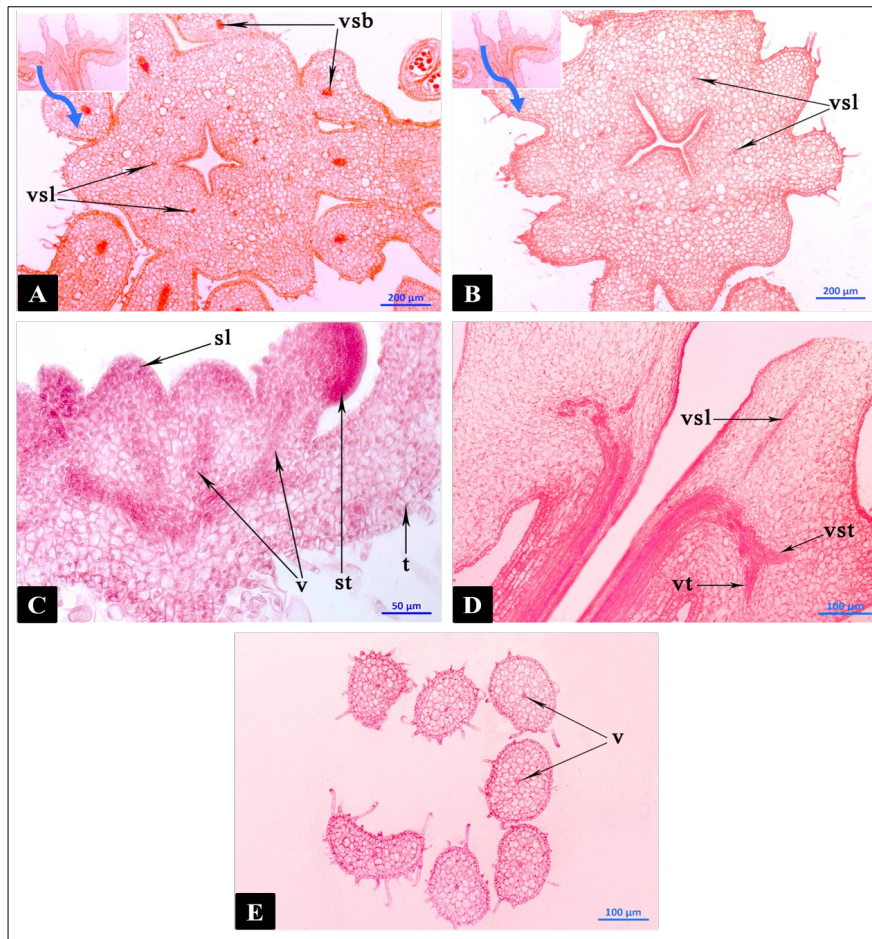


Fig. 4. Vascular pattern of gynostemium in *T. ponmudiana* (A) Cross-section (CS) of gynostemium through the base of stamen bundles; vsb: vascular traces towards the stamen bundles, vsl: vascular traces towards the stylar lobes, inset: LS of gynostemium; (B) The CS of gynostemium through the base of stylar lobes; vsl: vascular traces towards stylar lobes, inset: longitudinal section of gynostemium; (C) The LS of flower bud; t: tepal, st: stamen, sl: stylar lobe, v: vascular traces; (D) The LS of gynostemium- branching of vascular tissue; vt: vascular trace towards tepal, vst: vascular trace towards stamen; vsl: vascular trace towards stylar lobe; (E) CS of stylar lobes; v: vascular trace.

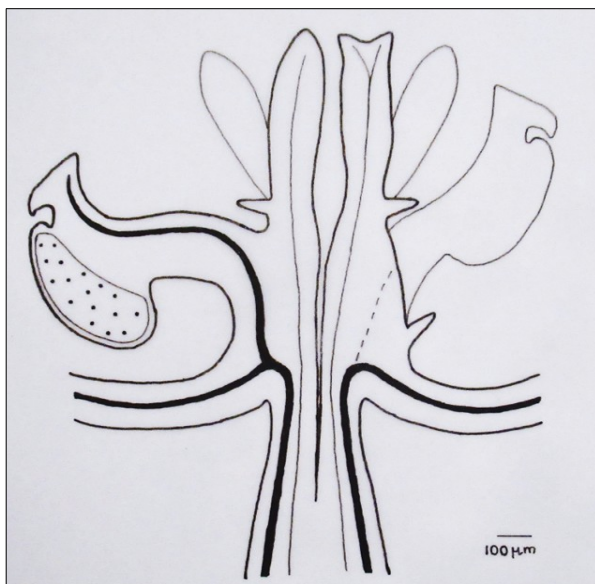


Fig. 5. Diagrammatic representation of vascular pattern of gynostemium in *Thottea ponmudiana*; d: dorsal vascular strand, p: placental vascular strand, t: tepal, ap1: solitary appendage, ap2: whorled appendage, st: stamen, sl: stylar lobe.

Development of gynostemium

Thottea ponmudiana possesses a cymose partial inflorescence which develops from the axils of the leaves. Here, the floral development initiates with the emergence of a floral meristem (Fig. 6A and 6B). From this floral meristem, different floral organs develop in the form of different floral organ primordia. Initially, from the dome-shaped floral meristem, three tepal primordia (forming the perianth) are formed (Fig. 6C). These tepal primordia later develop into 3 tepals. With the tepal formation process, the developing floral apex transforms into a triangular shape in outline, with a central hollow space that has square-shaped or widely cross-like borders. Then, the stamen primordia develop just inside and opposite to the tepals. These stamen primordia form 3 groups, each group with 2–4 stamen primordia (generally 3). They later develop into stamen bundles. With the further development stages of the stamen primordia, the floral apex becomes dish-like or concave in appearance. Concomitantly, primordia forming the solitary appendages (1–3 in number per flower, mostly 3) develop in alternate position to the groups of stamen primordia (Fig. 6D). Subsequently, the primordia for the

whorl of appendages (6–14 in number) develop from the region inner to the stamen bundles (Fig. 6E). Every solitary appendage primordium is positioned opposite to one of the whorled appendage primordia. Thereafter, primordia for the styler lobes begin to develop from the region inner to the whorled appendage primordia (Fig. 6F). Notably, one specimen exhibited a single stamen developing in the position of one of the solitary appendages (Fig. 7A). It was noted that in all the specimens, the hollow region at the centre persisted even after the development of all appendage primordia;

however, the floral apex became levelled or flatter with progress in development. Finally, the styler lobe primordia (6–10 in number) arise as a ring around the central hollow space and this hollow region becomes narrower. Then, numerous uncinat hairs and some filamentous hairs start to develop on the surface of the developing organ primordia. All the organ primordia undergo further steps of development, forming their respective organs, finally forming a complete, mature gynostemium (Fig. 7B and 7C).

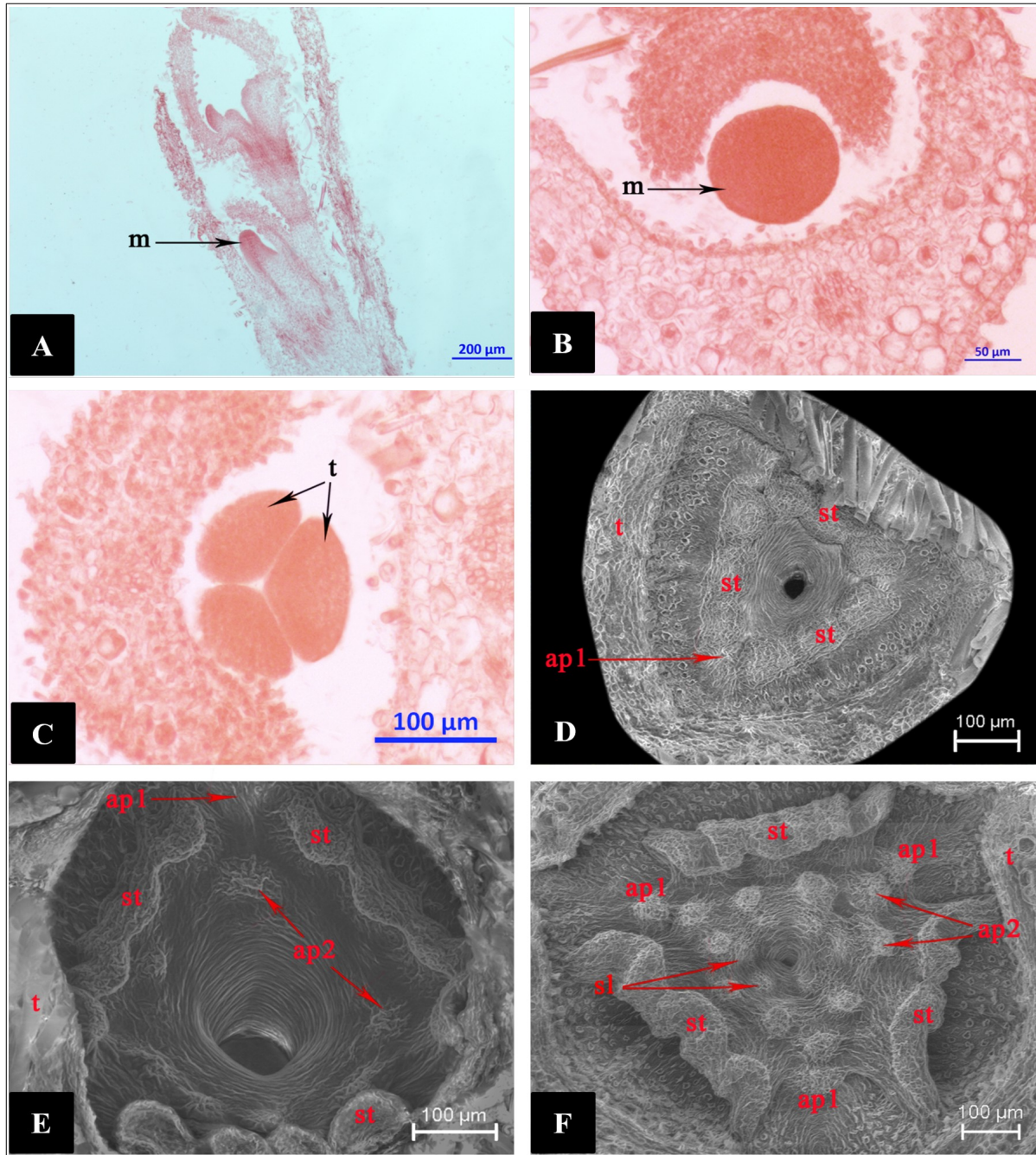


Fig. 6. Gynostemium development in *Thottea ponmudiana* (A) Longitudinal section (LS) of flower bud showing the development of floral meristem (m); (B) Cross-section (CS) of flower bud-development of floral meristem (m); (C) Formation of tepal primordia (t); (D) SEM image of flower bud-triangular floral apex showing the development of stamen primordia (st) and solitary appendage primordia (ap1); t: tepal; (E) Initiation of primordia for whorled appendages (ap2); t: tepal, ap1: solitary appendage primordium, st: stamen primordia; (F) Development of styler lobe primordia (sl); t: tepal, ap1: solitary appendage primordia, st: stamen primordia, ap2: whorled appendage primordia.

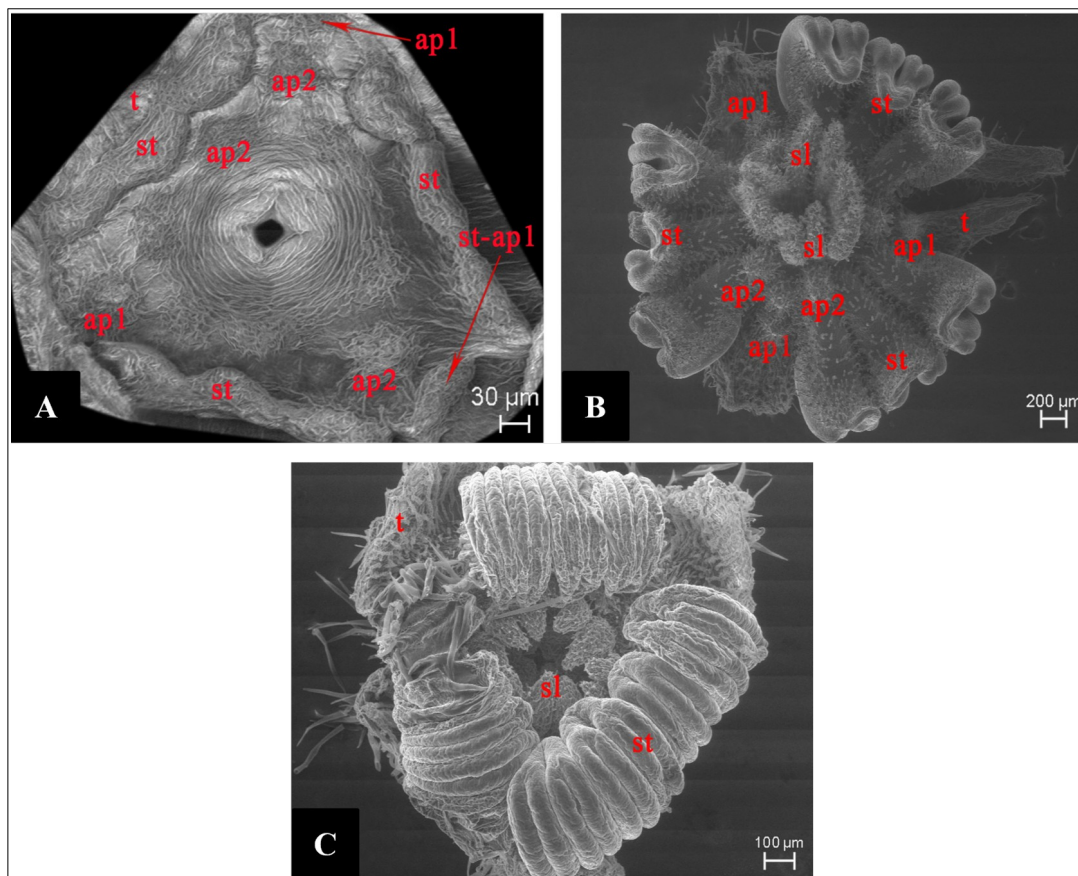


Fig. 7. Gynostemium development in *Thottea ponmudiana* (A) Development of a stamen (st-ap1) in the position of solitary appendage between the stamen bundles; t: tepal, ap1: solitary appendage primordia, st: stamen primordia; ap2: whorled appendage primordia; (B) Developing flower bud with all floral organs; t: tepal, ap1: solitary appendage, st: stamen, ap2: whorled appendage, sl: stylar lobe; (C) Gynostemium of a mature flower; t: tepal, st: stamen, sl: stylar lobe.

4. Discussion

The anatomical studies on the gynostemium of *T. ponmudiana* revealed that it is a simple, parenchymatous structure with vascular traces. The gynostemium does not contain nectary or other such specific secretory structures. Some studies have shown the presence of cells with ornamentations in the gynostemium of some Orchidaceae members (13). Similar characteristics were absent in the present study. However, the presence of multicellular unciniate hairs and filamentous hairs constituted two key observations on the surface of gynostemium in *T. ponmudiana*.

The development of vascular tissues in plants leads to the arrangement of vascular bundles within the plant body so that the whole vascular tissue lies in a specific way along the whole plant body. Some specific genetic and molecular mechanisms in plants lead to the particular arrangements of the vascular tissues inside the plant body (14). The vascular tissues are noteworthy because they link different major plant organs (15). The flower development and floral vascularisation studies in seven species of Pleurothallidinae (Orchidaceae) revealed that reduction in vascular traces (loss of traces) was an anatomic character in the stigmatic lobes and sterile whorls of gynostemium, more prominent loss in the former and these features did not affect the external appearance of the gynostemium (16). In the

present study, the dorsal vascular bundles contribute to the perianth and stamens and the placental vascular bundles supply the stylar lobes. However, earlier floral morphological and anatomical studies in *Apama siliquosa*, *Bragantia wallichii* and *Bragantia dalzellii* reported that the placental bundles in these flowers disappear before entering the ovules (17). However, in current study, the placental bundles did not disappear but directly entered the stigmatic lobes. Some earlier studies on the anatomical characteristics of rudimentary organs in plants have shown that some of these structures have vascular tissue, some others are without any vascular supply and still others do not have any vascular supply within them, but in the last type, remnants of vascular tissue meant for these structures are present in the plant axis (18).

The loss or fusion of vascular bundles or organs may lead to a reduction in the number of vascular bundles in plants, such as in Leguminosae. In the flowers of some plants, remnants of vascular traces corresponding to some particular organs once present in the past, but absent now, can be observed. In some other plants, even though certain organs are present, their corresponding vascular traces have disappeared either partially or completely (19). The former condition was reported earlier while studying the floral anatomy of *B. dalzellii*, *B. wallichii* and *A. siliquosa*, where the

vascular traces corresponding to the inner and outer whorl of appendages disappear when reaching above the receptacle; therefore, they do not enter the appendages (17). In another study on the flowers of *Carica papaya*, disappearing procambial vascular stubs were reported (20). *Piranhea trifoliata* of Picrodendraceae was also reported for the absence of vascularisation within the staminodes of pistillate flowers, but their remnants were observed (21). In the present study, even though there are 2 whorls of appendages in *T. ponmudiana*, the vascular traces corresponding to them are not observed anywhere in the floral vascular system. But the rudimentary stamen arising from the position of appendages possesses vascular tissues within them. In some plants, such as *Paronychia decandra* (Caryophyllaceae), *Suriana maritima* (Surianaceae), *Consolida regalis* (Ranunculaceae), *Ophiocaryon* spp. (Sabiaceae), *Leiothrix* (Eriocaulaceae), etc., vascular tissues are absent in the staminodes (22–26). In the palm tree *Chamaedorea tepejilote*, vascularisation is absent in both the male flower's pistillode and calyx and the female flower's staminode; therefore, these structures are considered extra rudimentary (27). In the staminate flowers of *P. trifoliata*, the nectary lacks any kind of vascular tissues (21). The absence of vascular tissues in the staminodes of *Paronychia* (Caryophyllaceae) led the authors to consider them as vestigial structures (28). The vascular plan of gynostemium in *T. ponmudiana* is dissimilar to that of some previously studied species (17). A sudden evolutionary reconfiguration of the floral base plan can result from genetic variations in plants and this results in the structural reorganisation of vascular tissue also (29–31). A reduction in the number of vascular bundles can result from the disappearance or fusion of the vascular bundles (19). The absence of vascular tissues within the staminodes of *T. ponmudiana* indicates that their anatomical features are more reduced and hence this species is evolving. Furthermore, the development of a rudimentary stamen in the position of a whorled appendage reveals that these appendages are staminodes.

In plants, the sexual stage commences with floral development and it is a crucial step in their life. Gynostemium development in different plant families occurs in different ways (32). Gynostemium in *Thottea* is formed through congenital development. The differential elongation of basal and apical regions of the organs undergoing congenital fusion helps in attaining different shapes of the resulting structure (33). The kind of synorganisation procedure and the position and number of the structural units involved in it influence the uniqueness of the resulting floral structure (34). An extremely complex level of synorganisation is present in families such as Apocynaceae and Orchidaceae with the formation of pollinaria (32), whereas only partial synorganisation occurs in different species of *Thottea* (3), where pollinaria and nectaries are absent. Here, the floral organ development is centripetal or acropetal, starting from the formation of the outermost whorl (perianth), serially followed by the inner ones and

completing the development by the formation of the innermost organs (stigmatic lobes). The centripetal floral organ development is regarded as an ancestral character in Angiosperms and the centrifugal one as an advanced character. Therefore, the early branched-off plant lineages lack centrifugal development (35, 36).

In *T. ponmudiana*, floral development starts with the formation of the perianth. Although it is a non-essential whorl, the perianth has utmost significance in plant taxonomic studies (37). In the present study, a change in the shape of the floral apex takes place along with the formation of a central hollow space. Some earlier floral development studies have also reported the occurrence of similar structural variations in 5 species of *Thottea* (38). During the initial stages of flower development, the apex of *T. ponmudiana* becomes concave. This kind of inward curvature can be due to the development of the inferior ovary below the concave area (39). There is a genetic level control over the order of development of various floral organs, their number and arrangement, all of which further determine the shape of the floral apex. Even small changes in their arrangement and shape can cause changes in the morphology of mature flowers. (23, 38, 40–43). The floral apex can be compared to the receptacle of a flower and is significant in playing a critical role in floral evolution (44).

The number of primordia for stamen, appendages and styler lobe seems to be different in different flowers of *T. ponmudiana*. Such instabilities are the common characteristics of most of the *Thottea* species (2–4, 7–9, 45–47). The peak level of stamen flexibility is considered an advanced character of the family Aristolochiaceae. The size of the primordium and the floral apex determine the position and number of the floral organs, including the stamens in *Thottea* (38). A stamen developing in the position of a solitary appendage in *T. ponmudiana* reveals that the solitary appendage is an actual staminode. Studies on certain orchid genera have disclosed the presence of some ornamental structures on their gynostemium, formed as a result of dorsal stamen outgrowth, or formed from staminodes, or from both staminodes and auricles (48, 49). However, such ornamentations were not present on the gynostemium of *T. ponmudiana*.

5. Conclusion

The anatomical studies reveal that the gynostemium of *Thottea ponmudiana* is simple parenchymatous with characteristic multicellular uncinatate and filamentous epidermal hairs. The developmental sequences indicate that the dorsal vascular bundles contribute to the perianth and stamens and the placental vascular bundles supply the styler lobes. Both the solitary appendages and whorled appendages are devoid of any vascular traces, except the rudimentary stamens. The comparative studies indicating the presence of the remnants of vascular traces corresponding to all the

appendages in some other species help to conclude that there is a complete reduction or absence of vascular traces in *T. ponmudiana* and this may be a point of evolutionary significance of this composite structure. The developmental studies help to identify various steps in the formation of a mature gynostemium, starting from a floral meristem and the variations in the shape of the floral apex during its development. The developmental studies on gynostemium also revealed the staminodial identity of the solitary appendages. The development of appendages to stamens indicates that the appendages are actual staminodes formed by the forward evolution of functional stamens, which were once present in their positions. The rare occurrence of stamens in the position of staminodes also indicates the instability with regard to their evolutionary development. A detailed floral evolutionary analysis is proposed as the future programme for arriving at a solid conclusion to trace out the phylogenetic trends in the genus *Thottea*.

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Authors' contributions

SPN, AKS and SKS conceived the idea and planned the experiments. SPN and SKS collected the plant samples. SKS performed the experiments under the supervision of SPN and AKS. All authors analysed the research data and drafted the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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