

Morpho-anatomical Diversity of *Angiopteris crassipes* Wall. ex C. Presl (Marattiaceae)

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Abstract

The paper describes for the first time the morpho-anatomical diversity of *Angiopteris crassipes* Wall. ex C. Presl from India. Morpho-anatomical characters such as hairs, scales epidermal features of pinnae (number of silica containing cells and arrangement of stomata), internal building of rhizome, root and petiole would be beneficial in taxonomic identification up to species level. In accordance to the IUCN (2019), most of the taxa of the genus *Angiopteris* are considered endangered worldwide. During the floristic survey authors found that natural population of Indian *Angiopteris* are under severe threat of anthropogenic pressure which need urgent attention for conservation.

Key words: Anatomy, Conservation, Fern, Morphology, Taxonomy

Introduction

Angiopteris is a well recognized giant evergreen fern genus in the family Marattiaceae with distribution of throughout the paleotropics from Madagascar to the South Pacific Islands. It was originally described by Hoffmann (1789). Thereafter the genus *Angiopteris* Hoffm. has been studied by various workers (Roxburgh 1814; Hooker & Greville 1827-31; Clarke 1880; Hanning 1889; Beddome 1892, 1893; Jeffrey 1901; Tansley 1907-1908; Sinnot 1911; Charles 1911; West 1917; Hieronymus 1919; Blomquist 1922; Bower 1923, 1926; Haupt 1940; Nozu 1956; Pichi Sermolli 1957, 1977; Foster 1966; Pant 1965; Thurston 1969; Bierhorst 1971; Ogura 1972; Bir 1976, 1977a,b; Holttum 1978; Li 1989; Rolleri et al. 1991; Tryon & Lugardon 1991; Liu et al. 2000; Rolleri 2002, 2003; Kingston et al. 2004; He & Chu 2006; Li & Lu 2006, 2007; Smith et al. 2006; Murdock 2008a, b; He & Christenhusz 2013; Roskov et al. 2018). The genus *Angiopteris* has distinctive characters combination, as the presence of short, broad and massive fleshy stock. Stipe swollen at base, frond bipinnate, pinnae with swollen base, pinnules narrowly oblong to elliptic with free veins, recurrent veins present in between two true veins running from margins, sori arranged in rows of sporangia attached

along veins (Holttum, 1954). Its sporangia are fused only at the base and having monolete spores while in all other genera of family Marattiaceae have trilete spores. The sporangia of *Angiopteris*, although discrete at maturity, have no morphological integrity when initiated (Murdock, 2008b).

The first and most comprehensive account on the morphology and anatomy of *Angiopteris* was by Harting (1853) as a part of joint work with De Variese who deals with taxonomy of species in the genus as mentioned in Holttum (1958). The morphological and anatomical features have generated a refined understanding of genus and species-level relationships within the family in terms of phylogenetic investigations. Murdock (2008 a,b) stated that the new taxonomy is congruent with the current understanding of the phylogeny of marattioid ferns based on both molecular and morphological data. The significance of morphological and anatomical features has been evaluated here. Keeping above facts in view, an attempt has been made to describe the morpho-anatomical diversity of *Angiopteris* from India in great detail. A preliminary account of the morpho-anatomical diversity of *Angiopteris* has already been reported by Yadav et al. (2022).

Botanical explorations was conducted in the region of Bailadila hills of Baster, Chhattisgarh and Pachmarhi Hills of Madhya Pradesh, a state of central India by one of us (RBY) and collected specimens for anatomical study. Earlier the population of *Angiopteris* in this region was taxonomically recognized as *A. evecta*, type of species (Dixit 1984). It is extremely widespread and encompasses a wide range of morphological variation (Murdock 2008b). The account by Fraser-Jenkins et al. (2017) records restricted distribution to the eastern part of the country (Assam & Arunachal Pradesh) in India. In addition to Pachmarhi Hills, earlier the population of *Angiopteris* in the Andaman and Nicobar Islands (ANIs) was also recognized as *A. evecta* (Dixit & Sinha 2001; Pandey & Diwakar 2008; Singh et al. 2014, 2021b). ANI's is one of the hotspots of biodiversity with 572 Islands (N 6°45' to 13°41' and E 92°12' to 93°37') and floristically very rich phyto-geographical region in the country with higher number of endemism (Singh et al. 2014, 2021a,b; Singh & Misra 2020; Singh & Ranjan 2021) where recent plant exploration in this region revealed discoveries of novelties in pteridophytic flora (Kholia et al 2016; Singh et al. 2016a,b,c) and other plant groups (Singh et al. 2014, 2021a, b; Singh 2017a, b, 2021, 2023; Naik et al. 2020, 2023; Singh & Misra 2020; Sivaramakrishna 2021). Although, Chen & Sun (2018) stated that field explorations on various habitat have been conducted across the world in order to provide information on the diversity of plants as well as document the unknown ones. More recently Singh et al. (2022) also stated that ongoing explorations will further enrich our knowledge on distributions of many more species. This is also necessary to alleviate the currently unprecedented frequency of biodiversity conservation (Naik et al. 2023).

India is one of the main world centers of biodiversity where pteridophytic flora was recorded time to time by various workers where currently, three species viz.: *A. crassipes* Wall. ex C. Presl, *A. evecta* (G.Forst.) Hoffm. and *A. helferiana* C. Presl are recognize in India. Among these the *A. crassipes* and *A. helferiana* are widely distributed throughout the country including ANIs. Based on the account by Fraser-Jenk et al. (2017), our specimen seems as *A. crassipes*. Therefore, we herein describe and

illustrate the morpho-anatomical diversity of *A. crassipes* in great detail from India.

Materials and methods

For the present morpho-anatomical investigations, plant materials were collected from the remote areas of Gulley Nala in Bastar district of state Chhattisgarh (Central India). Besides, it was also collected from the fern house of Botany Department of Allahabad University. Anatomy of rhizome, root and petiole was studied in serial microtome sections cut in different planes. For microtomy, small pieces of rhizomes, roots and petioles were fixed and stored in F.A.A which were later washed thoroughly in tap water and dehydrated in a graded series of tertiary butyl alcohol. Infiltration and embedding of the material was done in E. Merck paraffin wax. Serial microtome sections were cut at 10-15 μ thickness. Wherever, necessary hand sections were also cut. These sections were stained in the usual safranin fast green combination and mounted in D.P.X. for the study of epidermal feature leaves and petioles were subjected to light maceration in conc. Nitric acid and potassium chlorate and subsequently treated with dilute aqueous ammonia solution. Epidermal preparations were mounted in safranin-glycerin jelly and cover glasses were sealed by coating their margin with colourless copel varnish.

Nature of various depositions and cell contents was identified by special histochemical tests as suggested by Johansen (1940) and Foster (1941). Presence of cutin was confirmed by the appearance of red colour when fresh sections were treated with saturated solution of Sudan IV prepared in 70% ethanol (Margolena 1932).

Presence of starch grains were detected by appearance of blue colour when treated with potassium iodide solution. This was made by dissolving 3 gm of Iodine and 1.5 gm of potassium iodide in 100 ml of water. Lignin was tested by occurrence of red colour after treating lignified portion with phloroglucinol solution followed by 1-2 drops of 2.5% hydrochloric acid. Phloroglucinol solution was prepared by dissolving 1 gm phloroglucinol in 100 ml of 95% ethanol. To test the presence of tannin substances fresh sections were placed in 10% aqueous ferric chloride solution with a pinch of sodium carbonate and blue colour

of tannin substances was observed under the microscope. The presence of phlobaphene was detected by their natural brown colour as suggested by Reeves (1951). The descriptive terminology used is after Pant (1965) and Metcalfe & Chalk (1950).

Result

Growth Habit and External Morphology

The plants are usually found growing in moist shady places along the water streams. The height of the plant is usually 0.6-1.8 m, bearing globose, upright rhizome and numerous adventitious roots arising from its basal part. Rhizome is robust and may attain a diameter of up to 60 cm. The overall appearance of the plant is like a small tree. The petioles are spirally arranged and form a crown at the apex of the rhizome. Each petiole bears a pair of fleshy stipules at its base, which enclose the next younger petiole. Young petiole show typical circinate vernation. Pinnae are usually sessile or shortly stalked. A conspicuous swelling, called pulvinus, is present at the base of pinnae. The venation pattern of pinnae is open dichotomous. Segments of leaves show midrib and open dichotomizing lateral veins, which are branched once. Veinlets are usually sub-parallel. Free ends of the veins are often somewhat swollen.

Hairs and Scales

Multicellular branched hairs are present at the base of petiole. Hairs are hyaline or brown in colour with almost terminal cells. The density of hairs gradually decreases towards the upper side of petiole. At the base of petiole hairs are mixed with scales. Scales are large, brown and dense towards the base of the petiole. Marginal cells of scales are elongated and hair like with globose end. The distal side of scales is pointed while the proximal end is broad and blunt (Fig. 1 B, E).

Epidermis of Pinnae

Leaves are hypostomatic. The sides of epidermal cells on either face of leaves are more or less sinuous (Fig.2A, B). Leaves show irregularly scattered group of short, almost straight walled silica containing cell in their lower epidermis (Fig.2B). The Silica containing cells are mostly in pair but it may occur in group of up to 4 cells. Stomata are usually placed in the direction of the veins and are amphicyclic showing a ring of three or more clearly differentiated subsidiary cells (Fig.2B).

Anatomy

Rhizome

Transverse sections of rhizome show a single layered epidermis of thin walled cells towards the outer side. The epidermis is followed by a broad cortex. Cells of cortex are thin walled, parenchymatous and polygonal in shapes. Except for the outer 2-3 layers cells of cortex are filled with large number of starch grains. The starch grains are oval or spherical in shape (Fig.1F). Cortical region also shows the presence of many mucilage canals. Tannin filled cells are also scattered among the cortical cells. The stele is dictyostelic. Meristemes are arranged in a ring and their number varies according to the age and diameter of rhizome (Fig.1A). Each meristeme is enclosed by a single layered endodermis followed by layer of thin walled cells constituting the pericycle (Fig.1D). Metaxylem elements consist of scalariform tracheids and protoxylem tracheids show annular or spiral thickening (Fig.1C). Phloem consists of sieve cells and parenchyma.

Towards the inner side of meristeme is the pith, which is connected with the cortex through leaf gaps. Cells of pith are thin walled and parenchymatous filled with starch grains and contain mucilage canals similar those in the cortical region. Root traces arise from meristemes.

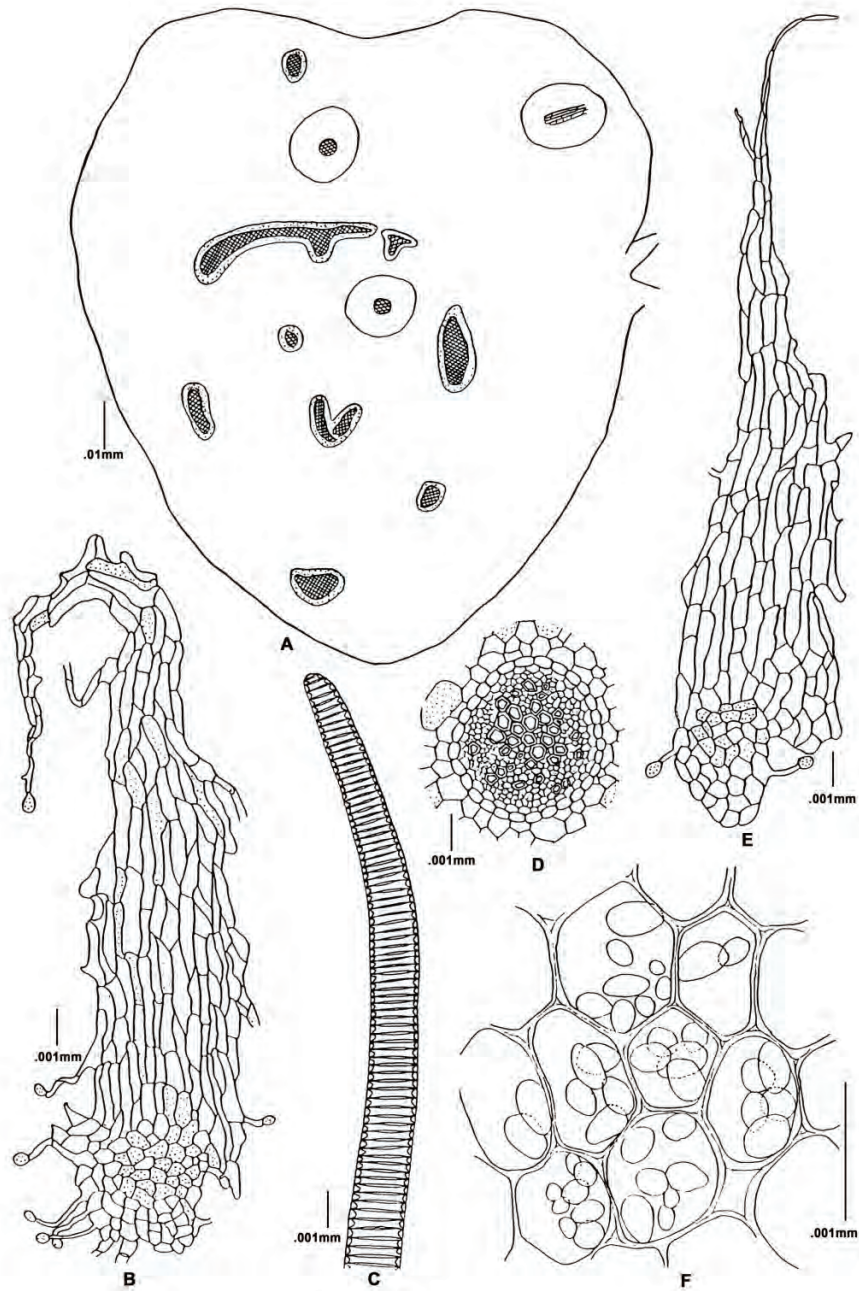


FIGURE-1

Fig.1. Angiopteris crassipes: A: Diagrammatic transaction of rhizome showing vascular strands and root traces; B: Scale from the rhizome surface; C: Single tracheid; D: Structural details of one of the vascular strand in A; E: Scale from the surface of young petiole; F: Cortical cells of rhizome showing starch grains.

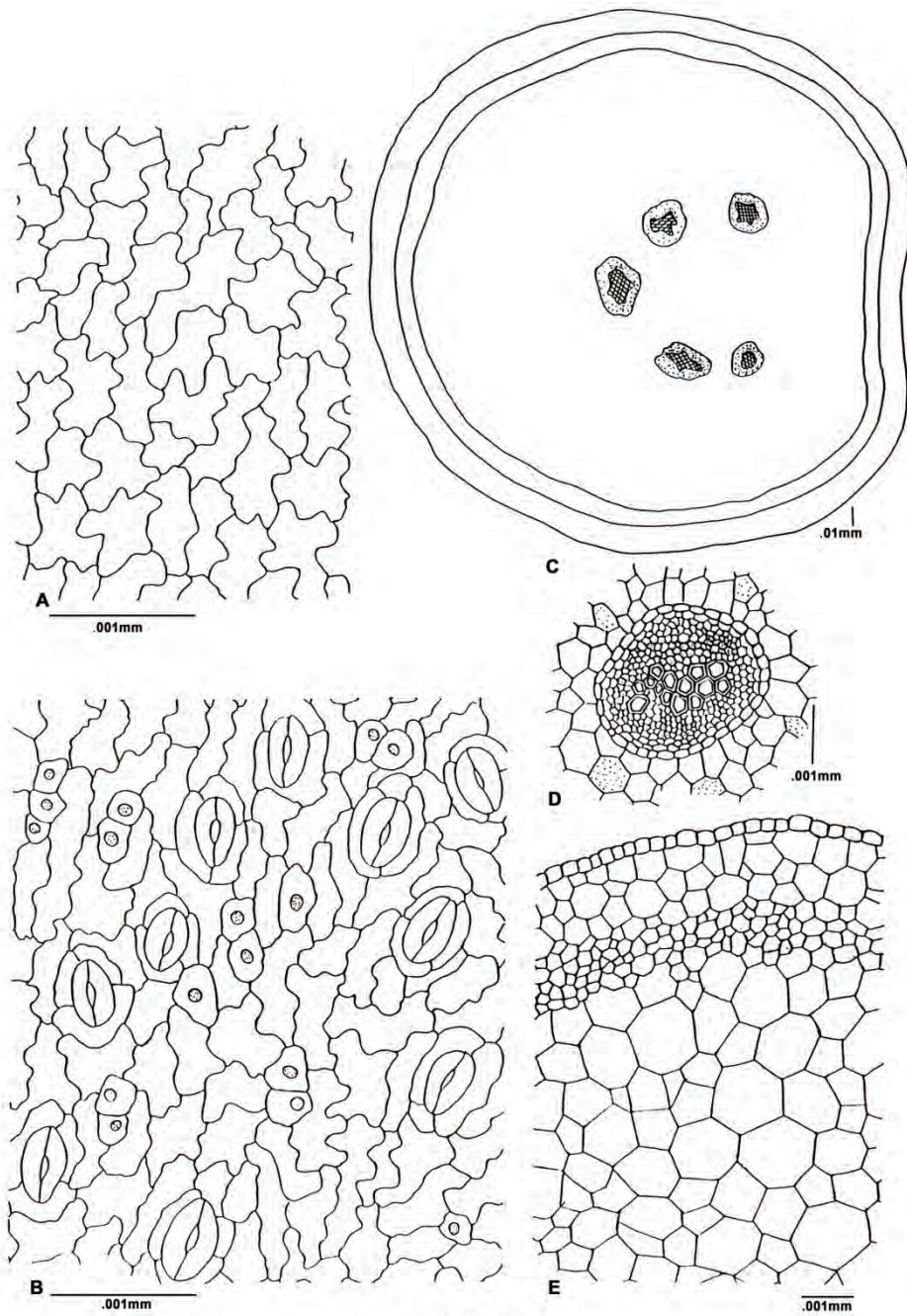


FIGURE-2

Fig.2. Angiopteris crassipes: A: Upper epidermis; B: Lower epidermis showing stomata and paired silica containing cells; C: diagrammatic transverse section of petiole at the base showing five vascular strands; D: Structural details of one of the vascular strands in C; E: a portion of transverse section of petiole showing epidermis and ground tissue.

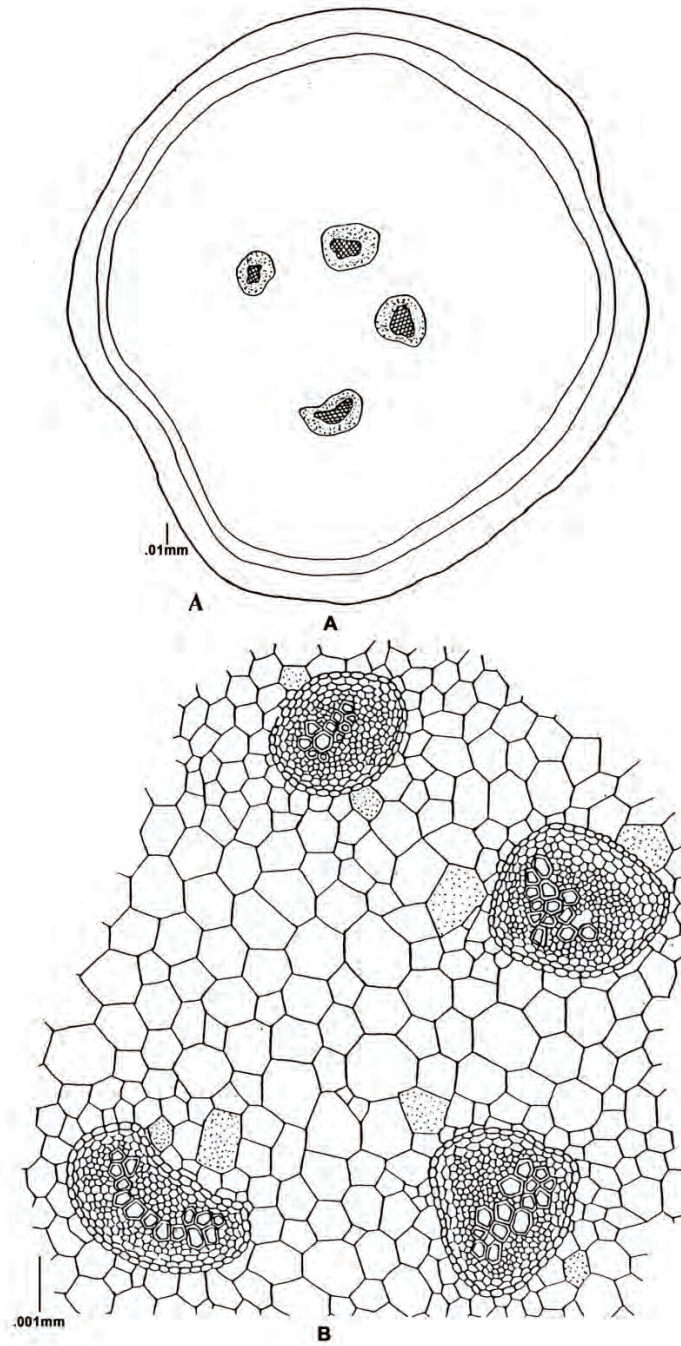


FIGURE-3

Fig.3. *Angiopteris crassipes*: A:Diagrammatic transaction of petiole at its middle portion showing four vascular strands; B: Structural details of vascular strands in A.

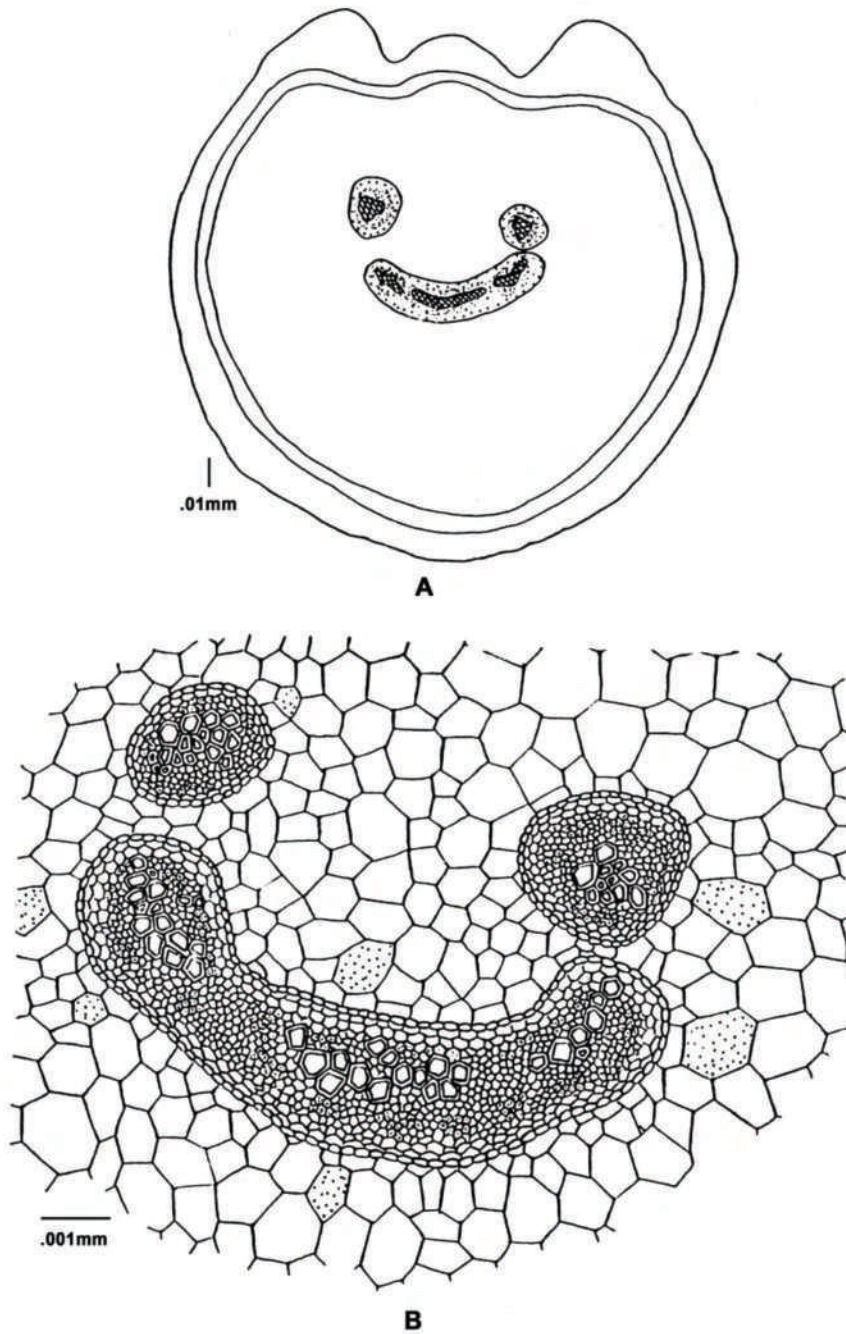


FIGURE-4

Fig.4. *Angiopteris crassipes*: A: Diagrammatic transverse section of petiole towards its tip showing three vascular strands;
B: Structural details of vascular strands in A.

Root

Transverse sections of roots show outer most layer of epidermis which is single layered and made up of thin walled barrel shaped cells. Young roots show multicellular root hairs. Epidermis is followed by a broad zone of the cortex. The cortical cells are thin walled parenchymatous and frequently filled with tannin. After cortex there is a single layered endodermis whose cells are thick walled and rectangular in shape. Next to endodermis is pericycle, which is composed of 1-2 layers of thin walled cells. Stele is polyarch and exarch with 9-10 protoxylem points. Small groups of phloem cells are present alternating the xylem arms. Small parenchymatous pith is present in the centre of the stele. Mucilage canals are frequently present in the pith and cortical region (Fig.6).

Petiole

In transverse section the petiole shows small thin walled epidermal cells which are covered by a thin cuticle. The bulk of petiole is composed of ground tissue. It is differentiated in to three zones, outer 3-4 layers of cells are made up of thin walled parenchyma, middle zone consisting of 3-4 layers of cells which are thick walled and sclerenchymatous, being comparatively smaller in size than the cells of outer and inner zone (Fig.2E). Inner zone of ground tissue consists of large, thin walled polygonal cells filled with starch grains. Starch grains are usually large and spherical or oval in shape. The concentrations of these grains are more towards the base of petiole and gradually decrease toward the apex. At the top of the petiole and in the rachis cells are usually devoid of starch grains. Some of the cells of inner zone contain tannin.

At the base of the petiole there are usually five widely separated vascular stands embedded in the parenchymatous ground tissue (Fig.2C). Each stand has a single layered endodermis. The pericycle is made up of thin walled cells, which are 1-3 layers in thickness. Xylem lies in the center of the vascular strand. It is plate like with several protoxylem points in exarch condition (Fig.2D). Xylem is surrounded by Phloem, Phloem consists of sieve cells and parenchyma and xylem has simple tracheids of various sizes. Metaxylemtracheids have scalariform and

pitted thickening while protoxylemtracheids have annular and spiral thickening.

The vascular strands during their upward course in the petiole gradually fuse with each other and ultimately there is a single strand at the tip. During the fusion, first the endodermis and at slightly higher level the pericycle and ultimately the phloem and xylem bundles of the two strands also fuse together. Vascular strand at this stage become somewhat 'C' shaped (Fig.3A,B; 4A,B & 5A, B).

Conservation status: During the course of our floristic surveys, the authors found that *A. crassipes* is sparsely scattered and confined to few localities of natural habitat with only few individuals. We observed that most of the habits were under anthropogenic pressure in India. We therefore assess the species here as 'Critically Endangered (CR)' based on the IUCN categories and criteria IUCN (2019).

As part of a conservation program, Indian species of *Angiopteris* is presently under *ex-situ* conservation at *Roxborough* Botanical Garden, Department of Botany of Allahabad University and Dhanikhari Experimental Garden-cum-Arboretum (DEGCA), Botanical Survey of India, Andaman and Nicobar Regional Centre, an excellent centre for collection and *ex-situ* as well *in-situ* conservation of wild species in the ANIs (Singh & Murugan 2014; Singh et al. 2014, 2021b). Live plants of *Angiopteris* was collected from natural population of Andaman and Nicobar Islands by one of us (LJS) in 2012 for conservation measures and introduced at DEGCA.

Discussion

Various studies indicated taxonomic significance of anatomical characters (Blomquist 1922; Pant 1965; Foster 1966; Pant & Khare 1969; Holttum 1978; Pant et al. 1984; Yadav 2000). In the present study morpho-anatomical diversity of *A. crassipes* has been described in great detail for the first time from India. Taxonomic significance of anatomical characters has also described in higher vascular plants by various authors (Metchalfe & Chalk 1950; Singh 2002; Singh & Misra 2012, 2015). *A. crassipes* is a robust fern with a globose upright rhizome with considerably thickened roots and spirally arranged

leaves. A conspicuous pulvinus is present at the base of Pinna. It is rather uncommon in occurrence and usually found in moist shady and humid places along the water

streams. The present investigation was made based on specimens collected from Pachmarhi hills and Baster areas.

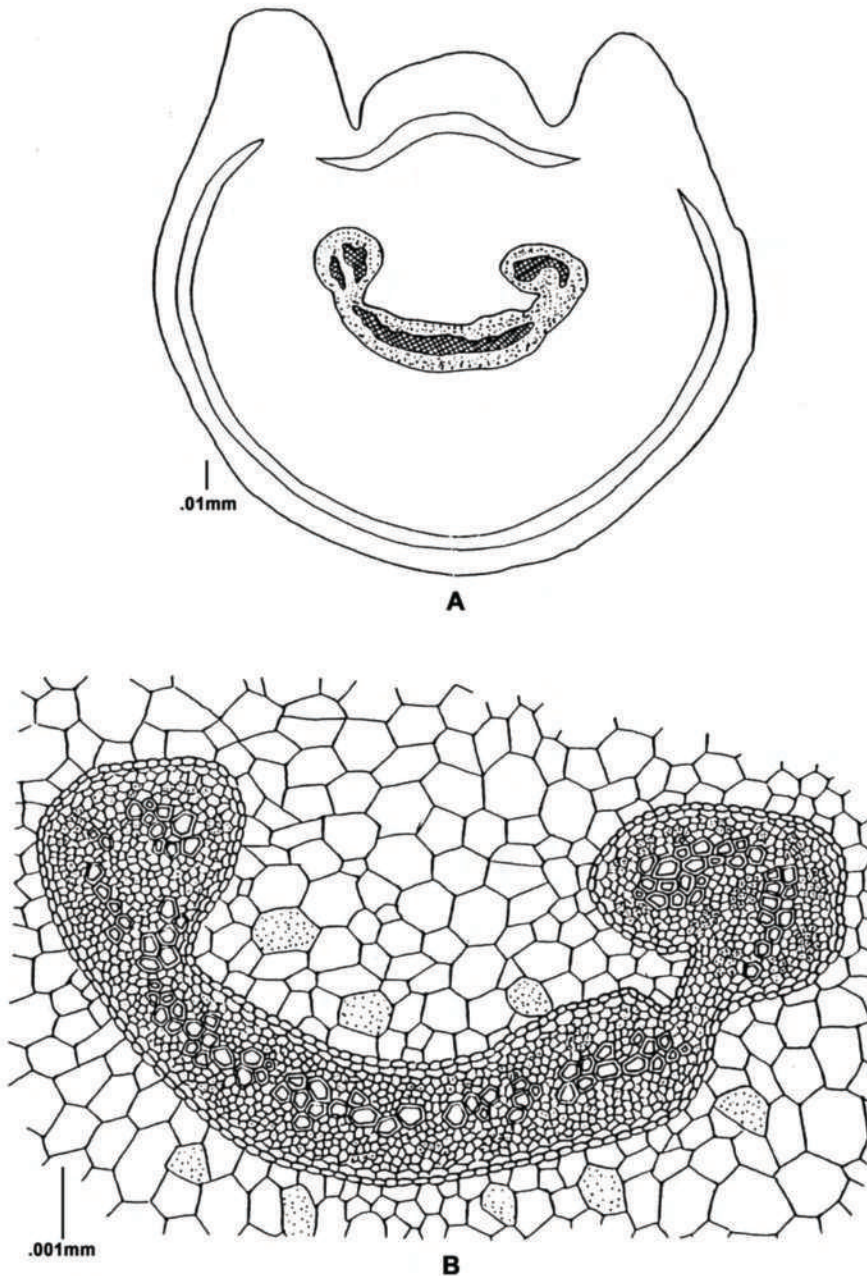


FIGURE-5

Fig. 5. *Angiopteris crassipes*: A: Diagrammatic transaction of petiole at its tip showing fused vascular strands; B: Structural details of A.

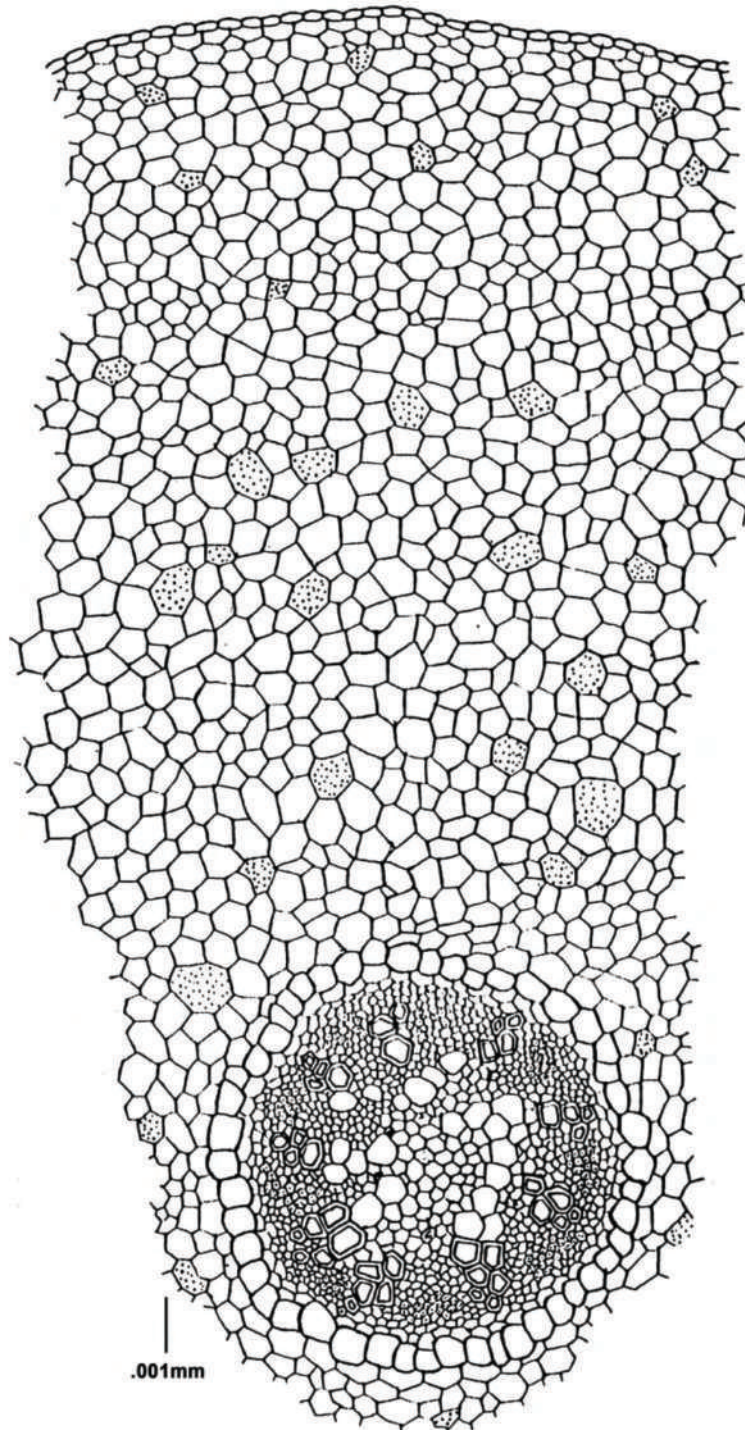


FIGURE-6

Fig.6. Angiopteris crassipes: Transaction of a portion of root showing steler structure.

Pinnae are hypostomatic, epidermal cells of both the surfaces are more or less sinuous walled. Epidermal cells show irregularly scattered groups of short almost straight walled silica containing cells in their lower epidermis. Silica containing cells are mostly in pair but may occur in groups of up to 4 cells. Stomata are usually oriented parallel to the veins and are amphicyclic showing a ring of three or four subsidiary cells. Details of epidermal features and development of stomata have been described by Pant & Khare (1969). According to them stomatal development is of mesoperigenous type, in which stomatal initial undergoes two divisions before producing the guard cells. The developmental pattern of stomata and also the appearance of mature stomata is highly different from other taxa of eusporangiate ferns.

Rhizome is dictyostelic with several vascular strands (meristeles) whose number depends on the age and size of the plants. Each meristele has mesarch xylem surrounded by phloem. The entire plant consists more of parenchyma and less mechanical tissues. Stem has many lyseogenous canals containing mucilage. The tanniferous cells are also of common occurrence. The parenchymatous cells of the cortex are usually filled with large number of starch grains. Roots too have tannin filled cells and several mucilage canals. The xylem of roots has several protoxylem points in exarch condition with groups of phloem cells alternating the xylem cells.

Petiole is also largely made up of parenchymatous tissue filled with starch. Usually five separate vascular strands enter the petiole which gradually fuses together during their upward course and ultimately at the base of rachis there remains a single vascular stand. Each vascular strand consists of several protoxylem points in exarch condition. Xylem is surrounded by phloem consists of sieve cells and parenchyma and xylem has simple tracheids of various size. Metaxylem tracheids have scalariform and pitted thickening while protoxylem tracheids have annular and spiral thickening. *A. crassipes* is a robust plant appearing like small tree fern sometimes reaching up to 2-3 meters in height but the entire plant is largely made up of parenchymatous tissue, which is traversed by large number of mucilage canals.

The morpho-anatomical studies of rhizome, petiole, root and epidermal features of pinnae have generated a refined understanding of genus and species-level relationships within the family in terms of phylogenetic investigations. Marattioids can be diagnosed morphologically on the basis of multiple characters and the new taxonomy is congruent with the current understanding of the phylogeny of marattioid ferns based on both molecular and morphological data. Therefore, in the present study, an attempt has been made to describe the morpho-anatomical features which indicate taxonomic significance. Because of complexity in identity of distributional range of *Angiopteris* taxa the findings of the present study are relevant for the correct identity of existing species in a particular geographical range. The present study also revealed that more systematic explorations will be required in India to elucidate what species of *Angiopteris* are still exist in India.

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