Comparative Anatomy of Stipe and Rhizophore in Four Selected Species of *Selaginella*

DISSERTATION

Submitted to the University of Kerala in partial fulfillment of the requirements for the degree of Bachelor of Science in Botany

By

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DECLARATION

We hereby declare that this project titled ***'Comparative Anatomy of Stipe and Rhizophore in Four Selected Species of Selaginella'*** is a bonafide record of work carried out by us under the supervision and guidance of **Dr. Nisha K. K.**, Assistant Professor, Department of Botany, All Saints' College, Thiruvananthapuram, and that no part of this work has been previously formed the basis for the award of any degree or diploma.

Thiruvananthapuram,
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CERTIFICATE

This is to certify that the project entitled ***'Comparative Anatomy of Stipe and Rhizophore in Four Selected Species of Selaginella'*** is an authentic work done by **Smrithi S. Nair, Adithya V., Hanna George, Amina S., Anju T., Aishwarya Lekshmi S. R., Greeshma R. S., Shibino S. Shibu, Krishna C. S., Dazrin S. Zahir, Aiswarya B. S., Anupama Santhosh and Praseetha T.** for the degree of Bachelor of Science in Botany of the University of Kerala, Thiruvananthapuram, during the course of their study in this college and that this has not previously formed the basis of the award of any degree, diploma or other similar title of recognition.

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Introduction & Review of Literature

Pteridophytes are unique in Plant Kingdom due to their peculiar location in between Bryophytes and Gymnosperms. This crytogamae group resembles bryophytes more due to characteristics like the existence of a sterile jacket around the antheridium and archegonium, the need for moisture or water for fertilisation, the alternating of generations, the development of spores, etc. In contrast, the sporophytic plant body, the differentiation of sporophyte into roots, stems, and leaves, the presence of vascular tissue for conduction, independent nature of the sporophyte, and other characteristics demonstrate its similarity to gymnosperms. Due to their highly developed vascular system, pteridophytes are best described as the most primitive vascular cryptogams. Pteridophyta was a prominent group of the plant kingdom in the Carboniferous era when tree-like forms were very common in forests (Dudani et al, 2009). Hence, it includes fossilised vascular plants. In tropical areas, Pteridophyte living representatives developed to their fullest potential.

Selaginella is a genus under Pteridophyta. Although having no characteristics of moss, *Selaginella* is commonly referred to as spike moss and club moss. According to Smith's classification (1955) *Selaginella* fall within the order Selaginellales of class Ligulopsida in the division Lycophyta. *Selaginella* is variously classified by various authors and Table 1 shows a comparison of significant morphological classifications proposed in the 20th century given by Kato (2005).

Table 1. Comparison of morphological classifications of *Selaginella*

Engler and Prantl (1902)	*Verdoorn (1938)*	*Pichi-Sermolli (1977)*	*Tryon and Tryon (1982)*	*Kramer and Green (1990)*
1. Lycopodiales	1. Lycopodiinae	1. Lycophytina	1. Lycopodiopsida	1. Lycopodiatae
1.1 Ligulatae				
1.1.1 Selaginellineae	1.1 Selaginellales	1.1 Selaginellales	1.1 Selaginellales	1.1 Selaginellales
1.1.2 Isoetineae	1.2 Isoetales	1.2 Isoetales	1.2 Isoetales	1.2 Isoetales
1.2 Eligulatae				
1.2.1 Lycopodineae	1.3 Lycopodiales	1.3 Lycopodiales	1.3 Lycopodiales	1.3 Lycopodiales
1.2.2 Psilotineae	2. Psilophytinae	2. Psilophyina		2. Psilotatae
2. Equisetales	3. Articulatae	3. Sphenophytina	2. Equisetopsida	3. Equisetatae
3. Sphenophyllales				
4. Filicales	4. Filicinae	4. Filicophytina	3. Filicopsida	4. Filicatae

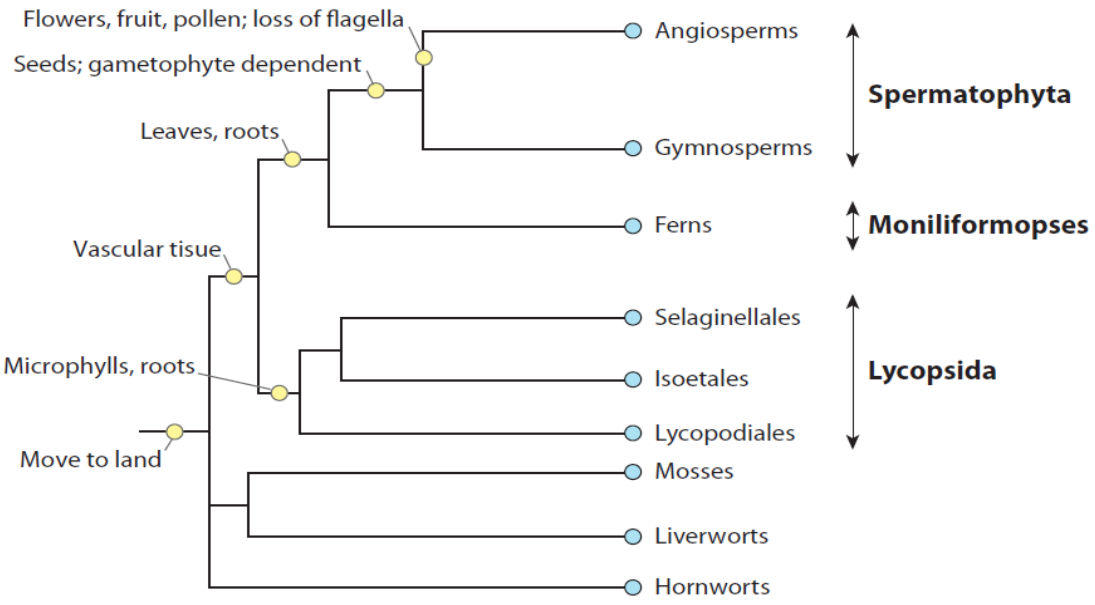
1.1. Evolutionary history

The oldest unequivocal *Selaginella* fossil dates from 333–350 million years ago (Alston 1946). Species of *Selaginella* from the upper Carboniferous are morphologically similar to extant species of *Selaginella*. *Selaginella* is an evolutionarily robust group of plants that was among the few to survive and thrive during the Permian-Triassic extinction, during which >90% of the earth's species became extinct (Thomas, 1992). At the present time, only one genus is recognized in the Selaginellaceae, but this genus is cosmopolitan in distribution. A molecular phylogenetic analysis of the genus has revealed that rates of molecular evolution among species are remarkably high compared with those of angiosperm families (Korall and Kenrick, 2004). *Selaginella* is particularly interesting from an evolutionary comparative perspective because it has retained the independent but water-dependent gametophyte generation that is typical of all non-seed plants.

The phylogenetic relationships of the lycophytes to other groups of extant land plants are illustrated in Figure 1.

1.1. Distribution and habitat

Selaginella P. Beauv. is a cosmopolitan genus with about 700 species all over the World. *Selaginella* are primarily found in tropical and subtropical areas of the world. Some species can be found in temperate areas also. Tropical rain forests, humid and shady habitats, damp shady sides of hills are the other habitat of *Selaginella*. Yet, some species are also xerophytes and epiphytes. Due to their delicate and fluffy fronds, which display different colours of



(Source: Annu. Rev. Plant Biol. 2009. 60:223–38)

Fig 1. The phylogenetic relationships of the lycophytes to other groups of extant land plants

green, blue, or bronze, several species are frequently seen as decorative plants. *Selaginella* species are widely grown as ornamental plants and are actively sold commercially.

In India *Selaginella* is represented by 65 species (Dixit, 1992; Madhusoodhanan and Nampy 1994; Antony et al, 2002; Nisha et al, 2010). Alston (1946) was the first person who enumerated 58 species of and provided a key for *Selaginella* from British India. Out of which, 44 species are confined to the present political boundaries of India. Alston (1952) made a revision of the West Indian species of *Selaginella*. Later Panigrahi and Dixit (1966, 1967, 1968) made extensive survey of Indian *Selaginella*. Afterwards Dixit (1992) published a monograph of "Selaginellaceae of India".

1.2. General characters

Selaginella are small herbaceous vascular plants with erect or creeping shoots, having small and ligulate leaves. Several species have rhizophore-based roots. Rhizophore, when present, is a special leafless branch produced from the stem towards the lower side. The plants are heterosporous and the sporophylls are grouped in the form of strobili. *Selaginella* gametophytes are dioecious, tiny, and severely reduced. Biflagellate antherozoids are present. At some point in the course of its development, the megagametophyte breaks loose from the megasporangium.

1.3. Morphological features

One of the most distinguishing features of *Selaginella* is its pattern of shoot and root branching. The *Selaginella* shoot apical meristem (SAM) regularly bifurcates to produce two new apices, and each bifurcation forms a Y-shaped branch junction. Bifurcation (or dichotomous tip branching) of the *Selaginella* apex is a plesiomorphic character that is shared with the earliest land plants known from the fossil record. As the shoot grows, one of the two newly formed apices is typically indeterminate and outgrows the other, which is either determinate or lags behind in growth. Alternating the pattern of dominant versus lagging apical growth at each new branch junction results in a zigzagged main stem axis. Although *Selaginella* can produce additional meristems at branch junctions, their development is considerably different from that of angiosperm axillary meristems. Lateral branching of the *Selaginella* root also does not occur, because all its roots branch only by bifurcation of the root tip.

In regard to leaf arrangement and shoot symmetry, *Selaginella* species are classified as either anisophyllous or isophyllous. Anisophyllous is with two ranks of smaller dorsal microphylls and two ranks of larger ventral microphlls. In isophyllous species, only one type of microphyll is present. Anisophylly gives the shoot dorsiventral asymmetry, with the smaller microphylls attached to the dorsal shoot axis and larger ventral microphylls attached to the ventral side of the shoot axis.

As in the case of the leaves of angiosperms, each *Selaginella* microphyll has distinct abaxial/ adaxial asymmetry. The abaxial epidermis of the dorsal microphyll and the adaxial epidermis of the ventral microphylls (i.e., the dorsal surfaces that face the light) have only one chloroplast per cell, whereas the opposing epidermal cells have multiple chloroplasts per cell. Stomata are limited to the abaxial surfaces of all microphylls and are located over their veins. Each microphyll typically has only a single unbranched vein that is directly connected to the vascular bundle of the stem. The absence of a leaf gap, rather than the size of the organ, distinguishes a microphyll from the megaphyll of the euphyllophytes (i.e., ferns and seed plants). In *Selaginella*, the ligule is a tiny, colorless, scale-like appendage attached to the adaxial surface at the base of each microphyll and is often fugacious. Another interesting feature of *Selaginella* is the angle meristem. After the apex of the sporophyte shoot bifurcates, a Y-shaped branch junction is formed. New meristems, referred to as angle meristems, arise de novo at these branch junctions and typically initiate in a branch junction below and after a new branch junction has formed at the apex.

The rhizophore is one of the most fascinating organs in *Selaginella.* The structure that first emerges from the angle meristem and ultimately gives rise to the root is the rhizophore, which is a simple leafless cylinder that lacks a root cap and root hairs. As in the case of roots, the rhizophore grows downward. In time, the rhizophore differentiates as a root with a root cap and root hairs.

1.4. Reproduction

Selaginella shows vegetative as well as sexual reproduction. Vegetative reproduction occurs through tubers, buds and fragmentation. Sexual reproduction occurs through spore formation.

Selaginella is heterosporous and produces strobili with microsporangia and megasporangia, which in turn produce the haploid microspores and megaspores, respectively. Prior to the

release of spores, the sporangia, which are always borne on the adaxial surface of each sporophyll of the strobilus, appear as thin clam-shaped structures that open along a seam to release the spores. The valves open and snap back together several times to eject all microspores from the microsporangium. The development of male and female gametophytes (prothalli) takes place from the haploid microspores and megaspores respectively i.e., microspores and megaspores are the unit of male and female gemetophytes, respectively.

1.5. Economic Importance

Selaginella is utilized conventionally for food, medicine, handicrafts and also as ornaments. *Selaginella*, is used as liver tonic, to promote blood circulation, treat cardiovascular diseases and to stimulate menstrual discharge in traditional Chinese medicine (Zhen-Ji and Tan 2005; Swamy et al. 2006). Recent developments in the Indian botanical research are suggestive of the concept that few species of the *Selaginella* (eg. *S. bryopteris*) are "Sanjeevani" (Sah et al. 2005; Sah 2008; Ganeshaiah et al. 2009, Antony and Thomas 2011). The *Selaginella* plants are usually used to cure many ailments including fever, jaundice, hepatic disorders, cirrhosis, diarrhea, cholecystitis, sore throat, cough of lungs. Furthermore, it promotes blood circulation and helps in removal of blood stasis and controlling external bleeding after injury etc (Singh and Singh 2015). They are considered to have the high content of numerous phytochemicals such as, carbohydrates, benzenoids, flavonoids, alkaloids, quinoids, chromones, lignans coumarins, phenylpropanoids, oxygen heterocycle, pigments and steroids. Therefore, crude extract and different bioactive compounds isolated from these plants have been evaluated in-vitro for their antimicrobial, antiviral, anti-diabetic, anti-mutagenic, anti-inflammatory, anti-nociceptive, anti-spasmodic and anticancer activities (Adnan et al 2021).

Selaginella kraussiana ("golden clubmoss"), *S. lepidophylla* ("resurrection plant" or "Jericho rose"), *S. uncinata* (peacock moss or peacock fern), *S. braunii*, *S. apoda* (meadow spike moss) are some common ornamental plants. *S. opaca*, *S. plana* and *S. wildenowii* are used as vegetables. *S. caudata* is used as a wrapping of fruits and vegetables from the garden.

1.6. Anatomical Peculiarities and its Significance

The sporophytic plant body of *Selaginella* consists of stem, leaves, and roots. The stem (stipe) is thin and branching, and it can be either upright or creeping. The stem is covered in small, scale-like leaves that overlap each other and are arranged in a spiral pattern. The leaves have a single, central vein, and their margins may be ciliate or entire. The roots of *Selaginella* are thin

and fibrous, and they grow from the stem nodes. They are primarily responsible for absorbing water and nutrients from the soil.

Internal structure of the stem (stipe) is distinguished into epidermis, cortex and stele. The epidermal cells are devoid of hairs and stomata. A thick layer of cuticle surrounds the epidermis on all sides. A distinct cortical zone is located inner to the epidermis. The cortex may or may not be distinguished as inner and outer cortex. In the parenchymatous cortex, the cells are often angular, meaning they lack intercellular spaces, but occasionally, the cells are rounded and given a few interstitial spaces. A well-developed stele occupies the central portion of the stem. The stele is of the protostelic type with xylem in the centre and is surrounded by phloem on all sides. A single-layered pericycle encircles the phloem. There is no pith. The stele remains suspended in the centre by radially elongated tubular, unicellular structures known as trabeculae. These are formed by the radial elongation of the endodermal cells. Trabeculae are provided with prominent casparian strips. There are enormous areas between the trabeculae that are called air spaces.

Different *Selaginella* species have varying numbers of stele. The way the stele is organised varies depending on the species. It can be siphonostele or protostele. A single-layered pericycle made up of parenchymatous cells surrounds the stele. Typically, the xylem is monarch, diarch, or multiarch. It is usually exarch but sometimes it may be mesarch. Tracheids often make up the xylem. There are no vessels at all. Phloem, which consists of sieve cells and phloem parenchyma, surrounds xylem on all sides. In phloem, companion cells are not present.

One unique feature of the stipe of *Selaginella* plants is the presence of small, scale-like leaves called microphylls that are arranged in a spiral pattern around the stem. The microphylls are attached to the stem by a small stalk called a ligule and have a single vein that runs through the center. The presence of microphylls is a defining characteristic of the Selaginellaceae family and distinguishes them from other lycophytes, which typically have larger leaves with multiple veins.

Another typical feature of *Selaginella* is the presence of rhizophore. Rhizophores are specialised root like structures arising from the base of the stem. They are characterized by a highly branched structure, with numerous lateral roots arising from a central axis. The lateral roots may be long and slender, or short and stubby, depending on the species. The anatomy of rhizophores is similar to that of regular roots, with a cortex, endodermis, and vascular tissue. However, the vascular tissue of rhizophores is often more highly branched and may be surrounded by a specialized sheath of cells.

Internal structure of root also has an epidermis followed by cortex and central stele. Epidermis is only one cell thick and the outermost covering layer. The cells are large and the unicellular root hairs arise from them. A wide zone of cortex is located just beneath the epidermis. The cortex may consist entirely of thin-walled parenchymatous cells or include a 3–5 mm thick sclerenchymatous outer cortex (hypodermis). The inner cortex is parenchymatous and thickly celled. Air spaces have also been reported in the inner cortex. It is traversed by trabeculae. Endodermis is followed by one to three layered pericycle. It is made up of parenchymatous cells. Root has a typical protostele. The xylem is exarch and monarch i.e., there is only one protoxylem group situated at the periphery. Xylem is surrounded by phloem on all sides. The xylem and phloem components have a stem-like structure.

Selaginella plants are heterosporous, which means that they produce two types of spores: microspores and megaspores. Microspores develop into male gametophytes, while megaspores develop into female gametophytes. Spores are formed in special cone like structures called strobili.

The strobili of *Selaginella* are specialized structures that are composed of modified leaves called sporophylls. The sporophylls are arranged in a spiral pattern around a central axis and are responsible for producing and releasing spores. The sporophylls of *Selaginella* strobili have a flattened shape and are attached to the central axis by a narrow base. The upper surface of the sporophylls bears sporangia, which are specialized structures that produce and release spores. The sporangia are typically clustered together in groups called sori, which are often covered by a protective flap of tissue called an indusium. The sori are arranged in a spiral

pattern around the central axis of the strobilus, with each sporophyll bearing a single sorus. The sori of *Selaginella* strobili can be either dorsal, meaning they are located on the upper surface of the sporophyll, or ventral, meaning they are located on the lower surface of the sporophyll.

The sporangia themselves have a unique structure that is characteristic of *Selaginella* plants. Each sporangium consists of a capsule that is filled with spores. The capsule is typically composed of several layers of cells that protect the spores and facilitate their release. In addition to the sporophylls, the strobili of *Selaginella* also contain specialized structures called ligules. Ligules are small, tongue-like structures that are located at the base of the sporophylls and are thought to play a role in protecting the sporangia from drying out.

1.7. Objectives of the study

Selaginella is a difficult genus to be classified. A large number of *Selaginella* species are morphologically polymorphic and have high morphological similarity among them. This confusion led to almost every species having more than one name, such as *S. ornata* and *S. involvens*, which have high morphological variation and with more than 25 synonyms each (Setyawan et al, 2016). Problems in species identification in *Selaginella* due to the similarities in morphological characteristics could be solved using anatomical characteristics.

The present work aims to study the anatomical variations in four selected species of *Selaginella* P. Beauv. with special emphasis on the stipe and rhizophore. Major morphological features were also studied.

Materials and Methods

2.1 Plant material

Four *Selaginella* species grown and maintained in the green house at All Saints' College, Thiruvananthapuram was selected for the present study. Plant specimens were observed and morphological details were recorded from the well preserved germplasm. Photographs of the habit and habitat were taken using Canon 1500 camera and the photographic image of the plant parts were taken using Labomed stereo zoom microscope with camera. Taxonomy and morphological descriptions were made in accordance with relevant literature survey (Dixit 1985; Antony et al 2002; Rekha & Krishnan 2017; POWO 2023; IPNI 2023.) Herbarium specimens were prepared and the plant material was identified with the help of Dr. Raju Antony, JNTBGRI, Palode.

2.2 Anatomical studies of the stipe

For anatomical studies, freshly collected specimens of the four species were used. Fine hand sections of the stipe were taken using standard procedures and was stained with 1% Aqueous Safranin O Solution and temporarily mounted in glycerine. The sections were observed under the low power and higher power using Leica DM500 Binocular research microscope and photographs taken with Leica LCC 50 HD camera.

2.3 Anatomical studies of the rhizophore

Rhizophore is present only in two of the species studied. For studying its anatomy, freshly collected specimens were used. Fine hand sections were taken using standard procedures and was stained with 1% Aqueous Safranin O Solution and temporarily mounted in glycerine. The sections were observed under the low power and higher power using Leica DM500 Binocular research microscope and photographs taken with Leica LCC 50 HD camera.

Results and Discussion

3.1 Plants selected for the study

In this study, four species of *Selaginella* were selected and the major morphological features were studied to analyse the variations among these species. Habit, nature and length of the stem, rhizophore and leaves were recorded and is tabulated in Table 2. The plants were identified as *Selaginella plana, S. braunii, S. ciliaris* and *S. vogelii.*

Table 2. Morphological features of selected *Selaginella* species

Characters	***S. plana***	***S. braunii***	***S. ciliaris***	***S. vogelii***
Habit	Herb	Herb	Creeping herb	Herb often large plants
Nature of stem	Erect	Erect	Erect	Erect
Stem length	20- 32cm	12.1- 20.5cm	1.4- 3.5cm	21- 69cm
Branching	Branched	Branched	Branched	Branched
Rhizophore	Ventral	Absent	Ventral	Absent
Rhizophore length	2.5 - 9 cm	-	0.2- 0.7 cm	-
Leaves	Heteromorphic	Heteromorphic	Heteromorphic	Heteromorphic
Strobili length	1 – 3.9 mm	0.03- 0.7 mm	0.2- 1.3 cm	0.3 - 1 cm
Sporophylls	Monomorphic	Monomorphic	Dimorphic	Monomorphic

i. ***Selaginella plana* (Desv.) Hieron** (Fig. 2)

Habit herb. Stems erect rarely prostrate to ascending, greenish to stramineous, 20- 32 cm long, 0.3–0.6 mm diam., exarticulate, not flagelliform or stoloniferous, 2- or 3-branched. Rhizophores ventral, borne on the proximal ⅔ of stems, filiform, 2.5 - 9 cm long and 0.1–0.2mm diam. Leaves heteromorphic throughout, chartaceous, both surfaces glabrous, upper surfaces green, lower surfaces silvery green to pale green. Lateral leaves distant, spreading to

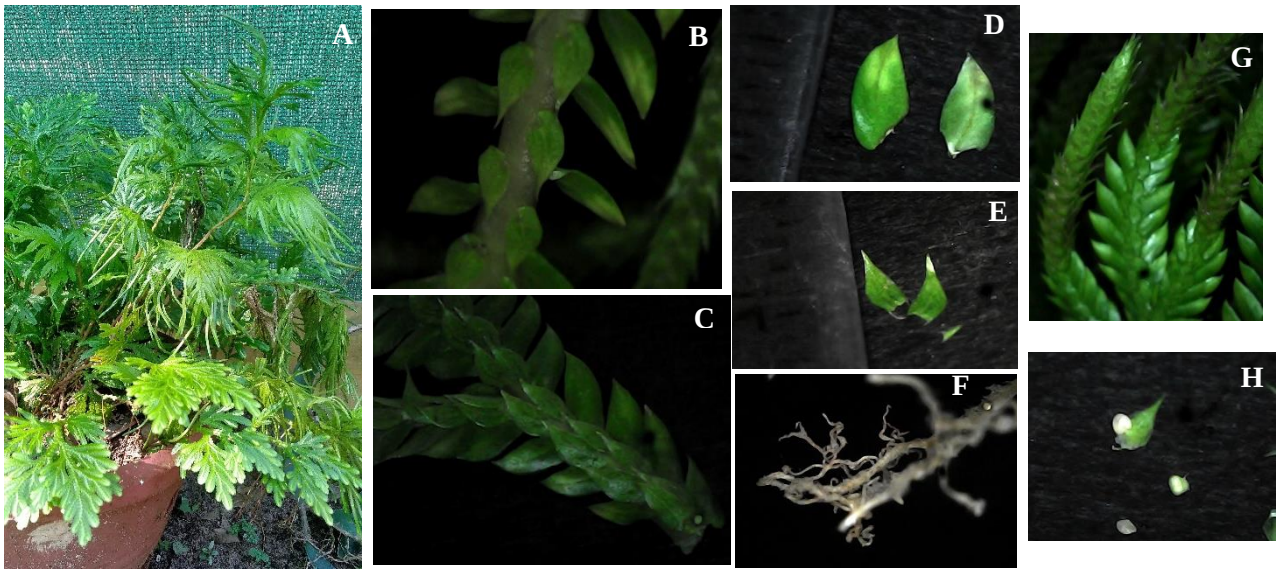


Fig. 2 *Selaginella plana* (Desv.) Hieron. **A-** Habit; **B- E-** Leaves; **F-** Rhizophore; **G-** Stobilus; **H-** Sporophyll with sporangium

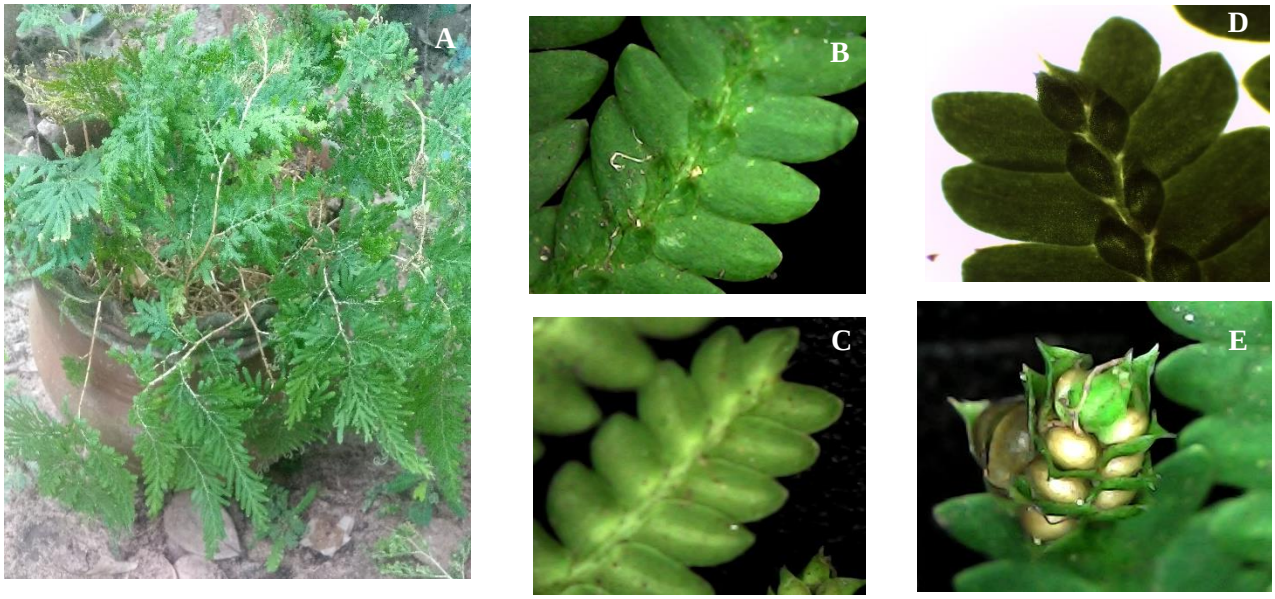


Fig 3. *Selaginella braunii* Baker. **A-** Habit; **B- D-** Leaves; **E-** Stobilus showing sporophyll with sporangium

slightly ascending, oblong, 1.1- 2.0 × 0.4- 1.0 mm; bases rounded, acroscopic bases overlapping stems, basiscopic bases free from stems; acroscopic margins on upper surfaces hyaline along proximal ½–¾ wide, basiscopic margins greenish on upper surfaces with rounded to quadrangular, entire along proximal ¾ and serrulate on distal ¼; apices acute to slightly cuspidate, each cusp 0.02–0.03 mm, tipped by 1–3 teeth; upper surface smooth, lower surface with scattered pappilae. Median leaves distant to slightly imbricate near the branch tips, ascending, elliptic to elliptic-lanceolate or ovate-elliptic, 0.7–1.4 × 0.4–0.7 mm; bases oblique; margins serrate to short-ciliate distally; apices acuminate to short-aristate, each acumen (arista) 0.15–0.2 mm, entire or obscurely tipped by 1–3 teeth; Axillary leaves similar to lateral leaves but with both margin ciliate along proximal ¼, otherwise short-ciliate to serrate distally. Strobili terminal on branch tips, compact, quadrangular, 1- 3.9 mm. Sporophylls monomorphic, without a laminar flap, ovate to ovate-lanceolate, 0.7–1.1 × 0.4–0.6 mm, each with a dentate (teeth often caducous) keel along distal ½ of the midribs; bases rounded; margins narrowly hyaline, serrate; apices acute, entire or obscurely tipped by 1–3 teeth; dorsal sporophylls with upper surfaces green and cells as in median leaves, except for the half that overlaps the ventral sporophylls, there hyaline with elongate, sinuate-walled cells, lower surfaces silvery green and comprising elongate, sinuate-walled cells; ventral sporophylls with both surfaces hyaline to faintly greenish hyaline, comprising elongate, sinuate-walled cells. Megasporangia in proximal portion in 2 ventral rows.

ii. ***Selaginella braunii*** **Baker** (Fig. 3)

Habit terrestrial herbs with erect stem, 12.1- 20.5cm cm long, 1-2 mm thick, stout, cylindrical, pale brownish. Branches many from middle to upper part. Rhizophores absent. Leaves heteromorphic throughout, chartaceous, those on and above first branch of stems with both surfaces usually glabrous and those below the first branch of stems often with few, caducous cilia-like or dentate projections on the upper surfaces of the median leaves and sporophylls and on the lower surfaces of lateral leaves, upper surfaces green or brownish (when old), lower surfaces silvery green or shiny brown (when old). Lateral leaves imbricate, spreading or ascending, broadly ovate to ovate-oblong, 0.9–1.0 × 0.4–0.6 mm; bases rounded to subcordate, acroscopic bases overlapping stems (more so on leaves below first branch), basiscopic bases free from stems; acroscopic margins broadly hyaline. Median leaves imbricate, ascending, broadly-ovate to ovate-elliptic, 0.4–1.0 × 0.2–0.4 mm; bases oblique, inner bases truncate,

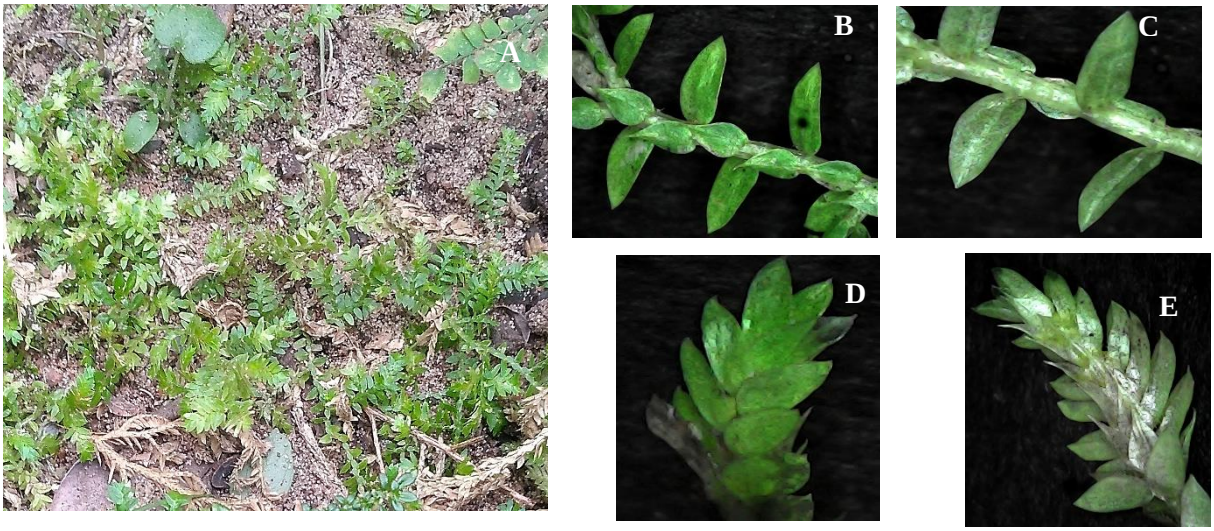


Fig 4. *Selaginella ciliaris* (Retzius) Spring. **A-** Habit; **B- C-** Leaves; **D-E-** Stobilus showing sporophyll

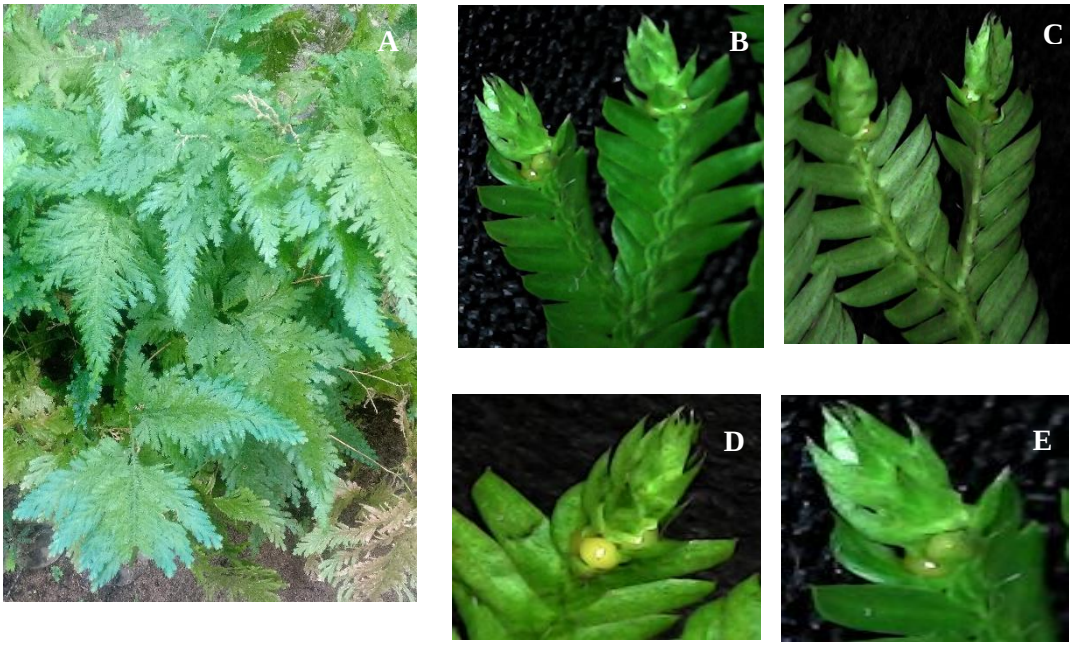


Fig 5. *Selaginella vogelii* Spring. **A-** Habit; **B- C-** Leaves; **E-D -** Stobilus showing sporophyll with sporangium

outer bases rounded and glabrous or these may also be ventricose (i.e., swollen) and each with a tuft of long cilia on leaves below first branch; margins broadly hyaline. Axillary leaves similar to lateral leaves. Strobili 0.03- 0.7 mm, terminal, sessile; sporophylls monomorphic, membranous, spiral, 1.5-1.7 x 0.5-0.7 mm, ovate-lanceolate, acuminate, minutely dentculate. Microspores 40 um in diameter, orange-red, trilete, tetrahedral, reticulate. Macrospores 450 µm in diameter, pale yellow, globose, tetrahedral, reticulate.

iii. ***Selaginella ciliaris*** **(Retzius) Spring** (Fig. 4)

Plants terrestrial, evergreen or seasonally green, shortly creeping, fertile erect stem 2-5 cm, with creeping or prostrate stems. Rhizophores restricted to lower part of erect fertile branches or to middle of main stem, borne on ventral side in axils of branches. Main stems branched throughout, stramineous, 0.3-0.4 mm in diam. in lower part, terete, not sulcate or sulcate; primary leafy branches 3 or 4 pairs, simple or forked or once pinnately branched, branchlets sparse, adjacent primary branches on main stem ca. 1 cm apart; leafy portion of main stem including leaves 3-4 mm wide at middle. Axillary leaves on branches symmetrical or slightly asymmetrical, ovate, 1.2-2 × 0.6-1 mm, base exauriculate, margin ciliolate in basal half, upward denticulate. Dorsal leaves ± symmetrical, those on main stems not obviously larger than those on branches; dorsal leaves on branches contiguous, ovate, 1.2-1.6 × 0.6-1 mm, slightly carinate, base subcordate or obtuse, margin minutely denticulate, apex acuminate or aristate. Ventral leaves asymmetrical; ventral leaves on branches spreading, ovate or ovate-lanceolate, 1.6-2 × 1.6-2 mm, apex acute; basiscopic margin subentire or minutely denticulate to apex; acroscopic base enlarged, broader, overlapping stem and branches, margin ciliolate. Strobili solitary, terminal, compact, dorsiventrally complanate, 4.5-13 × 2-4.5 mm; sporophylls strongly dimorphic, resupinate, white-margined; dorsal sporophylls minutely denticulate, with sporophyll-pteryx incomplete (ending midway to apex) and ciliolate; ventral sporophylls ovate-triangular, margin ciliolate, all known sporophylls megasporophylls; megaspores greenish-yellowish orange.

iv. ***Selaginella vogelii*** **Spring** (Fig. 5)

Herb often large plants reaching upto a height to 69 cm. Rhizome with profuse dichotomous branches, ca. 3 mm in diameter. Stem brownish to stramineous in colour, clothed with fimbriate

leaves densely on the apex and distantly on the older parts. Leafy branch shining pinkish-red throughout except upper branches where it is light pinkish to stramineous in colour, lower 2/3 unbranched; trichotomously divided at its apex; basal region cylindrical, hard, ca. 3.5 mm in diameter, glabrous, devoid of rhizophores, clothed with distantly placed isomorphic leaves. Isomorphic leaves broadly ovate, apex acute to acuminate, peltate, margin distinctly fimbriate. Lateral branches pinnate, broadly deltoid, clothed with contiguous heteromorphic leaves in four rows, stems of lateral branches covered by small hairs throughout on the lower surface. Heteromorphic leaves sessile, entire, shining green, iridescent to metallic blue, herbaceous: lateral leaf oblong, sub-acute; apex acute, median terminal, margin leaf isomorphic, ascending. elliptic ca. entire. Megaspore 1.5 mm, ovate with single. tetragonous.

3.2 Anatomy of stem (stipe)

i. ***Selaginella plana*** (Fig. 6A)

In *S. plana* the CS of the stem is elongated in outline. Epidermis is followed by hypodermis which is sclerenchymatous and 5-6 layered. It is followed by many layered thick walled parenchyma cells. A centrally located stele is connected to the cortex with the help of many long, radially elongated cells called trabeculae. The stele is separated from the cortex by air space. Stelar area is elliptical in outline and is tristelic in condition with three meristeles. Each meristele is protostelic with a solid central core of the xylem that is surrounded by phloem. Xylem is exarch in condition. Xylem is linear with metaxylem centrally localised and two protoxylem groups at the periphery.

ii. ***Selaginella braunii*** (Fig. 6B)

The CS of the stem is tetragonal in outline. Epidermis has many unicellular trichomes present. It is followed by a thick hypodermis made up of very thick sclerenchymatous cells and is several layered. It is followed by many layered thick walled parenchyma cells. Central stele is separated from the cortex by air space and is connected to the cortex with the help of radially elongated cells called trabeculae. Stele is monostelic and protostelic. The stelar area is elongated in outline. Xylem is exarch in condition. Metaxylem is elongated with 5 protoxylem groups.

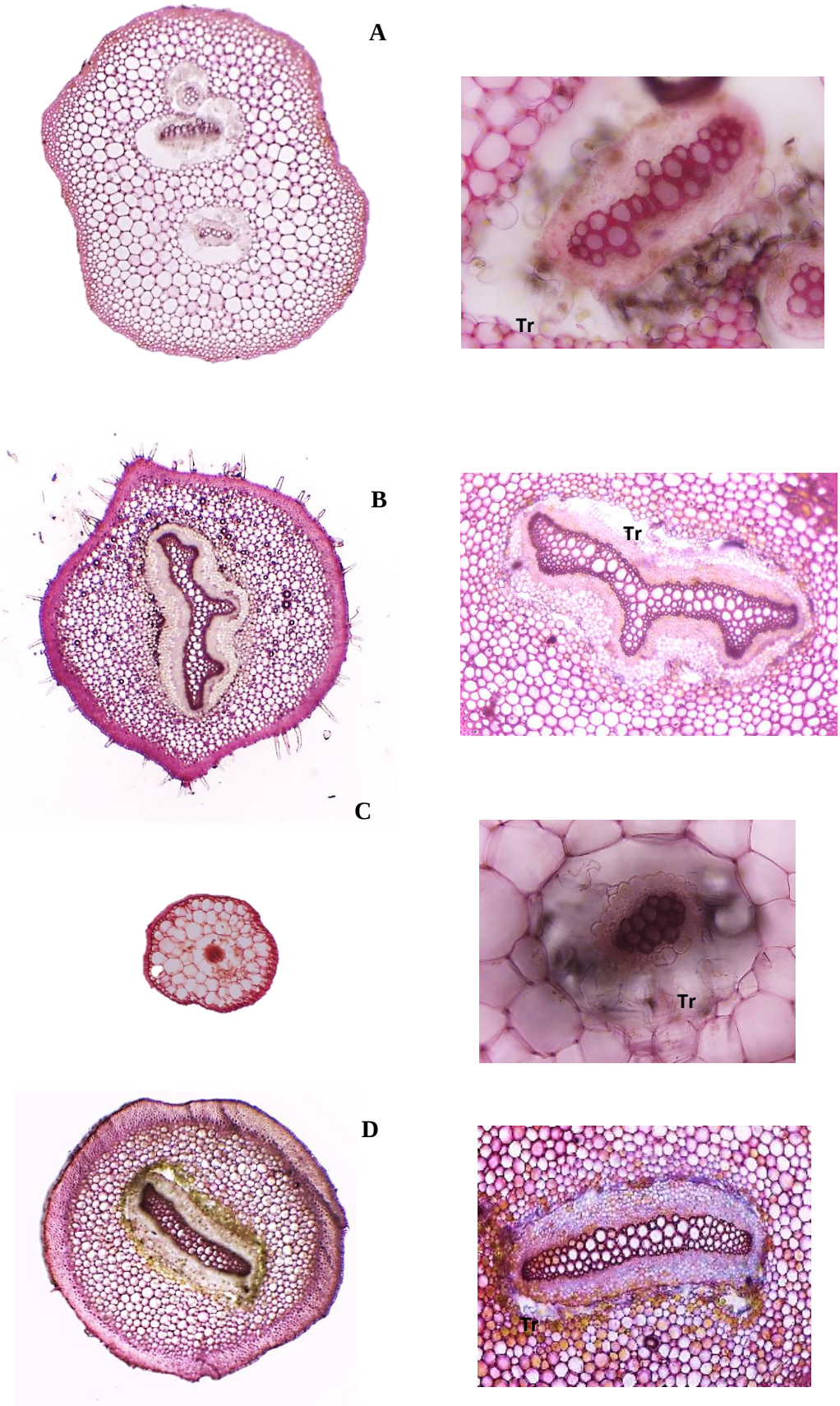


Fig 6. Stem anatomy of the selected *Selaginella* species- **A-** *S. plana*; **B-** *S. braunii*; **C**- *S. ciliaris*; **D**- *S. vogelii;* **Tr**- Trabeculae

Selaginella ciliaris (Fig. 6C)

Selaginella ciliaris stem is very small and the CS of the stem is circular in outline. Epidermis is followed by parenchymatous cortex. Sclerenchyma is completely absent. A large air space separates the stele from the cortex. The stele is connected to the cortex with the help of many radially elongated trabeculae. Stele is monostelic with circular outline. Stele is protostelic and xylem is exarch with a central mass of metaxylem and two small protoxylem groups at the periphery.

iii. ***Selaginella vogelii*** (Fig. 6D)

The CS of the stem is circular in outline. Epidermis is followed by many layered hypodermis made up of sclerenchyma. Rest of the cortex is parenchymatous. A small air space separates the stele from the cortex. The stele is connected to the cortex with the help of radially elongated trabeculae. Stele is monostelic and elongated in outline. Stele is protostelic with a central mass of metaxylem and two protoxylem groups at the periphery.

The C.S of the stem in all species of *Selaginella* showed epidermis, cortex, and central stele (Fig. 5). The outermost layer is the epidermis, which is a single layer of cells that covers the surface of the stem. Epidermis lacks stomata. Beneath the epidermis is a large cortex, which are responsible for storing starch and other nutrients. It surrounds the stele and is layered. The central core of the stem is stele made up of vascular tissue, which includes xylem and phloem. The xylem is responsible for transporting water and minerals from the roots to the rest of the plant, while the phloem is responsible for transporting sugars and other nutrients from the leaves to other parts of the plant.

The stele is separated from cortex by air space and is supported by trabeculae. The trabeculae are small, radial extensions of the sclerenchyma cells that protrude into the central cylinder of the stem. They are also known as medullary rays or medullary spokes. The trabeculae in *Selaginella* serve several functions, including providing structural support for the stem, facilitating the transport of water and nutrients between the central cylinder and the outer

cortex, and storing carbohydrates. The presence of trabeculae in the stem is a characteristic feature of *Selaginella* and helps distinguish it from other related plant species.

3.3 Anatomy of rhizophore

Rhizophores are specialized structures found in some species of *Selaginella*. These structures are modified stems that emerge from the nodes of the plant and grow downward into the soil, where they develop into new plantlets. The rhizophores are typically unbranched and cylindrical, and they have a distinct tip that functions in penetrating the soil. The tip of the rhizophore is covered with a layer of elongated cells that secrete mucilage, which helps the structure anchor into the soil and absorb water and nutrients. As the rhizophore grows downward into the soil, it develops adventitious roots along its length, which help anchor the plantlet and absorb nutrients.

Rhizophores were found only in two species studied, *Selaginella plana* (Fig. 7A) and *S. ciliaris* (Fig. 7B). Anatomically the rhizophore resembles the root. The CS of rhizophore showed epidermis, cortex and stele. Epidermis is single layered without any stomata and trichomes. The cortex is massive and is parenchymatous. Sclerenchymatous hypodermis (2-3 layers) is present in *S. plana* but is absent in *S. ciliaris*. The cortex also contains cells that secrete mucilage, which helps the rhizophore anchor in the soil and absorb water and nutrients. Unlike stem, air spaces and trabeculae are absent. The stele is monostelic and protostelic in nature with central mass of xylem surrounded by phloem. Xylem is exarch with metaxylem in the centre and a few protoxylem elements to one side. In *S. plana* a mass of metaxylem is present with a few protoxylem elements towards one side. *S. cilaris* has three large metaxylem elements with two small protoxylem towards one side.

3.4 Discussion

The largest genus of seed-free vascular plants *Selaginella* alone constitutes the family Selaginellaceae, the largest of the lycophyte families. The genus is estimated to contain ca. 800 species distributed on all continents except Antarctica, with the highest species diversity in tropical and subtropical regions (Weststrand and Korall 2016). Their differentiation requires

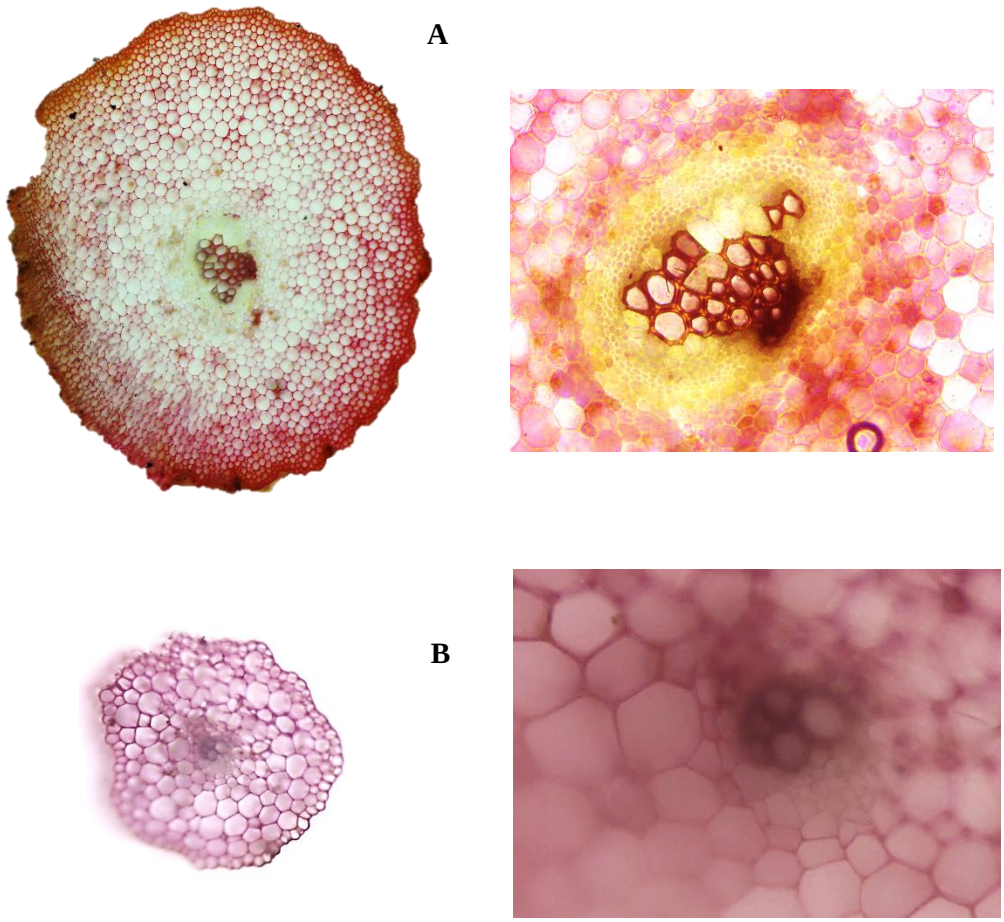


Fig 7. Rhizophore anatomy of the selected *Selaginella* species- **A-** *S. plana*; **B-** *S. ciliaris*

study of their minute morphological characteristics using microscopes. This makes their identification difficult. The identification of *Selaginella* depends much on the minute leaves which make it hard to differentiate because of the rather similar leaf morphology. Hence, the anatomical data could be useful to identify and to distinguish species in the genus.

The epidermis, cortex, and central stele are seen in the stem anatomy of all four species of *Selaginella*. The outline of the stem differed. In *S. plana, S. ciliaris*, and *S. vogelii*, the stem is circular; in *S. braunii*, it is tetragonal. Stomata are absent in the single-layered epidermis. In *S. plana*, *S. braunii*, and *S. vogelii*, the epidermis is followed by a well-defined multi-layered sclerenchymatous hypodermis followed by a massive parenchymatous cortex. In *S. ciliaris* sclerenchymatous hypodermis is not seen and parenchymatous cortex follows the epidermis.

The cortex encircles the stele which is large and multi-layered. The stele is separated from cortex by air space and is supported by trabeculae. The stele's outline is elongated in *Selaginella braunii* and *Selaginella vogelii*, whereas it is circular in *Selaginella ciliaris*. An elliptical outline is also observed in *S. plana*.

Stele was typical protostele in all the four species studied. Tristelic condition with three meristeles was found in *S. plana*, while in all other three species the stele was monostelic. Xylem is exarch in condition. Xylem is linear with metaxylem centrally localised and two protoxylem groups at the periphery in *S. plana.* Metaxylem is elongated with 5 protoxylem groups in *S. braunii.* While a central mass of metaxylem and two small protoxylem groups at the periphery was found in *S. ciliaris* and *S. vogelii.*

The vasculature of *Selaginella* has been generalized to be a protostele with meristeles, with the number and arrangement of the meristeles species- and organ- dependent. The overall stelar structure in *Selaginella* can be classified into several categories, although stelar type and vascular tissue arrangement can change during stem development. The simplest vascular system contains a single, usually dorsiventrally flattened stele (protostele), e.g., in *S. martensii*, *S. rupestris*, and *S. apoda*. In some species, shoot vasculature is composed of two meristeles (i.e., two protosteles, each enclosed within its own endodermis) running parallel to each other

in the stem (*S. kraussiana*) or of three or more meristeles as in *S. willdenovii*, *S. lyallii*, and *S. uliginosa*. The stele in the shoot can also form a complex network, resembling an actino-plectostele as in the giant *S. exaltata* (Mickel and Hellwig, 1969). Based on the stipe anatomical characteristics, Nurfarahain et al (2018) clustered *Selaginella* species into two groups based on the number of stele in the cross section namely; monostelic and tristelic groups. Regardless of the number of meristeles, they are located in a central air chamber (lacuna) and supported by short trabeculae.

Only the two species, *S. plana* and *S. ciliaris*, had rhizophores while it was absent in the other two species, *S. braunii* and S *vogelii*. With respect to rhizophore anatomy, the single-layered, epidermis is followed by a massive parenchymatous cortex that secretes mucilage. The stele is encircled by the cortex, which lacks air spaces and trabeculae in contrast to the stem. Sclerenchymatous hypodermis (2-3 layers) was present in *S. plana* but is absent in *S. ciliaris*. The stele is monostelic protostele. *S. ciliaris* had three large metaxylem elements and two small protoxylem elements on one side, whereas *S. plana* had a mass of metaxylem and a few protoxylem elements to one side. In contrast to shoots, the vascular system in rhizophores and roots is much simpler, usually composed of only one central protostele, and devoid of an air chamber.

A comparative account of anatomical characteristics of the species studied is presented in table 3. Problems in species identification in *Selaginella* due to the similarities in morphological characteristics could be solved using anatomical characteristics. By combining the morphological and anatomical features of each species in this family, an identification key to species will make the identification process becomes easier with the more available data provided to distinguish each species in this family.

Table 3. Comparative anatomical features in selected *Selaginella* species

Characters	***S. plana***	***S. braunii***	***S. ciliaris***	***S. vogelii***
Outline of the stem CS	Elongated	Tetragonal	Circular	Circular
Trichomes in stipe	Absent	Present	Absent	Absent
Hypodermis in stipe	Sclerenchyma	Sclerenchyma	Absent	Sclerenchyma
Air space and trabeculae in stipe	Present	Present	Present	Present
Nature of Stele in stipe	Tristelic & protostelic	Monostelic & protostelic	Monostelic & protostelic	Monostelic & protostelic
Number of protoxylem groups in stipe	Two	Five	Two	Two
Rhizophores	Present	Absent	Present	Absent
Outline of the rhizophore CS	Circular	-	Circular	-
Hypodermis in rhizophore	Sclerenchyma	-	Absent	-
Air space & trabeculae in rhizophore	Absent	-	Absent	-
Nature of Stele in rhizophore	Monostelic & protostelic	-	Monostelic & protostelic	-
Number of protoxylem groups in rhizophore	One	-	One	-

Summary and Conclusions

Selaginella P. Beauv. is a cosmopolitan genus with about 700 species all over the world. The genus *Selaginella* was first described by Palisot de Beauvois in 1804. Since then, a number of classifiations have been proposed that either recognized a single genus *Selaginella* P. Beauv. or divided the species into two to several genera or even included all species as subgenera under *Lycopodium* L. (Zhou and Zhang, 2015). Morphological features used to distinguish groups in classifications were, e.g., isophylly vs. anisophylly, phyllotaxy, habit, stelar arrangement, and spore ornamentation. Phylogenetic studies of the group have shown that these characters often are homoplastic, involving reversals and/or parallelisms, and that none of these morphology-based classifications properly reflect the phylogeny of the group (Weststrand and Korall, 2016). Problems in species identification in *Selaginella* due to the similarities in morphological characteristics could be solved using anatomical characteristics. Hence the present work aims to study the anatomical variations in four selected species of *Selaginella* P. Beauv. with special emphasis on the stipe and rhizophore.

Four *Selaginella* species grown and maintained in the green house at All Saints' College, Thiruvananthapuram was selected for the present study. The species selected for the study were *Selaginella plana, S. braunii, S. ciliaris* and *S. vogelii.* Plant specimens were observed and morphological details were recorded from the well preserved germplasm. Taxonomy and morphological descriptions were made in accordance with relevant literature survey (Dixit 1985; Antony et al 2002; Rekha & Krishnan 2017; POWO 2023; IPNI 2023.) Herbarium specimens were prepared and the plant material was identified with the help of Dr. Raju Antony, JNTBGRI, Palode. For anatomical studies, freshly collected specimens of the four species were used. Fine hand sections of the stipe and rhizophore were taken using standard procedures and was stained with 1% Aqueous Safranin O Solution and temporarily mounted in glycerine. The sections were observed under the low power and higher power using Leica DM500 Binocular research microscope and photographs taken with Leica LCC 50 HD camera.

The sporophytic plant body of *Selaginella* consists of stem, leaves, and roots. The stem (stipe) is thin and branching, and it can be either upright or creeping. The stem is covered in small, scale-like leaves that overlap each other and are arranged in a spiral pattern. The leaves have a single, central vein, and their margins may be ciliate or entire. The roots of Selaginella are thin

and fibrous, and they grow from the stem nodes. They are primarily responsible for absorbing water and nutrients from the soil. Another typical feature of *Selaginella* is the presence of rhizophore. Rhizophores are specialised root like structures arising from the base of the stem.

Internal structure of the stem (stipe) and rhizophore were studied in the present work. The C.S of the stem in all four species studied showed epidermis, cortex, and central stele (Fig. 5). The outline of the stem was circular in *S. plana*, *S. ciliaris*, and *S. vogelii*, while it was tetragonal in *S. braunii*. *S. braunii* also showed the presence of trichomes which was absent in other species. A well-defined multi-layered sclerenchymatous hypodermis followed by a massive parenchymatous cortex was found in *S. plana*, *S. braunii,* and *S. vogelii*. In *S. ciliaris* sclerenchymatous hypodermis is not seen and the cortex is entirely parenchymatous. The stele's outline was elongated in *Selaginella braunii* and *Selaginella vogelii*, whereas it was circular in *Selaginella ciliaris* and elliptical in *S. plana*. Stele was typical protostele in all the four species studied. Tristelic condition with three meristeles was found in *S. plana,* while in all other three species the stele was monostelic. Xylem was exarch with a central mass of metaxylem and two small protoxylem groups at the periphery in *S. plana, S. ciliaris* and *S. vogelii,* while elongated metaxylem with 5 protoxylem groups was found in *S. braunii.*

Only the two species, *S. plana* and *S. ciliaris*, had rhizophores while it was absent in the other two species, *S. braunii* and S *vogelii*. With respect to rhizophore anatomy, the single-layered, epidermis is followed by a massive parenchymatous cortex that secretes mucilage. The stele is encircled by the cortex, which lacks air spaces and trabeculae in contrast to the stem. Sclerenchymatous hypodermis (2-3 layers) was present in *S. plana* but is absent in *S. ciliaris*. The stele is monostelic protostele. *S. ciliaris* had three large metaxylem elements and two small protoxylem elements on one side, whereas *S. plana* had a mass of metaxylem and a few protoxylem elements to one side. In contrast to shoots, the vascular system in rhizophores and roots is much simpler, usually composed of only one central protostele, and devoid of an air chamber.

By combining the morphological and anatomical features of each species in this family, an identification key to species will make the identification process becomes easier with the more available data provided to distinguish each species in this family. Based on the stipe anatomical

characteristics, Nurfarahain et al (2018) clustered *Selaginella* species into two groups based on the number of stele in the cross section namely; monostelic and tristelic groups. In the present study we also found one species (*S. plana*) to be tristelic while all others were monostelic. The number and shape of stele and the number of protoxylem groups in the stipe vary with species and can be used for the confirmation of identity of the species. The presence of rhizophore and its anatomy is also useful in species confirmation.

References

1. Adnan M, Siddiqui A, Jamal A, Hamadou W, Awadelkareem A, Sachidanandan M & Patel M (2021). Evidence-Based Medicinal Potential and Possible Role of *Selaginella* in the Prevention of Modern Chronic Diseases: Ethnopharmacological and Ethnobotanical Perspective. Records of Natural Products. 15. 10.25135/rnp.222.20.11.1890.
2. Alston, A. H. G. (1946). An enumeration of the Indian species of *Selaginella*. Proceedings of the Indian National Science Academy, 9, 211-235.
3. Alston, A. H. G. (1952). A revision of the West Indian species. Bulletin of the British Museum (Natural History) Botany, 1, 27-47.
4. Antony, R. & Thomas R. (2011). A mini review on medicinal properties of the resurrecting plant *Selaginella bryopteris* (Sanjeevani), International Journal of Pharmacy and Life Sciences, 2, 933-939.
5. Antony, R., Kumar, E. S. S. & Khan A. E. S. (2002). *Selaginella camusii*, a new species of Selaginellaceae from India. Nordic Journal of Botany, 22, 337-340.
6. Dixit, R. D. (1985). Two New Species of *Selaginella* P. Beauv. from Kerala, India. Nelumbo, 27(1-4), 123-125.
7. Dixit, R. D. (1992). Selaginellaceae of India. Ed. Bishen Singh Mahendrapal Singh, Dehra Dun, India. pp. IX+1-196.
8. Dudani, S., Chandran, M. D. & Ramachandra, T. V. (2009). Pteridophytes of Western Ghats. In Biodiversity Documentation and Taxonomy, Ed. A. Biju Kumar, Narendra Publishing House, PP 343–351.
9. Ganeshaiah, K. N., Vasudeva, R. & Shaanker, R. U. (2009). In search of Sanjeevani. Current Science, 97, 484-489.
10. IPNI (2023). International Plant Names Index. Published on the Internet http://www.ipni.org, The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Herbarium. [Retrieved 05 April 2023].
11. Kato, M. (2005). Classification, Molecular Phylogeny, Divergence Time, and Morphological Evolution of Pteridophytes with Notes on Heterospory and Monophyletic and Paraphyletic Groups. Acta Phytotaxonomica et Geobotanica, 56 (2), 111-126.
12. Korall, P & Kenrick, P. (2004). The phylogenetic history of Selaginellaceae based on DNA sequences from the plastid and nucleus: extreme substitution rates and rate heterogeneity. Molecular Phylogenetics and Evolution, 31, 852-64.

13. Madhusoodanan, P. V. & Nampy, S. (1994). *Selaginella dixitii*, a new species of Selaginellaceae from India. Nordic Journal of Botany, 14, 527-529.
14. Mickel, J. T & Hellwig, R. L. (1969). Actino-plectostely, a complex new stelar pattern in *Selaginella*. American Fern Journal, 59(3), 123-134.
15. Nisha, P., Nampy, S. & Joby, P. (2010). *Selaginella lakkidiana* sp. nov. (Selaginellaceae) from India. Nordic Journa.l of Botany, 28, 665-666.
16. Nurfarahain Z., Haja M. K. M. & Shaharuddin M. I. (2018). The Study of the Species from Genus *Selaginella* (Selaginellaceae) in Forest of Peninsular Malaysia. International Journal of the Malay World and Civilisation 6 (Special Issue 1), 2018, 23-26.
17. Panigrahi, G. and Dixit, R. D. (1966). Studies in the systematics of Indian *Selaginella*-III. Proceedings of the Indian National Science Academy, 36, 102-108.
18. Panigrahi, G. and Dixit, R. D. (1967). Studies in the systematics of Indian Selaginella-II. The Journal of Indian Botanical Society, 46, 222-233.
19. Panigrahi, G. and Dixit, R. D. (1968). Studies in the systematics of Indian Selaginella-I. Proceedings of the Indian National Science Academy, B. 34, 191-209.
20. POWO (2023). "Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org/ Retrieved 22 April 2023."
21. Rekha, K., & Krishnan, A. (2017). Diversity of pteridophyte flora in Akamala forest station, Thrissur, Kerala. International Journal of Fauna and Biological Studies, 4(5), 1-3.
22. Sah P. (2008). Does the Magical Himalayan Herb “Sanjeevani Booti” really exist in Nature? Journal of American Science, 4, 1545-1003.
23. Sah, N. K., Singh, S. N. & Sahdev, S. (2005). Indian herb “Sanjeevani” (*Selaginella bryopteris*) can promote growth and protect against heat shock and apoptotic activities of ultra violet and oxidative stress. Journal of Biosciences, 30, 499-505.
24. Setyawan, A. D., Supriatna, J., Darnaedi, D. & Rokhmatuloh, S. S. (2016). Diversity of *Selaginella* across altitudinal gradient of the tropical region. Biodiversitas,17, 384-400.
25. Singh, S. and Singh, R. (2015). A review on endemic Indian resurrecting Herb *Selaginella bryopteris* (L.) Bak ‘Sanjeevani’. International Journal of Pharmaceutical Sciences and Research, 6, 50-56.
26. Smith, G. M. (1955). Cryptogamic Botany – Vol.II – Mc Graw Hill Co. NewDelhi

27. Swamy, R. C., Kunert, O. & Schühly, W. (2006). Structurally unique biflavonoids from *Selaginella chrysocaulos* and *Selaginella bryopteris*. Chemistry and Biodiversity, 3, 405-413.
28. Thomas, B. A. (1992). Paleozoic herbaceous Lycopsids and the beginnings of extant Lycopodium sens. lat. and Selaginella sens. lat. Annals of the Missouri Botanical Garden, 79, 623-31.
29. Weststrand, S., and Korall, P. (2016). A subgeneric classification of *Selaginella* (Selaginellaceae). American Journal of Botany, 103(12), 2160 -2169.
30. Zhen-Ji, L. & Tan, B. C. (2005). A review of the species diversity of *Selaginella* in Fujian Province of China. Acta Phytotaxonomica Sinica, 43, 50-59.
31. Zhou, X. M., and Zhang L. B. (2015). A classification of *Selaginella* (Selaginellaceae) based on molecular (chloroplast and nuclear), macromorphological, and spore features. Taxon, 64, 1117-1140.