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Comparative rhizome anatomy of polypodiaceous ferns from eastern Himalaya and its taxonomic implications

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ABSTRACT

Polypodiaceae is the second largest family of ferns characterized by creeping rhizomes covered with scales whose systematics however is mainly focused on frond and reproductive structures. Over the years, studies have demonstrated the significance of rhizome anatomical characters for fern taxonomy. However, work focusing on rhizome anatomy from the eastern Himalayan species is scanty. Morpho-anatomical characters of rhizome and rhizome scales of 30 fern species have been evaluated and compared for its taxonomic significance. Detailed observations were made using a light microscope (LM). Comparative micro-morphological and anatomical traits shared by close phylogenetically related species are also discussed. Anatomical traits like the presence and position of sclerenchymatic tissues, sclereids, thick lignified circumendodermal (CB) bands of vascular bundles, rhizome scales are taxonomically relevant. Characters of rhizome scale delimit several genera and are diagnostic for Polypodiaceae. The comparative rhizome anatomy helps us to better understand this fern family and the scale features outlined will aid in easy identification of several species. An artificial dichotomous key is constructed to discern the morpho-anatomical characters in the segregation of the examined species.

1. Introduction

The polygrammoid fern family (Polypodiaceae) is one of most species-rich fern families with 65 genera and 1652 species distributed across continents except Antarctica with maximum diversity in tropical and subtropical regions (Ching, 1978; Tryon and Tryon, 1982; PPG I, 2016). Many remarkable and noteworthy epiphytic specializations are observed within the family (Dubuisson et al., 2009). The nature of adaptation of epiphytes to the harsh and heterogeneous environments of forest canopies remains one of the most intriguing questions in plant ecology (Benzing, 2012; Lowman and Schowalter, 2012; Reyes-García et al., 2012). The sporophytic plant body mainly inhabit tree bark, junctions, or rocks and are capable of clonal growth usually via long, creeping rhizomes with adventitious roots (Zhang, 2012). Epiphytic ferns vary in morphology, physiology, and phenology (Schneider et al., 2004; Watkins et al., 2007), and it is likely that these epiphytic species have adapted to habitats using various strategies. Some epiphytic ferns produce elaborate rhizome scales and/or indeterminate rhizomes that help in trapping, storage of water and detritus (Watkins and Catherine, 2012). Epiphytism appears to be the ancestral life form in this family, but several shifts in the ecological preferences to saxicolous and rheophytic habitats are also observed (Schneider et al., 2010). The ferns despite having a long evolutionary history and wider ecological breadth have limited information about vascular attributes (Pittermann et al., 2013).

Polypodiaceae *s.l.* is an extant, monophyletic fern family that includes previously segregated families Grammitidaceae and Platyceriaceae (Smith et al., 2008). The familial delimitation and infrafamilial classification of Polypodiaceae is complex and represent a long-term controversy in fern systematics (Ching, 1940, 1978; Nayar, 1970; de la Sota, 1973; Hennipman and Roos, 1983; Schneider et al., 2004; Smith et al., 2006; PPG I, 2016). Several authors suggest frequent parallel evolution and homoplasy in members of Polypodiaceae (Hovenkamp, 1996; Schneider et al., 2009; He et al., 2018). A stable infrafamilial classification might be achieved by identifying more diagnostic combinations of characters that seems to be of systematic value including stelar arrangement in rhizomes and petioles, venation pattern, and spore ornamentation (de la Sota, 1973; van Uffelen and Hennipman, 1985; van Uffelen, 1993, 1997; Morton, 1993; Tejero-Díez et al., 2010; Chen et al., 2021; Wei and Zhang, 2022).

The systematic significance of rhizome morphology and anatomy in ferns at generic and familial rank is rarely explored. Earlier works

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related to rhizome morphology in taxonomic studies is limited to phrases like "rhizome long-creeping", "short-creeping" or "erect" (Price, 1983; Hovenkamp, 1990). Comprehensive survey on rhizome morphology is available only for the Davalliaceae and Aspleniaceae (Croxdale, 1976; Kato, 1974; Kato and Mitsuta, 1979; Mitsuta et al., 1980). In recent times, root anatomical investigation of *Asplenium* contributed to the fern systematics and phylogeny (Luna et al., 2020). Anatomical information of vegetative organs aids in identification and serves as an important taxonomic tool (Kowsalya et al., 2017). Within the Polypodiaceae *s.l.,* knowledge of rhizome morphology is restricted to a small number of scattered species. Hovenkamp (1990) investigated the variation in rhizome morphology present in the Polypodiaceae and related groups to determine the possible systematic significance and proposed three distinct types of rhizomes based on distinct and recurrent regularity in the position of branching points. Srivastava and Chandra (2009) studied the rhizome vasculature of four *Polypodium* species. However, the rhizome details and anatomical features of this family remain unexplored. Until further, details regarding many Polypodiaceae species become available, an evaluation of the significance of stelar architecture in this group will not be possible. Over the years, anatomical studies in ferns have provided important sources of characters for taxonomy and for testing phylogenetic hypothesis (Sundue et al., 2015; Wetzel et al., 2017; Shah et al., 2019; Luna et al., 2020; Jaimez et al., 2021). Among the members of Polypodiaceae and all its six subfamilies, availability of morphological features of the rhizome and lamina have proven to be relevant for a critical review of their evolutionary role (Sundue et al., 2010) or for better circumscription of genera (Smith and Tejero-Díez, 2014; Jaimez et al., 2021).

A total of 30 species growing in Darjeeling eastern Himalaya were analyzed. Most of these species also occur in the Indo-Himalayan and adjacent Sino-Himalayan regions (Fraser-Jenkins, 2008a). Darjeeling Himalayan region is one of the biodiversity rich zones of the eastern Himalayan landscape. The altitude range supports tropical to sub-alpine vegetation offering favorable micro-climatic conditions for luxuriant growth of the ferns. Several of the anatomical descriptions are made here for the first time. The aim of this work is to expand the studies, bring forth the data from a broader sampling and compare the rhizome morpho-anatomy in Polypodiaceae. Our goal is to assess the potential of the morpho-anatomical characteristics of rhizome for species delineation and ascertain its systematic significance for the phylogenetic relationships among the members of the genus.

2. Materials and methods

2.1. Species examined

The present study was conducted with specimens collected mostly from Darjeeling Himalaya- an integral part of the eastern Himalayan ecosystem. Geographically, the area extends between 27°2′9.62″N latitude and 88°15′45″E longitude covering wide altitudinal range between 130 and 3636 m asl. Fresh samples were collected from natural populations of the study area. Proper identification of the taxa was made following relevant literature (Fraser-Jenkins, 2008b; Mehra and Bir, 2008; Kholia, 2010; Frazer-Jenkins et al., 2021). Herbaria such as Lloyd Botanical Garden and Calcutta University Herbarium (CUH) were also consulted for confirming the identification. Correct nomenclature with authority was maintained following Plants of the World Online (2023) and Global Biodiversity Information Facility GBIF (2023). Herbarium exsiccates have been deposited at CUH for future study. We have focused on species under 10 genera belonging to four sub-families; Microsoroideae (genera *Goniophlebium, Lepisorus, Microsorum, Phymatosorus*), Crypsinoideae (*Drynaria, Arthromeris, Selliguea, Pichisermollodes*) Platycerioideae (*Pyrrosia*) and Loxogrammoideae (*Loxogramme*) under Polypodiaceae (PPG I, 2016; Wei and Zhang, 2022). These 30 species are found in eastern Himalaya mostly growing as luxuriant epiphytes and lithophytes, therefore can be considered as good representatives of the family for taxonomic considerations. The species collected and examined are listed in Table 1.

2.2. Anatomical procedures

External morphological details were studied under dissection microscope and observations were made under simple and compound light microscopes. Samples were rehydrated in hot water with a few drops of glycerin and kept at room temperature (Zarlavsky, 2014). The rhizome scales were carefully dissected using needle in order to keep them intact. For all selected specimens, transverse-sections of the rhizome were made. Subsequently, the sections were mounted unstained in 20 % glycerine, in order to register the color of the sclereid walls and later double stained to observe the tissue differentiation. The sections were cleared with sodium hypochlorite (10 %) and stained with safranine-alcian blue. After staining, the sections were gradually dehydrated with alcohol, and mounted with Canada balsam on conventional slides (Kraus et al., 1998; Ruzin, 1999). Anatomical descriptions are presented with specific terminology (Ogura, 1972; Payne, 1978; Tejero-Díez, 2005).

2.3. Measurement of cellular structures

Complete transverse sections of the roots and rhizomes were used for evaluation of structural parameters. Different anatomical traits were observed and photographs were taken with the aid of stereo microscope, Wild M3Heerbrugg and binocular microscope Leitz Laborlux D. The measurements of the hairs, scales, vascular bundles, and other cellular structures were performed with software ImageJ at appropriate magnifications.

3. Results

3.1. Root

On the ventral side of the rhizome, thin black or dark brown roots are scattered over, either densely in short-creeping rhizomes, or more sparsely in definite tufts, in case of long creeping rhizomes that aids in attachment with the substrate. Roots are present in irregular rows or generally in rows of two. The transverse section of the roots shows 1-layered epidermis with many unicellular root-hairs, followed by heterogeneous cortex with two zones, the outer cortex (oc) and inner cortex (ic), with vascular cylinder surrounded by endodermis. The inner cortex consists of several layers (4–10) of parenchymatous cells interrupted near the extreme end by two protoxylematic centers of the vascular bundles. The vascular cylinder is surrounded by an endodermis, characterized by rectangular cells with casparian band and 1, 2 layers of the pericycle. The vascular bundle is generally diarch with endarch metaxylem and exarch protoxylems (Fig. 1A, B). Scattered sclereids nests or sclerenchymatous tissues as observed in the rhizome cross-sections are not present in the root cross-sections (Fig. 1C–H).

3.2. Rhizome

The fern rhizomes vary in shape, size, color, thickness, growth habit among different taxa. Most species are lithophytes and epiphytes, while only four species are strictly epiphytic (Table 1).

Shape in TS and rhizome thickness: The transverse section of rhizome is generally circular in most species. However, it is elliptical in *Drynaria propinqua, D. quercifolia, Pyrrosia costata, P. heteractis, P. lanceolata* and *P. nuda*. In *Loxogramme porcata* and *Pyrrosia mannii,* the shape is elliptical but with a wavy outline (Fig. 2Q, 4I). Heart-shaped in *Lepisorus sublinearis* (Fig. 3M). *Lepisorus normalis*, being an epiphytic creeper, the rhizome is firmly attached to the host surface that it becomes flattened on one side leading to semicircular shape in cross section of rhizome (Fig. 3E).

Table 1
List of fern species collected and examined.

Taxon name	Habit	Altitudinal distribution (m)	Voucher no	Collection site	Collector(s) name
Arthromeris himalovata Fraser-Jenk. & Kandel	epiphyte	2400–2700	SM-0358	Third mile	S. Moktan
Arthromeris lehmannii (Mett.) Ching	epiphyte/ lithophyte	1800–2700	SM-0360	Sixth mile	S. Moktan
Arthromeris wallichiana (Spreng.) Ching	lithophyte/epiphyte	900–2700	SM-0363	Third mile	S. Mondal & S. Moktan
Drynaria propinqua (Wall. ex. Mett.) J.Sm. ex. Bedd.	epiphyte/lithophyte	1600–2400	SM-0501	Kurseong	S. Mondal
Drynaria quercifolia (L.) J.Sm.	lithophyte/ epiphyte	400–1000	SM-0550	Sukna	S. Mondal & S. Moktan
Goniophlebium amoenum (Wall. ex. Mett.) Bedd.	epiphyte/ lithophyte	100–1600	SM-0498	Kurseong	S. Mondal & S. Moktan
Goniophlebium argutum (Wall. ex Hook.) Bedd.	epiphyte/ lithophyte	1200–2200	SM-0582	Jorebunglow	S. Mondal & S. Moktan
Goniophlebium lachnopus (Wall. ex Hook.) Bedd.	epiphyte/ lithophyte	1500–2400	SM-0616	Third mile	S. Moktan
Lepisorus contortus (Christ) Ching	epiphyte	2200–2600	SM-0344	Third mile	S. Mondal
Lepisorus loriformis (Wall. ex. Mett.) Ching	epiphyte	2000–3000	SM-0493	Kaiyakatta	S. Moktan
Lepisorus mehrae Fraser-Jenk.	lithophyte/ epiphyte	1500–2400	SM-0365	Mungpoo	S. Mondal & S. Moktan
Lepisorus normalis (D.Don) C.F.Zhao, R.Wei & X.C.Zhang	epiphytic creeper	900–2600	SM-0599	Lebong	S. Mondal & S. Moktan
Lepisorus nudus (Hook.) Ching	epiphyte/ lithophyte	1500–2400	SM-0239	Lebong	S. Mondal
Lepisorus rostratus (Bedd.) C.F.Zhao, R.Wei & X.C.Zhang	epiphyte	1200–200	SM-0554	Rajahatta	S. Mondal & S. Moktan
Lepisorus scolopendrium (Ching) Mehra & Bir	epiphyte/ lithophyte	1400–2800	SM-0364	Chimney	S. Mondal
Lepisorus sublinearis (Baker ex. Takeda) Ching	epiphyte/ lithophyte	1800–2400	SM-0324	Third mile	S. Moktan
Loxogramme porcata M.G.Price	lithophyte/ epiphyte	800–2200	SM-0223	Mahanadi	S. Mondal & S. Moktan
Microsorum membranaceum (D.Don) Ching	lithophyte/ epiphyte	500–2600	SM-0464	Lebong	S. Mondal & S. Moktan
Microsorum punctatum (L.) Copel.	epiphyte/ lithophyte	300–750	SM-0512	Pankhabari	S. Mondal & S. Moktan
Phymatosorus cuspidatus (D.Don) Pic.Serm.	lithophyte/ epiphyte	1000–1850	SM-0211	Rohini	S. Mondal & S. Moktan
Pichisermollodes ebenipes (Hook.) Fraser-Jenk.	epiphyte/ lithophyte	300–1000	SM-0315	Third mile	S. Mondal & S. Moktan
Pichisermollodes nepalensis (Nakaike) Fraser-Jenk.	epiphyte/lithophyte	1200–2800	SM-0620	Senchal	S. Moktan
Pichisermollodes stewartii (Bedd.) Fraser-Jenk.	epiphyte/ lithophyte	1200–2000	SM-0614	Ghoom	S. Mondal & S. Moktan
Pyrrosia costata (C.Presl ex Bedd.) Tagawa & K.Iwats.	lithophyte/ epiphyte	1300–2200	SM-0533	Pankhabari	S. Mondal
Pyrrosia heteractis (Mett. ex. Kuhn) Ching	lithophyte/ epiphyte	300–2000	SM-0602	Bagora	S. Mondal
Pyrrosia lanceolata (L.) Farw.	lithophyte/ epiphyte	300–1500	SM-0386	Rongtong	S. Mondal & S. Moktan
Pyrrosia mannii (Giesenh.) Ching	lithophyte/ epiphyte	700–1400	SM-0546	Panighatta	S. Mondal
Pyrrosia nuda (Giesenh.) Ching	lithophyte/ epiphyte	400–1500	SM-0618	Teesta	S. Mondal & S. Moktan
Selliguea griffithiana (Hook.) Fraser-Jenk.	lithophyte/ epiphyte	1600–2800	SM-0355	Third mile	S. Mondal & S. Moktan
Selliguea oxyloba (Wall. ex. Kunze) Fraser-Jenk.	lithophyte/ epiphyte	1200–2800	SM-0371	Senchal	S. Moktan

In polypodiaceous ferns, rhizomes are long to short-creeping. The diameter of rhizome of each species was measured to assess its thickness (Table 2). In species of *Pyrrosia,* the rhizome type can be categorized based on thickness as *P. heteractis, P. lanceolata* and *P. nuda* have comparatively long creeping and thin rhizome whereas in *P. manni, P. costata,* the rhizomes are short tufts but thicker. *Arthromeris wallichiana, Drynaria propinqua, Drynaria quercifolia, Microsorum membranaceum, Phymatosorus cuspidatus* have bigger rhizome with diameter ≥10 mm. Rhizome diameter can help to distinguish species, especially in *Drynaria propinqua* and *Drynaria quercifolia* both having dimorphic fronds as *D. propinqua* usually have thinner rhizome of about 10–20 mm whereas *D. quercifolia* rhizome diameter is greater than 20 mm.

***Stele*:** The stele is arranged in a circular to elliptic pattern as observed in the transverse sections (Table 2). Anatomy of rhizomes in all the studied taxa shows dictyostelic with ring of bicollateral meristeles (vascular bundles) viewed in transverse section (Figs. 2–4). The vascular strands in species of *Arthromeris, Drynaria, Goniophlebium, Loxogramme, Microsorum, Phymatosorus, Pichisermollodes, Pyrrosia* and *Selliguea* are arranged in a circular pattern. In *Lepisorus* however, the arrangement of vascular bundle is in a circle with high number of scattered sclerenchyma strands except for *Lepisorus rostratus* (Fig. 3I). Meristeles or the vascular bundles usually have one layered endodermis, upto two layers of pericycle and xylem surrounded by phloem. Vascular cylinder is dorsiventral with leaf gaps closely placed at the dorsal surface and root traces are restricted to the ventral half.

From all the rhizome transverse sections, a single layered epidermis is observed, the primary xylem and phloem tissues are packed in discrete vascular strands surrounded by an endodermis often with an additional dark circumendodermal band (CB band). All the species within the family possess dictyostelic rhizome, however significant variations exist in its number, general form and arrangements of vascular bundles and sclerenchymatous tissues in the form of sclereids that are embedded in the parenchymatous cells of cortex (Table 2).

***Shape and number of vascular bundle (vb)*:** In all the examined species, the vascular strands are circular or circular-oval in shape within the ground tissue of cortex. The vascular bundles however, are elliptical in *Loxogramme porcata* (Fig. 2R).

The number of vascular strands ranges among species within different genera usually from 5 to 25. The general trend of species with thinner rhizomes and fewer vascular strands are observed in species like *Lepisorus rostratus* (6–8 vbs), *Pyrrosia heteractis* (6–10 vbs), *Pyrrosia lanceolata* (5–6 vbs), *Pyrrosia nuda* (5–6 vbs). Species having thick rhizomes like *Arthromeris wallichiana* (10–15 vbs), *Drynaria quercifolia* (18–20 vbs), *Phymatosorus cuspidatus* (15–20 vbs) and *Microsorum membranaceum* (18–25 vbs) have a greater number of vascular bundles. Comparatively, thinner rhizomes with greater than 15 vascular bundles are also observed in *Goniophlebium amoenum* (15–18 vbs), *G. argutum* (14–20 vbs), *G. lachnopus* (15–20 vbs) where rhizome diameter ranges from 5 to 7 mm. In *Lepisorus mehrae* (15–20 vbs), *Selliguea griffithiana* (12–20 vbs) and *S. oxyloba* (15–22 vbs), the rhizome diameter ranges from 3 to 5 mm, 3, 4 mm, and 4, 5 mm, respectively.

***Dark circumendodermal band*:** The presence of an additional dark circumendodermal band (CB band) along with an endoderm is in the vascular bundle of rhizome is observed in most of the taxa under study (Table 2). To observe the presence of the CB band, cross sections of rhizomes were taken not too close to the growing tip, as the point of sclerification does not start there. Under a microscope, the cross sections show a vascular bundle consisting of red-stained xylem and blue counter-stained phloem tissues, which are surrounded by an endodermis. In many species, a thick CB band is present, while it is absent in some. The CB band is very thick and prominent in species of *Arthromeris himalovata, A. lehmannii, A. wallichiana, Drynaria propinqua, D. quercifolia, Lepisorus loriformis, L. scolopendrium, L. normalis, L. nudus, L. rostratus, L. mehrae, Phymatosorus cuspidatus, Pichisermollodes ebenipes, Pyrrosia mannii, Selliguea griffithiana, Selliguea oxyloba* (Fig. 2A–J, 3A–P, M, N, I, J, Q–T). In *Goniophlebium amoenum, G. lachnopus, G. argutum, Loxogramme porcata, Lepisorus contortus, L. sublinearis, Microsorum membranaceum, M. punctatum, Pyrrosia heteractis, P. lanceolata, P. nuda,*

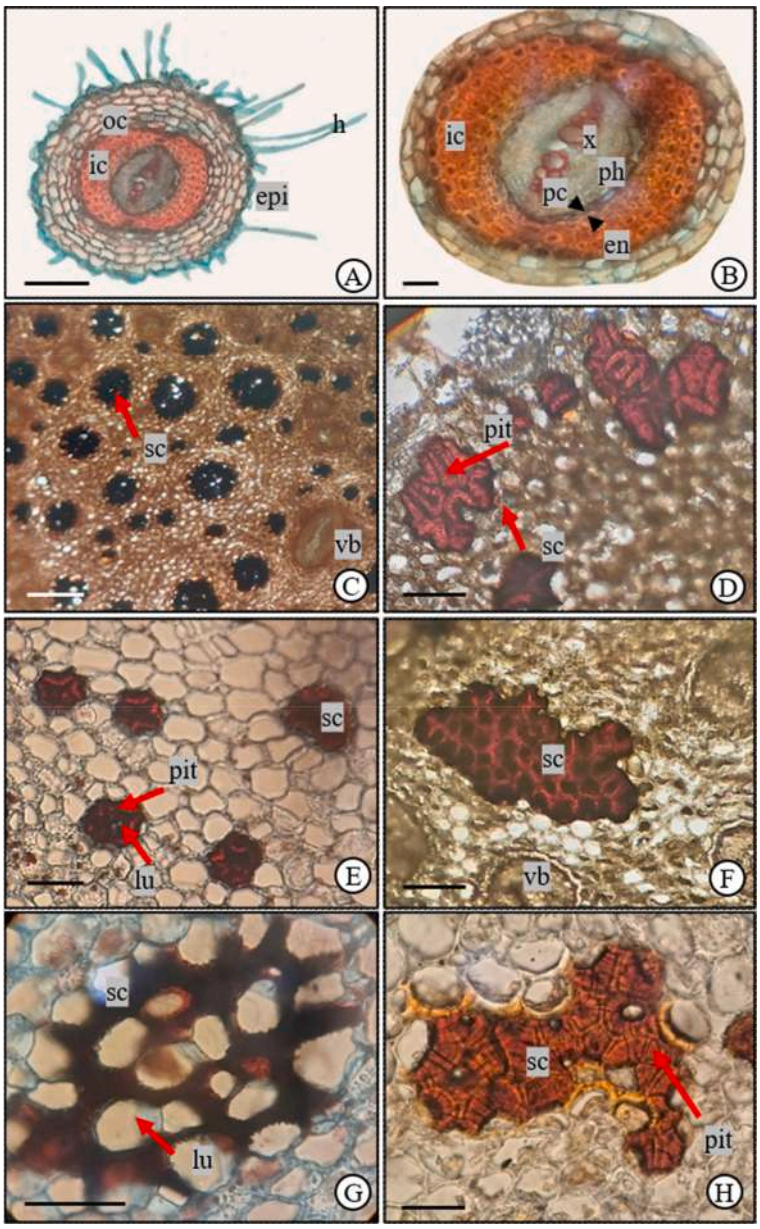

Fig. 1. A. Transverse section of the root of Polypodiaceae: A. *Selliguea oxyloba,* B. inner cortex, C. Sclereid nests of rhizome in *Lepisorus contortus,* D. *Pyrrosia mannii,* E. *Selliguea griffithiana*, F. *Pyrrosia lanceolata*, G. *Pichisermollodes ebenipes*, H. *Pyrrosia nuda,* (h, root hairs; en, endodermis; epi, epidermis; ic, inner cortex; lu, lumen; oc, outer cortex; pc, pericycle; ph, phloem sc, sclereids; vb, vascular bundles; x, xylem). Bars = 100 μm (A); 25 μm (B); 50 μm (C-F).

P. costata, Pichisermollodes stewartii and *P. nepalensis,* the CB band seems to be absent or non thick and dark (Fig. 2K–P, Q–T; 3M, N, Q-T; 4A-H, K, L, O, P).

Position of sclerenchyma: The nature and arrangement of sclerenchymatous tissues in the rhizome of most of our studied species like *Goniophlebium amoenum, G. lachnopus, G. argutum, Lepisorus contortus, L. loriformis, L. mehrae, L. nudus, L. scolopendrium, L. sublinearis, Microsorum membranaceum, M. punctatum, Pichisermollodes ebenipes, P. stewartii, P. nepalensis, Pyrrosia mannii, Selliguea griffithiana* and *S. oxyloba* show distinct sclerenchyma strands in the form of sclereid nests which lie randomly scattered in the ground tissue. In *Pyrrosia heteractis, P. lanceolata* and *P. nuda,* the sclerenchymatic tissues are present as sheaths in specific areas near subepidermal zone and in center near the meristeles. However, *Pyrrosia costata* exhibit an entirely different rhizome structure. Here, the rhizome is not differentiated into sclerenchyma and parenchyma and is entirely composed of not strongly thickened sclerenchymatous tissue (Fig. 4G, H). Only a thin parenchymatous peripheral zone occurs. Sclereids were absent in species of *Arthromeris himalovata, A. lehmanii, A. wallichiana, Drynaria propinqua, D. quercifolia, Loxogramme porcata* and *Phymatosorus cuspidatus* (Fig. 2A–C, G–I, Q; 3O).

***Number of sclerenchyma strands*:** The sclerenchyma strands or sclerotic nests passing longitudinally through the rhizomes of *Goniophlebium, Lepisorus, Microsorum, Pyrrosia, Pichisermollodes* and *Selliguea* varies in number from 1 to greater than 100. The species under study have been categorized as low (≤10) or high (≥100) based on number of sclerenchyma strands (Table 2). *Lepisorus normalis, L. rostratus, L. mehrae, Pyrrosia heteractis, P. lanceolata, P. nuda* have fewer sclereids in their ground tissues (Fig. 3E, I, K). Rhizomes of *Goniophlebium amoenum, G. argutum, G. lachnopus, Lepisorus contortus, L. loriformis, L. scolopendrium, L. nudus, L. sublinearis, Microsorum membranaceum, M. punctatum, Pichisermollodes ebenipes, P. nepalensis, P. stewartii, Selliguea griffithiana* and *S. oxyloba* contain more than 100 sclerenchyma strands.

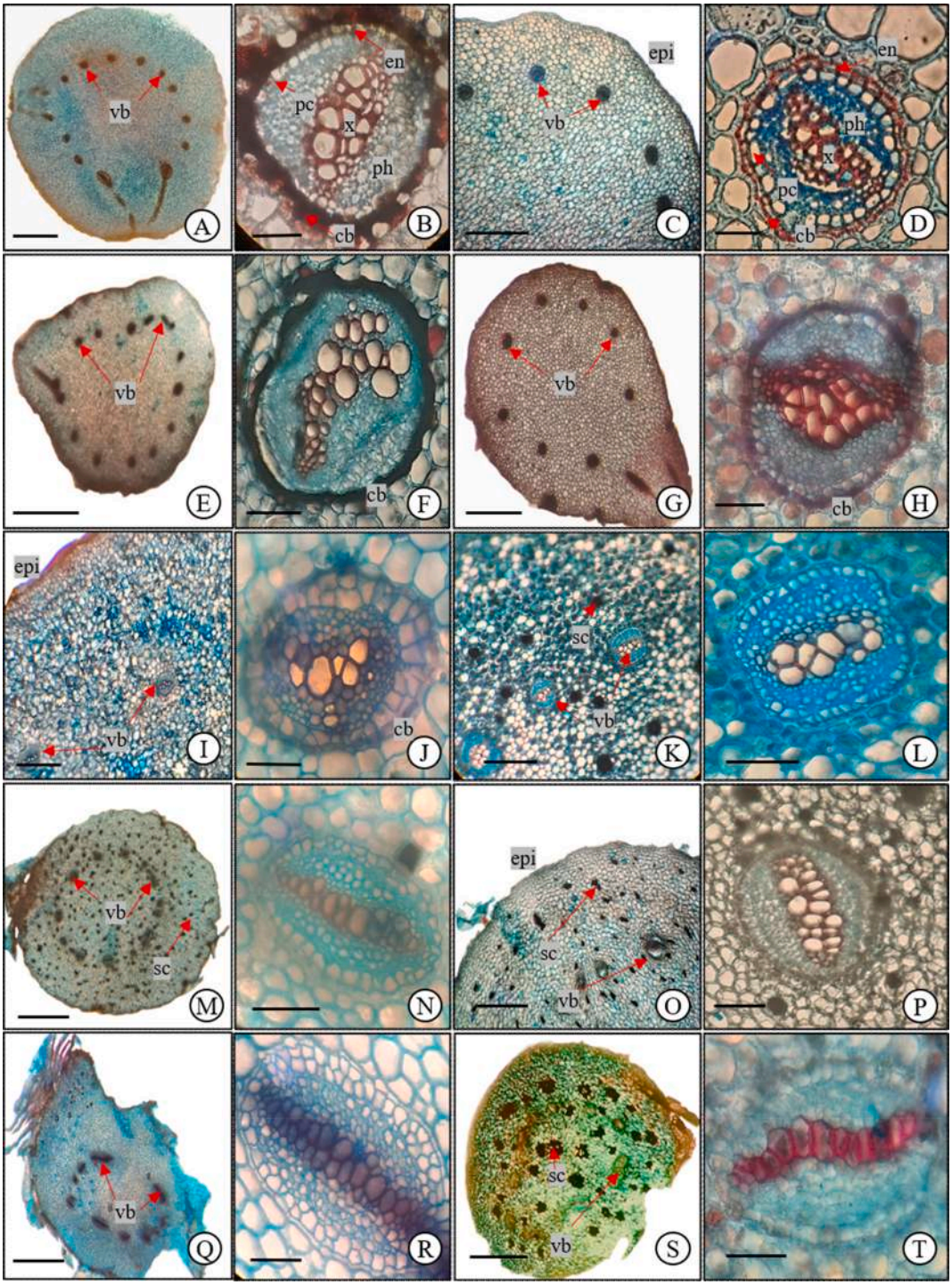

Fig. 2. Transverse section showing rhizome (left) and vascular bundles (right) of Polypodiaceae: A-B. *Arthomeris wallichiana,* C-D. *A. himalovata,* E-H. *A. lehmannii*, G-H. *Drynaria propinqua,* I-J. *D. quercifolia*, K-L. *Goniophlebium amoenum,* M-N. *G. lachnopus,* O-P. *G. argutum*, Q-R. *Loxogramme porcata,* S-T. *Lepisorus contortus*, (cb = circumendodermal band; epi = epidermis; en = endodermis; pc = pericycle; ph = phloem; sc, sclereids; vb, vascular bundles; x, xylem). Bars = 500 μm (A, E, G, M, Q, S); 100 μm (C, I, K, O); 50 μm (B, D, F, H, J, L, N, P, R, T).

3.3. Rhizome scales

The rhizome in Polypodiaceae under investigation is covered with scales that are dense and persistent on the short and long-creeping rhizomes. The shape, color, and size of these scales are highly variable among the species and therefore very diagnostic in nature (Figs. 5, 6). The following characters of the scales have great implications in diagnosis of both genera and species and the features are presented in the Table 3.

Type of attachment: The attachment of scales to the rhizome may be on their basal side (basifixed) or attached by a short stalk (peltate). Another intermediate form is a basally attached scale with large and overlapping auricles termed 'pseudopeltate'. Basifixed scales are observed in *Lepisorus contortus* (Fig. 5I), *L. loriformis* (Fig. 5J), *L. mehrae* (Fig. 5K), *L. nudus* (Fig. 5M), *Loxogramme porcata* (Fig. 5Q), *Pyrrosia costata* (Fig. 6H). Exclusively pseudopeltate are observed in *Microsorum*

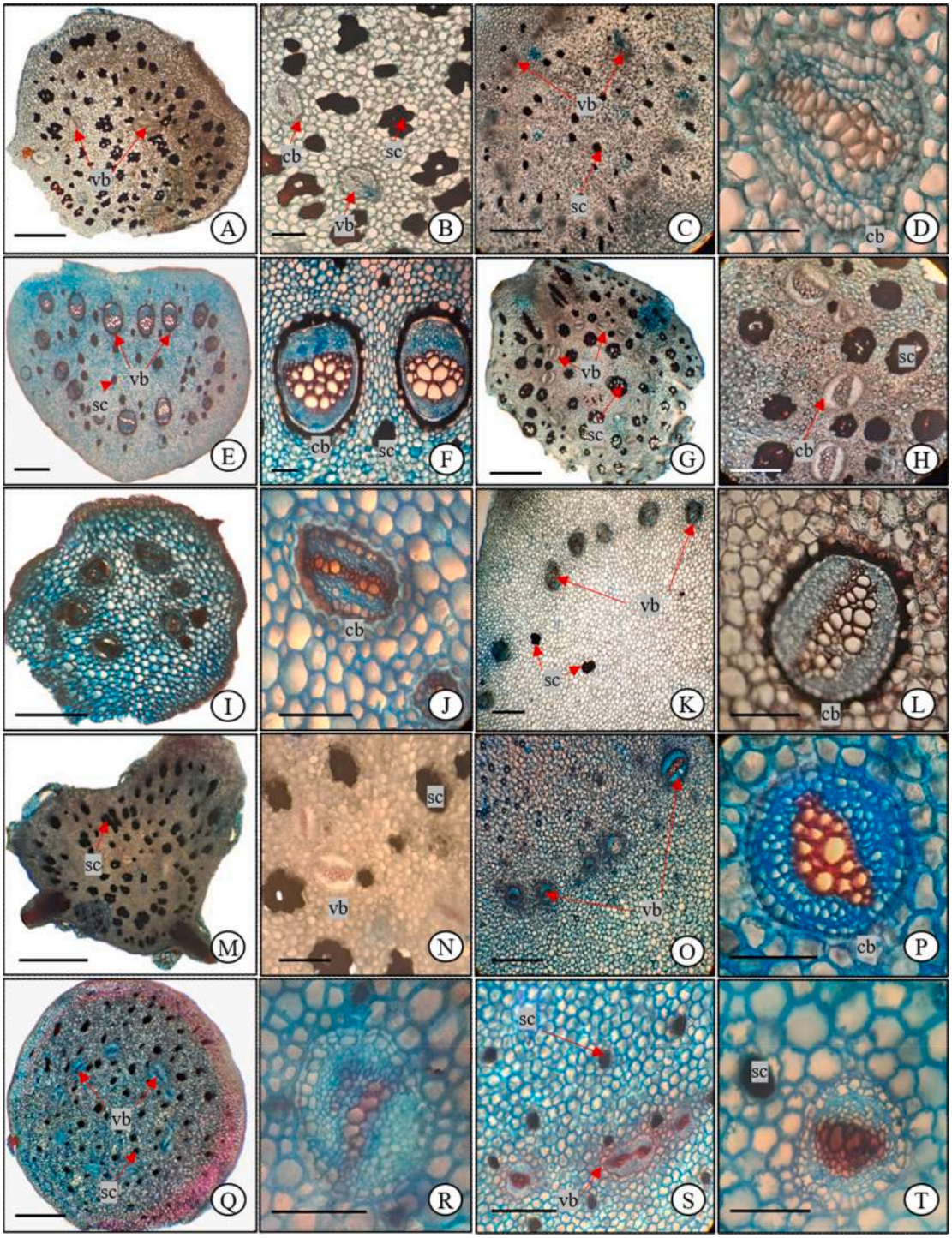

Fig. 3. Transverse section showing rhizome (left) and vascular bundles (right) of Polypodiaceae: A-B. *Lepisorus loriformis,* C-D. *L. scolopendrium,* E-F. *L. normalis*, G-H. *L. nudus,* I-J. *L. rostratus*, K-L. *L. mehrae,* M-N. *L. sublinearis*, O-P. *Phymatosorus cuspidatus*, Q-R. *Microsorum membranaceum,* S-T. *M. punctatum*. (cb = circumendodermal band; sc, sclereids; vb, vascular bundles). Bars = 500 μm (A, E, G, I, M, Q); 100 μm (C, K, O, S); 50 μm (B, D, F, H, J, L, N, P, R, T).

membranaceum (Fig. 6A) and *Pyrrosia mannii* (Fig. 6K) whereas exclusively peltate scales are observed in 21 species. In *Microsorum punctatum*, both type of scale attachment, peltate and pseudopeltate occurs (Fig. 6B, C).

Shape and color: The scales in shape are usually widest near the base or its point of attachment to the rhizome. Shape in most of the species is lanceolate. However, *Lepisorus normalis, Microsorum punctatum, Phymatosorus cuspidatus* shows orbicular scales (Fig. 5L, 6B-D). Subulate scales are observed in *Goniophlebium lachnopus* (Fig. 5G).

The color of the scale varies from whitish to golden brown to dark or blackish. The rhizome scales of most of the species are brown to dark brown, however in many species the scale color is very diagnostic. In *Arthromeris himalovata,* the scales are ferruginous, while in *A. lehmanni* and *A. wallichiana* it is light brown and golden brown respectively (Fig. 5A–C). In *Goniophlebium* genera, *G. amoenum* and *G. lachnopus* have brown and dark brown scales, while *G. argutum* shows blackish brown scales (Fig. 5F–H). The scales in *Lepisorus* species are remarkable and distinct. Dark brown scales are observed in *L. loriformis, L. nudus, L.*

Table 2
Comparative rhizome anatomical characteristics with stelar details of 30 species of Polypodiaceae.

Species	Shape of rhizome in cross section	Diameter (mm)	Number of vascular bundles (vb)	Shape of vascular bundles	Dark circumendodermal band	Number of sclerenchyma strands (High ≥100, Low ≤10)	Position of sclerenchyma
Arthromeris himalovata	circular	4–5	8–10	circular	+	–	–
Arthromeris lehmannii	circular	4–5	8–10	circular	+	–	–
Arthromeris wallichiana	circular	10–15	15–20	circular	+	–	–
Drynaria propinqua	elliptical	10–20	8–12	circular	+	–	–
Drynaria quercifolia	elliptical	20–30	18–20	circular	+	–	–
Goniophlebium amoenum	circular	5–7	15–18	circular - oval	–	high	scattered
Goniophlebium argutum	circular	5–7	14–20	circular - oval	–	high	scattered
Goniophlebium lachnopus	circular	5–7	15–20	circular - oval	–	high	scattered
Lepisorus contortus	circular	1.5–2.5	10–15	oval	–	high	scattered
Lepisorus loriformis	circular	1–2	8–10	oval	+	high	scattered
Lepisorus mehrae	circular	3–5	16–18	oval	+	low	scattered
Lepisorus normalis	semi-circular	3–4	5–17	circular	+	low	scattered
Lepisorus nudus	circular	1.5–2	8–10	circular - oval	+	high	scattered
Lepisorus rostratus	circular	1–2	6–8	oval	+	low	scattered
Lepisorus scolopendrium	circular	3–6	10–12	oval	+	high	scattered
Lepisorus sublinearis	heart-shaped	1.5–2.5	8–10	circular - oval	–	high	scattered
Loxogramme porcata	elliptical- wavy in outline	1–1.5	8–10	elliptical	–	–	–
Microsorum membranaceum	elliptical	3–10	18–25	circular	–	high	scattered
Microsorum punctatum	circular	4–8	10–12	circular	–	high	scattered
Phymatosorus cuspidatus	circular	10–20	15–20	circular	+	–	–
Pichisermollodes ebenipes	circular	3–6	8–10	circular	+	high	scattered
Pichisermollodes nepalensis	circular	2–3	6–8	circular - oval	–	high	scattered
Pichisermollodes stewartii	circular	3–4	10–12	circular - oval	–	high	scattered
Pyrrosia costata	elliptical	1–5	10–12	circular	–	high	not differentiated
Pyrrosia heteractis	elliptical	1–3.5	6–10	circular	–	low	central
Pyrrosia lanceolata	elliptical	1.2–2	5–6	circular	–	low	central
Pyrrosia mannii	elliptical- wavy in outline	2.5–3.5	8–10	oval	+	high	scattered
Pyrrosia nuda	elliptical	1.5–2	5–6	circular	–	low	central
Selliguea griffithiana	circular	3–4	12–20	circular - oval	+	high	scattered
Selliguea oxyloba	circular	4–5	15–22	circular - oval	+	high	scattered

sublinearis (Fig. 5J, M, P), golden brown in *L. normalis, L. rostratus* (Fig. 5L, N), light brown in *L. scolopendrium* and pale brown in *L. mehrae* (Fig. 5O, K). In *Lepisorus contortus,* the scales are pale brown with a narrow dark opaque central band near their point of attachment (Fig. 5I). The scales are orbicular in shape, dark brown at center and paler towards margins in *Phymatosorus cuspidatus* (Fig. 6D). In some cases, dark coloring may be formed as distinct darker spot near the apex like in *Drynaria quercifolia* (Fig. 5E) and *Pichisermollodes stewartii* (Fig. 6E). In *P. ebenipes* and *P. nepalensis,* scales are dark blackish brown and rusty brown respectively (Fig. 6F, G). In *Pyrrosia* genera, scales are shiny brown in *P. costata*, straw-colored in *P. mannii,* while light brown in *P. heteractis, P. lanceolata*, and *P. nuda* (Fig. 6H–N). Brown scales occur in *Selliguea griffithiana* while in *S. oxyloba* it is lanceolate in shape, dark brown at center and paler towards margins (Fig. 6P, O). *Loxogramme porcata* shows greyish brown scales (Fig. 5Q).

Scale apex: In several species, the scales are often contracted to acuminate in *Arthromeris wallichiana, Drynaria propinqua, Goniophlebium argutum, Lepisorus contortus, L. rostratus, L. scolopendrium, Loxogramme porcata, Pyrrosia costata, Selliguea oxyloba*, short acuminate in *Lepisorus nudus*, long acuminate in *Lepisorus loriformis, L. sublinearis, Pyrrosia mannii, Selliguea griffithiana,* long caudate in *Arthromeris himalovata, A. lehmanni, Goniophlebium lachnopus*. In *Drynaria quercifolia,* the apex of the scales is very long, narrowly caudate. In *Microsorum membranaceum, M. punctatum, Pichisermollodes ebenipes, P. nepalensis, P. stewartii, Pyrrosia heteractis, P. lanceolata*, and *P. nuda,* the apexes are acute.

Dimorphism and texture: In many species dimorphic scales exists. Triangular ovate and ovate scales in *Microsorum punctatum* (Fig. 6B, C), lanceolate and smaller ovate-lanceolate scales in *Pyrrosia heteractis* (Fig. 6M, N), bigger lanceolate and smaller lanceolate scales in *Pyrrosia lanceolata* (Fig. 6I, J). The texture of scales ranges from thin in species of *Arthromeris, Drynaria* and *Pyrrosia* to translucent and thickened, dark, and sclerified in species of *Lepisorus, Microsorum, Phymatosorus, Pichisermollodes* and *Selliguea*.

Indument and clathration: The surface of the scales may bear tufts of rhizoid-like hairs, near the point of attachment in *Goniophlebium amoenum* and *Lepisorus normalis* (Fig. 5F, L). The scales are clathrate, with distinct thickened cell walls forming a lattice-pattern or opaque. Clathrate scales are observed in majority of the species while opaque scales are present in species of *Pichisermollodes* and *Selliguea* (Fig. 6E–G, O,P).

***Scale margin*:** The marginal cells of scales generally have thinner walls with distinct hyaline margin that may be entire to variously dentate, or densely set with long and curled cilia like structures (Table 3). *Goniophlebium lachnopus, Lepisorus normalis, L. nudus, L. scolopendrium,*

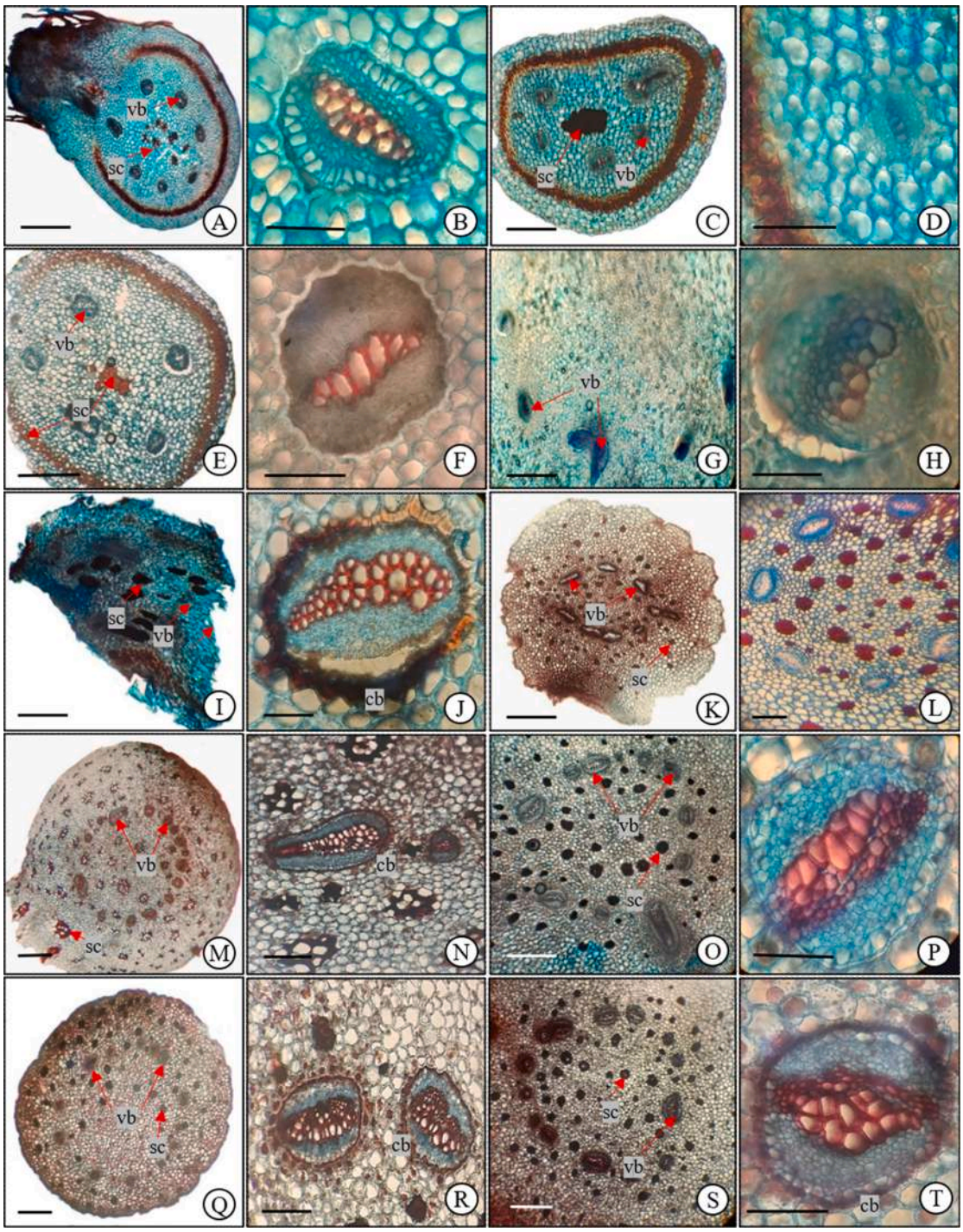

Fig. 4. Transverse section showing rhizome (left) and vascular bundles (right) of Polypodiaceae: A-B. *Pyrrosia heteractis,* C-D. *P. lanceolata,* E-F. *P. nuda,* G-H. *P. costata,* I-J. *P. mannii,* K-L. *Pichisermollodes stewartii*, M-N. *P. ebenipes,* O-P. *P. nepalensis*, Q-R. *Selliguea griffithiana*, S-T. *S. oxyloba*, (cb = circumendodermal band; sc, sclereids; vb, vascular bundles). Bars = 500 μm (A, C, E, I, K, M, Q); 100 μm (G, O, S); 50 μm (B, D, F, H, J, L, N, P, R, T).

Loxogramme porcata, Microsorum membranaceum, Phymatosorus cuspidatus, Pyrrosia heteractis, Selliguea griffithiana and *S. oxyloba* have scales with entire margins (Fig. 5F, L, M, O, Q, 6A, D, M–P). Toothed margin are observed in species of *Arthromeris,* with *A. himalovata, A. lehmanni* and *A. wallichiana* exhibiting toothed, minutely toothed and sparsely toothed respectively (Fig. 5A–C).

Scales in *Pyrrosia costata* have long toothed margin (Fig. 6H) while *Drynaria propinqua* and *Microsorum punctatum* shows dentate margins with strong dentation in *Drynaria quercifolia* (Fig. 5D, 6B, C, 5E). Denticulate scale margins are observed in *Goniophlebium amoenum, Lepisorus loriformis, L. sublinearis* (Fig. 5F, L, P) while the margins are serrated in *Lepisorus contortus* (Fig. 5I) and ciliate in rest of the species.

Key to the studied species based on rhizome characters

1a. Rhizome with sclerenchyma tissues in the ground tissue	8
b. Rhizome without sclerenchyma tissues in the ground tissue	2
2a. Scales orbicular in shape	*Phymatosorus cuspidatus*
b. Scales lanceolate in shape	3

(*continued on next page*)

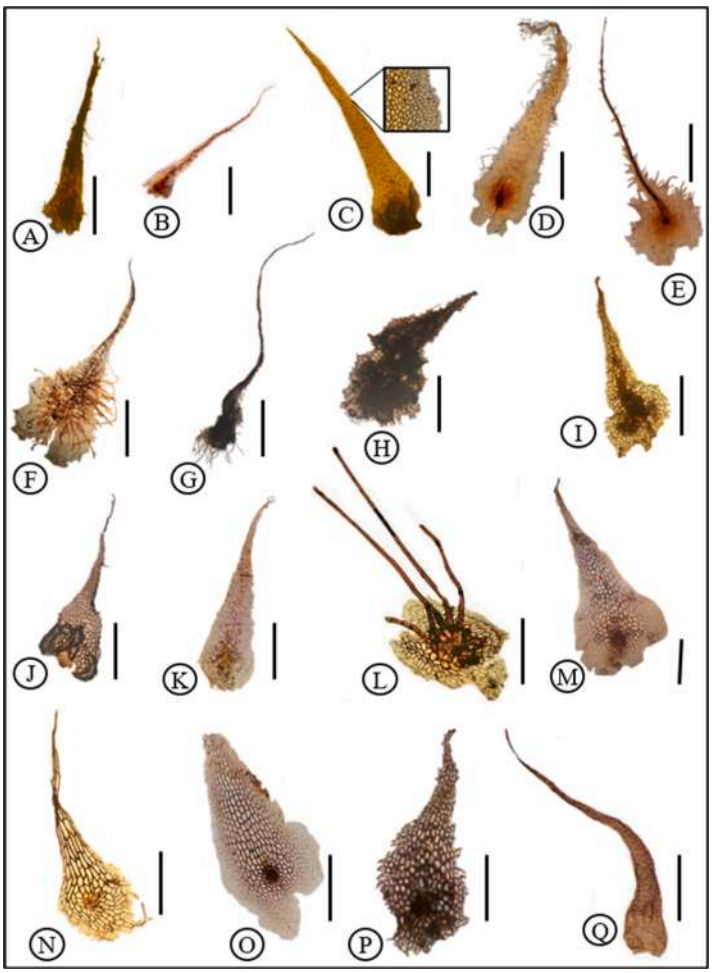

Fig. 5. Rhizome scales of Polypodiaceae. A. *Arthromeris himalovata*, B. *A. lehmannii*, C. *A. wallichiana,* D. *Drynaria propinqua*, E. *D. quercifolia*, F. *Goniophlebium amoenum,* G. *G. lachnopus,* H. *G. argutum*, I. *Lepisorus contortus*, J. *L. loriformis,* K. *L. mehrae*, L. *L. normalis*, M. *L. nudus*, N. *L. rostratus*, O. *L. scolopendrium*, P. *L. sublinearis*, Q. *Loxogramme porcata*. Bars =1 mm.

(*continued*)

3a. Scales peltate, golden brown to dark brown	4
b. Scales basifixed, greyish in color	*Loxogramme porcata*
4a. Rhizome covered with a whitish bloom	5
b. Rhizome without any whitish bloom	6
5a. Rhizome scales ferruginous, linear lanceolate	*Arthromeris himalovata*
b. Rhizome scales pale brown, lanceolate, base rounded	*Arthromeris lehmannii*
6a. Rhizome densely covered with golden brown scales with sparsely toothed margin	*Arthromeris wallichiana*
b. Rhizome densely covered with light or blackish brown scales with dentate margin	7
7a. Rhizome diameter >20 mm, covered with blackish brown scales having long narrow caudate apex	*Drynaria quercifolia*
b. Rhizome diameter <20 mm, covered with light brown, lanceolate scales	*Drynaria propinqua*
8a. Rhizome not differentiated into sclerenchyma and parenchyma, entirely composed of sclerenchymatous tissue	*Pyrrosia costata*
b. Rhizome differentiated into sclerenchyma and parenchyma tissues	9
9a. Sclerenchymatic tissues are present as sheaths in specific areas	10
b. Sclerenchymatic tissues scattered randomly	12
10a. Rhizome with many sclerenchyma strands in the central region	*Pyrrosia heteractis*
b. Rhizome with single sclerenchyma strand in the central region	11
11a. Rhizome scales dimorphic	*Pyrrosia lanceolata*
b. Rhizome scales monomorphic	*Pyrrosia nuda*

(*continued on next column*)

(*continued*)

12a. Rhizome short, covered with yellowish straw brown scales	*Pyrrosia manni*
b. Rhizome long, creeping covered with brown or blackish scales	13
13a. Rhizome scales clathrate	18
b. Rhizome scales opaque	14
14a. Scales linear lanceolate	17
b. Scales lanceolate with broad ovate base	15
15a. Scales glossy black, thickened circumendodermal band in vascular bundle	*Pichisermollodes ebenipes*
b. Scales brown, light circumendodermal band in vascular bundle	16
16a. Scales with a lighter brown near basal margin	*Pichisermollodes stewartii*
b. Scales uniformly rusty brown	*Pichisermollodes nepalensis*
17a. Scales dark brown, margin entire, apex acuminate	*Selliguea griffithiana*
b. Scales brown at center, paler toward margin, margin ciliate, apex acuminate	*Selliguea oxyloba*
18a. Scales pseudopeltate or peltate	22
b. Scales basifixed, lanceolate or ovate lanceolate	19
19a. Scales dark brown, ovate lanceolate	20
b. Scales pale brown, lanceolate, apex acuminate	21
20a. Scales with short acuminate apex, margin entire	*Lepisorus nudus*
b. Scales with long acuminate apex, margin denticulate	*Lepisorus loriformis*
21a. Scales pale brown with narrow dark opaque central band, margin serrate	*Lepisorus contortus*
b. Scales uniformly pale brown, ciliate margin	*Lepisorus mehrae*
22a. Presence of both pseudopeltate and peltate scales	23

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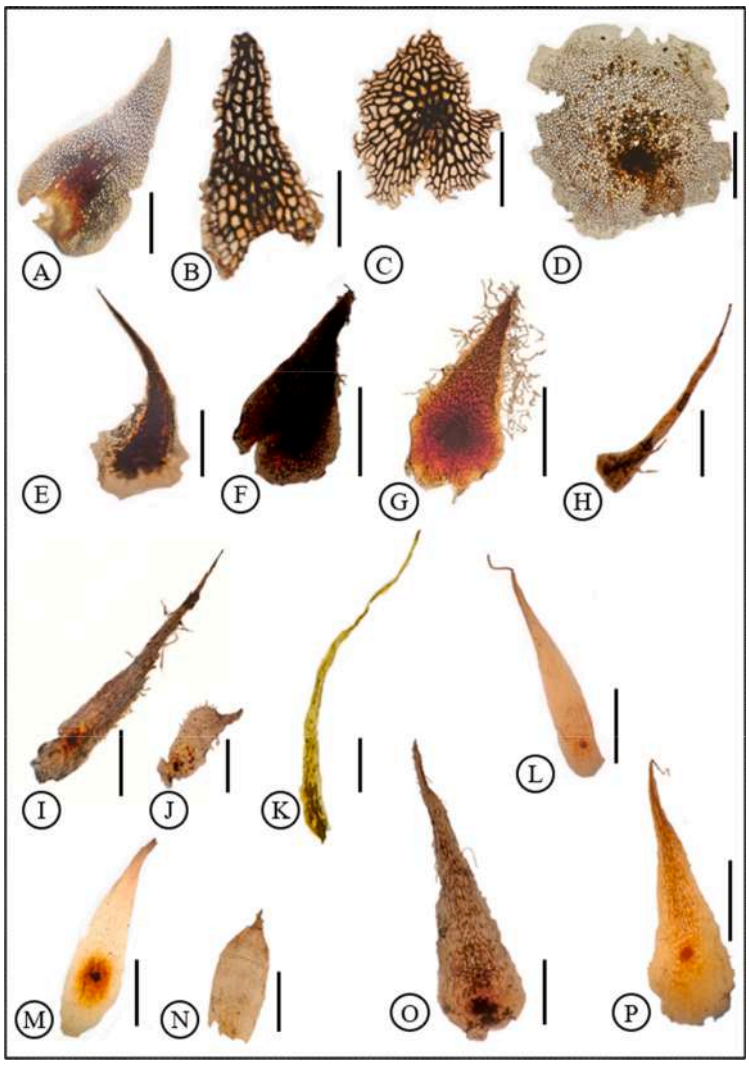

Fig. 6. Rhizome scales of Polypodiaceae. A. *Microsorum membranaceum,* B. triangular scale of *M. punctatum*, C. orbicular scale of *M. punctatum,* D. *Phymatosorus cuspidatus*, E. *Pichisermollodes stewartii,* F. *P. ebenipes,* G. *P. nepalensis,* H. *Pyrrosia costata,* I. *P. lanceolata,* J. smaller scales of *P. lanceolata,* K. *P. mannii*, L. *P. nuda,* M. *P. heteractis*, N. smaller scales of *P. heteractis,* O. *Selliguea oxyloba,* P. *S. griffithiana*. Bars = 1 mm.

(*continued*)

b. Presence of peltate scales only	24
23a. Ovate or triangular, margin entire, monomorphic	*Microsorum membranaceum*
b. Narrowly ovate or triangular, margin dentate, dimorphic	*Microsorum punctatum*
24a. Scales orbicular with tuft of hairs in the middle	*Lepisorus normalis*
b. Scales subulate to lanceolate	25
25a. Scales subulate, apex long caudate	*Goniophlebium lachnopus*
b. Scales lanceolate, apex acute or acuminate	26
26a. Scales brown, margin entire	27
b. Brown to blackish brown scales, margin denticulate or ciliate	28
27a. Scales golden brown, rhizome with 6–10 scattered sclerenchyma strands	*Lepisorus rostratus*
b. Scales light brown, rhizome with more than 10 scattered sclerenchyma strands	*Lepisorus scolopendrium*
28a. Scales brown with tuft of hairs	*Goniophlebium amoenum*
b. Scales dark to blackish brown without hairs	29
29a. Scales dark brown, apex long acuminate, margin denticulate	*Lepisorus sublinearis*
b. Scales blackish brown, apex acuminate, base margin ciliate	*Goniophlebium argutum*

4. Discussion

The vascular tissue arrangements in fern and fern allies have fascinated botanists worldwide ever since nineteenth century to modern times (Bower, 1923; Ogura, 1972; Pittermann et al., 2015). The root characters in the species appear to be uniform in all the members of the family and the minute variation may be an autapomorphy. Similar observations had been previously reported by Hovenkamp (1986).

The vascular morphology of the rhizome is well accepted conserved feature and is minimally influenced by the external environment and therefore a taxonomically significant trait useful for phyletic studies in homosporous ferns. Therefore, the structure and organization of the vascular system in ferns are often used in comparative studies (Holttum, 1964; Nayar and Chandra, 1967; Srivastava and Chandra, 2009; Vasco et al., 2013). Variation in stelar patterns has proven to be useful for taxonomic identification (Bower, 1923; Ogura, 1972). The general structure of the root is similar for the species in Polypodiaceae as described (Ogura, 1972; Lagoria et al., 2018).

Rhizomes in pteridophytes usually have species-or group-specific stele (Deroin and Rakotondrainibe, 2015; Nopun et al., 2016; Becari-Viana and Schwartsburd, 2017). In the classifications proposed by Pteridophyte Phylogeny Group (PPG I, 2016), Polypodiaceae is placed under suborder Polypodiineae (eupolypods I) and order Polypodiales.

Table 3
. Morpho-anatomical features of the rhizome scales in 30 species of Polypodiaceae.

Species	Attachment	Color	Dimorphism	Shape	Apex	Clathration ofcell walls	Margin
Arthromeris himalovata	peltate	ferruginous	–	linear lanceolate	long caudate	clathrate	toothed
Arthromeris lehmannii	peltate	light brown	–	linear lanceolate	long caudate	clathrate	minutely toothed
Arthromeris wallichiana	peltate	golden brown	–	lanceolate	acuminate	clathrate	sparsely toothed
Drynaria propinqua	peltate	light brown	–	lanceolate	acuminate	clathrate	dentate
Drynaria quercifolia	peltate	blackish brown	–	linear lanceolate	long narrow caudate	clathrate	strongly dentate
Goniophlebium amoenum	peltate	brown	–	lanceolate, with tuft of hairs	acuminate	clathrate	denticulate
Goniophlebium argutum	peltate	blackish brown	–	lanceolate	acuminate	clathrate	ciliate
Goniophlebium lachnopus	peltate	dark brown	–	subulate	long caudate	clathrate	entire
Lepisorus contortus	basifixed	pale brown with narrow dark opaque central band	–	lanceolate	acuminate	clathrate	serrate
Lepisorus loriformis	basifixed	dark brown	–	ovate-lanceolate	long acuminate	clathrate	denticulate
Lepisorus mehrae	basifixed	pale brown	–	lanceolate	acuminate	clathrate	ciliate
Lepisorus normalis	peltate	golden brown	–	orbicular with tuft of hairs in the middle	–	clathrate	entire
Lepisorus nudus	basifixed	dark brown	–	ovate-lanceolate	shortly acuminate	clathrate	entire
Lepisorus rostratus	peltate	golden brown	–	lanceolate	acuminate	clathrate	entire
Lepisorus scolopendrium	peltate	light brown	–	lanceolate	acuminate	clathrate	entire
Lepisorus sublinearis	peltate	dark brown	–	lanceolate	long acuminate	clathrate	denticulate
Loxogramme porcata	basified	greyish brown	–	lanceolate	acuminate	clathrate	entire
Microsorum membranaceum	pseudopeltate	dark brown	–	ovate or triangular	acute	clathrate	entire
Microsorum punctatum	peltate/ pseudopeltate	dark brown	dimorphic	ovate or narrowly ovate or triangular	acute	clathrate	dentate
Phymatosorus cuspidatus	peltate	scales dark brown at center, paler toward margin	–	orbicular	–	clathrate	entire
Pichisermollodes ebenipes	peltate	dark blackish brown	–	ovate-lanceolate	acute	opaque	ciliate
Pichisermollodes nepalensis	peltate	rusty brown	–	ovate-lanceolate	acute	opaque	ciliate
Pichisermollodes stewartii	peltate	dark brown	–	lanceolate	acute	opaque	ciliate
Pyrrosia costata	basifixed	shiny brown	–	lanceolate	acuminate	clathrate	long toothed
Pyrrosia heteractis	peltate	light brown	dimorphic	lanceolate and ovate	acute	clathrate	entire
Pyrrosia lanceolata	peltate	light brown	dimorphic	lanceolate and ovate	acute	clathrate	ciliate
Pyrrosia mannii	pseudopeltate	straw-colored	–	lanceolate	long acuminate	clathrate	dentate
Pyrrosia nuda	peltate	light brown	–	lanceolate	acute	clathrate	ciliate
Selliguea griffithiana	peltate	brown	–	lanceolate	long acuminate	opaque	entire
Selliguea oxyloba	peltate	brown at center, paler toward margin	–	lanceolate	acuminate	opaque	ciliate

Polypodiales is an evolutionary advanced order of monilophytes (ferns), based on recent phylogenetic analysis (Smith et al., 2006; PPG I, 2016). In the present investigation, all the Polypodiaceae members have a dictyostelic arrangement with atleast five meristele in the rhizomes. Most members of the family possess a perforated dictyostele. According to Nayar (1970) Polypodiaceae is further grouped into five subfamilies. We have focused on species from diverse groups like the microsoroids to platycerioids and loxogrammoideae in our study. The exclusive presence of dictyostele in the rhizome and petiole of the derived fern lineage indicates that xylem structure is an outcome of strong selection for harsh conditions and drought resistance during the Cenozoic era (Pittermann et al., 2015). The dictyosteles in the eupolypods I and II comprise of more than two vascular strands with wider conduits and broad range of tracheid sizes leading to alternative pathways for water movement and evade embolism (Brodersen and McElrone, 2013). However, complexities occur in the steles of drynarioids and the platycerioids ferns (Ogura, 1972; Schmid, 1982; Srivastava, 2009).

The major differences have been observed among the taxa in terms of presence and position of the sclerenchymatous tissues. The number of sclerenchyma strands varies in most species, however the arrangement of the strands and their position in the parenchyma is a reliable taxonomic character (Hovenkamp, 1986). The position of the sclerenchyma is very distinct among the *Pyrrosia* species of the platycerioids clade. The sclereids nests appear to be like the vesiculose sclereid reported in some angiospermic species, the term was proposed by Rao and Bhupal (1973) to a simple lobed sac-like base form with uneven outline, thin or thickened cell wall with a broad lumen or a narrow lumen. The sclerenchymatic cells are loaded with a brown pigment, phlobaphene, which might have protective function in preventing the tissue from decay (Ogura, 1972). Authors over the years paid relatively lesser attention to the variations in the distribution of sclerenchyma tissues in the rhizome. The presence or absence of sclerenchyma strands is diagnostic for several species (Hovenkamp, 1998).

In our study, we observe that species like *Arthromeris wallichiana,*

Arthromeris himalovata, Arthromeris lehmanii, Drynaria quercifolia, Drynaria propinqua of Drynarioideae or more appropriately Crypsinoideae subfamily (Wei and Zhang, 2022) lack the usual sclerenchyma or sclerotic nests in the ground tissues of cortex prevalent in the species under subfamilies Microsoroideae, Polypodioideae, Platycerioideae. However, we observe that species of *Selliguea* and *Pichisermollodes* of Crypsinoideae subfamily possess the sclerenchyma strands in a scattered arrangement in the ground tissue. Rhizomes of *Phymatosorus cuspidatus* of Microsoroideae also lack the sclerotic nests while it is prominent in species of *Goniophlebium*. The rhizome of *Loxogramme porcata* lacks any sclerenchyma. The presence of sclereids nests is a distinct trait among epiphyte species with narrow rhizome while species with the larger rhizome lack sclereid nests (Hernandez et al., 2006; Hernandez-Hernandez et al., 2007; Jaimez et al., 2021). Species of *Arthromeris, Drynaria quercifolia, Drynaria propinqua, Phymatosorus cuspidatus* are usually epiphytic and as well as lithophytic with rhizomes of larger diameter. Rao and Srivastava (1974) too reported that sclerenchyma strands were not seen in rhizomes of *Arthromeris wallichiana.*

Hernandez-Hernandez et al. (2012) indicated an increased occurrence of the circumendodermal band (CB) among the most derived ferns. The degree of cell wall thickening of the CB is variable among the studied taxa. The taxonomic significance of the CB requires further studies to evaluate at species level (Hernández-Hernández et al., 2012). Our investigation reveals, Polypodiaceae species have a thick CB, whereas it is either non thickened or absent in species of *Goniophlebium* with *Lepisorus contortus, L. sublinearis, Loxogramme porcata, Microsorum membranaceum, M. punctatum, Pichisermollodes stewartii, P. nepalensis, Pyrrosia costata, P. heteractis, P. lanceolata* and *P. nuda*. This band encircles the xylem and phloem tissues, however, its functions have not been fully understood. Greater number of studies involving larger number of species is required to investigate the role and the presence of this structure. This waxy layer might provide structural rigidity (Pittermann et al., 2015). It might have protective function like endodermis or a biomechanical function as it was predominant in the epiphytic or lithophytic ferns growing in challenging environments. Structural adaptation of vascular plants leads to evolution of several specialized vascular and mechanical tissues (Leroux et al., 2018).

The rhizomes are usually invested with induments like trichomes, scales, or both as epidermal appendages in leptosporangiate ferns (Pryer et al., 1995). The shape, color, and size of these scales are highly variable. The nature of scales like clathrate, margins entire or not, shape of the apex and base as basifixed, pseudopeltate or peltate are commonly used as taxonomic traits in species delineation (Hovenkamp, 1986; Kramer et al., 1990; Yu and Lin, 1996; Zhang et al., 2003; Liu et al., 2008; Qi and Zhang, 2009; Qi et al., 2013; Mondal and Moktan, 2022). Clathrate scales of rhizomes have been considered conservative and phylogenetically informative (Christenhusz and Chase, 2014). The scales are very supportive in delineating species under *Lepisorus, Pyrrosia, Arthromeris, Pichisermollodes, Microsorum*. They are unique and very diagnostic for each member in our study. The clathrate scales of varying shape from lanceolate in most species to strictly orbicular in *Phymatosorus cuspidatus*, size and features which are very specific aids in easy identification of the species. The scales of most members are mostly persistent and this feature is also observed in the epiphytic family Davalliaceae and it is suggested that the stalked scales may play a role in protection against desiccation, water storage, also in absorption of nutrient material (Tsutsumi and Kato, 2008). The function of the scales, its physiology and evolution need to be further analyzed for better understanding of the epiphytic fern groups.

5. Conclusion

In the present communication, the comparative morpho-anatomical data on the rhizomes of *Arthromeris himalovata, Arthromeris lehmannii, Drynaria propinqua, Goniophlebium amoenum, Goniophlebium argutum, Goniophlebium lachnopus, Loxogramme porcata, Pichisermollodes ebenipes, Pichisermollodes stewartii, Pichisermollodes nepalensis* are reported for the first time and have proven to be an important taxonomic tool. The data alone or in combination can be used for easy identification and comprehend their shared characteristics as well as their dissimilarities. The anatomical observations like the diameter, stelar arrangement, meristeles, circumendodermal band, presence or absence of sclerenchyma tissues and sclereids nests aids in species identification and the characters are relevant in phylogenetic aspects too. It can be claimed that morphological and anatomical characters are still pertinent in fern taxonomy. The vasculature being one element that contributes immensely to the success of vascular plants. The functions of several cellular structures like the CB bands, relevance of sclerenchyma strands with respects to ecological and physiological significance of the fern needs to be carried out for better understanding of the ferns and draw any reasonable inferences about their evolution.

CRediT authorship contribution statement

Sinjini Mondal: Conceptualization, Visualization, Methodology, Writing – original draft, Writing – review & editing. **Saurav Moktan:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Implications of stipe and midrib morpho-anatomy on the taxonomy of Polypodiaceous ferns

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Abstract

After decades of uncertainty, the current fern classification corroborates traditional morphology with molecular evidence and is widely accepted. However, in the complex family Polypodiaceae, some major generic recircumscriptions are still open. The present study aims to provide a comprehensive account of the stipe and midrib morpho-anatomical features of polypodiaceous ferns from the eastern Himalayan region. Fresh specimens belonging to 24 species were collected from the study area. Macro-morphological features like colour, size, and texture were observed in the field with the aid of a dissection microscope while micro-morphological traits like scales, indument, and anatomical details were observed under a compound microscope. The type of tissues, arrangement of vascular bundles, the shape of xylem, and circumendodermal band seem to be of taxonomic significance. The UPGMA reveals comparable correlations with previously published clades and relationships based on morphological as well as molecular data.

Key words: circumendodermal band, dictyosteles, pericycle, Polypodiaceae, stelar patterns, systematics, vascular tissue

Introduction

In fern systematics, the investigation of morphological traits plays an integral role in the advancement of the study (Hernández-Hernández *et al.* 2006, Smith *et al.* 2006, Arana *et al.* 2014, Prada *et al.* 2016). The nomenclatural history of fern taxonomy starts with Linnaeus (1753), who first attempted to organize ferns based on the shape and position of sori. Over the years, morphological characters have been utilized in fern classifications by many (Bower 1928, Sporne 1962, Holtum 1972, Hennipman 1990) and certain features are still relevant in recent times, especially for characterizing monophyletic groups (Smith *et al.* 2008, Vicent *et al.* 2014, Testo *et al.* 2019). Macro-morphological characters such as rhizome type, scales, hairs, arrangement of sori, etc., and micro-morphological traits like venation pattern, indument type, spore ornamentation, and anatomy of stipe (or petiole) are crucial for fern taxonomy (Ogura 1972, Moran *et al.* 2007, Passarelli *et al.* 2010, Hernández-Hernández *et al.* 2012). Anatomical details serve as valuable sources of characters for fern systematics in testing phylogenetic hypotheses (Sundue *et al.* 2015, Wetzel *et al.* 2017). Stipe anatomical characters have taxonomic significance for the variations observed in the stelar patterns, its shape and presence of sclerenchyma cells, and various other features (Ogura 1972, Bidin & Anita 1995, Bidin & Masturi 1996, Talip *et al.* 2014, Wetzel *et al.* 2017, Palacios-Rios *et al.* 2019, Jaimez *et al.* 2021). In ferns, the number, arrangement, and configuration of xylem tissues of vascular strands in each leaf trace have proven to be taxonomically significant (Ogura 1972, Lin & DeVol, 1977, 1978). The petiole or stipe serves as a diagnostic character in many ferns, especially the number of vascular strands (Christenhusz & Chase 2014). The vascular architecture of petioles is relatively conserved and thus can be considered an effective approach to elucidating the phylogeny of ferns (Hacke & Sperry 2001). It also helps in comprehending the evolutionary aspects of ferns through the reconstruction of character states using anatomical characters of the petiole, the number of vascular strands, and the presence and configuration of circumendodermal band (CB) (Hernández-Hernández *et al.* 2012). However, the stipe or petiolar anatomical details have been less utilized as a source of phylogenetically significant characters (Schuettpelz & Pryer 2008, Hernández-Hernández *et al.* 2012). In recent times, works on the stipe anatomy of fern have been performed and yielded valuable

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information on stipe anatomical details of some Gleicheniaceae from Malaysia (Yen 2006), in species of *Davallia* (Talib *et al.* 2012), *Selaginella* (Maideen *et al.* 2013), *Blechnum* (Talip *et al.* 2014) to name a few. Other studies related to stipe features include some common ferns of Indonesia (Sofiyanti *et al.* 2019), *Pteris* from Central America (Palacios-Rios *et al.* 2019). Subsequently, variations in the shape of vascular bundles correlating with the presence of circumendodermal bands were investigated (Hernández-Hernández *et al.* 2012). Furthermore, the stipe anatomy in species of *Adiantum*, *Drynaria*, *Lygodium*, *Marsilea*, *Pityrogramma* and *Pteris* from southern India has also been investigated (Resmi *et al.* 2016).

Polypodiaceae is one of the most diverse and widely distributed families of ferns in tropical and subtropical forests (Holttum 1972, Hennipman 1990, Wei & Zhang 2022). The familial delimitation and infrafamilial classification of Polypodiaceae represents an age-old controversy in fern systematics and multiple studies suggest the need for clarification of some generic boundaries within the family (Schneider *et al.* 2004, Smith *et al.* 2006, Christenhusz *et al.* 2011, Almeida *et al.* 2014, PPG I 2016). Phylogenetic studies based on molecular as well as morphological data have solved some of the existing problems within polygrammoid ferns (Schneider *et al*. 2004, Schuettpelz & Pryer 2007, Kreier *et al.* 2008, Otto *et al.* 2009, Wang *et al.* 2010, Sundue *et al.* 2014, Testo *et al.* 2019, Zhao *et al.* 2020), but many issues remain, especially among the Asian groups. Major generic-level re-circumscriptions have been proposed in the family with a redefinition of *Polypodium*, *Pleopeltis,* and allied genera (Smith *et al.* 2008), but the generic boundaries between *Polypodium* and *Microsorum* are unclear and require clarification (Smith *et al.* 2006). The genera *Tricholepidium* and *Loxogramme* lack published generic revisions (Christenhusz *et al.* 2011), and a comprehensive analysis of the genus *Arthromeris* is unavailable; the exact delimitation from *Microsorum* requires further study (Schneider *et al.* 2004, PPG 1 2016). Taxonomic complexities in regional and global context in relation to the genus *Pyrrosia* still exists (Wei *et al.* 2017). The systematic position of the genus *Lepisorus* is complex (Wei & Zhao *2*019).

Major fern classifications of Smith *et al*. (2006), Christenhusz *et al*. (2011) and PPG I, (2016) complied with a broad definition of Polypodiaceae by consolidating several closely related groups like the loxogrammoids, grammitids, Drynariaceae, and Platyceriaceae. However, the infrafamilial classification is still associated with complexities. A remedy for obtaining a stable infrafamilial classification is by identifying a set of diagnostic combinations of morphological and molecular traits that are systematically potent (Wei & Zhao 2022).

Over the years, several studies on petiolar or stipe anatomical features have been conducted that have aided in understanding the phylogenetic aspects of fern systematics (Sundue & Rothfels 2014, Pittermann *et al.* 2015). A broader sampling across the fern phylogeny is needed to gain a more nuanced perspective on the evolution of xylem structure and function in ferns, particularly those in the eupolypod I and II lineages (Pittermann *et al.* 2015).

Therefore, the objective of the present study is to investigate the morpho-anatomical features of stipe and costae/midrib of some members of Polypodiaceae from the eastern Himalayan region of India. Stipe anatomical features can serve as a tool in species identification and description of ferns when the sori are unavailable or immature. The characters might help to decipher and define the existing natural groups in published phylogenies. The findings will also aid in further understanding the relationships and accurate taxonomic delineation of the complex taxa within this large family.

Materials and methods

Field collection of samples

The specimens were collected from natural habitats during field visits to Darjeeling, which lies amidst the eastern Himalayan biodiversity hotspot, during the year 2020–2021. Mature samples were freshly collected from the field, and place, date, and voucher number were noted (Appendix 1). Voucher specimens have been deposited at Calcutta University Herbarium (CUH). For anatomical sections of stipe, costae/midrib, mature specimens were considered.

The species of Polypodiaceae that have been considered in this study are *Arthromeris himalovata* Fraser-Jenk. & Kandel, *Arthromeris wallichiana* (Spreng.) Ching, *Drynaria propinqua* (Wall. ex Mett.) J.Sm. ex Bedd., *Drynaria quercifolia* (L.) J.Sm., *Goniophlebium argutum* (Wall. ex Hook.) J.Sm., *Lepisorus contortus* (Christ) Ching, *Lepisorus scolopendrium* (Ching) Mehra & Bir, *Lepisorus loriformis* (Wall. ex Mett.) Ching, *Lepisorus normalis* (D.Don) C.F.Zhao, R.Wei & X.C.Zhang, *Lepisorus rostratus* (Bedd.) C.F.Zhao, *Lepisorus sublinearis* (Baker ex Takeda) Ching R.Wei & X.C.Zhang, *Loxogramme involuta* (D.Don) C.Presl, *Microsorum membranaceum* (D.Don) Ching, *Microsorum punctatum* (L.) Copel, *Phymatosorus cuspidatus* (D.Don) Pic.Serm., *Pichisermollodes ebenipes* (Hook.)

Fraser-Jenk., *Pichisermollodes stewartii* (Bedd.) Fraser-Jenk., *Polypodiodes amoena* (Wall. ex Mett.) Ching, *Pyrrosia costata* (C.Presl ex Bedd.) Tagawa & K.Iwats, *Pyrrosia heteractis* (Mett. ex Kuhn) Ching, *Pyrrosia lanceolata* (L.) Farw., *Pyrrosia mannii* (Giesenh.) Ching, *Selliguea griffithiana* (Hook.) Fraser-Jenk., and *Selliguea oxyloba* (Wall. ex Kunze) Fraser-Jenk.

For correct identification, relevant literature of Mehra & Bir (1964), Ghosh *et al.* (2004), Fraser-Jenkins (2008), Kholia (2010), and Fraser-Jenkins *et al.* (2021) were used. Herbaria such as Lloyd Botanical Garden and Calcutta University Herbarium (CUH) were also consulted for proper identification. Correct nomenclature was maintained following Plants of the World Online (POWO 2022).

Preparation, examination, and description of samples

For all the selected samples, the transverse section of the stipe and costae/midrib was done. The sections were cleared with sodium hypochlorite (10%) and stained with safranine-alcian blue. After staining, the sections were gradually dehydrated with alcohol and mounted with xylol or Canada balsam on conventional slides (Ruzin 1999). The observations and photographs were taken with the aid of a stereomicroscope (Wild M3 Heerbrugg) and binocular microscope (Leitz Laborlux D). Transverse sections of the base and the mid region of stipe from mature fern specimen were considered for the anatomical studies and significant differences observed were noted.

Several characters were considered in stipe and costae: morphological traits like size, colour, indument, and anatomical features, like the shape of the section, shape of vascular bundles, and type of tissues. The descriptions of the structures were made following the terminology of Sen & Mittra (1966), Ogura (1972), Sen & Hennipman (1981), Hovenkamp (1988), and Martínez &Vilte (2012).

Data analysis

The diagnostic characters for the fern stipe were assembled. Qualitative and quantitative traits of stipe, midrib, and scale were noted and scored for multivariate analysis. All the data sets were subjected to hierarchical clustering through UPGMA algorithm using PAST 4.03 (Hammer *et al.* 2001). A dendrogram based on Bray-Curtis degree of similarity was constructed.

Results

Stipe

The members of the family Polypodiaceae are usually epiphytic to lithophytic with rhizomes varying from long-creeping to short tufts covered with scales. The stipe of the studied species is articulate to a short phyllopodium, attached to the rhizome. Scales are often present towards the stipe base. The colour of the stipe varies from green, stramineous, various hues of brown to purple. Taxa like *Arthromeris wallichiana*, *Drynaria quercifolia*, *Pichisermollodes ebenipes*, *P. stewartii*, *Polypodiodes amoena* have long stipes whereas in species of *Lepisorus,* the stipes were short and in *Loxogramme involuta,* a very short and indistinct stipe is present. Species of *Drynaria have* long creeping rhizomes with brown lanceolate scales The stipes of the studied species vary from winged to terete, and scaly or glabrous. Prominent stipe wings were observed in *Drynaria quercifolia*, *Microsorum membranaceum*, *M. punctatum,* and *Pyrrosia costata.* Most of the species possess a glabrous stipe while in *Pichisermollodes ebenipes* and *Pyrrosia costata,* the stipes are scaly.

In *Goniophlebium argutum,* the stipes are distant on the rhizome, purplish, thick, glossy with sparse light brown hairs. In species of *Lepisorus,* stipes are articulated to the rhizome, approximate or distantly placed, generally short, often winged due to the decurrent lamina base, glabrous, or with deciduous scales. The surface is mostly smooth, scaly at the base and glabrous above in *Goniophlebium argutum* and *Polypodiodes amoena*. Stipes in species of *Pyrrosia* are scaly at the basal region. *Pyrrosia costata* has tomentose hairs only towards the upper part of the stipe while *P. heteractis*, *P. mannii,* and *P. lanceolata* are covered with hair throughout the stipe.

In general, the scales in rhizome and stipe bases are mostly peltate to pseudopeltate, clathrate, opaque, or hyaline with margin entire, toothed, dentate etc. However, they have a significant variation in shape, size, and colour. The scales in *Arthromeris himalovata* and *A. wallichiana* are golden brown, linear to lanceolate while it is pale brown in *Drynaria propinqua* to deep brown, peltate to pseudopeltate in *D. quercifolia*.

Bicolorous scales being dark brown at the center, pale brown towards the margin, and lanceolate in shape are observed in *Lepisorus scolopendrium* while greyish-brown, hollow scales, triangular in shape are observed in *Loxogramme involuta*. The shape of scales is almost circular or orbicular in *Phymatosorus cuspidatus* and distinct from others.

The cross section of the stipe shows an epidermal layer often with a cuticle, followed by 5-12 layers of hypodermis (Fig. 1). Below it is a cortex of 5–10 layers of parenchymatous tissue with several vascular bundles (VBs) scattered in U, V, or inverted **Ω** arrangement. The inverted **Ω** arrangement is observed in *Pyrrosia costata* and *P. heteractis* (Fig. 2). The two adaxial VBs are larger with hooked xylem strands while the other surrounding VBs are smaller with C-shaped xylem strands surrounded by phloem tissue. The U shaped arrangement is observed in *A. wallichiana*, *A. himalovata*, *D. propinqua*, *D. quercifolia*, *G. argutum*, *L. contortus*, *L. loriformis*, *L. sublinearis*, *M. membranaceum*, *M. punctatum*, *P. cuspidatus*, *P. amoena*, *P. stewartii*, *P. ebenipes*, *S. griffithiana*, *S. oxyloba*, *P. mannii*, while the V shaped arrangement is noted in *L. normalis*, *L. scolopendrium* and *P. lanceolata*.

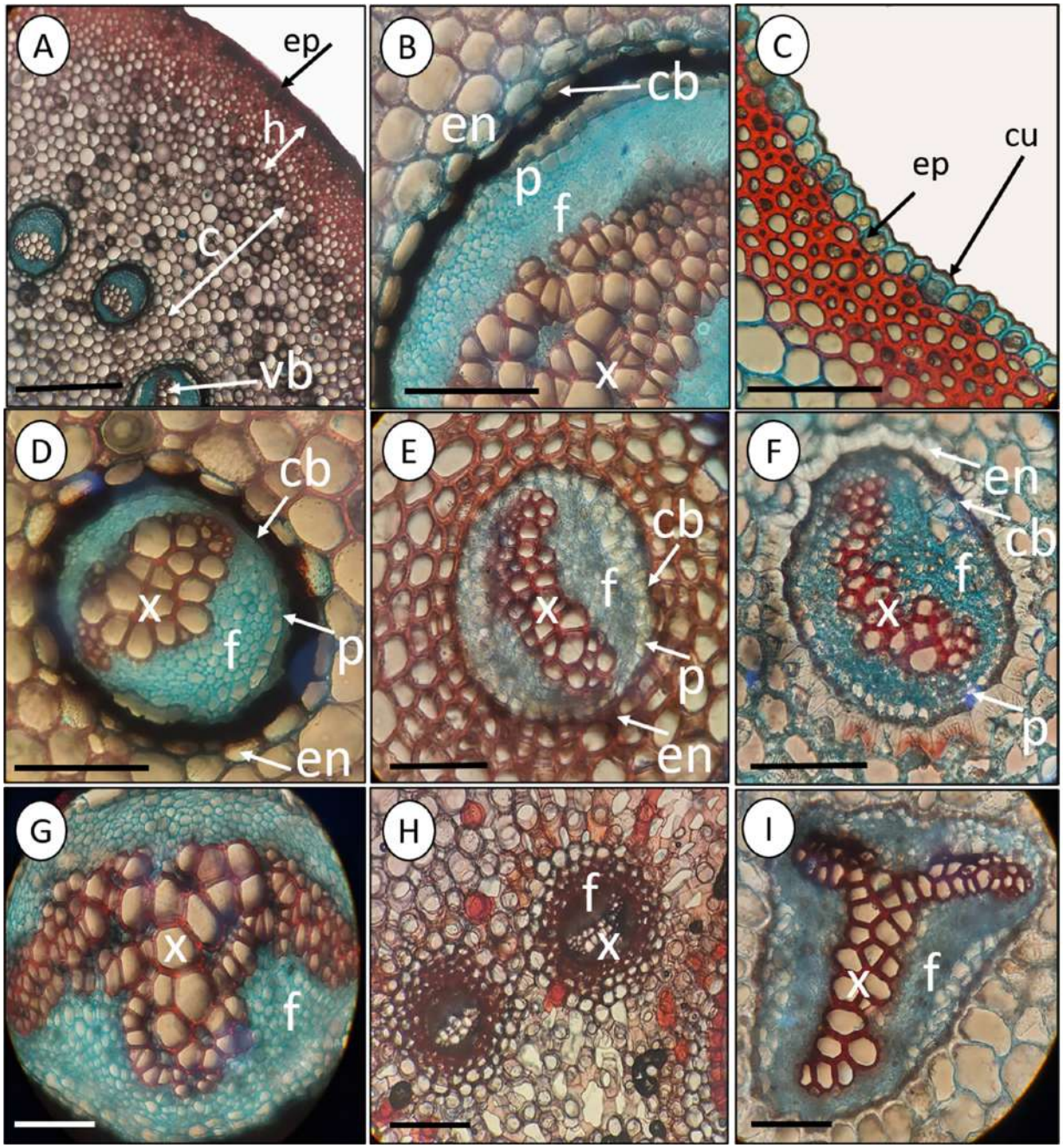

FIGURE 1. Anatomical details of studied Polypodiaceae. **A:** T.S. of stipe of *A. wallichiana* **B:** Enlarged view in cross section **C:** Portion of midrib in *P. heteractis* showing prominent cuticle **D – F:** VB of stipe in *A. wallichiana, L. involuta, P. heteractis* **G – I:** Enlarged view of midrib section in *A. wallichiana, L. involuta. P. heteractis,* with indications of xylem (x), phloem (f), endodermis (en), pericycle (p), cortex (c), hypodermis (h), epidermis (ep), circumendodermal band (cb), vascular bundle (vb), cuticle (cu). Bar = 200 μm.

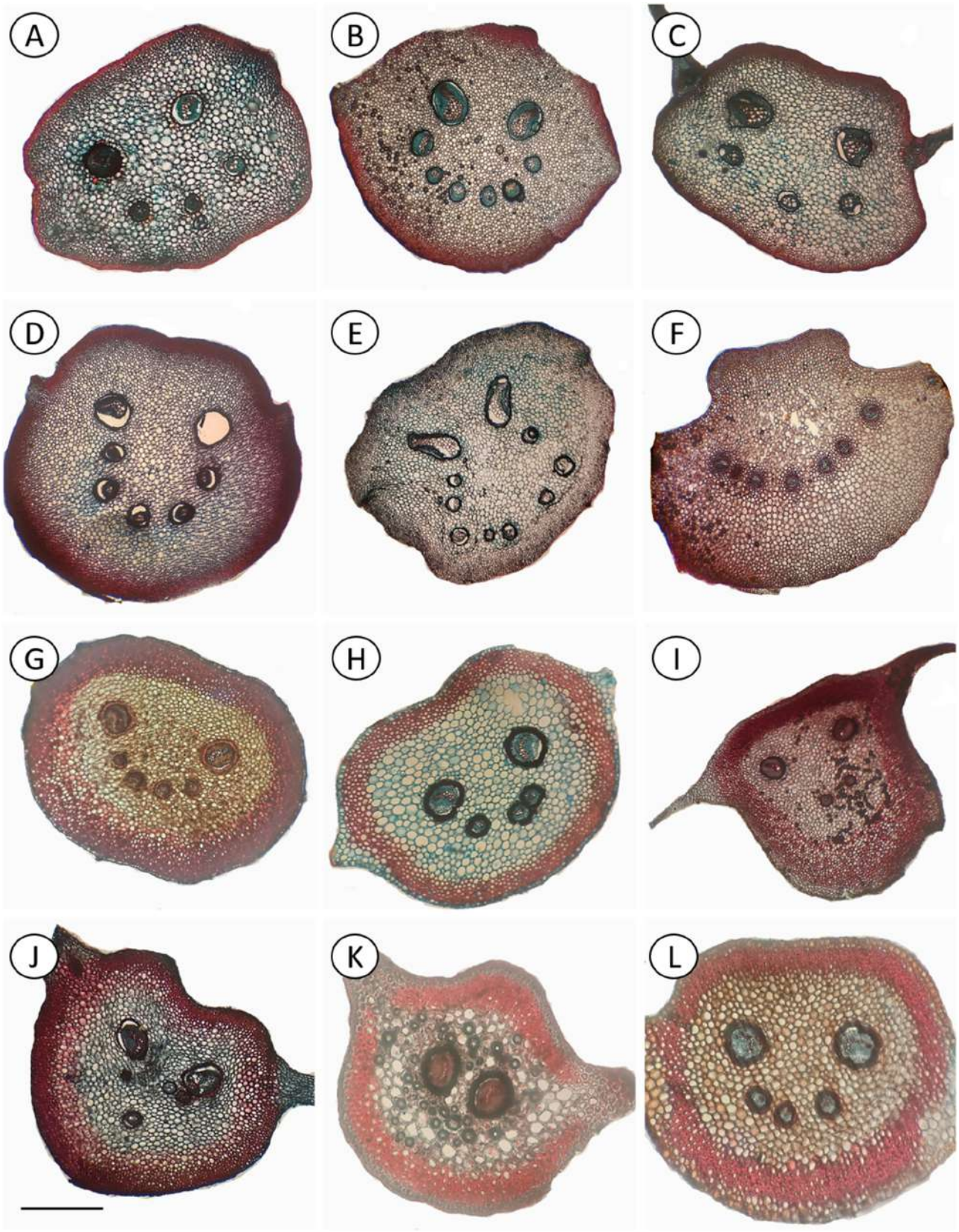

FIGURE 2. T.S. of stipe. **A:** *A. wallichiana* **B:** *A. himalovata* **C:** *D. propinqua* **D:***D. quercifolia* **E:** *G. argutum* **F:** *L. involuta* **G:** *L. contortus* **H:** *L. loriformis* **I:** *L. normalis* **J:** *L. scolopendrium* **K:** *L. rostratus;* **L:** *L. sublinearis.* Bar = 3 mm in A, B, C, D; 1 mm in E, H, I, J, L; 2 mm in F; 0.5 mm in G, K.

The stipe in cross-section was mostly oval or round in *Phymatosorus cuspidatus*, *Polypodiodes amoena*, and *Pichisermollodes ebenipes*, but heart-shaped in *Lepisorus loriformis*, *L. rostratus*, *L. scolopendrium*, *L. sublinearis, Microsorum membranaceum*, *M. punctatum*, *Pyrrosia costata,* and *P. lanceolata*. In *Loxogramme involuta,* the stipe is fan-shaped in cross-section, which sets it apart from other members in this study (Fig. 2F, 3).

The adaxial face of the stipe is usually terete or with a median groove. The vascular bundles of the studied taxa have circumendodermal band (CB). The CB is sclerenchymatic with a distinct colour that surrounds vascular bundles followed by endodermis. A heavily lignified endodermis is surrounded by the pericycle sometimes more than a single layer of large cells which lies in the phloem. The xylem consists of a metaxylem and a protoxylem (Fig. 1).

In *Arthromeris wallichiana* and *A. himalovata,* the stipe has a single-layered epidermis surrounding 2–8 layers of sclerenchymatous tissue and parenchymatous inner cortex (Fig. 2). There are three main and a varying number of lateral vascular bundles. The lateral bundle is composed of an exarch xylem that is made up of 10–15 and 20–30 tracheids in *A. himalovata* and *A. wallichiana,* respectively. The other median vascular bundles have diarch xylem. The endodermis and circumendodermal band (CB) are highly lignified, followed by 1–4 layers of pericycle with phloem surrounding the xylem except at the protoxylem points. In *A. himalovata,* around 5–6 vascular bundles and in *A. wallichiana,* around 8–15 vascular bundles are arranged in U- shape (Fig. 2 A–B).

The transverse section of the mid portion of the stipe in *Drynaria quercifolia* shows an ovate outline and is winged towards the base, while it is circular and slightly winged in *Drynaria propinqua*. The single epidermal layer is followed by sclerenchymatic outer cortex and inner cortical parenchyma with 5–15 vascular bundles arranged in U-shape, opening towards the adaxial surface of the stipe with two main kidney-shaped VBs with hooked xylem strands.

Midrib and Costae

Lamina dissection ranges from entire, lobed to pinnatifid and costate, or simply pinnate among various taxa. The cross-section of the midrib and costae has been studied in the species with simple and complex lamina respectively.

The costae/midribs are green and stramineous in *Loxogramme involuta*. The diameter of the costae varies from *ca.* 0.2–0.3 mm in *Goniophlebium argutum* and *Polypodoides amoena* to 5–15 mm in *Pyrrosia costata* and *Loxogramme involuta*. Although the intraspecific variation occurs due to age and level of maturity of the fronds, specimen at a similar stage of maturity has been considered in our study.

The transverse section of the midrib or costae is round in most species (Fig. 4,5). Midrib of *Loxogramme involuta* is raised adaxially and flat abaxially. The transverse section of the midrib in *Lepisorus normalis* is triangular shaped (Fig. 4I). In *Microsorum membranaceum*, *Selliguea griffithiana,* and *Pyrrosia lanceolata,* the abaxial surface appears to be grooved while in *Pyrrosia costata*, *P. heteractis,* and *P. mannii,* it appears flat abaxially and raised adaxially.

A transverse section of the frond in the apical portion of the lamina shows that the midrib has a T-shaped xylem in the centre and heavily lignified endodermis (Fig. 5). This T shape arrangement of the xylem varies in each species. For instance, it is short and thick in *Arthromeris wallichiana* and thinner and elongated in *Pyrrosia heteractis.* Similarly, in *Selliguea oxyloba* and *Pichisermollodes ebenipes,* the T shape almost appears to be X in shape (Fig. 5 F, H). The xylem tissue is non-hippocampiform, T-shaped, surrounded by a compactly arranged phloem with a layer of upper and lowers epidermis. The upper epidermis is thick and is accompanied by a hypodermis composed of compactly arranged cells, while the inner cortical parenchyma is made up of loosely arranged cells. The dendrogram obtained from UPGMA clustering based on the similarity matrix (Fig. 6) shows close relationships between *A. wallichiana*, *S. griffithiana* and *A. himalovata* (> 0.9); *G. argutum* and *P. amoena* (> 0.9); *P. ebenipes* and *S. oxyloba* (≥ 0.9). The two species of *Drynaria* grouped more closely with the species of *Arthromeris*, *Selliguea,* and *Pichisermollodes*. *Pyrrosia mannii* and *P. lanceolata* show lesser similarity with *P. costata* and *P. heteractis*. Among the *Lepisorus* species, *L. normalis* and *L. loriformis* share more (> 0.9) similarity while *L. contortus*, *L. rostratus,* and *L. sublinearis* are closer to each other. *Loxogramme involuta* was chosen as outgroup because the short stipe having a distinct fan shaped cross section and fan-shaped xylem strand arrangement differs from the rest of the Polypodiaceae members, which exhibit T-shaped xylem strands.

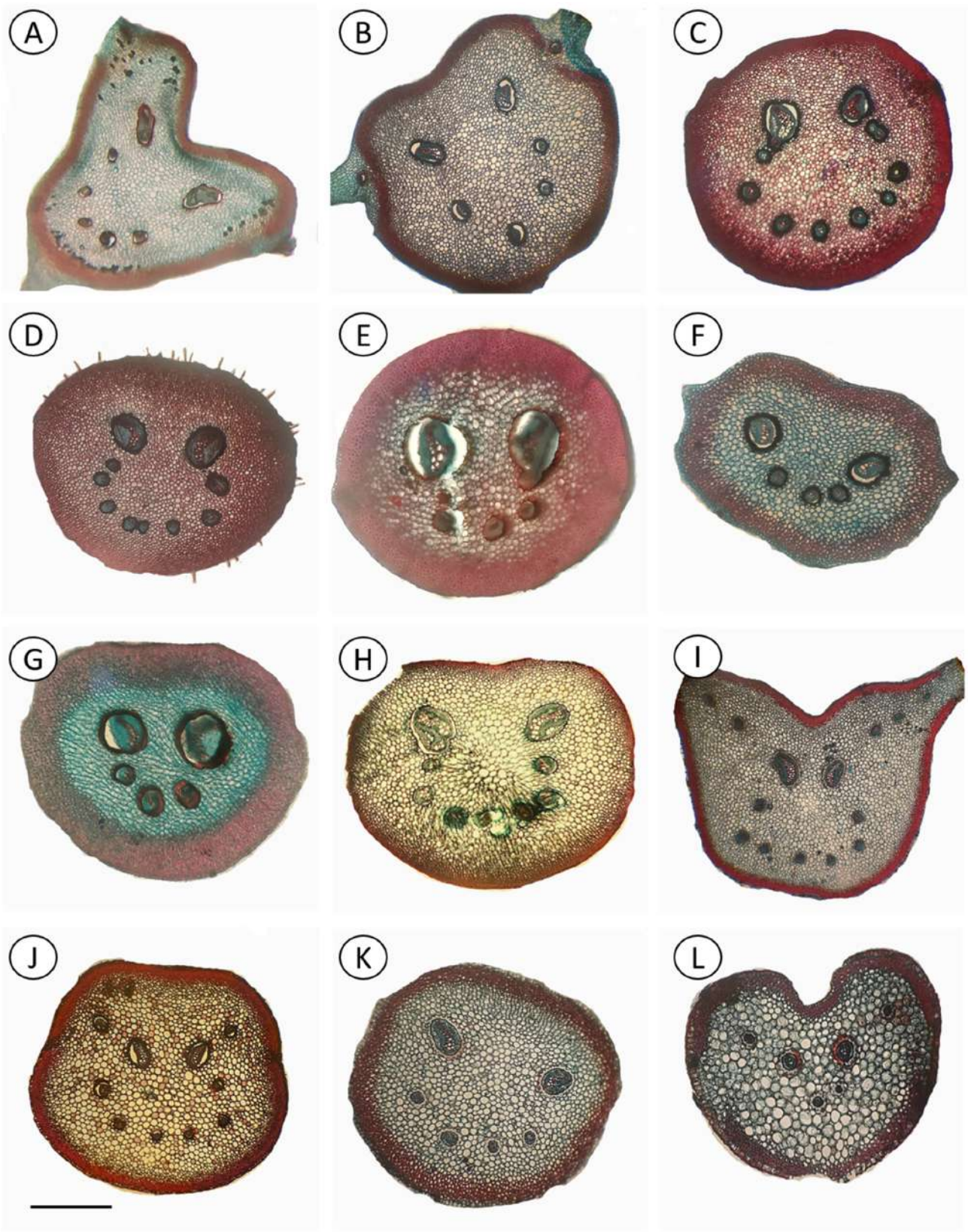

FIGURE 3. T.S. of stipe. **A:** *M. membranaceum* **B:** *M. punctatum* **C:** *P. cuspidatus* **D:** *P. amoena* **E:** *P. stewartii* **F:** *P. ebenipes* **G:** *S. griffithiana* **H:** *S. oxyloba* **I:** *P. costata*; **J:** *P. heteractis* **K:** *P. mannii* **L:** *P. lanceolata.* Bar = 3 mm in A, B, C, I; 1 mm in D, E, H, J, K; 0.5 mm in L.

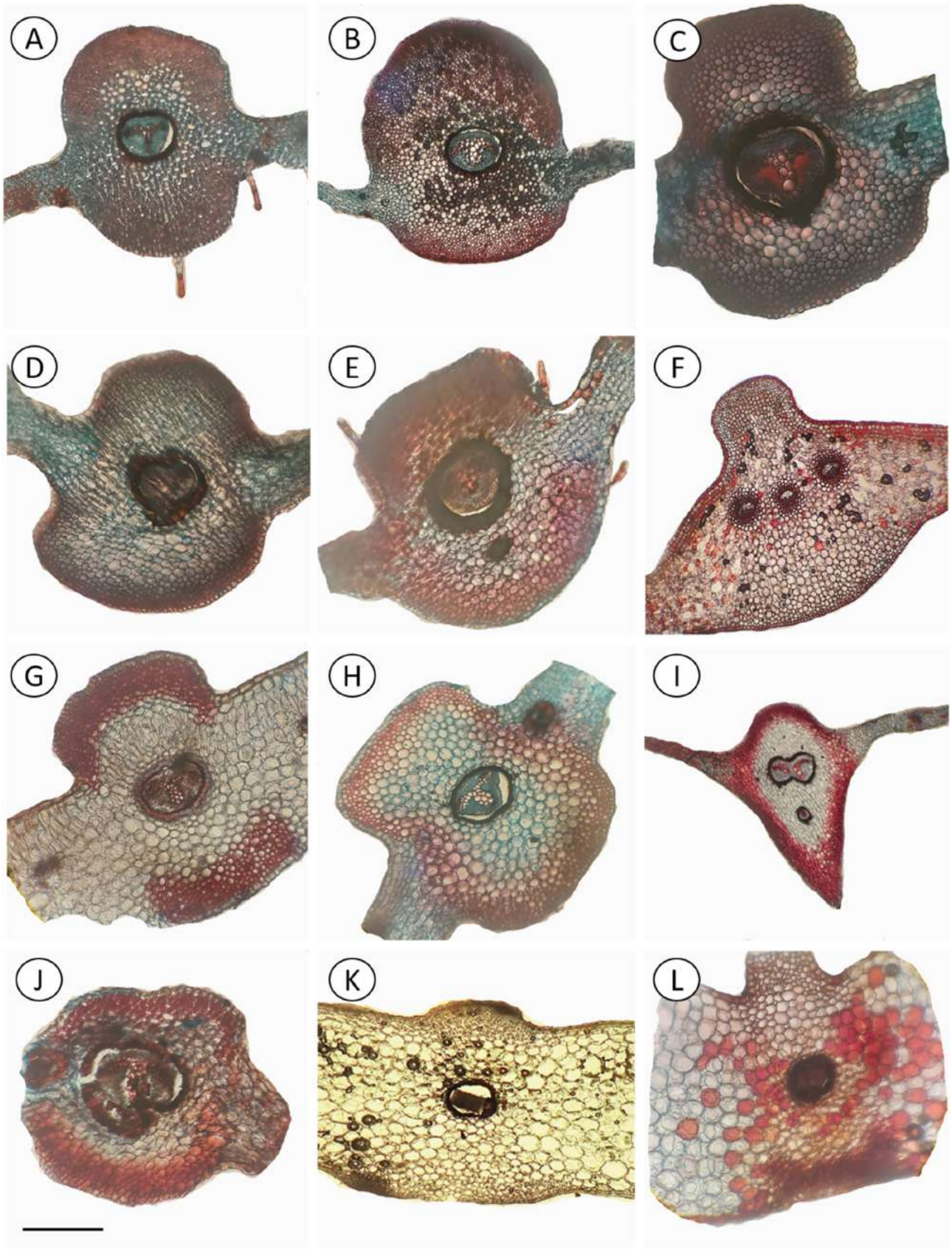

FIGURE 4. T.S. of midrib. **A:** *A. wallichiana* **B:** *A. himalovata* **C:** *D. propinqua* **D:** *D. quercifolia* **E:** *G. argutum* **F:** *L. involuta* **G:** *L. contortus* **H:** *L . loriformis* **I:** *L. normalis* **J:** *L. scolopendrium* **K:** *L. rostratus* **L:** *L. sublinearis.* Bar = 2 mm in A, B, C, D, F; 1 mm in H, I, J, L 0.5 mm in E, G, K.

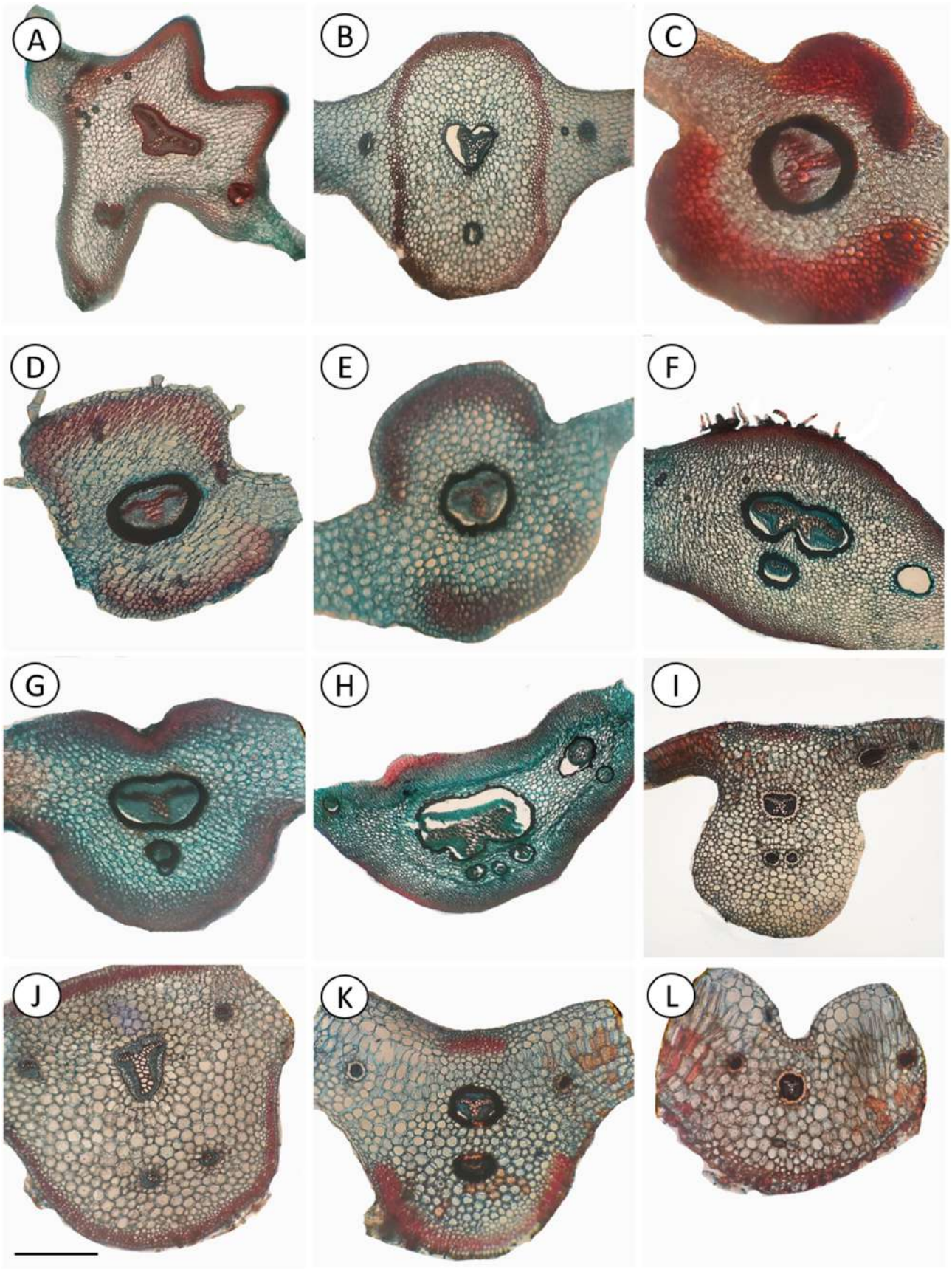

FIGURE 5. T.S. of midrib. **A:** *M. membranaceum* **B:** *M. punctatum* **C:** *P. cuspidatus* **D:** *P. amoena* **E:** *P. stewartii* **F:** *P. ebenipes;* **G:** *S. griffithiana* **H:** *S. oxyloba* **I:** *P. costata* **J:** *P. heteractis* **K:** *P. mannii* **L:** *P. lanceolata.* Bar = 3 mm in A, B, C, I; 1 mm in F, G, H, J, K; 0.5 mm in D, E, L.

Discussion

The anatomical details of the vegetative organs in plants are taxonomically valuable and have aided in species delineation at different hierarchical levels (Rio *et al.* 2005, Lens *et al.* 2008, 2009, Carvalho *et al.* 2017). Stipe anatomical features were established to be a powerful tool for the systematic study of ferns because of the variations observed in the shape of stele and the presence of special tissues like sclerenchyma (Ogura 1972, White 1984, Bidin & Anita 1995, Masturi 1996). In this work, the anatomical investigation of the stipe, midrib, or the costae in 24 Polypodiaceous ferns was carried out because available detailed observations of stipe or petiolar features of this group has been scanty (Hovenkamp 1986, 1998).

We intend to contribute novel information in fern systematics for all the studied taxa which adds to some previously known data on some of the genera. Reconstruction of petiole anatomical characters like the number of vascular strands, the presence and configuration of the circumendodermal band (CB), and several other features improve our understanding of fern evolution (Hernández-Hernández *et al.* 2012).

Hacke and Sperry (2001) reported that the dictyostelic vascular architecture in the stipe was conserved through the species belonging to eupolypods I. The taxa grouped under eupolypods I possess a dictyostelic structure with several vascular bundles scattered, generally two large ones on the adaxial portion and one large vascular bundle on the abaxial portion with smaller bundles on the side (Tan *et al.* 2020). In our study, the results indicates that all 24 species have a dictyostelic nature. Only in *Lepisorus rostratus* we observe two large vascular bundles in the adaxial region, which can be interpreted as a primitive trait. The presence of sectored dictyosteles with at least two vascular strands in the petioles of more derived fern lineages indicate that xylem structure faced strong selection pressure for drought resistance during the Cenozoic era (Pittermann *et al.* 2015). Greater number of xylem strands and broad ranges in tracheid sizes would provide alternative pathways for water movement and help prevent embolism (Wheeler *et al.* 2005, Christman *et al.* 2009, Brodersen *et al.* 2012). For subtle conclusions on the evolution of xylem structure and function in ferns, broader sampling covering the fern phylogeny is required (Pittermann *et al.* 2015).

All our studied taxa show the presence of a prominent circumendodermal band (CB). The CB has been reported previously for species of six families in the eupolypods I (Hernández-Hernández *et al.* 2012) and the Polypodiaceae is among them (PPG I, 2016). The degree of cell wall thickening of the circumendodermal band (CB) is variable among the studied species and might be specific for certain species or at least species groups. The taxonomic significance of the CB requires further evaluation at the species level (Hernández-Hernández *et al.* 2012). The function of the CB is still unresolved, however, possibilities of it having a protective function like an endodermis or having a biomechanical function have been suggested (Hernández-Hernández *et al.* 2012), as it was predominant in epiphytic or lithophytic ferns. These are stressful habitats as the plants are more exposed to wind or falling rocks, respectively. Polypodiaceous ferns are mostly epiphytic with smaller to mid-sized, simple or pinnatisect (only rarely more divided) leaves, which are exposed to lower bending shear forces. Therefore, the presence of CB may also be interpreted as evidence of the biomechanical function as reported by Hernández-Hernández *et al.* (2012).

The different anatomical characters observed in this study (i.e., and number of vascular bundles, shape, xylem type, presence of CB and the presence or absence of adaxial grooves) are similar to the observations reported earlier for petiole and stipe (Hernández-Hernández *et al.* 2012, Noraini *et al.* 2012, 2014, Palacios-Rios *et al.* 2019; Tan *et al.* 2020).

The C-shaped xylem in the vascular bundles was observed in only few species of Polypodiaceae (Tan *et al.* 2020) but was prominent in our set of species except *Loxogramme involuta*. The C-shaped vascular bundle character in both Nephrolepidaceae and Polypodiaceae has been reported earlier by Kramer (1990). This strengthens the relationship of Nephrolepidaceae and Polypodiaceae as possible sister families (Kuo *et al.* 2011, Lehtonen 2011, Liu *et al.* 2013, Tan *et al.* 2020).

In species with rigid stipes, the cortex may be partially sclerenchymatous by forming hypodermal sclerenchyma (Parihar 1965), which Ogura (1972) found only in erect or sub-erect species and while it was in the creeping ones. The hypodermal sclerenchyma was present in our examined species which were also erect in nature. A T-shaped xylem in the strand midrib is a characteristic trait of the family Polypodiaceae (Rao 1973), and was generally present in the studied species. The length, breadth, and thickness of the xylem in its T shape varies among the species: in *Arthromeris,* the T-shaped xylem strand is thick and shorter in length whereas in *Pyrrosia mannii* it is much longer. In other species like *Lepisorus normalis*, *Pichisermollodes ebenipes*, *Selliguea oxyloba,* and *Pyrrosia costata,* the xylem strands tend to take an X shape. Adaxial grooves are observed in *P. mannii*, *P. lanceolata* and *L. involuta*. Adaxial grooves are significant features in ferns used to describe and delineate taxa by several authors (Stockey *et al.* 1999, Kramer 1990, Regalado *et al.* 2018).

The presence of a distinct well-developed cuticle on the epidermis is observed in the midrib of *P. heteractis* and *P. lanceolata.* Some species of *Pyrrosia* s.l. are known to be drought tolerant and morphological specialisations such the coriaceous, thick, succulent texture of the lamina and the presence of a thick cuticle on the epidermis, sunken stomata and sori are some of the xerophytic adaptations (Wei *et al.* 2017). From our field survey it was observed that the occurrence of *Pyrrosia* mostly in the warm tropical, subtropical to utmost subtemperate forest zones between 400 – 1600m asl with abundant sun exposure may attribute to the presence of thick cuticle. However, further investigation is needed for definitive conclusion in this perspective. *Pyrrosia* species are reported to have two distinct growth forms for drought resistence, poikilohydrous and succulent forms. Poikilohydrous forms have short rhizomes, stelar induments forming dense mat, thin hypodermis, and their fronds roll and stretch in response to water shortages in dry seasons. Succulent plants of *Pyrrosia* possess long-creeping rhizomes, thick adaxial epidermis and sunken stomata (Hovenkamp 1986, Wei *et al.* 2017). In our study, *P. heteractis* and *P. lanceolata* can be categorised as succulent species whereas *P. costata* and *P.mannii* are poikilohydrous species as they exhibit similar morpho-anatomical features as discussed above. The cuticle seems to be prominent in case of the succulent species.

Leptosporangiate ferns were classified based on sorus position and the presence or absence of an indusium into four broad groups (Lloyd 1971). The gymnogrammoid ferns have marginal sori with modified leaf margin as false indusium whereas Polypodioid ferns bear sori on the abaxial side of the frond and lack an indusium. However, the position of genus *Loxogramme* has turned out to be complex due to its sporangial structure. They possess one-row sporangial stalk only at the base which is similar to that of grammatid ferns (=Polypodiaceae *pro parte*) ferns (Wilson 1959). The position of this genus has been subjected to frequent alterations. According to Christensen (1938), it was "distinct genus of very doubtful relationship" with closer relations to *Grammitis*. Nayar (1955) suggested that *Loxogramme* be placed under Pteridaceae. Recent classifications based on molecular phylogeny place the genus under subfamily Loxogrammoideae within Polypodiaceae (PPG I 2016, Wei & Zhang 2022). The traits of genus *Loxogramme* is a blend between gymnogrammoid and polypodiaceous ferns on account of adherent fronds, spores either globose and trilete or oblong and monolete but stipe vascular structure like the general vasculature pattern of the Polypodiaceae (Nayar 1955). Our findings suggest that the shape of the xylem strands observed in *Loxogramme involuta* is unique from the rest of the Polypodiaceae. The genus *Loxogramme* may have evolved as an offshoot in the line of descent of the Polypodiaceae from Davalliaceae (Srivastava *et al.* 2007).

Pittermann *et al*. (2015) mapped the petiole type onto the fern phylogeny proposed by Smith *et al.* (2006), where Polypodiaceae and Davalliaceae are seen to be closely related, which is mirrored in the shared dictyostelic stelar organisation with two major vascular bundles. The dendrogram obtained in our study shows that *Loxogramme involuta* is out grouped from the rest of the Polypodiaceae members. This information on stipe and midrib characters on similar species has been compared with respect to previously published clades resulting from molecular phylogenetic study by Wei & Zhang *et al.* (2022). *Loxogramme involuta* as an outgroup and its position as an outlier among Polypodiaceae due to its unique anatomy is not unexpected. Molecular phylogenetic studies consistently retrieve *Loxogramme* as sister to the rest of the Polypodiaceae (Schneider *et al.* 2004, Kreier & Schneider 2006, Wei *et al.* 2021, Wei & Zhang 2022).

Furthermore, the studied species of *Arthromeris*, *Drynaria, Pichisermollodes,* and *Selliguea* share >0.8 similarity (Fig. 6), which is in accordance with earlier classifications (Smith *et al*. 2006, PPG I 2016, Wei & Zhang 2022), where they were together with *Synammia* in the subfamily Crypsinoideae. However, the lack of resolution between the taxa of *Selliguea, Arthromeris* and *Pichisermollodes* would either suggest a redefinition of the genera or the more inclusive concept of *Selliguea* for all these taxa as proposed by Wei & Zhang (2022).

Wei & Zhang (2022) further treated *Phymatosorus*, *Microsorum,* and some *Lepisorus* under the subfamily Microsoroideae. Our dendrogram puts *Lepisorus loriformis* and *L. normalis* in closer affinity with *Microsorum membranaceum* and *M. punctatum*. However, all *Lepisorus* and *Phymatosorus* are resolved as a polyphylum (Fig. 6). *Goniophlebium argutum* and *Polypodoides amoena* reveal more than 98% similarity between them, which is interesting as *Polypodoides amoena* was previously carried under *Goniophlebium* (Ching 1978).

Advances in molecular phylogenetic studies have contributed to a better understanding of the highly diverse and complex family Polypodiaceae and in resolving the perplexing relationship between the genera and species. The taxonomic significance of micromorphological and detailed anatomical traits in species delineation is immense. Stipe characteristics, including the shape of the outline, thickness of sclerenchyma, parenchyma, the number and arrangement of vascular bundles, and shape and size of the vascular bundle in combination with others were found to be reliable enough to distinguish species.

The stipe and midrib morpho-anatomical characters like shape, stelar arrangement, the thickness of sclerenchyma layers, and xylem arrangement can be used in combination with other diagnostic characters. Details of stelar arrangement

in rhizomes, venation pattern, and spore ornamentation have served as taxonomically valuable parameters (de la Sota 1973, van Uffelen & Hennipman 1985, van Uffelen 1997, Tejero-Diez *et al.* 2010, Chen *et al.* 2021). Parallel evolution and homoplasy in morphology have been noted in Polypodiaceae (Hovenkamp 1996, Schneider *et al.* 2009). However, identifying a set of diagnostic characters of taxonomic significance will help in better understanding of the interrelationships among taxa and infrafamilial classifications.

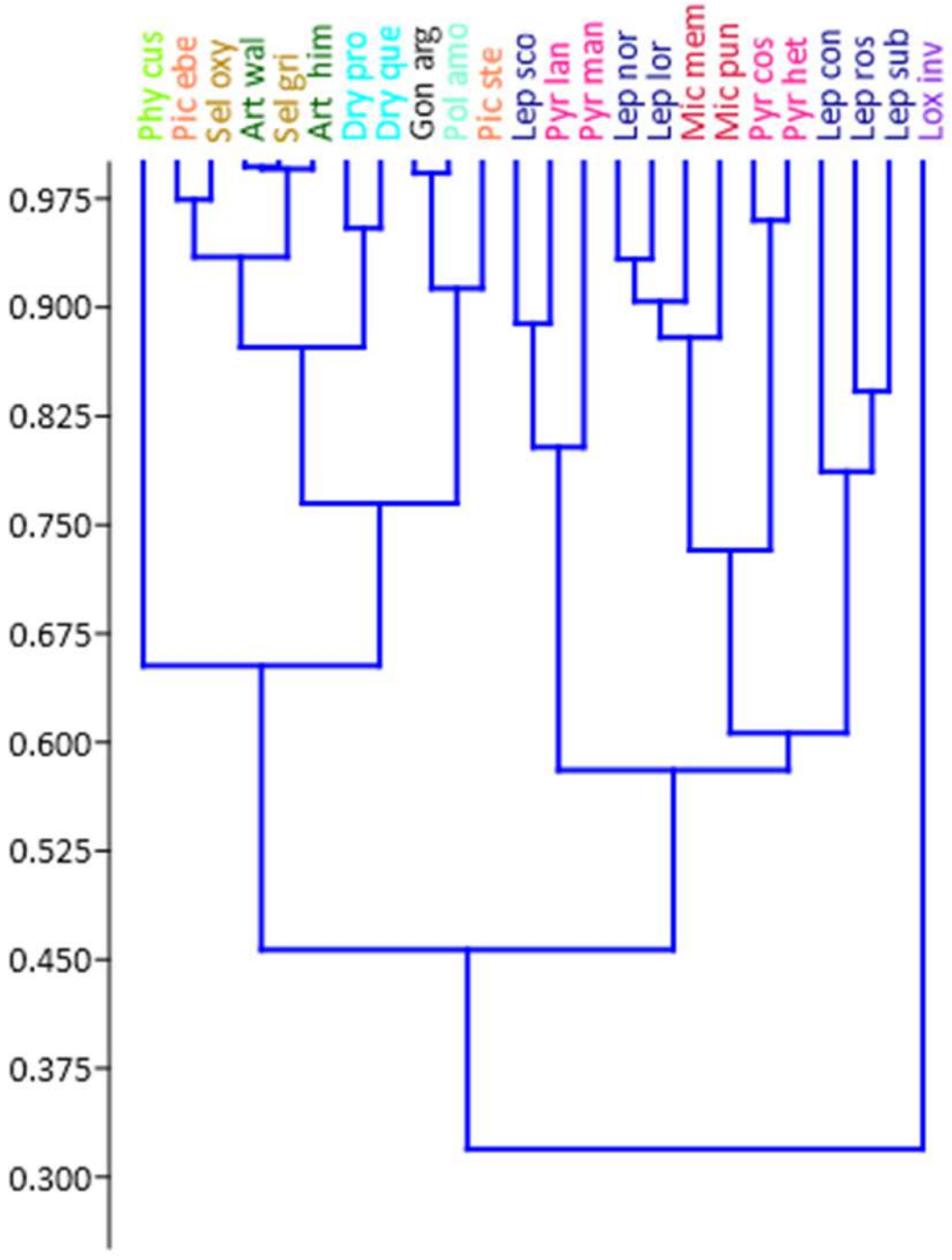

FIGURE 6. UPGMA tree of the studied taxa based on Bray-Curtis similarity coefficient

TABLE 1. Summary of morpho-anatomical details of stipe and midrib in species examined.

Taxa	Stipe colour	Stipe length (cm)	Rhizome scale morphology	Stipe features	Xylem type	Arrangement of VBs	Shape of stipe in TS	Shape of midrib in TS	Shape of xylem strand in VBs
Arthromeris himalovata	dark stramineous	≥15	ferruginous, linear-lanceolate	glabrous	exarch/ diarch	U	oval	oval	T
A. wallichiana	stramineous	20–40	golden- brown, lanceolate	glabrous	exarch/ diarch	U	oval	oval	T
Drynaria propinqua	brown	10–20	pale, appressed, margin dentate	glabrous, slightly winged	exarch/ diarch	U	oval	oval	T
D. quercifolia	light brown	≤ 30	deep brown, linear, pseudopeltate or peltate toward apex, strongly dentate	glabrous, shortly winged	exarch/ diarch	U	oval	oval	T
Goniophlebium argutum	stipe purplish	6–13	dark brown, peltate, ovate, ciliate at margin, acuminate	scaly at base glabrous above	exarch/ diarch	U	oval	oval	T
Lepisorus contortus	stramineous	2–2.5	brown, peltate, lanceolate,	glabrous	diarch	U	oval	oval	T
L. scolopendrium	light green	0.5–1	bicolorous,dark brown at the center, pale-brown towards margin, lanceolate	sparsly scaly	diarch	V	heart shaped	oval	T
L. loriformis	stipe palish green,	2–4	brown, ovate-lanceolate	glabrous, lamina decurrent.	diarch	U	heart shaped	oval	T
L. normalis	straw coloured	2–5	brown, orbicular with tuft of hairs	stipe thick, short	diarch	V	oval	triangular	T
L. rostratus	straw coloured	1–3	brown, subulate-lanceolate	glabrous	diarch	U	heart shaped	straight	T
L. sublinearis	light green	1–5	brown, lanceolate		diarch	U	heart shaped	straight	T
Loxogramme involuta	greenish brown	very short	brown with whitish- yellow, ovate	glabrous, winged	diarch	U	fan shaped	triangular	Fan

...continued on the next page

TABLE 1. (Continued)

Taxa	Stipe colour	Stipe length (cm)	Rhizome scale morphology	Stipe features	Xylem type	Arrangement of VBs	Shape of stipe in TS	Shape of midrib in TS	Shape of xylem strand in VBs
Microsorum membranaceum	stramineous	1–6	brown, ovate	glabrous, grooved and winged	diarch	U	heart shaped	oval	T
M. punctatum	stramineous	3–4	dark brown, ovate-lanceolate	glabrous, small winged	exarch/ diarch	U	heart shaped	oval	T
Phymatosorus cuspidatus	brown	2–50	dark brown, orbicular to circular	stipe articulated, glabrous, grooved dorsally, castanecous	diarch	U	round	oval	T
Pichisermollodes ebenipes	purple	5–10	dark brown, ovate-lanceolate	scaly at base, winged	diarch	U	oval	oval (rachis winged)	T-X
P. stewartii	light brown	3–8	dark brown, ovate-lanceolate	articulated, glabrous	diarch	U	oval	oval (rachis winged)	T
Polypodiodes amoena	brown	10–20	dark-brown, ovate-lanceolate, acuminate, denticulate	castaneous	diarch	U	oval	oval (rachis winged)	T
Pyrrosia costata	light brown	1–4	brown, lanceolate	winged, scaly at base, tomentose above	diarch	inverted Ω	heart shaped	oval	T
P. heteractis	stramineous	4–7	brown, lanceolate	scaly	diarch	inverted Ω	oval	oval	T
P. lanceolata	brown	≤ 1	brown, lanceolate	winged at base scaly above	diarch	V	heart shaped	straight	T
P. mannii	brown	1	brown, lanceolate	densely scaly	diarch	U	oval	oval	T
Selliguea griffithiana	stramineous	4–10	dark brown, lanceolate	glabrous	diarch	U	oval	oval	T
S. oxyloba	light brown	8–20	dark brown, lanceolate	glabrous	diarch	U	oval	oval (rachis winged)	T-X

TABLE 2. Character and character states used in numerical analysis of stipe and midrib.

Character		Character states
1.	Stipe length (cm)	Very short (< 1): 0; Short (1–3):1; Medium (3–15): 2; Long (> 15): 3
2.	Shape of scales	Lanceolate: 0; Linear lanceolate:1; Ovate lanceolate: 2; Ovate: 3; Orbicular: 4
3.	Colour of stipe	Stramineous: 0; light brown to brown: 1; light green to greenish brown: 2; purple or slightly purple: 3
4.	Texture of stipe	Glabrous: 0; Scaly: 1
5.	Stipe wings	Not winged: 0; Winged: 1
6.	Arrangement of VB in cortex	U pattern: 0; V pattern: 1; inverted Ω: 2
7.	Shape of stipe in cross section	Round: 0; Oval: 1; Heart shaped: 2
8.	Midrib texture	Glabrous: 0; Scaly: 1
9.	Wings on midrib/rachis	Not winged: 0; Winged: 1
10.	Shape of midrib/rachis in cross section	Oval: 0; Triangular: 1
11.	Shape of xylem strand in midrib	T shaped: 0; T-X shaped: 1; Fan shaped: 2
12.	Cuticle in midrib	Absent: 0; Presence: 1
13.	Thickness of sclerenchyma layers in stipe (μm)	Thin (< 500): 0; Thick (≥ 500): 1
14.	Thickness of CB (μm)	Thin (< 50): 0; Thick (≥ 50): 1
15.	Number of VBs in stipe	Few (2–3): 0; Many (≥ 4): 1

Conclusion

It is observed that stipe and midrib characters have a systematic significance at the generic as well as the species level. Although not perfectly fitting, it reflects a definitive correlation with certain taxonomic groups and clades established by molecular and other morphological evidence. Thus, the information obtained from our study will aid in further investigation for phylogenetics and systematics of the diverse and less explored fern family Polypodiaceae.

Conflict of interest

No conflict of interest exists.

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Appendix 1: Material studied

Arthomeris himalovata* Fraser-Jenk. & Kandel.:** INDIA: Darjeeling, Third mile, SM-0358 (CUH). ***Arthromeris wallichiana* (Spreng.) Ching.:** INDIA: Darjeeling, Third mile, SM-0363 (CUH). ***Drynaria propinqua* (Wall. ex Mett.) J.Sm. ex Bedd.:** INDIA: Darjeeling, Kurseong, SM-0501 (CUH).Drynaria quercifolia* (L.) J.Sm.:** INDIA: Darjeeling, Sukna, SM-0550 (CUH). ***Goniophlebium argutum* (Wall. ex Hook.) J.Sm.:** INDIA: Darjeeling, Jorebunglow, SM-0582 (CUH). ***Lepisorus contortus* (Christ) Ching.:** INDIA: Darjeeling, Third mile, SM-0344 (CUH).***Lepisorus scolopendrium* (Ching) Mehra & Bir.:** INDIA: Darjeeling, Chimney, SM-0364 (CUH). ***Lepisorus loriformis* (Wall. ex Mett.) Ching.:** INDIA: Darjeeling, Kaiyakatta, SM-0493 (CUH). ***Lepisorus normalis* (D.Don) C.F.Zhao, R.Wei&X.C.Zhang.:** INDIA: Darjeeling, Lebong, SM-0599 (CUH). ***Lepisorus rostratus* (Bedd.) C.F.Zhao.: INDIA:** Darjeeling, Rajahatta, SM-0554 (CUH). ***Lepisorus sublinearis* (Baker ex Takeda) Ching R.Wei & X.C.Zhang.:** INDIA: Darjeeling, Third mile, SM-0324 (CUH). ***Loxogramme involuta* (D.Don) C.Presl.: INDIA:** Darjeeling, Mahanadi, SM-0223 (CUH). ***Microsorum membranaceum* (D.Don) Ching.:** INDIA: Darjeeling, Lebong, SM-0464 (CUH). ***Microsorum punctatum* (L.) Copel: INDIA.:** Darjeeling, Pankhabari, SM-0512 (CUH). ***Phymatosorus cuspidatus* (D.Don) Pic.Serm.:** INDIA: Darjeeling, Rohini, SM-0211 (CUH). ***Pichisermollodes ebenipes* (Hook.) Fraser-Jenk.:** INDIA: Darjeeling, Third mile, SM-0315 (CUH). ***Pichisermollodes stewartii* (Bedd.) Fraser-Jenk.:** INDIA: Darjeeling, Ghoom, SM-0614 (CUH). ***Polypodiodes amoena* (Wall. ex Mett.) Ching.:** INDIA: Darjeeling, Kurseong, SM-0498 (CUH). ***Pyrrosia costata* (C.Presl ex Bedd.) Tagawa & K.Iwats.:** INDIA: Darjeeling, Pankhabari, SM-0533 (CUH). ***Pyrrosia heteractis* (Mett. ex Kuhn) Ching.:** INDIA: Darjeeling, Bagora, SM-0602 (CUH). ***Pyrrosia lanceolata* (L.) Farw.:** INDIA: Darjeeling, Rongtong, SM-0386 (CUH). ***Pyrrosia mannii* (Giesenh.) Ching.:** INDIA: Darjeeling, Panighatta, SM-0546 (CUH). ***Selliguea griffithiana* (Hook.) Fraser-Jenk.:** INDIA: Darjeeling, Third mile, SM-0355(CUH). ***Selliguea oxyloba* (Wall. ex Kunze) Fraser-Jenk.:** INDIA: Darjeeling, Senchal, SM-0371 (CUH).

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RESEARCH ARTICLE

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Study on the morpho-anatomy of *Lepisorus* species through light microscopy and scanning electron microscopy and its systematic implications

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Abstract

The genus *Lepisorus* is often associated with complex lineages in Polypodiaceae, which leads to difficulty in understanding taxonomic relationships among the species. The taxa is mostly epiphytic with some distinct features like rhizomes covered with clathrate scales, simple fronds, sori in a single row, intermixed with peltate paraphyses. In this study, we precisely focus on the *Lepisorus* species and seek a broader understanding of the taxonomic relationships that prevail in this genus. The morpho-anatomical traits of rhizomes, stipe, lamina, and sporangia were explored in nine species with the aid of light microscopy and scanning electron microscopy techniques for gathering the qualitative and quantitative data sets. These character traits were resolved into groups based on degree of similarity and principal component analysis to comprehend the covariance among the variables. The data were then employed to prepare an artificial dichotomous key and hierarchical cluster analysis was established that revealed five related clades with eight members whereas one member emerged as outgroup. The results correspond with other recent phylogenetic studies of the concerned genus hence confirming immense reliability and thrust of light microscopy, scanning electron microscopy, and morphology-based studies that are being less utilized in fern taxonomy.

KEYWORDS
Lepisorus, light microscopy, morpho-anatomy, scanning electron microscopy, taxonomy

1 | INTRODUCTION

Polypodiaceae is considered as a major family of leptosporangiate ferns and the fourth largest epiphytic family of vascular plants (Christenhusz & Chase, 2014). The family is considered paraphyletic, while the grammitids are treated separately under Grammitidaceae (Smith et al., 2006). Some major recircumscriptions with the need for new definitions have been recommended for Polypodiaceae, particularly for *Polypodium*, *Pleopeltis*, and allied genera (Schneider et al., 2004; Smith et al., 2006). Earlier records show the placement of leptosporangiatae within a single family (Polypodiaceae or Dennstaedtiaceae). These families have been classified into close natural groups in recent classifications (Smith et al., 2006, 2008; Christenhusz et al., 2011; Christenhunz & Chase., 2014). However, in depth relationships are yet to be resolved. The systematic position of the genus *Lepisorus* (J.Smith) Ching has been of complex nature. Frequently, it has been segregated as a distinct genus (Bir & Trikha, 1968, 1974) or often merged with *Pleopeltis* (Copeland, 1947; Panigrahi & Patnaik, 1965). *Lepisorus s.l* was treated by J. Smith in 1846 (Zink, 1993) as a section of heterogeneous species *Drynaria*. Ching (1933) further raised the section *Lepisorus* to generic rank. The distinct features of *Lepisorus* are epiphytic, epilithic or terrestrial ferns with creeping rhizome ranging from short to long (Hennipman et al., 1990). Molecular studies also confirm the distinctiveness among the

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paleotropical *Lepisorus* (Schneider et al., 2004). Works by (Bir & Trikha, 1974; Ching & Wu, 1980; Hovenkamp & Franken, 1993; Wu & Ching, 1991; Zhang et al., 2003) consider a stringent boundary of *Lepisorus* and segregated close associates into independent genera. *Lepisorus*, *Paragramma*, *Belvisia*, *Drymotaenium*, and *Platygyria* are morphologically characterized by the shape of frond, scales, paraphyses, and soral features (Ching & Wu, 1980; Copeland, 1947; Hovenkamp & Franken, 1993). Over the years, various investigations lead by (Fraser-Jenkins, 2008; Hennipman et al., 1990; Kreier et al., 2008; PPG I, 2016; Wang et al., 2010; Yu & Lin, 1997) recognized *Lepisorus* in a wider lens by including other smaller genera such as *Paragramma*, *Belvisia*, *Drymotaenium*, and *Platygyria*. The fern genus *Lepisorus* under family Polypodiaceae comprises about 80 species worldwide (PPG I, 2016; Qi et al., 2013). Majority of the species from this genus are distributed along China, northern India, Japan, Malaysia, and a few species upto Afro-Madagascar and Hawaiian Islands (Wang et al., 2010), naturally inhabiting the tropical and subtropical Old World regions (Wang et al., 2010, 2011). Earlier in India, 30 species of the genus are known to occur from the Himalayan region (Bir, 1988). However the number was lowered between 13 and 16 species (Fraser-Jenkins, 2008). Around 17 *Lepisorus* species were listed particularly in the eastern India and 7 in the state of West Bengal (Ghosh et al., 2004). Phylogenetic analyses based on the molecular dataset and several morphological synapomorphies recovered *Neolepisorus*, *Lemmaphyllum*, *Tricholepidium*, *Neocheiropteris*, and *Lepidomicrosorium* and included within the genus *Lepisorus* (Wei & Zhao, 2019). The genus is associated with taxonomic tangles and difficulty in species level identification because of close similarity and variations (Bir & Trikha, 1974). Its close ally *Pleopeltis* has certain similarities with genera *Microsorum* and *Phymatodes* (PPG, 2016). The generic circumscription of *Lepisorus* and its allies is a matter of controversy since its recognition (Zhao et al., 2020). In reference to the new classification of *Lepisorus* by Zhao et al. (2020) and Global Biodiversity Information Facility (GBIF, 2021), we have included *Lepisorus normalis* (D.Don) C. F. Zhao, R. Wei, & X. C. Zhang and *Lepisorus rostratus* (Bedd.) C. F. Zhao, R. Wei & X. C. Zhang in our study. *Tricholepidium normale* (D.Don) Ching and *Lemmaphyllum rostratum* (Bedd.) Tagawa are now considered as their synonyms, respectively (Wei & Zhao, 2019). In recent works, SEM and LM observations were useful for evaluation of micro-morphological features in certain species (Adeonipekun et al., 2021; Hameed et al., 2022; Rashid et al., 2018; Usma et al., 2020). Characters such as rhizome anatomy, frond venation pattern, type of indusium, indusial presence, spore type, and ornamentation are of significant weightage. Dimorphism and blade dissection when given importance in delimitation of genera are highly homoplastic (Smith et al., 2008). The present work is an attempt to create an inclusive account with an aim to carry out an extensive study to understand and compare the morpho-taxonomic and anatomical aspects in *Lepisorus* with the aid of microscopy techniques.

2 | MATERIALS AND METHODS

2.1 | Materials

The study was conducted with nine species of *Lepisorus* under the family Polypodiaceae. Specimens were collected from various locations of Darjeeling Himalayan region that extends between 27°13′10“ N to 26°27′05” N latitude and 88°53′ E to 87°30″ E longitude covering an altitudinal range between 130 and 3636 m asl in the lap of the

TABLE 1 Code, location, coordinates, voucher number, and collectors of taxa under study.

Species	Species code	Location within study area	Coordinates	Voucher no.	Collectors
Lepisorus clathratus (C.B.Clarke) Ching	LCL	Chitrey	27° 3′5.66”N 88°16′13.05″E	SM-0097	S. Mondal and S. Moktan
Lepisorus contortus (H.Christ) Ching	LCO	Third Mile, Senchal, Lava	27° 0′25.63”N 88°15′51.64″E	SM-0344	S. Mondal
Lepisorus loriformis (Wall. ex Mett.) Ching	LLO	Kayakatta, Gairibans,	27° 3′26.57”N 88° 1′32.51″E	SM-0493	S. Mondal and S. Moktan
Lepisorus mehrae Fraser-Jenk.	LME	Senchal, Mungpoo	26°59′42.53”N 88°17′0.42″E	SM-0365	S. Mondal
Lepisorus normalis (D.Don) C.F.Zhao, R.Wei & X.C.Zhang	LNO	Third mile, Lebong	27° 0′18.94”N 88°17′21.29″E	SM-0599	S. Mondal
Lepisorus nudus (Hook.) Ching	LNU	Lebong	27° 3′4.29”N 88°16′19.72″E	SM-0239	S. Mondal
Lepisorus rostratus (Bedd.) C.F.Zhao, R.Wei & X.C.Zhang	LRO	Rajahatta	26°57′7.51”N 88°17′50.99″E	SM-0554	S. Mondal and S. Moktan
Lepisorus scolopendrium (Buch.-Ham. ex D.Don) Tagawa	LSC	Chimney, Ghoom	27° 0′0.31”N 88°15′37.85″E	SM-0364	S. Mondal
Lepisorus sublinearis (Bak. ex Takeda) Ching	LSU	Third mile, Mungpoo	27° 0′18.94”N 88°17′21.29″E	SM-0324	S. Mondal and S. Moktan

eastern Himalaya hotspot. Specimens were freshly collected from the field and the identification of the taxa was made following available literature and correct nomenclature was considered following Global Biodiversity Information Facility (GBIF, 2021) and Plants of the World Online (POWO, 2021). The species were assigned with a code where the first letter of generic name and first two letters of the specific epithet was used (Table 1).

2.2 | Methods

2.2.1 | Light microscopy (LM)

Light microscopic observations were made for morpho-anatomical studies for which fresh materials were examined. For each species, 2–3 mature specimens were selected per character. Macro-morphological traits were measured using a ruler and protractor, whereas micro-morphological characters like rhizome scales, leaf scales, and paraphyses were measured in stereo microscope Wild M3 Heerbrugg. The characters observed were given suitable codes for easier designation (Figure 1). The qualitative traits were scored as binary or multistate coding while the quantitative characters were studied following Wei and Zhang (2013). The morphological terminologies were followed as per Lellinger et al. (2002). For anatomical study, mature and fresh samples were selected and transverse sections were studied for each trait. For epidermal study, mature leaves were boiled in water before being macerated in 35% NaOCl solution. Pieces of epidermis were prepared and mounted in Canada balsam. The terminology for epidermis was followed as per Zhang et al. (1999) and Sun and Zhang (2009). The photographs of the sections were taken under a Stereo microscope Wild M3 Heerbrugg and binocular microscope Leitz Laborlux D.

2.2.2 | Scanning electron microscopy (SEM)

SEM study was made to understand the spore morphology. Mature spores were stuck to aluminium stubs with double-sided tape, sputter-coated with gold, observed and photographed using scanning electron microscope. Terminology for spore morphology and ornamentation was followed as per Quanxi and Jing (2003) and Tryon and Lugardon (2012).

2.3 | Data analysis

The diagnostic characters for the fern sample were assembled. For each trait, an average of three observations was made and the data were standardized. Qualitative and quantitative characters were assembled separately. A total of 14 qualitative and 23 quantitative traits were measured and scored for multivariate analysis. All the character data set was subjected to Principal components analysis (PCA) using R version 4.1.1 (R Core Team, 2013) and Hierarchical Clustering through UPGMA algorithm using PAST 4.3 (Hammer et al., 2001). Subsequently, dendrogram based on degree of similarity of studied characters were constructed. Additionally, a set of dichotomous artificial key to the species was constructed in the bracketed/parallel key format.

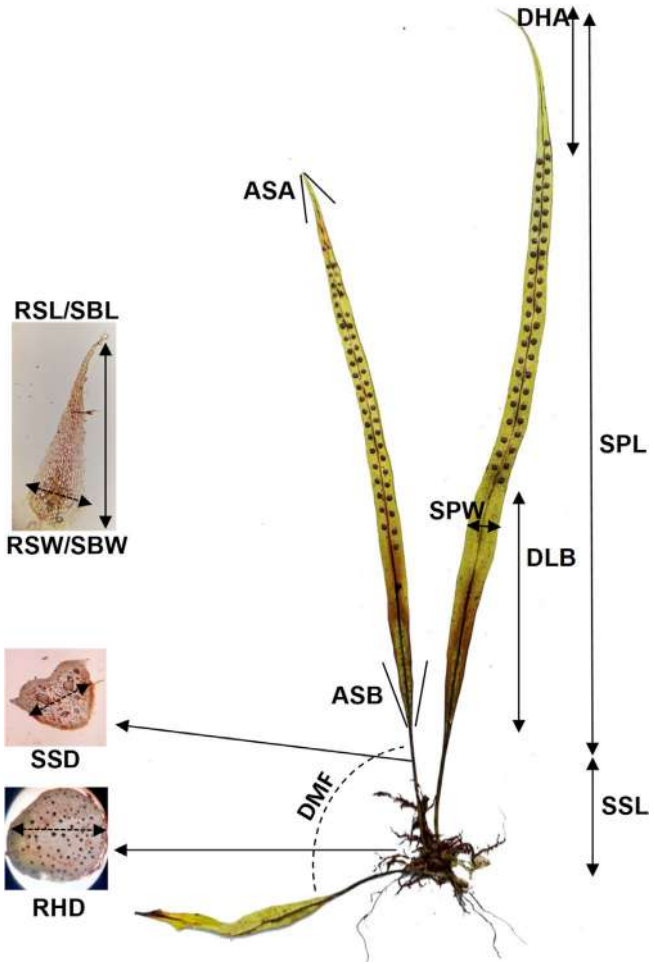

FIGURE 1 Morphometric characters in *Lepisorus*; ASA: Sporophyll apex angle; ASB: Sporophyll base angle; DHA: Distance between apical sori and frond apex; DLB: Distance between basal sori and frond base; DMF: Distance between closest frond; RHD: Rhizome diameter; RSL: Rhizome scale length; RSW: Rhizome scale width; SBL: Stipe base scale length; SBW: Stipe base scale width; SPL: Sporophyll length; SPW: Sporophyll width; SSD: Sporophyll stipe diameter; SSL: Sporophyll stipe length.

3 | RESULTS

The purpose of this study was to delineate the morpho-anatomical traits of the fern taxa so as to understand the relationship with the help of hierarchical clustering based on the degree of similarity. The results of our observations are enumerated in the following sections.

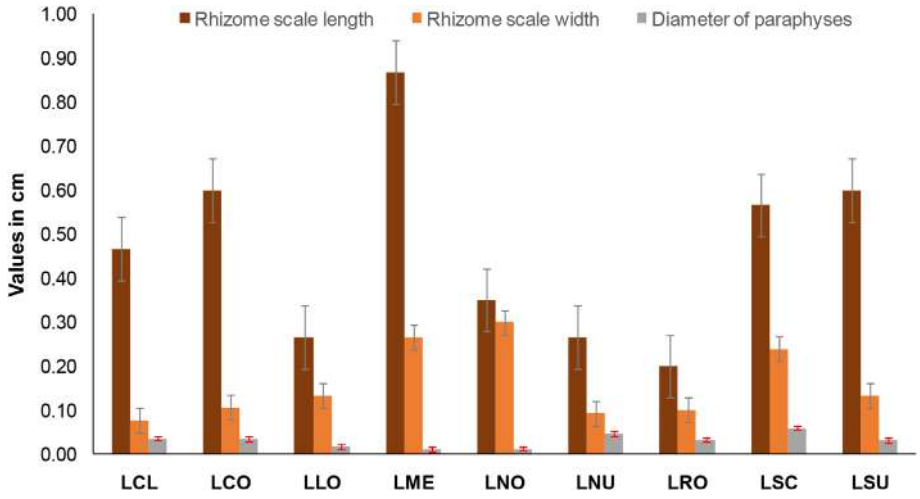

FIGURE 2 Comparative representation of rhizome scale length/ width and paraphyses diameter.

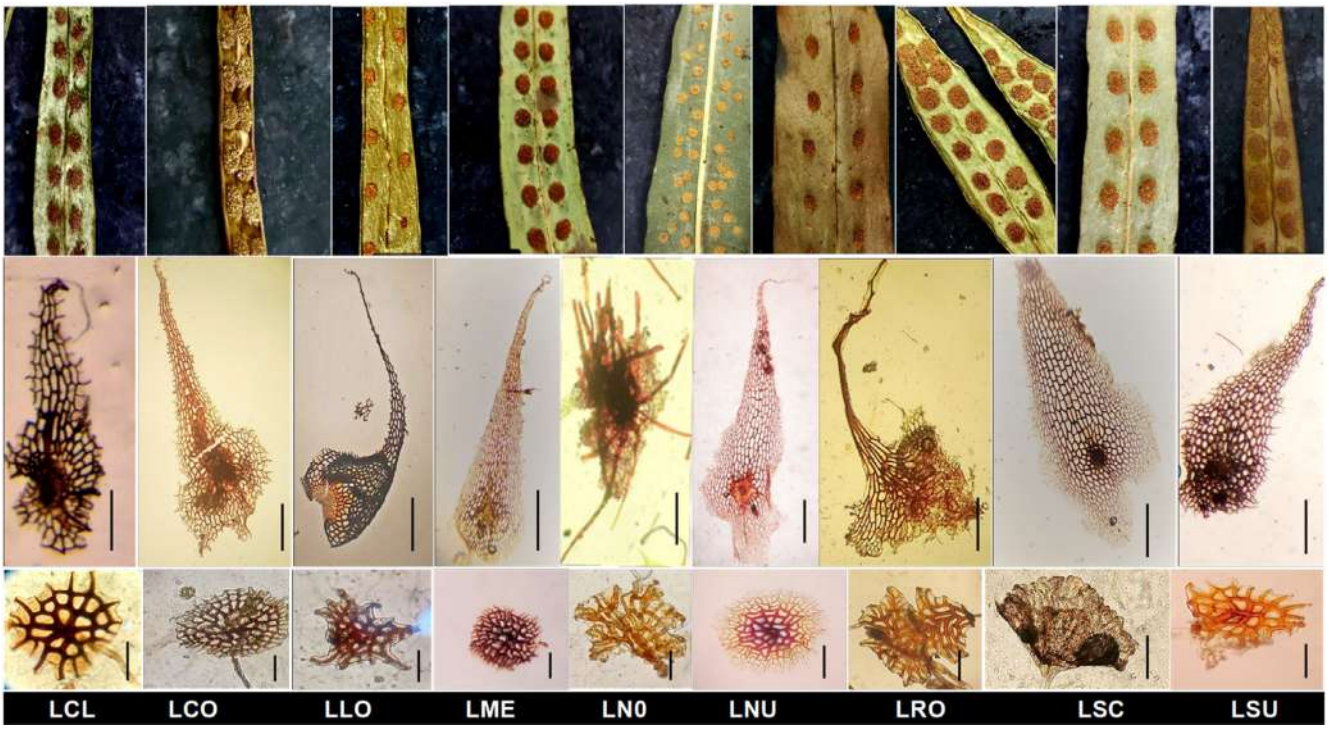

FIGURE 3 From top: Soral arrangement; rhizome scales; Soral paraphyses *(scale bar-0.5 mm)*.

TABLE 2 Anatomical characters of stipe and rhizome.

	Stipe				Rhizome	
Species code	Shape of TS	Shape of VB	No of VB	No of layers of sclerenchymatous sheath	Arrangement of sclerenchymatous strands	Vascular bundles
LCL	Globose	V	2–4	4–5	Scattered	15–20
LCO	Globose	U	5–6	5–6	Scattered	10–15
LLO	Round	U	7–8	10–12	Scattered	8–10
LME	Globose	V	3–4	3–4	Scattered	16–18
LNO	Round	V	4–5	15–20	Scattered	5–7
LNU	Heart shaped	U	5–6	7–8	Scattered	8–10
LRO	Heart shaped	L	2	5–8	Scattered	11–15
LSC	Heart shaped	U	4–5	8–10	Scattered	10–12
LSU	Heart shaped	V	4–5	8–9	Scattered	8–10

Abbreviations: TS, transverse section; VB, vascular bundle.

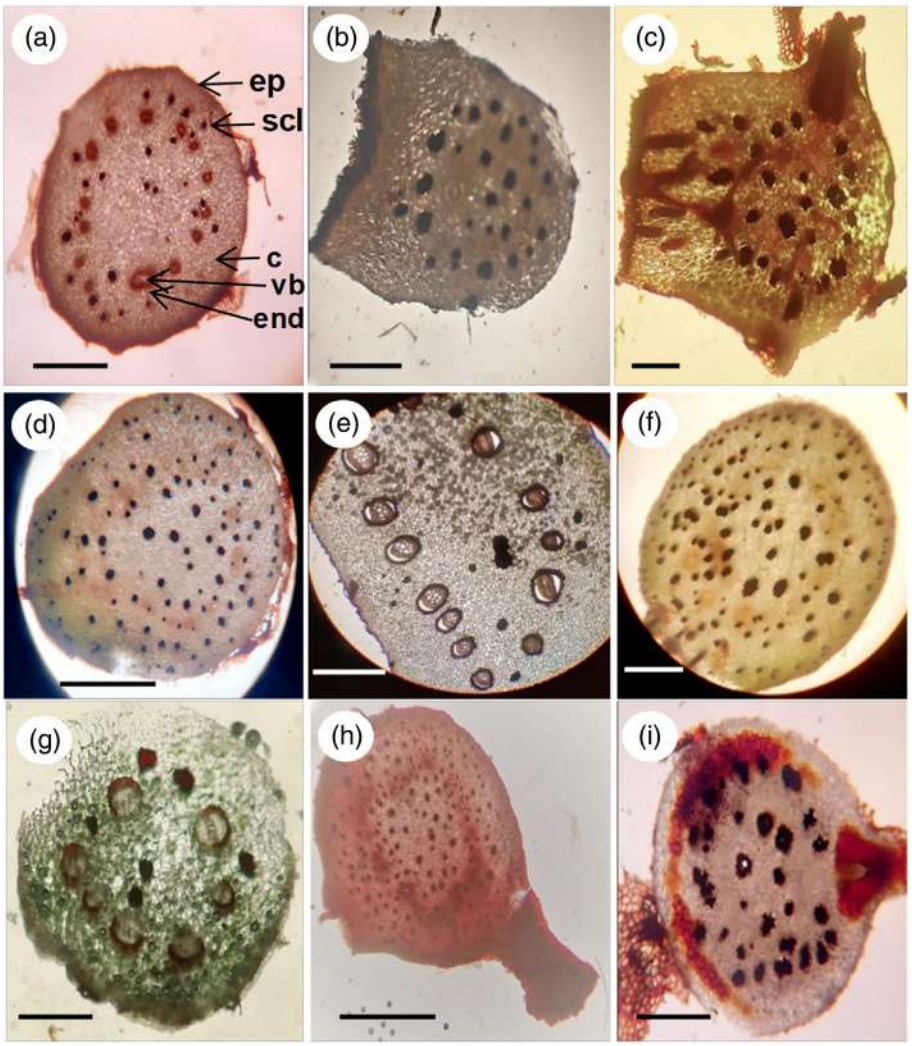

FIGURE 4 T.S. of rhizome; (a) *L. clathratus*; (b) *L. contortus*; (c) *L. loriformis*; (d) *L. mehrae*; (e) *L. normalis*; (f) *L. nudus*; (g) *L. rostratus*; (h) *L. scolopendrium*; (i) *L. sublinearis* (ep-epidermis; scl-sclerenchyma; c-cortex; end-endodermis; vb-vascular bundles; *scale bar-500 μm*).

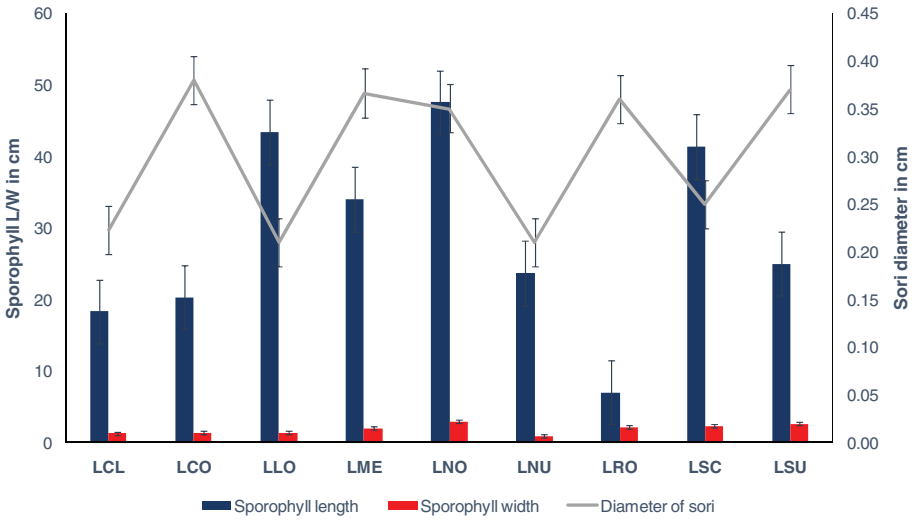

FIGURE 5 Comparative representation of sporophyll length/width and sori diameter.

3.1 | Morpho-anatomical characters under LM

3.1.1 | Rhizome and scales

It was generally observed that in *Lepisorus*, the rhizomes were densely covered with scales. In *L. contortus* and *L. sublinearis*, it was densely scaly at earlier phase but naked with maturity. The rhizome of *L. scolopendrium* were relatively thick and strong whereas slender in *L. clathratus*, *L. contortus*, *L. loriformis*, *L. nudus*, and *L. rostratus*. In *L. rostratus*, it was distinct by its green color and was covered with scales. The length ranged from 2 to around 9 mm and width from 1 mm being slender to around 3 mm (Figure 2).

TABLE 3 Anatomical characters of frond epidermis and midrib.

	Epidermis				Stomata		Midrib	
	Shape		Anticlinal wall					
Species code	Up	Lo	Up	Lo	Type	Level	Hypodermis	Sclerenchymatous sheath
LCL	IR	IR	SI	SI	Polocytic	SUP	+	+
LCO	IR	IR	SI	SI	Polocytic	SUP	+	+
LLO	QU	QU	SI	SI	Polocytic	SUP	+	+
LME	IR	IR	SI	SI	Polocytic	SUP	+	+
LNO	IR	IR	SI	SI	Copolocytic	SUP	+	+
LNU	QU	QU	SI	SI	Polocytic	SUP	+	+
LRO	IR	IR	SI	SI	Polocytic	SUP	+	+
LSC	IR	IR	SI	SI	Polocytic	SUP	+	+
LSU	IR	IR	SI	SI	Polocytic	SUP	+	+

Abbreviations: IR, irregular; Lo, lower; QU, 5-6 sided; SI, sinuous; SUP, superficial; Up, upper.

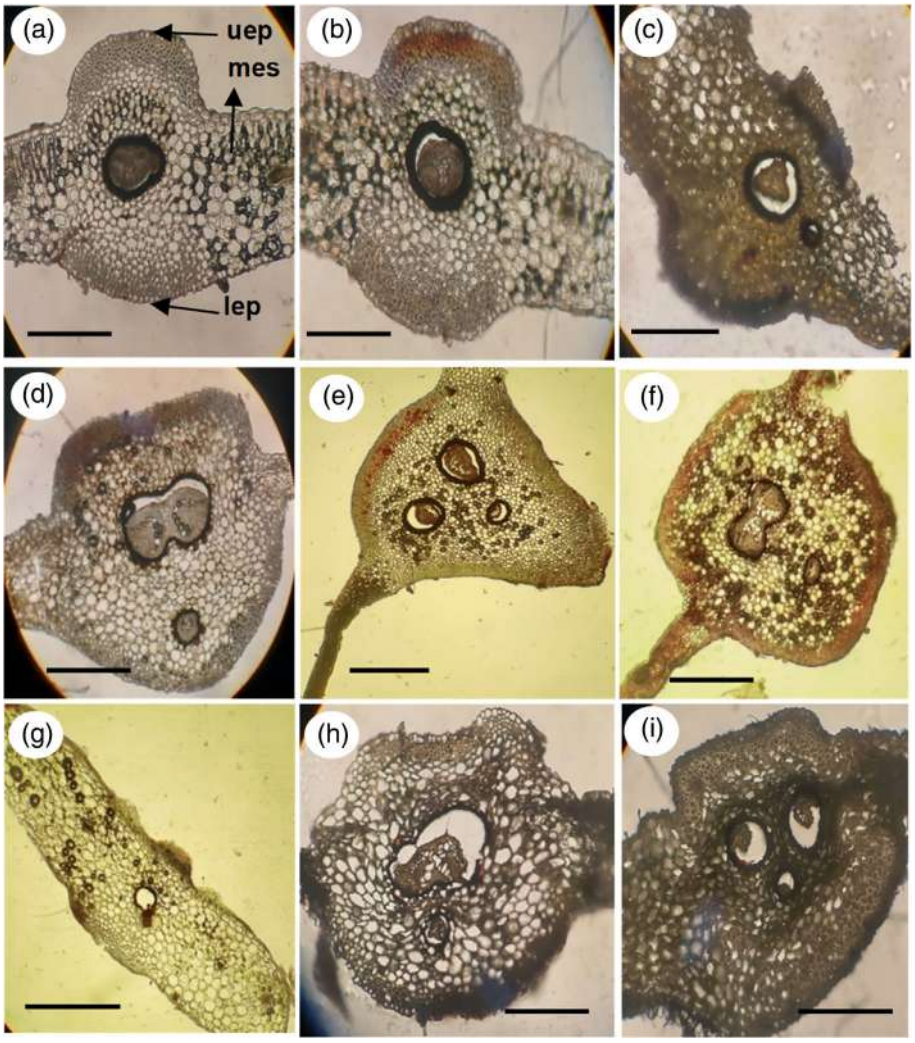

FIGURE 6 T.S. of midrib; (a) *L. clathratus*; (b) *L. contortus*; (c) *L. loriformis*; (d) *L. mehrae*; (e) *L. normalis*; (f) *L. nudus*; (g) *L. rostratus*; (h) *L. scolopendrium*; (i) *L. sublinearis* (uep-upper epidermis; mes-mesophyll; lep-lower epidermis; *scale bar-500 μm*).

The rhizome scales were long, creeping, clathrate, and translucent with entire margin and shape varying from ovate to lanceolate. In species like *L. clathratus*, *L. contortus*, *L. rostratus*, *L. scolopendrium*, and *L. sublinearis*, the scale shape tends to be lanceolate, ovate in *L. normalis* and ovate-lanceolate in *L. loriformis*. The color ranged mostly from deep brown in *L. clathratus*, *L. nudus* to pale brown with a

narrow distinct dark opaque central band in *L. contortus*. Scales of *L. mehrae* were thick, brown to dark brown with lighter margins while in *L. normalis*, the rhizome scales were thick, brown, orbicular with tuft of hairs in the middle (Figure 3).

Anatomically, the rhizomes in most species were dictyostelic with varying number of meristeles arranged in a ring. Numerous sclerenchymatous strands were scattered in the parenchymatous ground tissue (Table 2). The diarch meristeles have a heavily lignified endodermis, a pericycle some cells thick, a plate-like xylem composed of tracheids and phloem surrounding it (Figure 4).

3.1.2 | Frond and stipe

The fronds in observed species were simple, lanceolate, glabrous to sub-glabrous with a single midrib and margin entire. In *L. contortus* and *L. loriformis*, the margins often revolute when dried. The sporophyll length and width of the studied species were of wide range with distinct sori arrangement on the abaxial surface. In *L. loriformis* and *L. normalis*, the sporophylls were long, often beyond 40 cm. *Lepisorus rostratus* sporophylls were smaller up to 10 cm. A graph has been plotted for the visualization of this variation (Figure 5). The lateral venations were mostly obscure except in *L. clathratus* and *L. rostratus*. The frond surface varied from sub-coriaceous to herbaceous with different colouration.

The laminar surface color change was observed as the freshly collected specimen dried up. In *L. clathratus*, the greenish frond turned brownish. The abaxial surface of *L. contortus* was grayish to yellowish green, whereas adaxial surface was greenish. In *L. loriformis*, it was yellowish on both the sides when dried, and the margin strongly curled up while in *L. mehrae* the green specimen turned pale or reddish. *L. normalis* retained its green or yellowish green color. In case of *L. nudus*, it was grayish green on both surfaces when dried. In *L. rostratus* the green fronds turned pale brown. Similarly, *L. scolopendrium* turned reddish brown on both surfaces and *L. sublinearis*, turned grayish green or brownish. The transverse sections of the stipe were mostly round and globose, while heart-shaped in *L. nudus*, *L. rostratus*, *L. scolopendrium*, and *L. sublinearis* with number of vascular bundles ranging from 2 to 8 (Table 2).

The anatomy of the midrib showed a weak differentiated or undifferentiated mesophyll with a hypodermis consisting of a single layer of enlarged cells; upper epidermis, one cell thick and the lower epidermis with many stomata (Table 3). The midrib consists of thick-walled outer cortex that merged gradually into the thin-walled inner cortex. There were around 1-3 main bundles with a thick endodermis, a pericycle, a plate-like xylem with phloem on both sides. A T-shaped xylem was noticed as the two main lateral bundles unite towards the tip and eventually fused with one of the median dorsal bundle (Figure 6).

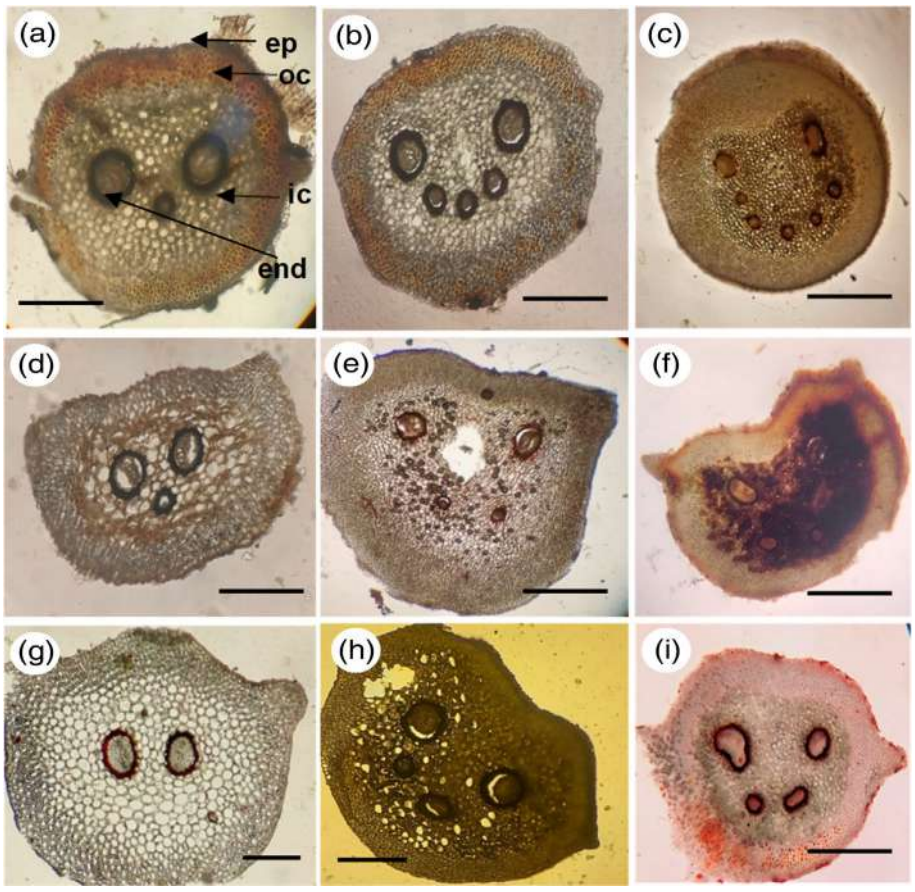

FIGURE 7 T.S. of stipe; (a) *L. clathratus*; (b) *L. contortus*; (c) *L. loriformis*; (d) *L. mehrae*; (e) *L. normalis*; (f) *L. nudus*; (g) *L. rostratus*; (h) *L. scolopendrium*; (i) *L. sublinearis* (ep-epidermis: oc-outer cortex; ic-inner cortex; end-endodermis; *scale bar-500 μm*).

The stipe was usually straw colored and slender but sometimes deep brown in case of *L. nudus*. Below the epidermis was the sclerenchymatous sheath which was around 3–10 layers (Figure 7).

The leaf epidermal cells of *Lepisorus* were usually irregular in shape, with sinuate anticlinal walls. The shape of the epidermal cells was similar on both the adaxial and abaxial surface. Stomata were polocytic and copolocytic, superficial, found on the abaxial side in all the studied taxa (Figure 8).

3.1.3 | Soral arrangement, sporangia, and paraphyses

The sorus was superficial, abaxial and mixed. The clathrate peltate paraphyses were intermixed with the sporangia that were arranged in discrete sori having distinct distribution in each species. In *L. clathratus*, the sub-orbicular sori were arranged along the length, between costa and margins, covered dark brown, stellate long spines, lumina transparent, and clathrate paraphyses. In *L. contortus*, the sori was orbicular distributed in distal half, slightly towards costa, covered with paraphyses brown at center, peltate. Only in *L. loriformis*, the sori remained close to margin, usually covered with paraphyses, deep brown to black, transparent margin with long spines. Round sori of *L. mehrae* were positioned slightly towards the midvein (Table 4). The sori in *L. normalis* were orbicular, arranged in 1–3 irregular rows on each side of midrib with peltate paraphyses. In *L. nudus*, sori were arranged in distal one third of lamina, sub-orbicular covered with deep brown, orbicular paraphyses (Figure 2). The sporangium varied from globose to spherical, brown, golden brown in *L. loriformis*, *L. sublinearis*, *L. scolopendrium*, and deep brown in *L. normalis*. The spores were monolete and pale yellow to light brown in color (Figure 9). The quantitative measurements of spores in equatorial plane have been tabulated in Table 5.

3.2 | Spore morphological characters under SEM

The spores were bilateral, monolete, ranged from light to dark brown in color. *L. clathratus* spores showed smooth exine while *L. contortus* and *L. loriformis* spores had reticulate and tuberculate exine surface, respectively. Spore with rugulate surface ornamentation in *L. normalis* and deep rugulate surface ornamentation in *L. rostratus* have been

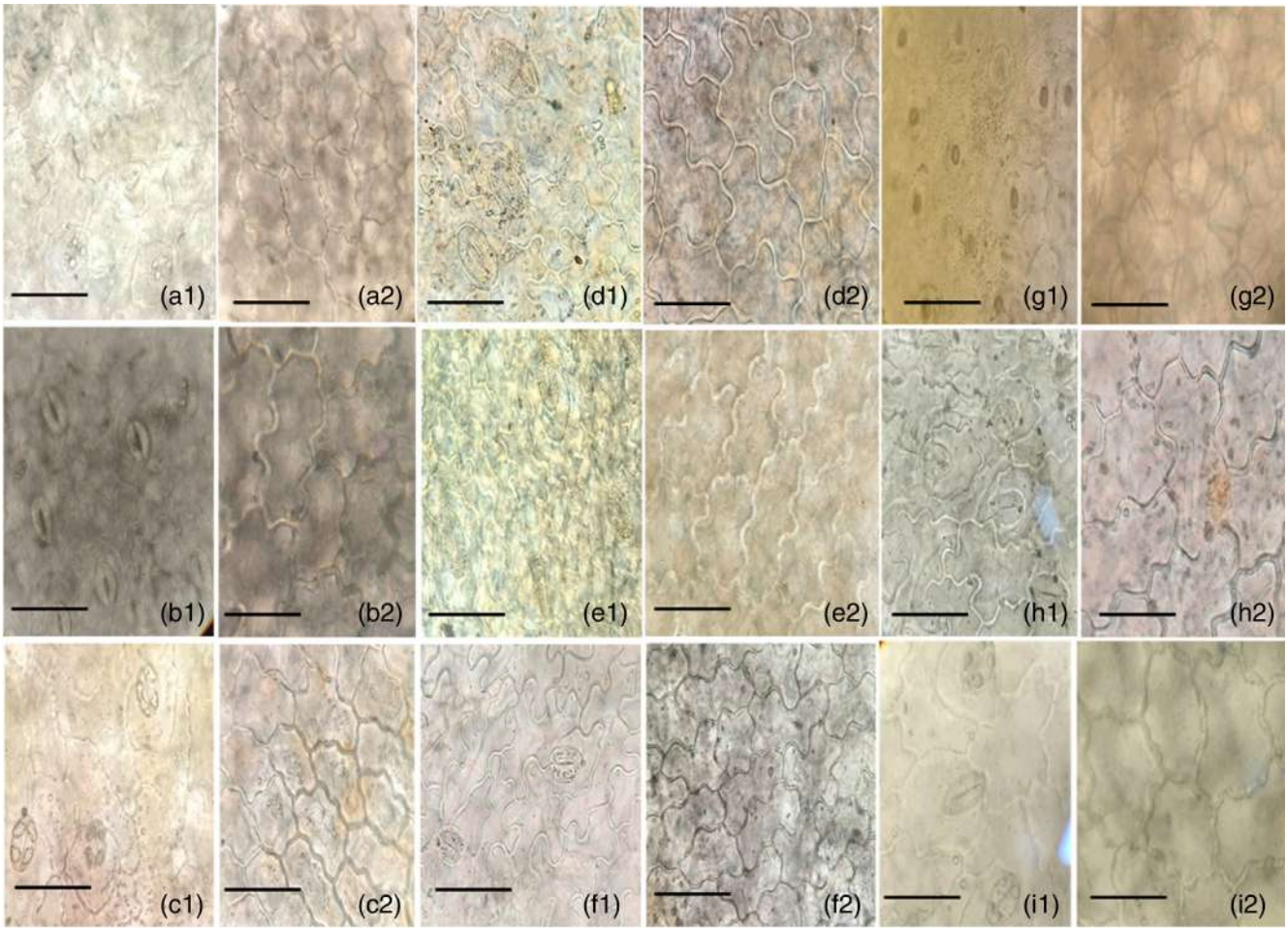

FIGURE 8 Frond epidermis; (a) *L. clathratus*; (b) *L. contortus*; (c) *L. loriformis*; (d) *L. mehrae*; (e) *L. normalis*; (f) *L. nudus*; (g) *L. rostratus*; (h) *L. scolopendrium*; (i) *L. sublinearis* (1-abaxial; 2-adaxial; *scale bar-10 μm*).

TABLE 4 Qualitative characteristics of taxa under study.

Characters	Character code	LCL	LCO	LLO	LME	LNO	LNU	LRO	LSC	LSU
Frond type	FSM	Monomorphic	Monomorphic	Monomorphic	Monomorphic	Monomorphic	Monomorphic	Monomorphic	Monomorphic	Monomorphic
Sporophyll apex	SPA	Acuminate	Long acuminate	Long acuminate	Acuminate	Acuminate	Acuminate	Acuminate	Acuminate	Acute
Sporophyll base	SBA	Cuneate	Decurrent	Gradually attenuated	Decurrent	Gradually attenuated	Cuneate	Cuneate	Attenuated	Attenuated
Frond widest position	FWP	At or below middle	Middle	Middle	Middle	Middle	Middle	Below middle	Below middle	One third from base
Lamina shape	LAS	Lanceolate	Linear-lanceolate	Linear	Lanceolate	Linear	Lanceolate	Elliptic	Lanceolate	Lanceolate
Lamina texture	LAT	Herbaceous	Subcoriaceous	Subcoriaceous	Subcoriaceous	Herbaceous	Fleshy	Fleshy	Herbaceous	Subcoriaceous
Venation	VEN	Visible	Hidden	Hidden	Hidden	Hidden	Hidden	Hidden	Slightly visible	Hidden
Scale shape	SCS	Lanceolate	Broad lanceolate	Ovate lanceolate	Lanceolate	Orbicular with tuft of hair	Ovate lanceolate	Lanceolate	Lanceolate	Lanceolate
Scale apex	UPS	Shortly acute	Acuminate	Acuminate	Acuminate	Orbicular	Acuminate	Acuminate	Acuminate	Acuminate
Sorus type	SOT	Suborbicular	Orbicular	Orbicular	Round	Orbicular	Suborbicular	Orbicular	Elliptic	Orbicular
Sorus position	SOP	Medial	Distal half of lamina	Submarginal	Slightly towards midvein	Irregular	Distal one third of lamina	Above widest part of lamina	Distal half of lamina	Distal half of lamina
Density of rhizome scales	DRS	Densely scaly	Densely scaly when young	Densely scaly	Densely scaly	Densely scaly	Densely scaly	Densely scaly	Densely scaly	Densely scaly
Soral paraphysis	SPP	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Arrangement of sori	ASO	Close	Close	Close	Close	Close	Close	Close	Close	Close

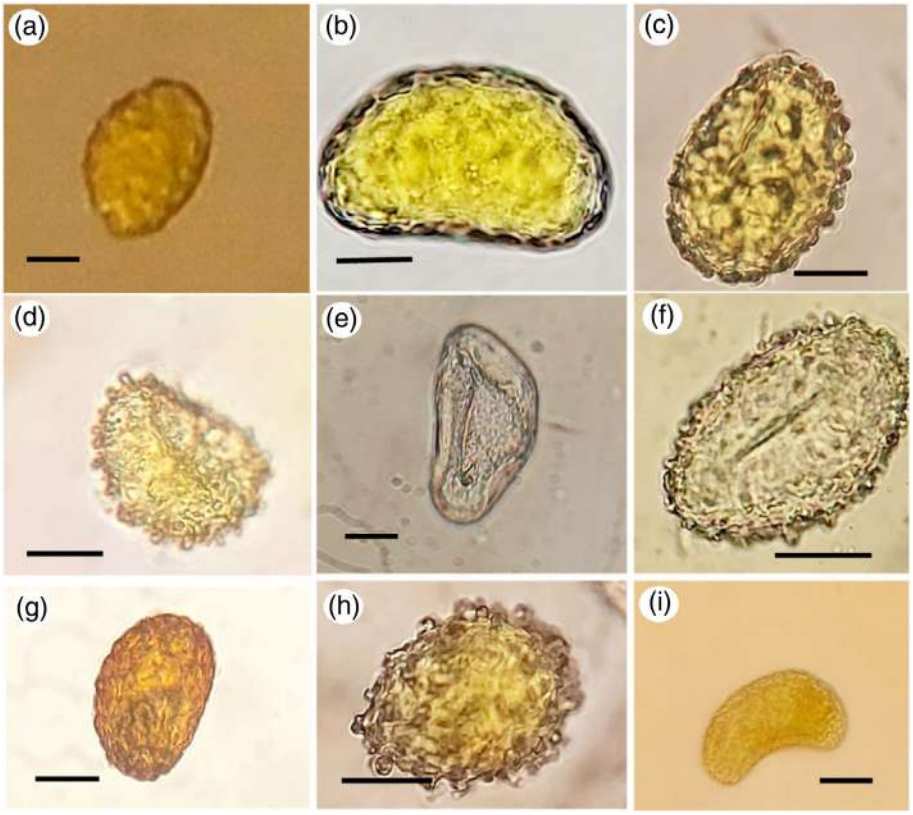

FIGURE 9 LM images of spores; (a) *L. clathratus*; (b) *L. contortus*; (c) *L. loriformis*; (d) *L. mehrae*; (e) *L. normalis*; (f) *L. nudus*; (g) *L. rostratus*; (h) *L. scolopendrium*; (i) *L. sublinearis* (*scale bar-10 μm*).

observed. In *L. nudus*, spore exine was spinulose and in *L. sublinearis* it was granulose while *L. mehrae* and *L. scolopendrium* spores had verrucose exine (Figure 10).

3.3 | Principal component analysis

The first 5 PCs (Principal Components) or Dim (Dimension) have eigenvalue >1 and thus provides a good approximation of the variation present in the original data set. From the variable PCA plot and loading plot, we can see that the variables of RSL, RSW, SBL, SBW, and SSD are highly clustered with angle between the variable vectors small as observed in the biplot (Figure 11). The length of PCs in biplot refers to the amount of variance contributed by the PCs. The longer the length of PC, the higher the variance contributed and well represented in the space. The RHD, DLB, DMF, SPL, SSL, and ASA characters were observed having long length in the PCA analysis. Therefore, it can be concluded that the rhizome diameter, distance between basal sori and frond base, distance between closest frond, sporophyll length, sporophyll stipe length, and sporophyll apex angle vary among the studied species and hence serve as a taxonomically distinguishing feature. PCA of morphological characters revealed that the first two principal components were used to analyze the structure of the data in a PCA bi-plot accounting for 33.8% of the total variance. The variance explained by the data points on Dim 1 (33.8%) is more than the variance of data points on Dim 2 (20.9%). In biplot graph, sporophyll apex angle (ASA), distance between basal sori and frond base (DLB), rhizome scale length (RSL), and many other characters exhibited positive loading, while SSL, DMF, were negatively loaded.

3.4 | Interrelatedness among taxa

The interrelationships of the studied taxa were delineated via two methods; first, a dichotomous key was developed on the basis of the qualitative morphological data, secondly, hierarchical clustering based on the quantitative morphological data and visualization through dendrogram based on degree of similarity was drawn.

Dichotomous taxonomic keys.

1a) Fronds lanceolate, oblong-lanceolate, sickle shaped, ovate to elliptic.....3.

b) Fronds loriform .....2.

2a) Fronds loriform with marginal sori ..... *L. loriformis*.

b) Fronds loriform with irregular rows on each side of midrib.....*L. normalis*.

3a) Fronds ovate to elliptic, 2–2.5 cm wide discrete sori.....*L. rostratus*.

b) Fronds lanceolate to broadly lanceolate, sickle shaped, apex acuminate.....4.

4a) Fronds broadly lanceolate .....5.

TABLE 5 Quantitative characteristics of taxa under study.

Characters	Character code	LCL	LCO	LLO	LME	LNO	LNU	LRO	LSC	LSU
Rhizome diameter	RHD	0.43 ± 0.07	0.30 ± 0.04	0.31 ± 0.01	0.80 ± 0.28	0.55 ± 0.45	0.26 ± 0.45	0.15 ± 0.01	0.4 ± 0.57	0.4.33 ± 0.33
Distance between closest frond	DMF	0.11 ± 0.10	1.03 ± 0.48	0.43 ± 0.03	0.16 ± 0.03	0.65 ± 0.01	0.6 ± 0.1	2.50 ± 0.88	1.03 ± 0.52	0.60 ± 0.10
Sporophyll length	SPL	18.33 ± 4.40	20.33 ± 4.91	43.33 ± 11.66	34 ± 2.08	47.50 ± 3.45	23.66 ± 4.48	7 ± 3.87	41.33 ± 3.48	25 ± 2.88
Sporophyll width	SPW	1.33 ± 0.33	1.40 ± 0.20	1.46 ± 0.26	2 ± 0.76	3 ± 0.36	1.36 ± 0.63	2.25 ± 0.53	2.33 ± 0.16	2.66 ± 0.35
Sporophyll length: width ratio	SLW	10:1	20:1	35:1	8:1	15:1	25:1	3:1	10:1	7.5:1
Sporophyll length above and below widest point	SAB	1.3 ± 0.5	0.73 ± 0.20	2 ± 0.03	0.63 ± 0.6	0.56 ± 0.51	0.7 ± 0.66	2.33 ± 0.34	0.59 ± 1	1.88 ± 0.66
Sporophyll stipe length	SSL	5.83 ± 0.14	2.33 ± 0.166	3.16 ± 0.66	2.16 ± 1	1.75 ± 0.33	3.17 ± 0.78	1.25 ± 0.65	0.65 ± 0.33	2.33 ± 0.02
Sporophyll diameter	SSD	1.66 ± 0.33	0.13 ± 0.03	0.64 ± 0.33	0.13 ± 0.14	0.15 ± 0.125	0.93 ± 0.03	0.1 ± 0.22	0.1 ± 0.06	9.66 ± 0.33
Sporophyll apex angle	ASA	12.33 ± 1.45	8 ± 1.527	7.33 ± 0.66	39.33 ± 0.16	3 ± 1.32	10.66 ± 0.66	13 ± 0.67	21 ± 1	15 ± 1.73
Sporophyll base angle	ASB	11 ± 0.57	8.33 ± 0.88	7.66 ± 0.33	36 ± 0.56	11 ± 0.79	21.56 ± 1.66	41 ± 2.4	19.66 ± 0.33	20 ± 1
Distance between apical sori and frond apex	DHA	0.73 ± 0.14	2.66 ± 0.16	1.76 ± 0.14	2.76 ± 0.16	4.25 ± 0.15	1.83 ± 0.16	3.25 ± 0.07	1.43 ± 0.67	0.54 ± 0.8
Distance between basal sori and frond base	DLB	4.5 ± 0.28	4.33 ± 0.16	6.84 ± 0.16	12.16 ± 0.96	6.5 ± 0.85	14.33 ± 0.86	1.25 ± 0.06	16 ± 0.57	8.4 ± 0.20
Rhizome scale length	RSL	0.46 ± 0.12	0.6 ± 0.06	0.26 ± 0.06	0.86 ± 0.06	0.35 ± 0.16	0.26 ± 0.33	0.2 ± 0.66	0.56 ± 0.06	0.6 ± 0.114
Rhizome scale width	RSW	0.56 ± 0.06	0.10 ± 0.03	0.13 ± 0.03	0.26 ± 0.55	0.3 ± 0.05	0.09 ± 0.17	0.1 ± 0.04	0.24 ± 0.33	0.13 ± 0.03
Stipe base scales length	SBL	0.58 ± 0.15	0.63 ± 0.8	0.93 ± 0.08	0.93 ± 0.33	0.3 ± 0.83	0.23 ± 0.33	0.15 ± 0.14	0.4 ± 0.2	0.43 ± 0.13
Stipe base scale width	SBW	0.60 ± 0.05	0.13 ± 0.03	0.13 ± 0.03	0.30 ± 0.1	0.30 ± 0.01	1.10 ± 0.26	0.10 ± 0.33	0.16 ± 0.66	0.1 ± 0.99
Diameter of sori	DOS	0.22 ± 0.011	0.38 ± 0.04	0.21 ± 0.03	0.36 ± 0.02	0.35 ± 0.02	0.21 ± 0.01	0.36 ± 0.03	0.25 ± 0.02	0.37 ± 0.03
Sori apart from midvein	SAM	0.21 ± 0.007	0.11 ± 0.007	0.15 ± 0.02	0.23 ± 0.02	0.13 ± 0.02	0.25 ± 0.07	0.30 ± 0.005	0.26 ± 0.02	0.15 ± 0.04
Sori apart from leaf margin	SAL	0.21 ± 0.008	0.12 ± 0.011	0	0.55 ± 0.02	0.43 ± 0.007	0.46 ± 0.02	0.3 ± 0.04	0.37 ± 0.03	0.13 ± 0.007
Sporangium size (μm)	SGS	129.33 ± 4.74	145 ± 7.07	310 ± 4.71	368.33 ± 4.90	285 ± 2.3	348.33 ± 1.36	178.33 ± 3.60	294 ± 1.69	335 ± 2.35
Spore length (μm)	SRL	30.66 ± 1.9	41.33 ± 0.27	34.66 ± 0.5	47.33 ± 1.18	32 ± 1.247	41.66 ± 0.72	35 ± 0.054	62.66 ± 1.18	51.33 ± 0.72
Spore breadth (μm)	SRB	26.66 ± 0.7	34.33 ± 0.9	25.33 ± 0.7	35.33 ± 0.27	23.33 ± 0.72	36 ± 0.47	31 ± 0.54	41 ± 0.47	38.33 ± 1.65
Diameter of paraphyses	DOP	0.03 ± 0.007	0.03 ± 0.004	0.01 ± 0.004	0.01 ± 0.001	0.01 ± 0.0007	0.46 ± 0.03	0.33 ± 0.01	0.60 ± 0.04	0.32 ± 0.01

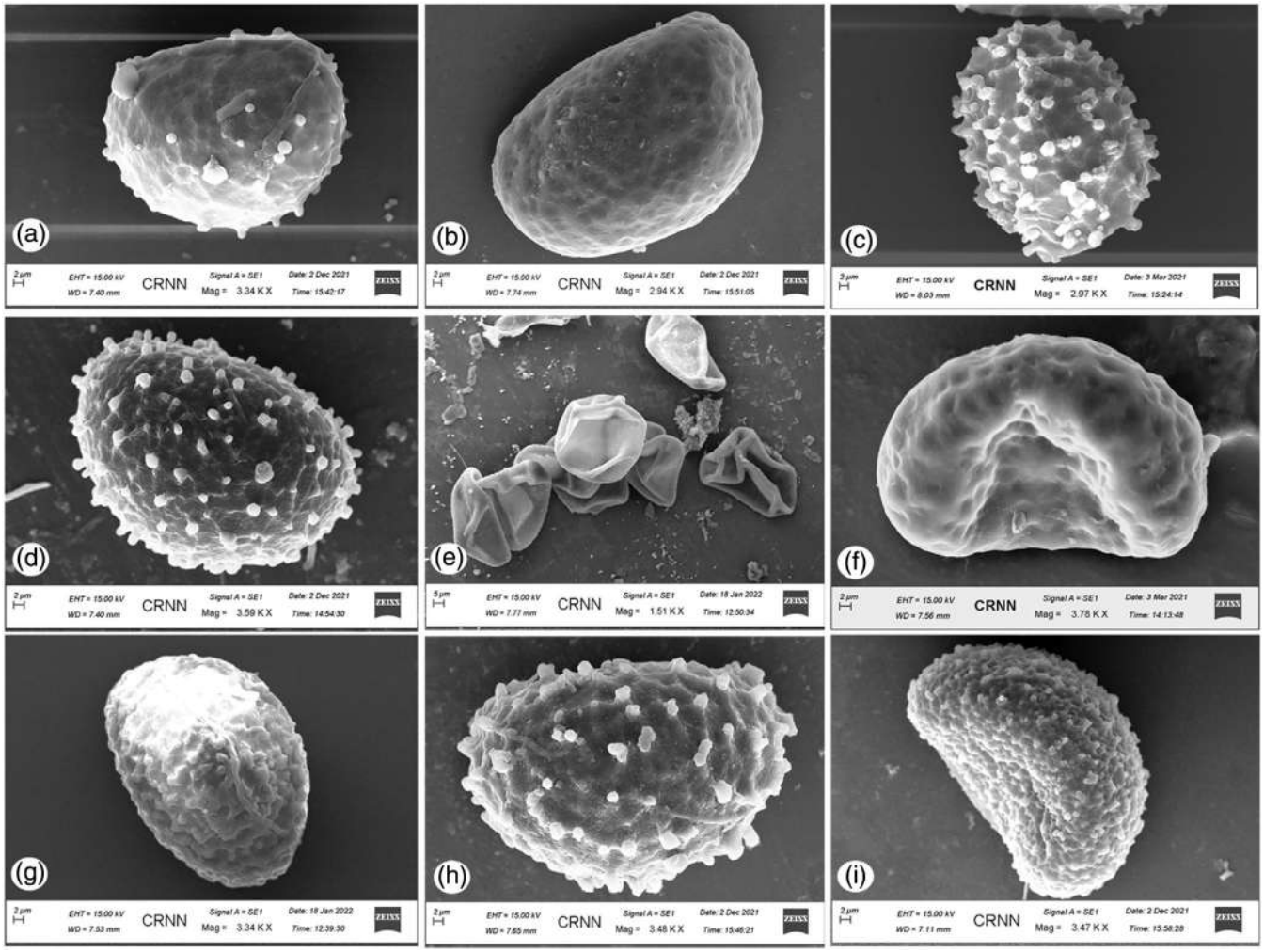

FIGURE 10 SEM images of spores; (a) *L. clathratus*; (b) *L. contortus*; (c) *L. loriformis*; (d) *L. mehrae*; (e) *L. normalis*; (f) *L. nudus*; (g) *L. rostratus*; (h) *L. scolopendrium*; (i) *L. sublinearis*.

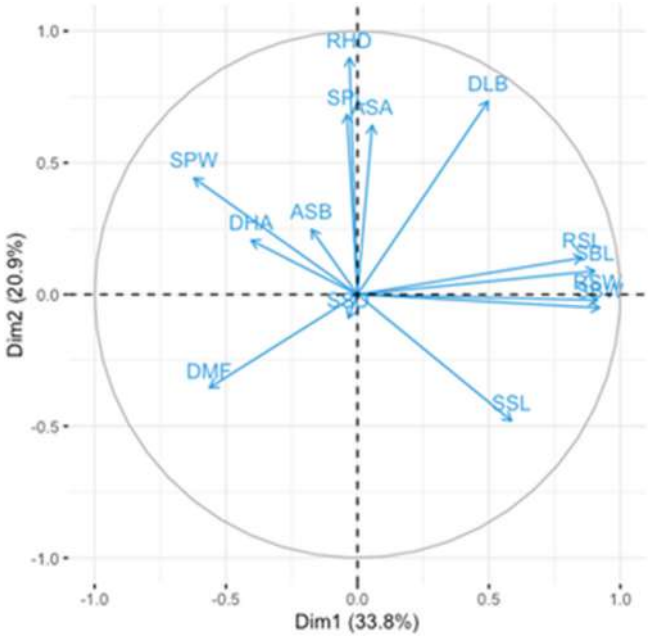

FIGURE 11 PCA scatter biplot of the studied taxa.

b) Fronds lanceolate, rhizome scales lanceolate.....6.

5a) Fronds broadly lanceolate, 30–40 x 2–2.5 cm..... *L. mehrae*.

b) Fronds broadly lanceolate, 20–30 x 2-5 cm..... *L. sublinearis*.

6a) Rhizome scales broadly lanceolate, gradually acuminate..... *L. scolopendrium*.

b) Rhizome scales ovate lanceolate to narrowly lanceolate .....7.

7a) Rhizome scales narrowly lanceolate, deep brown to black..... *L. clathratus*.

b) Rhizome scales brown.....8.

8a) Rhizome scales ovate-lanceolate, with only a narrow central opaque.
band..... *L. contortus*.

b) Rhizome scale margin pale brown, centre brown..... *L. nudus*.

3.5 | Hierarchical clustering and dendrogram

The above discussed variations in the considered morphological characteristics has led to the grouping of the nine species under study into two major clades based on their degree of similarity with eight taxa under one clade and one taxa *L. rostratus* under another clade forming outgroups. One of the major clades further consists of two nested groups with around 82% similarity while the other clade also formed two other groups with around 75%–82% similarity (Figure 12). *L. clathratus* and *L. contortus* formed one group while the latter group showed species like *L. nudus*, *L. sublinearis*, and *L. scolopendrium* with high degree of similarity.

4 | DISCUSSION

The present study was initiated to taxonomically distinguish and shed light on the relationships of *Lepisorus* species on the basis of certain morpho-anatomical characters with the aid of morphometric data through light and scanning electron microscopy technique. The study focuses on the rhizome features, rhizome scales, frond and stipe anatomical characters, soral arrangement and paraphyses, spores and other parameters.

Over the years, we observe that the generic status of *Lepisorus* has been reinstated based on rhizome anatomy, induments on various parts, venation pattern, and spore details (Zink, 1993). Chinese species of genus *Lepisorus* have been grouped into two sections *Lepisorus* and *Hymenophyton* (Lin, 2000). Another nine major clads were drawn studying its phylogeny (Wang et al., 2010) and was supported by Kholia et al. (2008). Nine major clades were recovered through a phylogenetic study combining only a few morphological characters of *Lepisorus*, mostly Asian and African species (Wang et al., 2010). Some *Lepisorus* species of the Kumaon Himalayas were segregated on the basis of thickness of rhizome and deciduousness of the lamina (Kholia et al., 2012). The shape and structure of the rhizome scales serve as important characters for species delineation (Yu & Lin, 1996). Rhizome scales, lamina features, paraphyses, sori and spores characters have been significant in generic and infrageneric classification of genus

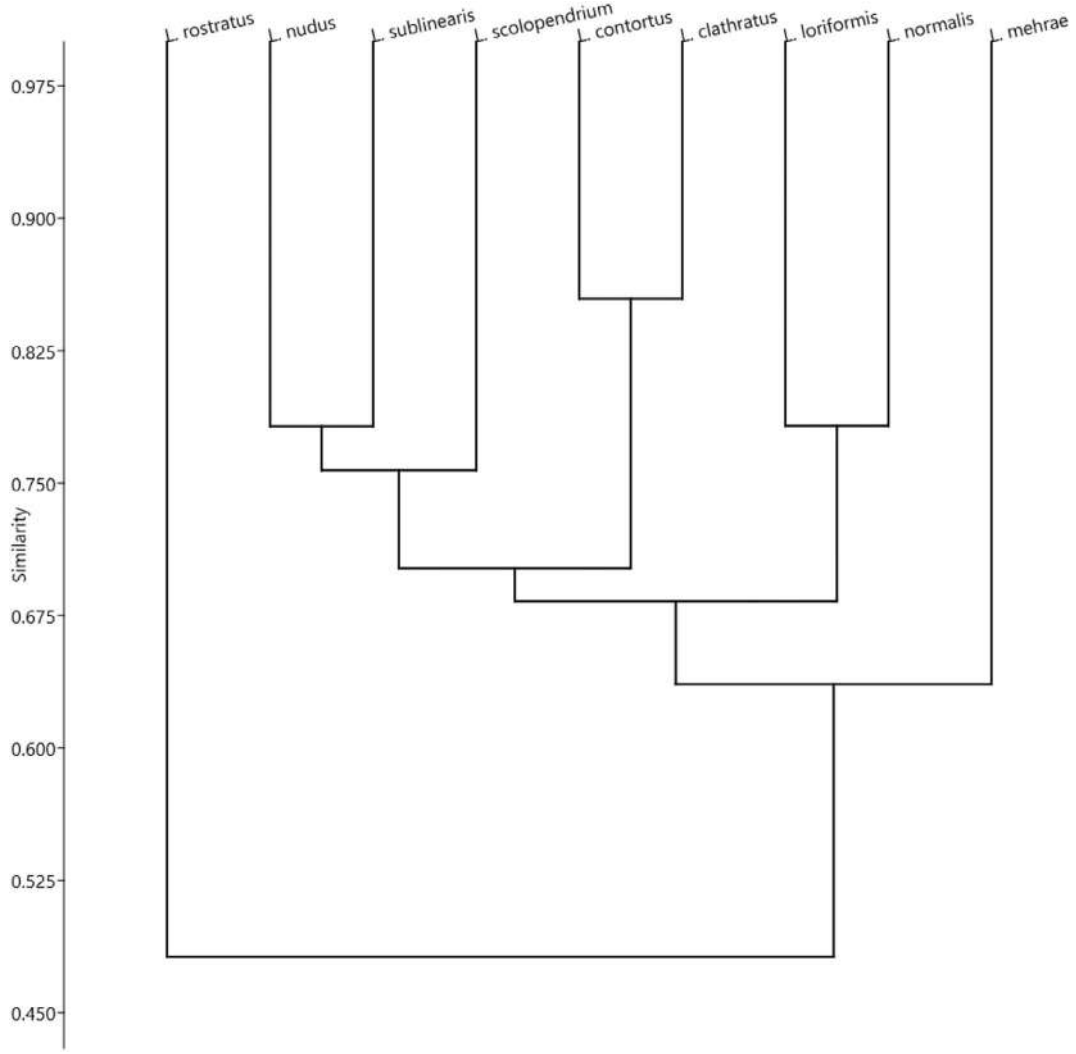

FIGURE 12 Dendrogram based on cluster analysis of studied taxa.

Lepisorus (Chatan et al., 2009; Lin, 2000; Liu et al., 2008; Qi et al., 2013; Qi & Zhang, 2009; Zhang et al., 2003). Scales of rhizomes have been demonstrated to be conservative and phylogenetically informative, especially if clathrate scales are present (Christenhusz & Chase, 2014). In our study, we observed that the shape and size of rhizome scales, diameter of the paraphyses and position of the sorus seems to be an impactful character. Frond shape is considerably a useful character as it varies from mostly lanceolate or linear-lanceolate. The presence of venation is of little importance as it is either obscure or visible. The morphological and anatomical features of rhizome and stipe have been observed and seem to be useful in taxonomic investigations. In earlier studies, the stipe features were considered as diagnostic character and in particular the number of vascular strands (Christenhusz & Chase, 2014). The transverse sections of the midrib sheds light on the T-shaped xylem that turns to be exclusive to family Polypodiaceae (Rao & Srivastava, 1973). The importance of leaf micro-morphology in fern taxonomy and systematics is highlighted in works of (Chuang & Liu, 2003; Nayar, 1962; Sen & Hennipman, 1981; Sen & Mukhopadhay, 2011; Shah et al., 2019; Shah et al., 2020; Sundue, 2009; Viane & Van Cotthem, 1977). In our study, the soral arrangements, sori diameter, paraphyses and spore characters have been significant taxonomic traits to understand the variations among the species. The use of soral and indusium shape to delineate fern genera dates back to Linnaeus (1753) and Smith (1793), and has since been relied upon heavily as a diagnostic character in eupolypod ferns (Tryon, 1952). Holttum (1966) reinstated the need of work on spores of pteridophytes and has made an evaluation on the role of spores in fern taxonomy. In recent works of Shah et al. (2019), multivariate analysis on the basis of qualitative and quantitative morphological trait was used in the discrimination of species and genera in Thelypteridaceae. The morphometric characters in our study also reflect the importance of microscopic studies as a significant tool for taxonomic segregations.

From the present investigation, it can be stated that morpho-anatomical characters observed under LM and a SEM has a diagnostic value for the *Lepisorus* species. The qualitative and quantitative morphometric variations within the taxa establish the importance of morpho-anatomical studies in taxonomic discourse. It is observed from previous studies that UPGMA and PCA based on certain qualitative and quantitative morphological traits can help in the discrimination of species and genera in ferns. In the present study also the UPGMA proved to be precisely significant for segregation of taxa.

5 | CONCLUSIONS

The morphological characters taken into account under LM and SEM studies have previously aided in providing valuable information at family, generic, and specific levels. In the present work, qualitative and quantitative morpho-anatomical data proved to be an important taxonomic tool for distinguishing *Lepisorus* species. It was possible to distinguish the species with ease and comprehend their shared characteristics as well as their dissimilarities. It can be affirmed that morphological and anatomical characters are still relevant tool in fern systematics especially in cases where the specimen seem apparently ambiguous. Characters like spore morphology as observed under LM and SEM has a diagnostic value at generic as well as specific level. The PCA, UPGMA cluster analyses based on qualitative and quantitative morphological traits can be used widely in the taxonomic delimitation of fern species.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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Micro-morphological characters in Polypodiaceae and its taxonomic significance

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Abstract. The present study insights into the interrelationships and taxonomic segregation of some Polypodiaceous fern taxa based on leaf architecture and foliar micro-morphology. Twenty-seven fern species were examined and valuable qualitative and quantitative data were obtained to generate UPGMA dendrogram. A dichotomous key differentiating the taxa was prepared. Results reveal that even though species have overlapping characters, certain specific traits prove taxonomically valuable. The results proved that traits like leaf shape, higher order leaf venation, stomatal and epidermal features are indeed important diagnostic characters and hence can be used for the identification of fern species in their immature stage or even in absence of sori. These data sets often combined with other morphological as well molecular data would contribute to fern phylogenetic study particularly of the large and complex family Polypodiaceae.

Keywords: Epidermis, ferns, leaf architecture, stomata.

INTRODUCTION

Polypodiaceae *s.l.* is an extant, monophyletic family of ferns that includes Polypodiaceae and previously segregated families Grammitidaceae and Platyceriaceae (Smith et al. 2008). As per PPGI (2016), Polypodiaceae comprise 6 sub-families, 65 genera, and 1,652 species and it is the second largest family of ferns (Hori et al. 2022). However, earlier reports estimate approximately 50 genera under the family worldwide (Tryon and Tryon 1982; Hennipman et al. 1990; Parris 1990; Smith et al. 2008). The family is a sub-cosmopolitan group mainly characterized by creeping stems covered with varying scales, fronds attached to phyllopodia, venation free or sometimes areolate with free or included veins, round to globose exindusiate, sori on abaxial lamina surface with yellowish to greenish monolete spores (Tryon and Tryon 1982; Hennipman et al. 1990; Parris 1990; Smith et al. 2008).

Kubitzki (1990) initially coined the term "polygrammoid ferns" until a phylogenetic study established the name Polypodiaceae (Schneider et al. 2004). Phylogenetic studies of major derived fern groups, such as asplenioid, dryopteroid, and polygrammoid ferns, have been of great importance since the ferns are an integral component of the tropical vegetation (Schneider et al. 2004). Major generic-level recircumscriptions have been suggested

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Comparative morpho-anatomical study in *Pyrrosia* (Polypodiaceae) from Darjeeling Himalaya, India

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ABSTRACT

The aim of the study is to compare the morpho-anatomical characters in *Pyrrosia* and create a comprehensive account to fulfil the persisting taxonomic lacuna. The genus *Pyrrosia* under Polypodiaceae is widely distributed in Darjeeling eastern Himalaya. However, detailed morpho-anatomical investigation of the species from the region is limited. The specimens were carefully observed and photomicrographed using light microscope and SEM. Principal Component Analysis have been carried out using quantitative morpho-anatomical traits and based on their differences, a taxonomic key and UPGMA dendrogram is constructed to assess the interrelatedness among the taxa. The results revealed several morphological variations in rhizome type, rhizome scales, lamina type, sori distribution *etc*. Variations in anatomical characters were also observed in laminar indument, stomata and epidermal cell type, presence or absence of sclerenchyma, stipe shape, distribution of sclerenchyma tissue strands, sclerenchymatous sheaths and spore shape and sporoderm ornamentations. The PCA results showed a variance explained by the data points on component 1(53.022%) which is greater than the variance of data points on component 2 (23.631%). Traits like number of branches in stellate hairs, size, lamina length, stipe length, rhizome scale length, rhizome scale width, number of sori in each areole shows positive loading therefore being taxonomically significant.

Keywords: morpho-anatomy, polypodiaceae, *Pyrrosia*, taxonomy

INTRODUCTION

Pyrrosia Mirbel (Polypodiaceae) is an old-world fern genus having terrestrial and epi-

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Taxonomic study on the subfamily Crypsinoideae of Polypodiaceae from Darjeeling Himalaya, India

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Abstract

The present communication is an insight into the fern taxa under the subfamily Crypsinoideae (Polypodiaceae) from Darjeeling Himalaya. Ten species under the subfamily have been characterized and analyzed with the aid of light microscopy for diagnostic traits. A compact taxonomic treatment, habit, sporulation, distribution and important notes have been provided. Morphological traits like rhizome features, frond organisation, sori arrangement and micro-morphological details on rhizome scale shape, size and colour provide valuable information in fern systematics. A dichotomous key to the taxa have been prepared for smooth identification.

Key words: fern, taxonomy, morphology, Crypsinoideae.

INTRODUCTION

Polypodiaceae is considered as the second largest family of ferns encompassing 65 genera and 1,652 species (PPG I 2016; Hori *et al.* 2022) which are diverse and widely distributed (Almeida *et al.* 2017; Wei & Zhang 2022). The family is often considered paraphyletic, while the grammatid ferns are treated separately under Grammitidaceae (Smith *et al.* 2006). Several previous records reflect the placement of many leptosporangiate ferns within big families like Polypodiaceae or Dennstaedtiaceae. Some major recircumscriptions and new definitions have been suggested for larger families like Polypodiaceae (Schneider *et al.* 2004; Smith *et al.* 2006). Nayar (1970) was the first to put forward the concept of subfamily within Polypodiaceae recognizing five subfamilies being Platycerioideae, Pleopeltidoideae, Polypodioideae, Microsoroideae and Crypsinoideae excluding the loxogrammoid and grammitid ferns. Hovenkamp (1998) reports the *Arthromeris* and *Phymatopteris* clades occur in the northern India, Indo-China, throughout the Himalayas extending up to Japan while the *Selliguea* clade mainly occurs in the Malay Archipelago and the Pacific, with only few species crossing the region of southern Indo-China and southern China. Smith *et al.* (2006) classifies Polypodiaceae (Polygrams) into an aggregation of smaller families like Drynariaceae, Grammitidaceae (grammitids), Gymnogrammitidaceae, Loxogrammaceae, Platyceriaceae, Pleurisoriopsidaceae. Christenhusz *et al.* (2011) recognised the subfamily Drynarioideae that included the Drynarioid and Selligueoid ferns. Furthermore, Schneider *et al.* (2004) recognised the Drynarioid and the Selligueoid ferns into two sub-clades Drynarioid and Selligueoid. The latter comprised four genera viz. *Arthromeris* (T.Moore) J.Sm., *Gymnogrammitis* Griff. *Polypodiopteris* C.F. Reed and *Selliguea* Bory while the Drynarioid fern group included *Drynaria* (Bory)J.Sm. and *Aglaomorpha* Schott. PPG I (2016) recognised several genera like *Drynaria, Arthromeris, Selliguea* and three others under subfamily Drynarioideae. Nayar (1970) first recognised the subfamily Crypsinoideae which was published later as Drynarioideae (Crabbe *et al.* 1975). However, Wei & Zhang (2022) based on plastome sequence, nuclear ribosomal and morphological evidence grouped three genera, *Drynaria*, *Synammia* and *Selliguea* (including *Arthromeris Gymnogrammitis, Paraselliguea, Pichisermollodes, Phymatopteris*) under subfamily Crypsinoideae.

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Chapter 7

Spore Morphology and Ornamentation of Fern and Fern-Allies from West Bengal, India

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Abstract

Over the years numerous works have established the implications of spore morphological characters in fern taxonomy. In this chapter, we extensively studied 25 species under 19 families and subjected to qualitative and quantitative analysis to resolve them into groups based on the degree of similarity. The study was conducted with the aid of light microscopy and scanning electron microscopy techniques for gathering the qualitative and quantitative traits. The qualitative data on key characters of spores like variations in the spore type, shape in polar and equatorial axis, colour, class and surface ornamentation were examined. The spore shape varied from ellipsoidal to tetrahedral with monolete or trilete aperture and mostly medium sized. The polar diameter ranges from 17.76±1.1µm to 858±16.8µm while the equatorial diameter varied from 22.20±1.1µm to 799±15.8µm with surface ornamentation varying from granulose to verrucate and tuberculate. The data were subjected to multivariate analysis and an artificial dichotomous key were prepared. Principal component analysis was performed to comprehend the covariance among the variables followed by hierarchical clustering to deduce the patterns of correlation.

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Introduction

Pteridophytes i.e., ferns and fern-allies are the pioneer plants to appear on the earth about 438 million years ago in the Silurian period of mid-Paleozoic era (Dudani et al. 2014). The fern lineages are considered to have diverged "in the shadow of angiosperms" (Schneider et al. 2004). They are the first vascular plants to successfully invade terrestrial habitats but also exhibit a diverse distributional range. They grow mostly in damp and shady places of temperate and tropical zones (Lakshminarasimhan and Arisdason, 2018). Ferns are spore-bearing plants that have a life cycle with free-living, independent gametophyte and sporophyte. The spores are capable of developing into a new individual that may be adapted for dispersion and survival in unfavourable conditions (Erdtman, 1957). Spore bearing sporangia of ferns known as sori are aggregated in clusters that are circular to linear and distributed on the abaxial surface of the fronds (Yatskievych, 2003). In fern-allies and some primitive ferns, sporangia are also borne either on stem or axil of the leaves. Pteridophytes are categorised into two types on the basis of spores, homosporous (one type of spore) and heterosporous (two types, microspores and megaspores). In some fern and fern-allies thin walled, green and photosynthetically active spores are present (Yatskievych, 2003). Cholorophyll-bearing spores are found in a few unrelated families i.e., Equisetaceae, Osmundaceae, Blechnaceae. These spores germinate in less than 3 days or viability is retained over a year. Non-chlorophyll bearing spores found in majority of species have longer germination period and viability time (Lloyd et al. 1970). Spore morphology, particularly exine ornamentation of ferns aids in the classification and taxonomic delineation. Characters of spores have been used in the construction of diagnostic keys especially at the genus level (Erdtman, 1952, 1957). Colour, ornamentation, exine thickness are significant traits (Agashe and Caulton, 2009). Fern spores are of two types-trilete (tetrahedral, primitive characters) and monolete (bilateral, advanced characters) (Vijayakanth et al. 2016). In due course of time the spore morphological traits have been pivotal, thus playing a vital role in classification, identification and establishing phylogenetic relationships in ferns (Sorsa, 1964; Liu et al. 2000; Zenkteler, 2012; Dutra and Gasparino, 2018; Esfandani-Bozchaloyi and Zaman, 2018; Gul et al. 2019; Sofiyanti et al. 2019; Ullah et al. 2019). Due to the importance of spore in ferns, this plant

group is widely used as the model for studies related to spore taxonomical studies (Adeonipekun et al. 2021). Spore morphological aspects have been investigated since early days and major fern families studied were Aspleniaceae, Blechnaceae, Polypodiaceae, Pteridaceae and Grammitidaceae (Nayar and Devi, 1963). Over the years, spore morphology in fern and fern-allies have been observed and studied with the aid of light microscopy and scanning electron microscopy (Tryon and Lugardon, 1991; Shaojun et al. 2002; Zenkteler, 2012; Li et al. 2013; Vaganov et al. 2017; Mazumdar, 2018; Shah et al. 2019; Ullah et al. 2018, Adeonipekun et al. 2021).

External features and characters of Polypodiaceae ferns in India were examined by Pal and Pal, (1970). Systematic description of spore of ferns from north-western Kutch was observed by Saxena, (1978), external sculpture of spores from Kolli hills (Vijayakanth and Sathish, 2016). The state of West Bengal lies towards the eastern part of India, surrounded by the Himalayas in the north and the Bay of Bengal to the south. The state has been divided into five phyto-ecological zones *viz.* Darjeeling Himalayan zone (500-3600 m), sub-montane Terai region, Alluvial plain surrounding river Bhagirathi and its tributaries, the western Chhotanagpur plateau region and the mangrove forests of Sundarban in South 24-Parganas (Bhattacharyya, 1997). Comprehensive descriptions of modern fern spores from the region have not been carried out earlier. The present chapter focuses on the study of spore morphology in 25 species of fern and fern-allies under 19 families to serve taxonomic purpose utilising LM and SEM techniques. Apart from creating a taxonomic tool for accurate species identification, it also serves as a template for comparison within the same or related taxa from other tropical regions.

Materials and Methods

Study Area

Field collection of fertile fern specimens was conducted over a period of time from different locations in the state of West Bengal (22. 9868°N, 87. 8550°E) located on the east coast of India with special focus on Darjeeling Himalaya for its rich diversity of Pteridophytes. The total area of the state is 88,752 sq km, with recorded forest cover of 11,879 sq km that comprises 13.38% of its geographical area out of which 7,054 sq km are Reserved Forest, 3,772 sq km are Protected Forest and 1,053 sq km as Unclassed Forests. The state houses

6 National Parks, 16 Wildlife Sanctuaries and 5 Conservation Reserves which are a part of the Protected Area Network. As per classification of forest types (Champion and Seth, 1968), the forests in West Bengal belongs to eight type groups, further classified into 30 forest types (SFR, 2019). The identification of the collected taxa was made following available literatures (Ghosh, 2004; Fraser-Jenkins, 2008; Kholia, 2010) and correct nomenclature was maintained following Pteridophyte Phylogeny Group (PPG, 2016), Global Biodiversity Information Facility (GBIF, 2022) and World Flora Online (WFO, 2022).

Preparation of Samples

For light microscopy (LM), fresh and mature spores of 25 species of fern and fern-allies were examined under 50% glycerol (Pal and Pal, 1970). Approximately, 10-20 spores from each taxa were randomly selected for qualitative and quantitative characterization of trait. The morphological characters that were taken into consideration include type, shape, colour, size, surface ornamentation and exine thickness. For size measurements, spores were selected at random and a minimum of three readings for each sample by measuring polar axis (PA) and equatorial axis (EA) in µm were calculated (Devi, 1977). The size class was categorized as per (Erdtman, 1957); very small < 10µm, small 10-25µm, medium 25-50µm, large 50-100µm, very large 100-200µm and gigantic >200µm. The surface ornamentation pattern was studied following Devi, (1977) and for necessary spore terminology Tryon and Lugardon, (2012); Punt et al. (2007) was followed.

Spore morphological and micro-morphological observations and measurements were made with a graduated ocular under light microscope. Photographs were taken under binocular microscope Leitz Laborlux D. For SEM analysis, mature spores were gathered from collected samples. The spores were stuck to aluminium stubs with double-sided tape, sputter-coated, observed and photographed using scanning electron microscope Zeiss EVO 18 Special Edition.

Data Analysis

For data analysis, the qualitative and quantitative spore traits were tabulated and evaluated. For qualitative characters multistate coding was assigned for spore type, shape, colour, class and surface. A principal component analysis

was performed using R version 4.1.1 (R Core Team, 2013) to detect taxonomically significant characters. Dendrogram based on similarity matrix was constructed using cluster analysis in PAST version 4.03 (Hammer et al. 2001). Additionally, a dichotomous artificial key to the species was also constructed in the bracketed/parallel key format.

Results

In the present chapter, spores from 25 species of fern and fern-allies under 19 families have been documented. The colour and size of the spores varied in great range from small, medium, large to very large (Figure 1).

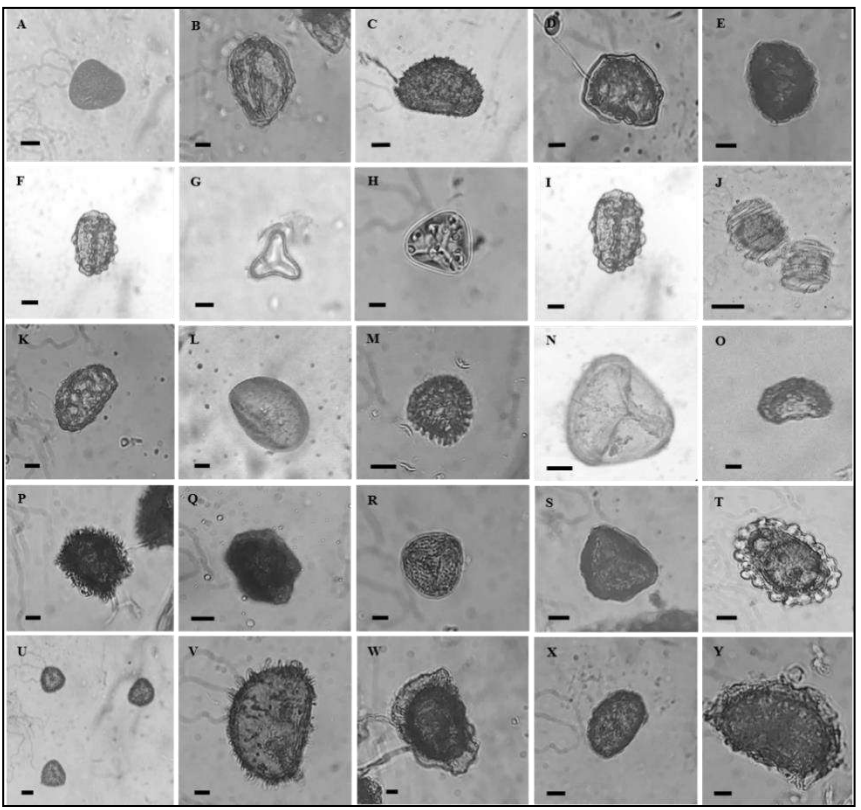

Figure 1. (Continued).

Figure 1. LM images of the spores (A) *Adiantum lunulatum* (B) *Angiopteris helferiana* (C) *Arthromeris wallichiana* (D) *Asplenium yoshinagae* (E) *Athyrium pectinatum* (F) *Blechnum orientale* (G) *Cyathea spinulosa* (H) *Diplopterygium giganteum* (I) *Elaphoglossum stelligerum* (J) *Equisetum arvense* (K) *Leucostegia pallida* (L) *Lindsaea odorata* (M) *Lycopodium clavatum* (N) *Lygodium flexuosum* (O) *Nephrolepis cordifolia* (P) *Oleandra wallichii* (Q) *Polystichum lentum* (R) *Pteridium aquilinum* (S) *Pteris biaurita* (T) *Pyrrosia lanceolata* (U) *Sellaginella repanda* (V) *Selliguea griffithiana* (W) *Tectaria gemmifera* (X) *Vittaria flexuosa* (Y) *Woodwardia unigemmata* (scale bar = 10µm).

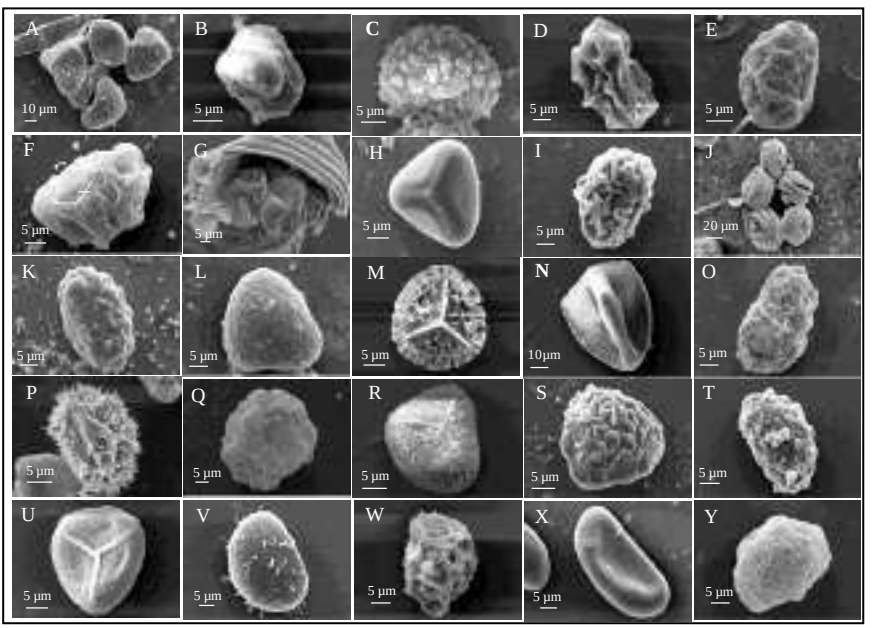

Figure 2. SEM images of the spores (A) *Adiantum lunulatum* (B) *Angiopteris helferiana* (C) *Arthromeris wallichiana* (D) *Asplenium yoshinagae* (E) *Athyrium pectinatum* (F) *Blechnum orientale* (G) *Cyathea spinulosa* (H) *Diplopterygium giganteum* (I) *Elaphoglossum stelligerum* (J) *Equisetum arvense* (K) *Leucostegia pallida* (L) *Lindsaea odorata* (M) *Lycopodium clavatum* (N) *Lygodium flexuosum* (O) *Nephrolepis cordifolia* (P) *Oleandra wallichii* (Q) *Polystichum lentum* (R) *Pteridium aquilinum* (S) *Pteris biaurita* (T) *Pyrrosia lanceolata* (U) *Sellaginella repanda* (V) *Selliguea griffithiana* (W) *Tectaria gemmifera* (X) *Vittaria flexuosa* (Y) *Woodwardia unigemmata*

Spore Aperture

The spores have been divided into three aperture types as trilete, monolete and alete. Most of the species are homosporous while *Selaginella* contains both microspores and megaspores. Monolete spores were found in 15 studied species, trilete in 9 species and single taxa *Equisetum arvense* without any lete. The monolete spores were bilaterally symmetry and ellipsoidal or elliptic in shape while trilete spores were radially symmetrical with globose, spherical and tetrahedral in shape (Figure 2). In species *Asplenium yoshinagae* Makino, the spores have monolete surture, ellipsoidal in both equatorial and polar views. The mean polar axis length is 25.53±1.1µm while the equatorial mean is 42.18±2.2µm. Spores of *Athyrium pectinatum* (Wall. *ex* Mett.) C. Presl *ex* T. Moore were monolete, bilateral, ellipsoidal in shape, yellowish-brown in colour and medium sized. The mean length is 22.20±1.1µm and 31.08±1.1µm in polar and equatorial axis respectively. Two species were examined under family Blechnaceae, *Blechnum orientale* L. and *Woodwardia unigemmata* (Makino) Nakai. The spores of *Blechnum orientale* were monolete, ellipsoidal in shape, yellowish-brown, medium in size with mean PA 27.75±1.1µm and EA 36.63±1.9µm. The *Woodwardia unigemmata* spores were monolete, ellipsoidal in shape, yellowish-brown coloured and medium sized (Table 1). The mean PA and EA were 26.64±1.9µm and 41.07±2.2µm respectively. *Cyathea spinulosa* Wall. *ex* Hook. was studied under Cyatheaceae. The spores were trilete, tetrahedral in shape and triangular, medium in size with mean PA 33.30±3.8µm and EA 34.41±2.2µm in length (Figure 3). Two species were studied under Dryopteridaceae family, *Elaphoglossum stelligerum* (Wall. *ex* Baker) T. Moore *ex* Salomon and *Polystichum lentum* (D. Don) T. Moore. The spores of *Polystichum lentum* and *Elaphoglossum stelligerum* were monolete, elliptic shape, pale brown and brown, medium sized (PA 25.53±1.1µm and EA 34.41 ± 1.1µm) and (PA 20.80 ± 1.3µm and EA 27.71±1.1µm) respectively. Spores of *Pteridium aquilinum* (L.) Kuhn belonging to family Dennstaedtiaceae was trilete, tetrahedral, yellowish-brown in colour with mean size of PA 24.42 ± 1.1µm and EA 27.75 ± 1.1µm.

The spores of the studied taxa *Equisetum arvense* L. were globose in shape, medium sized with mean PA 31.08±1.1µm and EA 27.75±1.1µm in length. The surface of the spores was granulose and yellowish-brown in colour. The spores have four elaters, green ribbon-like appendages coiled around spore body. The welcome fern *Diplopterygium giganteum* (Wall. *ex* Hook.) Nakai spores were trilete, tetrahedral in shape. Colour of spores were

Table 1. Qualitative and quantitative characters of studied taxa

Taxon	Spore Type	Shape	Colour	Size (µm)			Surface
				P.A	E.A	Class	
Adiantum lunulatum	Trilete	Tetrahedral	Brown	47.73 ± 2.2	41.07 ± 1.1	Medium	Granulose
Angiopteris helferiana	Trilete	Globose	Yellowish white	23.31 ± 1.1	22.20 ± 1.1	Small	Granulose with perinous
Arthromeris wallichiana	Monolete	Ellipsoidal	Yellowish brown	29.97 ± 1.9	44.40 ± 4.0	Medium	Granulose-spinulose
Asplenium yoshinagae	Monolete	Ellipsoidal	Yellowish brown	25.53 ± 1.1	42.18 ± 2.2	Medium	Rugulate
Athyrium pectinatum	Monolete	Ellipsoidal	Yellowish brown	22.20 ± 1.1	31.08 ± 1.1	Medium	Reticulate, folded perispore
Blechnum orientale	Monolete	Ellipsoidal	Yellowish brown	27.75 ± 1.1	36.63 ± 1.9	Medium	Plain to irregularly granulose
Cyathea spinulosa	Trilete	Tetrahedral	Light brown	33.30 ± 3.8	34.41 ± 2.2	Medium	Granulose
Diplopterygium giganteum	Trilete	Tetrahedral	Yellowish white	26.64 ± 1.9	24.42 ± 1.1	Medium	Granulose
Elaphoglossum stelligerum	Monolete	Elliptic	Pale Brown	20.80 ± 1.3	27.71 ± 1.1	Medium	Faintly granulose
Equisetum arvense	Alete	Globose	Yellowish brown	31.08 ± 1.1	27.75 ± 1.1	Medium	Granulose
Leucostegia pallida	Monolete	Ellipsoidal	Yellowish brown	25.53 ± 1.1	38.85 ± 2.9	Medium	Verrucate
Lindsaea odorata	Monolete	Ellipsoidal	Brown	36.82 ± 1.5	47.34 ± 1.5	Medium	Granulose
Lycopodium clavatum	Trilete	Globose	Yellowish white	24.42 ± 1.1	23.31 ± 1.9	Small	Granulose and reticulate
Lygodium flexuosum	Trilete	Tetrahedral	Yellowish brown	67.71 ± 2.9	67.71 ± 2.9	Large	Granulose-verrucate
Nephrolepis cordifolia	Monolete	Ellipsoidal	Dark brown	17.76 ± 1.1	25.53 ± 2.2	Medium	Verrucate
Oleandra wallichii	Monolete	Ellipsoidal	Yellowish brown	33.19 ± 1.1	42.18 ± 1.1	Medium	Spinulose
Polystichum lentum	Monolete	Ellipsoidal	Brown	25.53 ± 1.1	34.41 ± 1.1	Medium	Reticulate with perinous
Pteridium aquilinum	Trilete	Tetrahedral	Yellowish brown	24.42 ± 1.1	27.75 ± 1.1	Medium	Tuberculate
Pteris biaurita	Trilete	Tetrahedral	Reddish brown	39.96 ± 1.9	35.52 ± 1.1	Medium	Tuberculate
Pyrrosia lanceolata	Monolete	Elliptic	Pale brown	45.58 ± 3.8	55.23 ± 4.0	Large	Verrucate

Taxon	Spore Type	Shape	Colour	Size (µm)			Surface
				P.A	E.A	Class	
Selaginella repanda	Trilete (mic) Trilete (meg)	Globose Globose	Yellowish brown Dark brown	27.75 ± 2.9 858 ± 16.8	24.42 ± 2.9 799 ± 15.8	Medium Gigantic	Verrucate Verrucate
Selliguea griffithiana	Monolete	Ellipsoidal	Dark yellowish brown	34.41 ± 1.1	87.69 ± 2.9	Large	Tuberculate- spinulose
Tectaria gemmifera	Monolete	Ellipsoidal	Dark brown	36.63 ± 1.9	31.08 ± 1.1	Medium	Rugulate with irregular folds perinous
Vittaria flexuosa	Monolete	Ellipsoidal	Yellowish brown	27.17 ± 2.3	44.71 ± 3.0	Medium	Faintly granulose with perinous
Woodwardia unigemmata	Monolete	Ellipsoidal	Yellowish brown	26.64 ± 1.9	41.07 ± 2.2	Medium	Granulose

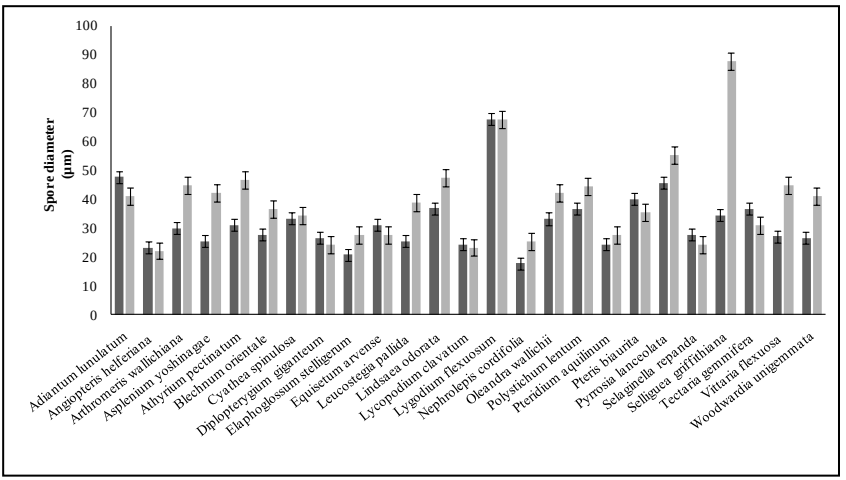

Figure 3. Spore diameter in polar and equatorial axis

yellowish-white, medium sized having mean length of 26.64±1.9µm and 24.42±1.1µm along polar and equatorial axis. Only one species was studied from Hypodematiaceae family, *Leucostegia pallida* (Mett.) Copel. The spores were monolete, ellipsoidal in shape, medium in size. The mean length of PA and EA were 25.53±1.1µm and 38.85±2.9µm respectively. The taxa *Lindsaea odorata* Roxb. was studied under Lindsaeaceae. The spores were monolete, ellipsoidal in shape, medium in size with mean PA 36.82±1.5µm and EA 47.34±1.5µm in length. The spores of *Lycopodium clavatum* L. were trilete, globose shaped, yellowish-white in colour, exine thick, small sized. The mean polar and the equatorial length were 24.42±1.1µm and 23.31±1.9µm respectively. Spores of the climbing fern *Lygodium flexuosum* (L.) Sw. were trilete, tetrahedral in shape, yellowish-brown in colour. The spores were large in size with mean PA 67.71±2.9µm and EA 67.71±2.9µm. *Angiopteris helferiana* C. Presl was studied under Marattiaceae family. The spores were trilete, globose in shape, smaller in size with mean PA 23.31±1.1µm and EA 22.20±1.1µm. *Nephrolepis cordifolia* (L.) C. Presl is the species studied under Nephrolepidaceae. The spores were monolete, bilateral, ellipsoidal in shape and medium in size. The mean PA and EA were 17.76±1.1µm and 25.53±2.2µm respectively. *Oleandra wallichii* (Hook.) C.Presl was studied under family Oleandraceae. Spores monolete, ellipsoidal in shape, yellowish-brown, medium in size with PA 33.19±1.1µm and EA 42.18±1.1µm in mean length. Three species were studied under Polypodiaceae family, *Arthromeris wallichiana* (Spreng.) Ching, *Pyrrosia lanceolata* (L.) Farw., and *Selliguea*

griffithiana (Hook.) Fraser-Jenk. The spores of all the species were monolete and ellipsoidal or elliptic shaped. Spores of *Selliguea griffithiana* were large in size (PA 34.41±1.1µm and EA 87.69±2.9µm). *Arthromeris wallichiana* spores were medium sized with mean length of 29.97±1.9µm and 44.40±4.0µm along polar and equatorial axis respectively. Subsequently, the spores of *Pyrrosia lanceolata* were pale brown in colour, large in size with mean PA 45.58±3.8µm and EA 55.23±4.0µm.

Spores of three taxa were investigated under Pteridaceae family, *Pteris biaurita* L., *Adiantum lunulatum* L. and *Vittaria flexuosa* Fée. The spores of both *Pteris biaurita* and *Adiantum lunulatum* were trilete, tetrahedral in shape, medium in size with PA 39.96±1.9µm and EA 35.52±1.1µm in *Pteris biaurita* and PA 47.73±2.2µm and EA 41.07±1.1µm in *Adiantum lunulatum*. In *Vittaria flexuosa* the spores were monolete, ellipsoidal, yellowish-brown in colour with size PA 27.17±2.3µm and EA 44.71±3.0µm in mean length. Heterosporous *Selaginella repanda* (Desv. *ex* Poir.) Spring was studied under Sellaginellaceae. The microspores are trilete, tetrahedral, medium in size and globose in shape and size of mean PA 27.75±2.9µm and EA 24.42±2.9µm. The megaspores were gigantic in size (PA 858±16.8µm and EA 799±15.8µm), dark brown, trilete and globose. The spores of *Tectaria gemmifera* (Fée) Alston under Tectariaceae were monolete, bilateral, eillipsoidal in shape, dark brown in colour with mean size of PA 36.63±1.9µm and EA 31.08±1.1µm.

Surface Ornamentation

The present study suggests that the spore surface ornamentation can be divided into different types *viz.* granulose, verrucate, tuberculate, rugulate, reticulate, wrinkled, spinulose, tuberculate-spinulose, granulose-spinulose (Figure 2).

Asplenium yoshinagae spores are medium sized and exine appears deep brown while perine light brown under LM. The surface appears rugulate with irregular sharp folds under SEM. The perispore layers in *Athyrium pectinatum* were light brown, wrinkled and reticulate with folded perispore. The perine layers in *Blechnum orientale* were light yellow, almost smoothy, with plain to irregularly granulose surface with one or two short folds. The perine layers in *Woodwardia unigemmata* were hyaline, smooth and light yellow with densely granulose surface. *Cyathea spinulosa* exine is light brown in colour and surface granulose. *Pteridium aquilinum* spores were tuberculate and yellowish-brown with perine absent. *Polystichum lentum* and *Elaphoglossum stelligerum* spore surface were dark brown, reticulate and faintly granulose

respectively. The surface of *Equisetum arvense* spores was granulose and yellowish-brown in colour while *Diplopterygium giganteum* showed prominent granulose structure. Verrucate and yellowish-brown spore surface was observed in *Leucostegia pallida* and brown granulose in taxa *Lindsaea odorata*. *Lycopodium clavatum* showed granulose-reticulate surface all over while in *Lygodium flexuosum* surface ornamentation appears granulose-verrucate and that of *Angiopteris helferiana* granulose and yellowish-white in colour. *Nephrolepis cordifolia* outer surfaces were verrucate and dark brown in colour whereas in *Oleandra wallichii,* prominent spinulose, dark brown straight and sharp spines adhering to exine with light brown, wrinkled and irregularly folded perine was observed. The outer surface of *Selliguea griffithiana* was dark yellowish-brown, tuberculate and spinulose while surface of *Arthromeris wallichiana* spores were granulose and spinulose and that of *Pyrrosia lanceolata* were verrucate. Reddish-brown spores with tuberculate surface were observed in *Pteris biaurita* while in *Adiantum lunulatum,* the outer surface of the spores were densely granulose. *Vittaria flexuosa* possess thick exine, perine present with granulose ornamentation. Both the microspores and megaspores of *Selaginella repanda* were verrucate in surface ornamentation while in *Tectaria gemmifera* the perispore layer were thick, light brown, with irregular folds protruding through rugulate surface structure.

Key to the Studied Taxa

1a. Alete, globose, yellowish brown, elaters present ….. *Equisetum arvense*
b. Monolete or trilete, size small, large or medium ……..…… 2
2a. Trilete, medium or large, tetrahedral or globose ……..…..…. 12
b. Monolete, ellipsoidal, elliptic, medium to large ……..……..…. 3
3a. Elliptic, medium, pale brown ……..……. *Elaphoglossum stelligerum*
b. Elliptic, large, yellowish brown ……..…… *Pyrrosia lanceolata*
4a. Ellipsoidal, medium, yellowish brown ……..…… 5
b. Ellipsoidal, brown or dark brown, medium or large ……..……..... 9
5a. Yellowish brown, surface ornamentation smooth …… *Vittaria flexuosa*
b. Yellowish brown, verrucate …… *Leucostegia pallida*
6a. Yellowish brown, rugulate ……..…… *Asplenium yoshinagae*
b. Yellowish brown reticulate with folded perispore..*Athyrium pectinatum*
7a. Yellowish brown, granulose ……..……. *Woodwardia unigemmata*
b. Yellowish brown irregularly granulose ……..…… *Blechnum orientale*

..8a. Yellowish brown, granulose-spinulose …..…. *Arthromeris wallichiana*
b. Yellowish brown, spinulose …….……..……. *Oleandra wallichii*
9a. Dark yellowish brown, large, tuberculate-spinulose ……..…..…
Selliguea griffithiana
b. Dark brown to brown, medium ……...…… 10
10a. Dark brown, rugulate with irregular folds ……..….. *Tectaria gemmifera*
b. Dark brown, verrucate …… *Nephrolepis cordifolia*
11a. Brown, granulose ……... *Lindsaea odorata*
b. Brown, reticulate with folded pernous ……..…. *Polystichum lentum*
12a. Trilete, tetrahedral ……..…… 15
b. Trilete, globose ……..…….. 13
13a. Globose, medium, yellowish brown, verrucate ..… *Selaginella repanda*
b. Globose, small, yellowish white ……..……..…… 14
14a. Trilete, globose, yellowish white, granulose ….. *Angiopteris helferiana*
b. Trilete, globose, yellowish white, granulose-reticulate ……..……..
Lycopodium clavatum
15a. Tedrahedral, medium, brown, light brown to reddish brown ……. 16
b. Tetrahedral, large, yellowish brown, granulose-verrucate ……..…
Lygodium flexuosum
16a. Tetrahedral, light brown granulose …….. *Cyathea spinulosa*
b. Tetrahedral, brown, granulose ..……..…… *Adiantum lunulatum*
17a. Tetrahedral, yellowish white, granulose ….. *Diplopterygium giganteum*
b. Tetrahedral, medium, tuberculate ……..……..… 18
18a. Tuberculate, reddish brown ……. *Pteris biaurita*
b. Tuberculate, yellowish brown ……..…. *Pteridium aquilinum*

Table 2. PCA variable-loading qualitative and quantitative

	Dim1	Dim2
Spore Type	-0.337	0.675
Shape	0.251	0.840
Colour	0.794	0.152
PA	0.457	-0.190
EA	0.531	-0.689
Class	0.685	0.316
Surface	0.562	0.234

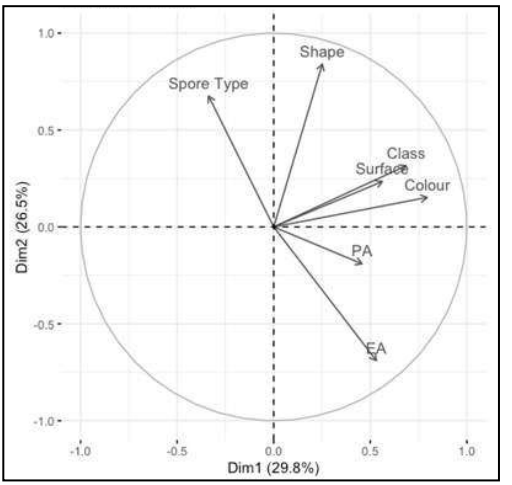

Figure 4. PCA scatter biplot for the observed traits.

Multivariate Analysis

The qualitative and quantitative traits of the spores under study were analysed through Principal Component Analysis (PCA). The first two components (Dim1 and Dim2) of PCA for the spore data were significant and explained 56% of the total variance (Figure 4). The variation along the first axis was explained by polar axis (PA) and equatorial axis (EA) of the spore while the variation along the second axis was explained by spore characters including spore shape, class, colour and surface ornamentation. Among the loaded characters in PCA, the important trait that contributed significantly were spore type, colour as well as spore class and surface ornamentation (Table 2).

The UPGMA dendrogram has led to the grouping of 25 genera under study into 3 major clades based on their degree of similarity with 16 taxa under one clade and 8 taxa forming second clade with *Selliguea griffithiana* remaining outgroup (Figure 5). One of the major clades further consists of two nested groups with around 80% similarity while the other clade also formed two other groups. Taxa such as *Lindsaea odorata, Polystichum lentum, Adiantum lunulatum, Cyathea spinulosa, Pteris biaurita, Lygodium flexuosum, Tectaria gemmifera, Leucostegia pallida, Woodwardia unigemmata, Asplenium yoshinagae, Blechnum orientale, Athyrium pectinatum, Vittaria flexuosa, Arthromeris wallichiana, Oleandra wallichi* in one group considering about 85% similarity and *Pyrrosia lanceolata* was

separated. Another group showed species like *Pteridium aquilinum, Diploterygium giganteum, Equisetum arvense, Lycopodium clavatum, Angiopteris helferiana, Sellaginella repanda, Nephrolepis cordifolia* and *Elaphoglossum stelligerum.* Closer relationship was observed between *Pteris biaurita* and *Lygodium flexuosum, Woodwardia unigemmata* and *Asplenium yoshinagae* and between *Lycopodium clavatum* and *Angiopteris helferiana.*

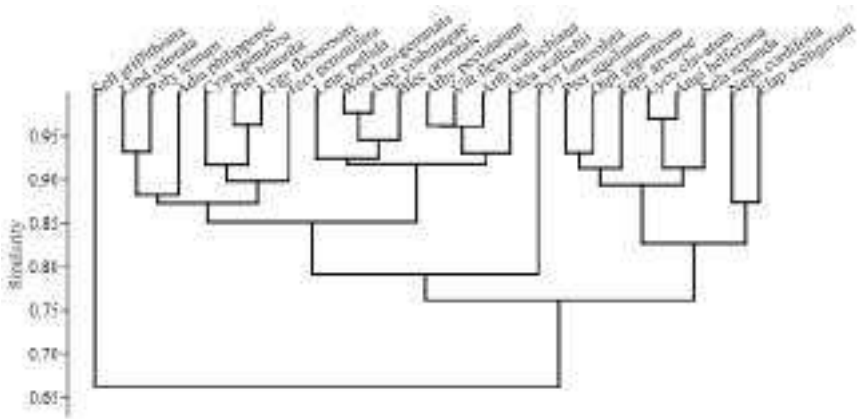

Figure 5. UPGMA dendogram based on similarity matrix.

It is interesting to note from these results that *Selliguea griffithiana, Arthromeris wallichiana* and *Pyrrosia lanceolata* all members of family Polypodiaceae show lesser degree of similarity with each other *Arthomeris wallichiana* shows a higher degree of similarity with *Vittaria flexuosa* of family Pteridaceae and *Athyrium pectinatum* of family Athyriaceae (>0.95). According to Kreier et al. (2008) several genera of the family Polypodiaceae are controversial with further studies required. *Polystichum lentum* and *Elaphoglossum stelligerum* of Dryopteridaceae also shows lesser degree of similarity while *Polystichum lentum* seems closer to *Lindsaea odorata* of Lindsaeaceae and *Elaphoglossum stelligerum* is in proximity to *Nephrolepis cordifolia* of Nephrolepidaceae family. Studies at the generic level especially of *Polystichum* were suggested for the correct placement of genera and confirmation of monophyly (Christenhusz et al. 2011). Three species studied under Pteridaceae family, *Adiantum lunulatum, Pteris biaurita* and *Vittaria flexuosa* shows about 0.85 degree of similarity.

Discussion

Pteridophytic spore studies involve a broad characterization of spore morphology. The spore characters play a significant role in the classification and the phylogenetic study of Pteridophytes (Vijayakanth and Sathish, 2016). Though the spores of all the taxa under consideration contain some common features they also differ in certain characteristics. In the present study which includes 25 species, it was found that most species were homosporous with one heterosporous. A monolete spore is considered to be more advanced compared to the trilete spore (Devi, 1977). Based on the spore type, 15 species exhibited monolete spores while 9 species were trilete. Out of 15 species, the spores of 12 were ellipsoidal in shape with elliptic in *Elaphoglossum stelligerum* and *Pyrrosia lanceolata* while the trilete spores were tetrahedral in 7 species with globose in 2 taxa. The colour of the spores varied from yellowish-brown to brown, dark brown, white, pale brown to yellow. Trilete spores varied in colour like dark brown, light brown, brown, yellowish-brown, whitish, yellowish-white to reddish-brown. Spore size class ranged from 17 x 25µm to 907 x 845µm. Smallest spores were observed in *Nephrolepis cordifolia* and *Angiopteris helferiana* whereas larger were seen in *Lygodium flexuosum* and gigantic megaspores in *Sellaginella repanda.* Similar trends of the fern spore sizes were also noted in other studies (Makgomol, 2006; Zenkteler, 2012; Shaikh and Madhav, 2019; Morajkar et al. 2021). Various types of surface ornamentation were found in different pteridophytic species within same genera. Smooth exine patterns with non-perine, trilete spore represent the most primitive spore within the fern taxa and ornamented exine, monolete spore with perine represents the most advanced (Devi, 1977). In the present study, trilete with mostly non-perinous spores were found in the primitive fern genera *Lycopodium* and monolete, perinous spores were found in advanced fern genera like *Tectaria*. Devi, (1977) reported that in *Lygodium*, most of the spore ornamentation were tuberculate, while in the present study it was observed that, spores of *Lygodium flexuosum* contain some variation such as some spores showed granulose surface while some verrucate. *Selaginella repanda* contained gigantic sized megaspores with verrucate surface as recorded in earlier works Wang et al. (2018). Nayar and Devi, (1968) mentioned that the spores of Pteridaceae is of typical trilete, tetrahedral with rugulose-tuberculate type of ornamentation, similarly in the present study also it was found that out of 3 species investigated under Pteridaceae, *Pteris biaurita* showed tuberculate with a prominent collar like ridge and *Adiantum lunulatum* spores with reticulate and granulose surface. As reported by

Kachhiyapatel et al. (2019), lady fern genus *Athyrium* spores were monolete, bilateral, perispore with or without fold, rugulate or rugose surface ornamentation and granulose. The investigated spores of *Athyrium pectinatum*, in the present study showed almost the same result with reticulate and folded perispore. Xing et al. (2013) examined *Tectaria* spores as monolete, elliptic with folded wing like perispore, verrucose, rugose and spinose surface morphology. In the present study, *Tectaria gemmifera* showed similar morphological characters with irregularly folded perispore and rugulate surface. The spores of *Asplenium yoshinagae* observed was monolete, ellipsoidal and sharply folded rugulate ornamentation and similar results were reported by Morbelli and Guidice, (2005). Rarely distributed fern species *Angiopteris helferiana* has trilete spore, globose shaped with yellowish-white and granulose surface. Majority of spore surface ornamentation observed were granulose (7spp.) and the remaining spores were verrucate (4 spp.), tuberculate (3 spp.), spinulose (1 sp.), rugulate (2 spp.), reticulate (2 spp.), smooth (1 sp.) with some of the spores exhibiting combinations of ornamentations like tuberculate-spinulose, granulose-spinulose, granulose-verrucate to granulose-reticulate. Medium sized spores were found in most of the species (19 spp.) and rest of the spores size were large (3 spp.), small (2 spp.) and gigantic (1 spp.). Most of the monolete spores showed ellipsoidal shape (52%) while some others elliptic (8%). In case of trilete spores some were tetrahedral shape (28%) and remaining globose shaped (8%). In this work, it was observed that monolete aperture spores mostly appeared ellipsoidal or elliptic and trilete apertures were tetrahedral or globose in polar view which supports the relationship as observed by Makgomol, (2006) and Zenkteler, (2012).

The results of the study indicate that the fern within a family have the same aperture type. In the family Polypodiaceae, all the studied three species have monolete aperture. In the Dryopteridaceae family both *Elaphoglossum stelligerum* and *Polystichum lentum* has monolete spores. Similarly, in *Blechnum orientale* and *Woodwardia unigemmata* of Blechnaceae, both of them have monolete spores. However, the spores of *Vittaria flexuosa* were found to have monolete aperture type, while *A. lunulatum* and *Pteris biaurita* under Pteridaceae had a trilete spore aperture. Similar kind of variations was noted by Morajkar et al. (2021) where they studied the spore morphological aspects of ferns in Western Ghats. *Pteridium aquilinum* according to some reports is considered to be absent in the Indian subcontinent (Ranil et al. 2010). This species is sometimes misidentified as it is related to *Pteris revolutum* (Fraser-Jenkins, 2008). Vijayakanth and Sathish, (2016) and Morajkar et al.

(2021), suggested the occurrence of *P. aquilium* from eastern and western ghats respectively. *Pteridium aquilinum* was found to be the most diverse species occurring in elevation along 500-3300m, followed by *Polystichum lentum* and *Nephrolepis cordifolia* of the eastern Himalayan region (Sharma et al. 2019).

The morphology of spores suggest that they possess a significant phylogenetic value at the species level particularly in the ornamentation, size and present or absence of perispore (Moran et al. 2007). The spore characters reflect their taxonomic significance with reference to ornamentation in segregating the taxa at specific level (Liu et al. 2000; Vijayakanth and Sathish, 2016). Forest resources have been serving the mankind ever since the growth of civilisations. Ferns and fern-allies forms an integral part of the forest areas. The local and indigenous people still depend on the resources for daily living. Several species included in our study have ethnobotanical purposes in the form of food, medicines, ornamentals, etc. The rhizome of *Angiopteris helferiana* is edible. *Adiantum lunulatum, Equisetum arvense, Nephrolepis cordifolia, Pteris biaurita* have medicinal values to the local people (Thapa et al. 2016). The fronds of *Pteris* spp. are used to cure cuts and wounds. Decoction of *Adiantum lunulatum* is used as diuretic, to cure dysentery. *Lygodium flexuosum* along mustard oil is used for treating rheumatism. The ferns also serve an important role in waste water bioremediation. Stem portions of *Cyathea* are used as ideal base for growing orchids. Several ornamentals such as *Dryopteris, Adiantum, Cyathea, Nephrolepis* spp. have aesthetic value (Rawat and Satyanarayana, 2015). The spore morphological characters under LM and SEM taken into account in this study yielded significant information for the fern taxa. The traits like spore aperture and surface has proven to be an excellent taxonomic tool for understanding and segregating the species of fern and fern-allies. Nevertheless, the study on the morphology of spore could be an impeccable tool in fern taxonomy and systematics.

Conclusion

Using the spore morphology characters as a taxonomic tool for the family fern and fern-allies has proved to be reliable in the works of various scientists since many decades. Variations in the spore characters taken into account for this study like spore shape, size and ornamentation have yielded valuable information for segregating species under study. It was possible to distinguish among the genera as well and readily understand their shared characteristics

as well as their dissimilarities. It was observed that particular families share certain characteristic features of the spore and these served as parameters for further classification. It can be affirmed that spore characters of fern and fern-allies together with morphological features can be considered as an infallible tool in fern systematics. From the above communication, it is clear that from the systematic point of view, the morphology of spore is of great value and could be employed for better understanding of the taxa. For solving the problems of taxonomy and phylogeny, morphological characters of spores could be very useful.

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The fate of *Diplomeris hirsuta* (Lindl.) Lindl.: A vulnerable Orchid in Darjeeling region of eastern Himalaya, India

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ABSTRACT

The present communication investigates the population and habitat assessment of *Diplomeris hirsuta* (Lindl.) Lindl. (Orchidaceae) in Darjeeling region of eastern Himalaya, India over a period of three years. The method of quadrat sampling was followed and it was observed that the data show a reduction in the average population count of the species from 13.58 to 9.41 with a decline of about 20 to 25 percent including variation in the importance value index from 116.73 to 98.34 over a study period. The fate of the orchid is under tremendous threat and is likely to become extinct from its present vulnerable categorization if the frequency of population decrease continues at this rate. Proper conservation strategy is utmost necessary at this point for its survivability.

Key words: *Diplomeris hirsuta, Vulnerable, Darjeeling, eastern Himalaya*

Introduction

Orchids are the second largest angiospermic families of flowering plants after Asteraceae (Chase *et al.,* 2015). It possesses one of the most highly evolved floral specializations and is well diversified monocotyledons. Around 31,000 species are present globally (Joppa *et al.,* 2010) out of which 29,199 species have been accepted (Govaerts *et al.,* 2017). Each year hundreds of new species names are being published (Schuiteman, 2017). Most of the orchid taxa are annual or perennial with epiphytic, lithophytic or terrestrial in their habitat. They have fascinated people as they possess highly ornamental flowers (Handa, 1986).

The orchid *Diplomeris* was first established in 1825 in works of *Prodromus Florae Nepalensis* (Hamilton and Don, 1825). The genus comprises four species in the Eastern Himalayan region, occurring in India, Nepal and China. One of the terrestrial species, *Diplomeris hirsuta,* is native to ranges of Himalaya to Central China, Nepal, Western Bhutan and Eastern India. It is also referred to as *Snow Orchid*, and its etymology originates from the Greek word '*diplo'* and '*merus'* meaning double and portion respectively, due to the presence of the divided stigma,appendix-like projections on the column (Pearce and Cribb, 2002). Hirsuta means *'hairy'*, in Latin, with reference to the leaves, stem and ovary of the species (Jalal, 2012).

The accepted nomenclature for the species is *Diplomeris hirsuta* (Lindl.) Lindl. Gen. Sp. Orchid. PL: 330 (1835). Type: Nepal, Gossainthan, Wallich's collectors Wall. Cat. 7065, with synonym *Diplochilus hirsuta* Lindl.(Ohashi, 1972). The ecological niche for the species is on mossy rocks, beside brooklets or road sides in moist open walls with shady habitat. This species is noted for its few scattered distribu-

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tion within a limited range. It was initially reported from Nainital by (Rau and Arora, 1973), the only known locality of *Diplomeris* in the Western Himalaya. In eastern Himalayan region the species has been reported from West Kameng district of Arunachal Pradesh (Chowdhery, 1998). Earlier record of the taxa is from Nepal and Bhutan but with no proper identified location and four occurrences have been confined to the present study area itself (GBIF, 2020). This orchid has been categorized as Vulnerable in the IUCN Red Data Book (Nayarand Sastry, 1987) and it is also banned under CITES Appendix II. However, under the Biological Diversity Act 2002, with reference to Schedule-38 of the Act, out of large number of orchid taxa that were proposed to be declared as threatened, 12 species have been categorized as threatened and another 8 have been proposed among which *Diplomeris hirsuta* is one of the species (Agarwala and Singh, 2013). Its vulnerability through landslides and habitat encroachment was first recorded in the mid-70s (Pradhan, 1974).

Materials and Methods

Study Area

The Darjeeling Himalaya is located as a spur in the lap of the eastern Himalaya and extends between 27° 13' 10'' N to 26° 27' 05'' N Latitudes and 88° 53' E to 87° 59' 30'' E Longitudes. It is a hilly region in the northernmost end of the Eastern India situated in the form of an inverted wedge. Darjeeling Himalayan region is drained by numerous streams and rivers like Teesta, Rangeet, Mahananda, Jaldhaka, Balason, Mechi, Lish, Gish, Murti etc. The pristine beauty and the floristic diversity of Darjeeling Hills is well known and is considered to be a treasure house of myriad of floral and faunal elements. Not only the resident species, but huge numbers of exotic species are also found to be naturalized in this Himalayan belt due to the greater number of tourists who are attracted to the natural beauty and wealth of the hill. Due to the distinct altitudinal variations between the plains and the mountainous regions, the species richness varies within short altitudinal ranges. The Darjeeling Himalaya harbors around 311 orchid species under 85 genera, out of which around 77 species are terrestrial (Yonzone *et al.*, 2012), of which *Diplomeris hirsuta* is one of the species with limited ecological niche.

The present study was conducted at the only known habitat of *Diplomeris hirsuta* in Darjeeling Himalaya along National Highway 31A on the left between Coronation Bridge (26°54'9.50''N to 88°28'20.39''E), Sevoke uphill upto around 2.5 – 3 km on the road side walls parallel to river Teesta (Fig. 1). The distributional niche for the species lies on the sub-tropical zone with altitudinal range between 250 to 800 m amsl. The mean annual temperature for the area varies between 24 °C to 28 °C with around 31 °C during the growing season for the species. The average annual precipitation remains 3200 mm. The forest surrounding the habitat harbors a mixture of sub-tropical tree species like *Tetrameles nudiflora, Pandanus furcatus, Trema orientalis, Macaranga denticulata, Ailanthus integrifolia, Duabanga grandiflora, Gynocardia odorata, Gmelina arborea, Diploknema butyracea, Callicarpa arborea* and *Litsea cubeba.* Shrubs and climbers in the sub-tropical habitat include species like *Woodfordia fruticosa, Dioscorea alata, Mikania micrantha, Leea guineensis Thunbergia grandiflora, Ampelocissus sikkimensis, Ficus hederacea,*

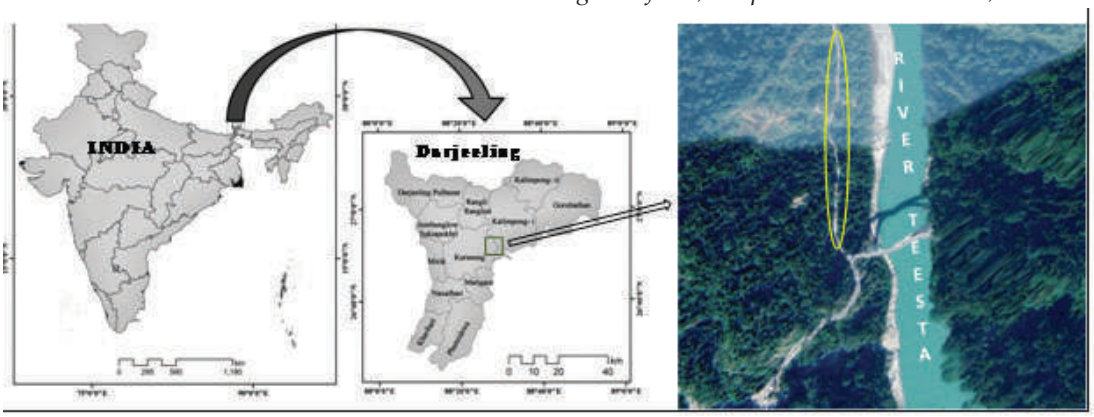

Fig. 1. Map showing study area with orchid habitat (*marked yellow)*

Debregesia longifolia, Stephania glabra, Tetrastigma serrulatum, Bauhinia vahlii and the herb and grasses include species such as *Commelina suffructicosa, Globba racemosa, Globba teesta, Pogonatherum crinitum, Elatostema lineolatum, Colocasia affinis, Adiantum lunulatum, Utricularia brachiata, Saccharum longesetosum, Begonia hatacoa, Aleuritopteris bicolor, Diplazium esculentum* etc. The realized niche for *Diplomeris hirsuta* is mostly associated with species of *Colocasia, Elatostema, Chrysopogon, Begonia, Globba, Adiantum* and *Selaginella* including some hepatics.

Population Sampling

The population and habitat assessment for *Diplomeris hirsuta* was conducted during 2016 and 2019 during its peak growing season July-August. A quadrat sampling method was followed for estimating the frequency, density and abundance of the species along with the associated species within the quadrat. A plot size of 1 x 1 m was placed on its habitat with the help of yard stick and the number of individual count of *Diplomeris hirsuta* was done along with the number of other associated taxa. The morpho-taxonomic discourses have been carried out in the field itself. A total of 12 quadrats were placed within the limited distributional range both in the year 2016 and 2019. These fixed quadrats were placed in such a way so as to include at least 7-8 individuals of the species.

The individual data count was then tabulated and pooled by plots to compute and estimate frequency, density, abundance, and abundance to frequency ratio (Curtis and McIntosh, 1950; Phillips, 1959). The relative values were summed up to determine importance value index. The abundance to frequency ratio (A/F) was estimated to understand the spatial distribution pattern of the species. A brief account of the nature of population of the species within the time frame of three years was studied and analysed.

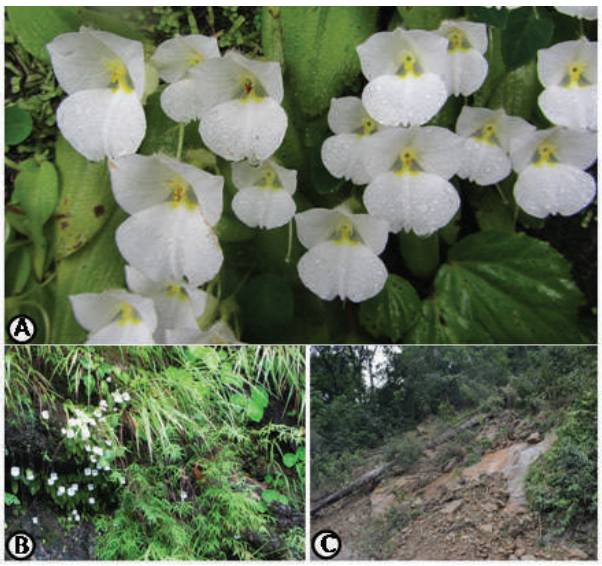

Fig. 2. **A :** *Diplomeris hirsuta* in full bloom **B:** Orchid habitat **C:** Landslide at the habitat site

Results

Taxonomy

The species is terrestrial and mostly lithophytic on moist and damp rocks on the walls. Plant height about 5 – 7 cm; tuber spherical-ellipsoid with 0.5 – 1 by 0.5 – 0.8 cm. Stem are short, sparsely pubescent, 2 – 3 cm. Single leaf (sometimes with another small sub-opposite leaf) at the basal position with oblong shape and sub-acute apex, sessile, stiffly pubescent, sheathing. Inflorescence with l to 2 flowered; floral bract ovate, acute, stiffly pubescent. Flower 2 – 2.5 cm wide, white; pedicel and ovary narrowly oblong, pubescent. Dorsal sepal ovate-oblong, sub-acute, sparsely pubescent, 1 – 1.3 cm; lateral sepals oblong-ovate, pubescent (Fig. 2A). Petals orbicular-reniform, sub-acute, 1 – 2.2 by 0.8 – 1.8 cm. Lip simple, spurred, clawed at base; apical lobe broadly ovate-orbicular; apex bi-lobed, emarginate, mucronate; spur long, funnel shaped at base tapering below and curved, pubescent 3.5 – 5 cm long. Column 6 – 7 mm; stigma 2, oblong, erect, broadly ovate with irregularly lobed margin; anther locules triangular with long tubes, 5 – 6 mm long; staminodes small, oblong; pollinia cylindric; caudicle long. The flowering period falls during the month of June to August.

Habitat Assessment

The result obtained through habitat study of *Diplomeris hirsuta* showed 10 associated species under 9 genera belonging to 7 families that remained within the orchid habitat, with *Elatostema lineolatum* and *Colocasia affinis* showing much dominance followed by *Begonia hatacoa* and *Chrysopogon gryllus.* Two species of fern *Adiantum lunulatum* and *Aleuritopteris bicolor* were also found to be associated closely along with *Selaginella* sp. and one species of minute epiphyte *Utricularia brachiata* (Fig. 2B).

However, the calculations made on the observed data showed that there have been tremendous de-

cline in the population count of the species within this span of time. It was observed within the fixed quadrat, there was a reduction in the average population count of the species from 13.58 to 9.41 with decrease of about 20 to 25 percent in population in the placed quadrat within the study period. The calculation showed a decline in the density and abundance also from 13.6 to 9.4 with change in importance value from 116.73 to 98.34 (Table 1). Among the associated species, *Elatostema lineolatum* and *Colocasia affinis* expressed maximum IVI score of 31.07 and 24.14 respectively followed by *Begonia hatacoa* and *Chrysopogon gryllus.* The minimum score of 8.44 was estimated for *Utricularia brachiata* with only three individual count during the year 2016 which however increased slightly later. The calculations made thereafter three years, showed similar score for the associated species with slight increase in the IVI score of *Elatostema lineolatum* and *Colocasia affinis.* However, the calculations showed that fern *Adiantum lunulatum* scored slightly more and species like *Chrysopogon gryllus* and *Begonia hatacoa* expressed reduced IVI during 2019. Based on the abundance to frequency ratio, it was observed that the distribution pattern for the orchid was contiguous in its niche.

Discussion

The habitat for the orchid *Diplomeris hirsuta* is very limited to the study site and there are no any records of its occurrence in any other places in Darjeeling Himalaya. As it was observed in the present study, there has been a decline in the population of this vulnerable species with limited ecological amplitude. The Darjeeling Himalaya is one of the several marked avalanche zones in the Himalayan region (Bhandari, 2004). The mountainous terrains are characterized by high energy with instability and variability of the masses. Landslides are the significant form of natural disaster that causes the loss of properties and lives (Gerrard, 1994). The variable geomorphology and neo-tectonic activities resulting in the region being highly prone to earthquakes. The zone is geologically very fragile and noted as seismic zone IV (Negi, 2018). The pivotal causes of the landslides are also the variable degree of intensive rainfall including earthquake (De, 2004). The heavy concentration of rainfall during short span, especially during June to August is one of the main reasons for the landslides to occur in the habitats of the orchid along NH 31A thereby affecting the species population (Fig. 2C). However, it was observed that some of the associated taxa in the orchid habitat showed quick regeneration than the orchid itself. The Teesta basin in recent times is one of the most landslide prone areas of the nation, a huge sediment load to the river is contributed. The vegetation is being continuously disturbed due to soil erosion that affects the peak growing season of *Diplomeris hirsuta* along the highway which is the only known niche for the orchid.

While several Hydel power projects have been set up on the river Teesta at different sites causing a major threat to the biodiversity loss in this region with social impact on the inhabitants of the villages adjoining the river. The stage IV dam proposed by NHPC at Coronation Bridge are low and run off river dams that would submerge around 359.89 ha forests and would also result in the realignment of

Table 1. Community association of orchid with other taxa in the habitat

Species	2016					2019				
	F	D	A	A/F	IVI	F	D	A	A/F	IVI
Adiantum lunulatum Burm. *f.*	41.7	0.9	2.2	0.053	17.83	41.7	1.3	3.2	0.077	24.93
Aleuritopteris bicolor (Roxb.) Fraser-Jenk.	25.0	0.5	2.0	0.080	12.54	25.0	0.6	2.3	0.093	15.20
Begonia hatacoa Buch.-Ham. *ex.* D.Don	66.7	1.3	1.9	0.028	22.62	66.7	1.0	1.5	0.023	21.86
Colocasia affinis Schott	75.0	1.3	1.8	0.024	24.14	75.0	1.3	1.8	0.024	25.99
Diplomeris hirsuta (Lindl.) Lindl.	100	13.6	13.6	0.136	116.73	100	9.4	9.4	0.094	98.34
Elatostema lineolatum Wight	75.0	2.2	2.9	0.039	31.07	75.0	1.9	2.6	0.034	31.65
Globba racemosa Smith	25.0	0.5	2.0	0.080	12.54	25.0	0.6	2.3	0.093	15.20
Globba teesta Nirola & Das	33.3	0.6	1.8	0.053	13.59	33.3	0.7	2.0	0.060	15.97
Chrysopogon gryllus (L.) Trin.	50.0	1.2	2.3	0.047	20.75	50.0	0.8	1.7	0.033	18.64
Pogonatherum crinitum (Thunb.) Kunth	58.3	1.0	1.7	0.029	19.62	58.3	0.9	1.6	0.027	20.21
Utrcicularia brachiata Oliv.	25.0	0.3	1.0	0.040	8.44	25.0	0.4	1.7	0.067	12.10

NH 31A (Rudra, 2003). The huge construction works and the reservoirs built would enhance the risk of seismicity. As the habitat of the orchid is along the walls on the road sides, the frequency of daily transportation is extremely high on this route, as it is the only route within Darjeeling Terai that connects the state of Sikkim with the rest of the country. Since the geological formation in this zone is fragile, the vibration that occurs due to extreme transportation is also one of the chief reasons for loosening of the rocks on the walls and erosion of the soil and landslides in this hilly tract. Beside these, air and noise pollution is also a considerable problem in the area and the disposal of sewages in and around the construction places where the workers reside have also become a major cause of pollution in the river affecting aquatic ecology (Lakra *et al.,* 2010). The threats that are being associated to the diversity of orchid are continuing at an alarming rate. The decline in the population of *Diplomeris hirsuta* is affected severely due to such multiple activities, be it natural or anthropogenic. The species in already in distress condition in the Western part of the Himalaya too with limited distribution and if proper strategies are not approached at this point, then the day is not too far for the species to be categorized from vulnerable to critically endangered towards extinct.

Conclusion and Recommendation

The present communication that highlights the status of *Diplomeris hirsuta* expresses a matter of concern. The survivability and fate of the orchid in its fragile habitat ecosystem is at high risk with possible decline of the population annually. If the frequency of population deteriorates at the present estimated percent, then the time is not so far for the species to be on the verge of extinction from its present vulnerable condition in this part of the Himalaya. Active participation of environmentalist, conservationist, orchidologist is must to keep an eye on the species with proper consultation with the surrounding local community. Introduction of the species in nearby forest and similar habitats, multiplication through tissue culture and micro-propagation and thereby hardening and planting in likely environment or suitable horticultural practices is the only option that remains for the species to conserve in future. Study on regeneration behavior, seedling ecology, reproductive morphology will act as a boon for understanding the species survival. Else, at this frequency of threats and rate of population loss, the species that is already under vulnerable category will be on the threshold of becoming extinct from this part of the Himalaya.

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Review Article

A Paradoxically Significant Medicinal Plant *Carapichea ipecacuanha*: A Review

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ABSTRACT

Background: *Carapichea ipecacuanha* (Brot.) L. Andersson is the botanical source of Ipecac drug and contains major alkaloids emetine, cephaline that are pharmaceutically used against bronchitis associated with cough in children, severe diarrhea (amoebic dysentery) and also cancer. Ipecac serves as an expectorant to thin mucous and easy coughing. Low doses are used to enhance appetite and it is administered orally to cause vomiting after suspected poisoning. **Materials and Methods:** The review highlights the taxonomy, nomenclature, distribution, medicinal uses and major pharmacological activities including side effects of Ipecac drug reported in recent years consulting various published papers dealing with Ipecac. **Results and Conclusion:** The species is rarely distributed due to disturbances in their habitats in natural growing condition. Further studies are required to scientifically evaluate the traditional uses of this plant through extraction and identification of their active ingredients and the mechanisms and mode of action that would serve as a source of collective information on this plant.

Key words: *Carapichea ipecacuanha*, Taxonomy, Distribution, Pharmacology, Medicine.

INTRODUCTION

Human beings are biological species existing in symbiotic relationships with a significant number of other biological species of plants and animals. We are dependent on biological diversity of plants and animals we consume and also raw materials and medicines that we use.[1] Medicinal plants are considered as high yielding resources of ingredients that can be used in drug development either pharmacopoeial, non-pharmacopoeial or synthetic and thereby play a critical role in the development of human cultures and civilizations globally.[2] The World Health Organization (WHO) defined Traditional Medicine as the sum total of all knowledge and practices, used in diagnosis, prevention and elimination of physical, mental, or social imbalance relying exclusively on practical experiences and observations translated from generation to generation.[3] As per WHO, around 80 percent of people globally rely on herbal medicines for significant aspect of their primary health care. The "Green Wave" triggered by rising bionomical consciousness has given rise to increased involvement in herbal formulations all over the world. Consumption of medicinal plants has gone twice up in the western countries. The quantity of plant-derived medicaments or health foods has increased slowly to meet global demands.[4] Around 21,000 plant species have the potential for being used as medicinal plants as per reports of WHO.[5] Among ancient civilizations, India has been known to be rich repository of medicinal plants. Forests in India is the principal repository of numerous medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and other products.[6] Approximately 8,000 herbal remedies have been databased in AYUSH systems. Ayurveda, Unani, Siddha and Folk medicines are the major systems out of which Ayurveda and Unani are most developed and widely practiced in

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India.[7] Medicinal plants may gift three crucial benefits: health benefits to the people who consuming plants as medicines; monetary benefits to people who harvest, process and distribute them for commerce; and society-wide benefits, creating job opportunities, tax revenues and a healthier labour force.[8] Where medicinal plants are harvested from the wild rather instead of cultivation, they are exposed to both general and specific threats. General threats includes climate change and habitat loss to development and agriculture. A specific threat is over-collection to cope up with the demand for medicines.[9]

In Tropical regions, the practice of using plants against treatment of diseases is widespread and their importance and uses has been well documented ever since the old world met the native Americans.[10] In Southern America, two of the important species, popularly known as cinchona (*Cinchona* spp.)[11] and *ipecacuanha* (*Carapichea ipecacuanha* (Brot.) L.Andersson)[12] have been reported for treatment of various diseases and ailments.[13] The species *C. ipecacuanha* is recognized one of the world's most important medicinal plants and Brazilian ipecac is considered the most valuable as it shows the highest emetine content.[14]

Nomenclature

Back in 1648 although Piso had referred the taxa *C. ipecacuanha* in Historia Naturalis Brasiliae, the notes were not fulfilling to be named legitimately. On the basis of material supplied by Joseph Celestine Mutis, a physician to the then Viceroy of New Granada, Linnaeus (1781) eventually described this material as *Psychotria emetica* L. f. in Supplementum Plantarum Systematis Vegetabilium.[15] Félix Avellar Brotero of the University of Coimbra (Portugal) later in 1802 described this plant as *Callicocca ipecacuanha*.[16] Persoon (1805), remembered best for his work on fungi, named this plant as *Cephaelis ipecacuanha*.[17] The origin of the term *ipecacuanha* comes from the Brazilian Indians.[18] The etymology comes from the Indian words *ipe* (bark), *caa* (plant), *cua* (fragnant), *nha* (grooved), i.e. *"bark of fragnant and striated plant"*.[19] Six synonyms have been designated for *P. ipecacuanha,* presently the common referred synonym is *Cephaelis ipecacuanha*.[14] However, the most recent revised named as *Carapichea ipecacuanha* (Brot.) L. Andersson, which is actually accepted for the species.[12]

Cephaelis ipecacuanha was based on a collection by Bernardino Antonio Gomes in Brazil.[20] The incorrectly given protologue, Bernard. Bnt. Gomes, led to the incorrect abbreviation B.B. Gomes and it was cited *s.n.* in LISU Herbaria (University of Lisbon) as holotype of *Callicocca ipecacuanha*.[21] However, a precise study in LISU went futile and an excellent illustration of Gomes' material, present in the protologue, was therefore selected as lectotype.[12]

Distribution

C. *ipecacuanha* is native to forests of South and Cental America.[22] It has a disjunctive distribution in rainforests of Central (Nicaragua, Costa Rica and Panama) and South America (Colombia and Brazil).The original inhabitants of the Brazilian territory already used ipecacuanha as medicine and were aware of its emetic properties.[23] This indigenous knowledge was passed on to the European colonizers, who used the ipeca for decades as an emetic for patients who ingested poison and also for children who had ingested toxic substance.[24] *Carapichea* is commonly known as Rio or Brazilian ipecac, indigenous to Brazil, particularly especially the moist and shady forests of Matto Grosso and Minas Geraes.[25] It is also cultivated to some extent in Malaysia, Burma and the Darjeeling Hills of West Bengal, India.[26] Ipecac in India was cultivated by the British in 1866 and 1872 from material sent from Brazil but proved to be unsuccessful. Over the years, limited production has been established at Malaya, Burma and Darjeeling district to Nilgiri and Sikkim in recent years. Ipecac has been produced along with companion crop *Cinchona*. The first written account about ipecacuanha can be traced back to 1601 by a Portuguese Jesuit who studied Brazilian history and recorded a drug named *igpecaya* or *pigay*.[23] Ipecacuanha is a slow-growing plant and hence it has less economic appeal as a crop plant. It is rarely cultivated in South America but it has been cultivated in India to New Zealand.[27]

Taxonomy

Carapichea ipecacuanha (Brot.) L. Andersson is a species of plant belonging to the family Rubiaceae, one of the largest families of plants. This angiospermic family harbors around 13,000 species belonging to 650 genera 30.[28] Ipecac plant cultivation is not easy outside its natural rainforest habitat, in southeast Asia several attempts have been made but was not of much success.[29] In natural growth conditions, the plant species are distributed in circular as well as elliptically shaped clusters with very well delimited borders and occupy humid, shady areas under the forest canopy.[30]

Morphologically, the plant bears opposite leaves above, but usually naked below with pubescent toward the apex. Leaves are petiolate, entire, oblong, dark green with rough texture. Roots are branched and covered with a thick, transverse ringed bark, that becomes a diagnostic character for the drug. Colour shows reddish brown, with characteristic round ridges that are linked to

subterranean stem by a ground distinct filament. At the base of leaf stalks, a pair of whitish stipules is seen. The inflorescence is capitate, enclosed by a large one-leafed involucres. Flowers are small, white, funnel-shaped, enclosed within four large ovate bracts, corolla white with reflexed limb segment, stamens 5, slightly exserted (Figure 4). The stamens and pistils are dimorphic with some flowers bearing long stamens and short pistils and in contrast, other flowers short stamens and long pistils. Fruit are berry, dark violet, crowned by the limb of the calyx, 2-celled, 2-seeded.[31,32] Vernacular names *Eng:* Ipecac; *Brazil:* Rio ipecac.[33] Other such popular names in Brazil include *ipeca, poaia, poalha*[34] and *pepaconha*[35] and also termed *raicilla* in Central America countries.[36] *Kannada:* Ipikaakyunaoushadhi; *Tamil:* Ipika, ipikakku, ipikakkucceti; *Urdu:* Gurmarbuti.[37]

Medicinal uses of Ipecac drug

The importance of C. *ipecacuanha* in Historia Naturalis Brasiliae, probably, the oldest formal documentation of C. *ipecacuanha* use was highlighted.[38] Roots of C. *ipecacuanha* were brought by Piso from Brazil to Europe in early 1649. However, the material was sceptically used by the Europeans until the early decades of the 1700s. Johann Schweitzer was the pioneer to use the material against dysentery suffered by Dauphin Louis, the eldest son of Louis XIV and Crown Prince of France.[39] The roots were used especially against coughs, bronchitis, whooping cough and amoebic dysentery. The roots were usually harvested from 3 years old plants and dried. The plant is also used in homeopathy in the treatment of nausea.[40] During World War I, approximately 4-7% of the total number of patients admitted in military hospitals in North Africa suffered acute dysentery caused by *Entamoeba histolytica,* resulting in large number of casualties.[41] By the year 1925, about 10% of the European and American population acquired *E.histolytica* cysts.[42] Until 1960s, surgeons used root extracts of *Carapichea* to treat patients suffering from amoebic dysentery. An extensive study evaluating the utility of large doses of ipecacuanha by Joseph Ewart of the Bengal Medical Service has been published in the Indian Annals of Medical Science.[43] Edward Scott Docker of the Indian Army Medical Service, while his stay in Mauritius, first tried large doses of ipecacuanha for treatment of dysentery in 1858 and succeeded in reducing patient mortality from 18 to 25 %.[17] However, large doses of Ipecac through mouth were complicated by severe nausea and vomiting. Over the years, an alternative therapy was discovered by Leonard Rogers in India, that the principal alkaloid in ipecac killed amoebae in mucus of stools from patients with dysentery at dilution as high as 1/100000. In 1912, he successfully treated three patients in Calcutta,who had been unable to tolerate oral ipecac, by injection of emetine.[44] In due course of time, surgeons in India used large doses of *C. ipecacuanha* root extracts. Dysentery was a prevalent disease and continues to be so within tropical country especially India.[45] The World Health Organization (WHO) in compiling a global inventory of medicinal plants. It is an remarkable effort and if adopted by the Primary Health Care (PHC) as strategy, it could provide treatment of people worldwide, especially in the developing countries with comprehensive health care.

Ipecac, or Syrup of Ipecac (SOI) is a drug used to induce vomiting and in higher doses it is a rapidly acting emetic. Excessive use of SOI as a purgative in eating disorders is increasing, even though its medicinal importance has lessened over the years.[46] This drug was previously used as an expectorant in mild doses. Study was conducted to describe how *"poaieiros"* in Brazil maintained the cultural memory of *P. ipecacuanha*.[47] The root is the most utilized part and its mode of preparation is tincture or in mixture with tobacco, wine or sugarcane. The loss of knowledge associated with ipecac is caused by rural exodus, habitat due to deforestation and agricultural practices.

In the 19th century, ipecacuanha was registered as an emetic and an expectorant in the pharmacies of Benedictine monasteries of Rio de Janeiro and Olinda, Brazil.[48] For therapeutic uses and in treatment for dysentary, ipecac remained in India and Europe.[49] The principal constituents in ipecac roots are emetine, a non-phenolic alkaloid and cephaeline, a phenolic alkaloid and the total content of the two alkaloids accounts for more than 84%.[50] Decoction of leaf is used as an expectorant and powdered form are used against dysentery. The alkaloids emetine and cephaline have proven pharmacologically active as emetics, anti-amoebics and anti-diarrheal.[51] Several uses of ipecac have been found in recent studies, including treatment against dysentery, bronchitis, worms, blood disorders, leukemia, teething children, cancer, induction of vomiting, expectorant and as an anti-amoebic.[36] It is also applied externally on the site of bites by the venomous insects and scorpions.[52] Paradoxically, ipecac is itself a poison as it promptly induces vomiting. However, there is less concern for its intrinsically poisonous nature.[46]

Recommended Dosage

Ipecac syrup, consisting of total alkaloids 123 to 157 mg per 100 mL, has been administered to induce vomiting. Dosage range normally for the syrup is 10 to 30 mL, yielding a dose of alkaloids of 12 to 48 mg. The syrup and fluid extract of ipecac have distinct properties, the

extract is 14 times stronger than the syrup. Ipecac, is not recommended for routine use by the American Academy of Clinical Toxicology (AACT), the European Association of Poison Centres, Clinical Toxicologists (EAPCCT) and the American Academy of Pediatrics (AAP).[53] However, for cumulative toxicity, for amoebic dysentery, administration of emetine in small doses for a short time span is given with intervals of some weeks then followed by further treatment.[54] Ipecac is itself a poison as it readily induces vomiting.[46] In human the most exhibited complication related to ipecac administration are diarrhea, lethargy, depression and prolonged vomiting. Therefore use of the emetic is not routinely recommended.[55]

Production And Technology of Ipecac

Ipecac cultivation is suitable in well-drained soil, rich in humus, with enough moisture, humidity and shade and it is difficult to cultivate outside natural habitat. During late spring season, propagation via green wood is usually done, in sandy soil compost at temperature around 21-24°C. Ipecac can also be propagated via root cutting during seasons of harvesting. When the plants bear flowers, the roots are dug and then dried for use by the pharmaceutical industry. Cultivated plant are eventually replanted after partial removal of roots. The principal source of drug at present is Costa Rica.The global production of Ipecac is approximately 100 tonnes per year,which comes mostly from Nicaragua,Brazil and India.[56] Cenargen initiated a program for the recollection and conservation of the genetic variability. During 1988 to 1991, five expeditions for collections were undertaken, in the States of Rondonia, MatoGrosso, Pernambuco, Bahia, Espirito Santo, Rio de Janeiro, Minas Gerais and 86 accessions were collected as well as maintained in field germplasm banks at Embrapa-Ocidental Amazon, Belém, Para and at Florestas Rio doce, Linhares and Espirito Santo.[34] In due course of time other germplasm collections was established at the University of North Fluminense.[57] *C. ipecacuanha* species could be successfully regenerated by means of callus culture with 2,4-D and NAA along with kinetin promoting callus induction growth.[58]

The Brazilian medicinal species were challenged from intense extractivism (root harvesting and gradual loss of its habitat). A discussion to evaluate the three localization strategies of Mata Atalantica population and also to survey cultural and ethnobotanical aspects of the species was conducted. The species localization strategies were based on popular information-PL; Localization herbarium referred-HR and random localization-RL.[59] Conservation and production of ipecac plants from long term shoot cultures have been established and due to high pharmacological value of emetine and high risk of extinction together with great market demand, a need for alternative cultivation methods is necessary.[60] Data on the development of an *in vitro* root culture protocol for *P. ipecacuanha*. Leaf, nodal, intermodal rood segments were introduced in culture media containing different concentrations of Indol Butyric Acid (IBA).[61]

Chemical Constituents

In the year 1817, Pelletier and his group separated the "emetic principle" of ipecacuanha and named it as emetine.[62] The active principle of ipecac was formed of many different bases.[63] Firstly, the non-crystalline base which forms crystalline salts was emetine, the second one formed crystalline salts and was called cephaeline and another alkaloid was also identified as psycotrine. The constituents of the drug was mainly emetine (1-2 %), cephaeline, psychotrine, tannic acid called ipecacuanhic or cephaelic acid with starch, resin, etc.[32] With the ratio of emetine to cephaeline content (i.e., 2-3:1), samples were indeed *Cephaelis ipecacuanha* and the standard current pharmaceutical substance was confirmed to be *Cephaelis acuminata* (with ratio emetine : cephaeline, 1:1). The active principles exist only in the bark of the root and probably in the thin, outer layer of cork cell.[64] Alcohol extraction of the plants *Cephaelis acuminata* and *Cephaelis ipecacuanha* yield ipecac or Syrup of Ipecac (SOI). The extract is mainly a mixture with glycerin, sugar (syrup) and methyl paraben. The active ingredients are plant alkaloids, cephaeline and methyl-cephaeline (emetine).[46]

Gradually, emetine, $C_{15}H_{22}N_2O_5$ and cephaeline, $C_{14}H_{20}NO_2$, which were formerly supposed to be same were differentiated.[65] The botanical source of Ipecac is cited in Pharmacopoeias as the dried roots of *Carapichea ipecacuanha* and *Cephaelis acuminata*.[66] The roots of ipecac contain a number of medically active constituents including isoquinoline, alkaloids, tannins and glycosides. From the dried roots (crude drug "ipecac"), of *C. ipecacuanha* isolation of 6-*O*-methylipecoside, ipecosidic acid, neo-ipecoside, 7-*O*-methylneoipecoside, 3,4-dehydro neoipecoside and demethylalangi-side was done.[67] Figure 1 represents the compounds isolated from roots of ipecac.[68]

Ipecacuanha obtained from *C.ipecacuanha* is a chemical compound with white crystalline bitter alkaloid, emetine named after its peculiar emetic principle. The chemical structure and stereochemistry of emetine were first studied and illustrated by chemical degradation experiments.[69] Emetine was chemically characterized[70]

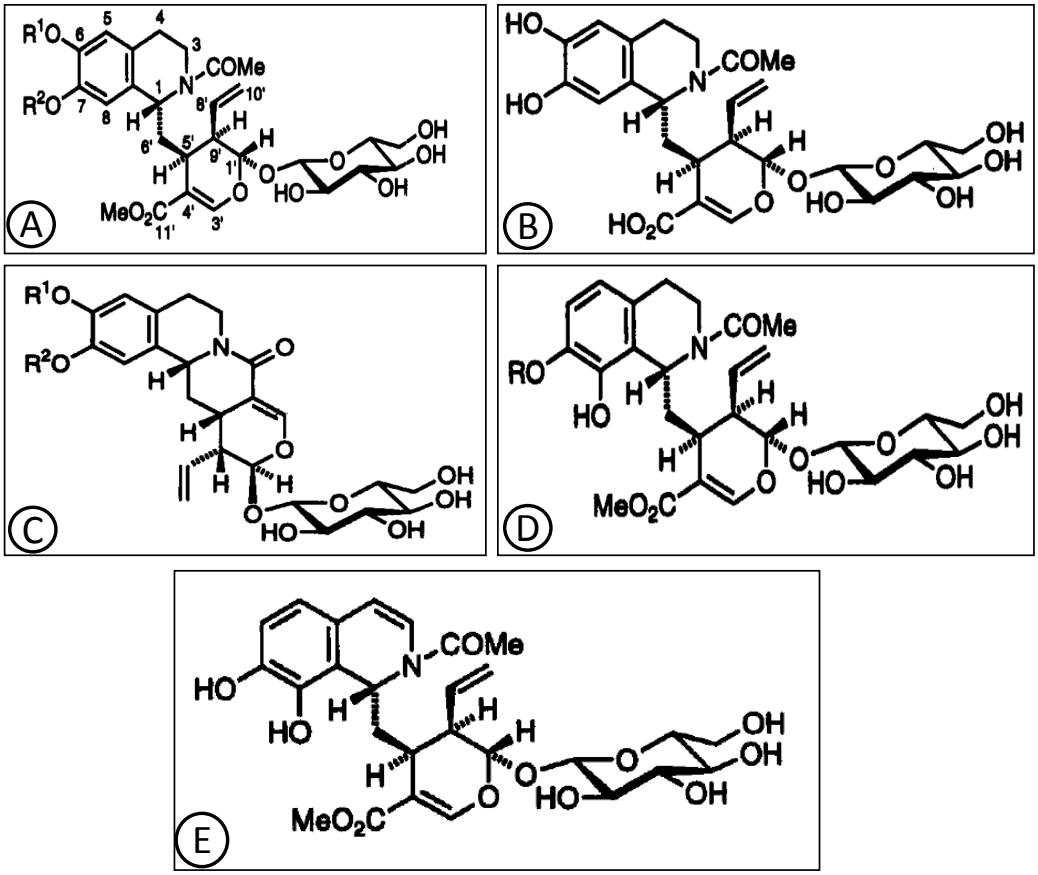

Figure 1: Chemical compounds isolated from roots of Ipecac; A: 6-*O*-Methylipecoside R1 = Me, R^2 = H. B: Ipecosidic acid, C: Demetylalangiside R^1=R^2=H, D: Neoipecoside R=H, 7-*O*-Methylneoipecoside R = Me. E: 3,4-dehydroneoipecoside.

and molecular structure of emetine was also provided as ($C_{30}H_{40}N_2O_5$)[71] which remains valid today.[65] The highest level of emetine is in the roots than in stems and leaves and none in the seeds of *C. ipecacuanha*.[72] Emetine hydrochlorate have been obtained in crystalline form that became popular as a medicine.[73] Figure 2 shows the chemical structure of emetine and cephaeline.[74] Later, isolation and characterization of the other alkaloid cephaeline from *C.ipecacuanha* roots was also performed.[6]

Biosynthesis of Emetine and Cephaeline

The biosynthesis of emetine and cephaeline comes from two main biosynthetic pathways, the biosynthesis of dopamine from L-tyrosine and that of secologanin from geranyldiphosphate.[75,76] Site of dopamine is cytosol, accumulated in the vacuole. The first step of the pathway is the condensation of dopamine and secologanin, two epimers, (S)-deacetylisoipecoside and the (R)-deacetylipecoside are formed as a result of condensation. The condensation reactions of dopamine and secologanin and of dopamine and protoemetine are supposed to occur in the vacuole. Then (S)-epimer is further converted to ipecac alkaloids such as cephaeline and emetine, the (R)-epimer gives rise to alkaloid alglucosides such as ipecoside and alangiside. Biosynthesis Emetine branches off from N-deacetylisoipecoside through its 6-O-methylation by IpeOMT1, with assistance by IpeOMT2, further by deglucosylation by IpeGlu1. The 7-hydroxy group of the isoquinoline skeleton of the aglycon is methylated by IpeOMT3 before the formation of proemetine, followed by sequential O-methylations by IpeOMT2 and IpeOMT1 to form cephaeline and emetine, respectively. In addition to this central pathway of ipecac alkaloid biosynthesis, formation of all methyl derivatives of ipecac alkaloids could be explained by the enzymatic activities of IpeOMT1–IpeOMT3, exhibiting in Figure 3 that they are necessary for all O-methylation reactions of ipecac alkaloid biosynthesis.[75] Biosynthesis of emetine and cephaeline[77] and the ecology of variations in alkaloid production in *C. ipecacuanha* populations in widespread geographical regions have been since carried out.[78]

Uses of Emetine and Cephaeline

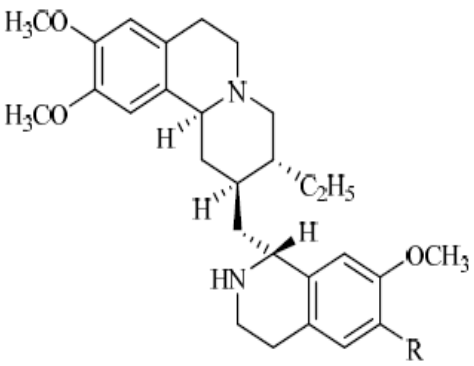

Figure 2: Chemical structure of Emetine (R=OCH_3) and Cephaline (R=OH).

Emetine is mainly used as an emetics to induce vomiting until the stomach turns empty, making it suitable for treating drug overdoses. Low doses acts as expectorant and excess dose leads to severe vomiting and diarrhoea. The gastric and bronchial systems are stimulated by emetine, curing fevers and cyst formation during amoebic dysentery.[40] Emetine causes an increased secretion in the trachea in minute dose and therefore is recommended to clear throat. However, Cephaeline shows stronger emetic activity and higher toxicity. Emetine can kill protozoa, even at a concentration of 0.5-1.0 mg/mL, therefore, it is used as a specific medicine targeted for treating amoebic dysentery.[79]

Emetine also exhibits cytotoxic activity, inhibiting protein synthesis, which makes it suitable for applications in drug-induced apoptosis.[24] Emetine, has been found to have anti-helminthic and antiamoebic properties.[46] In recent times,synthetic analogues of emetine with less adverse effects are used in the treatment of amoebiasis.[80]

Biological Activities of Emetine and Cephaline

Investigations to determine the specific roles of emetine and cephaeline indicated that emetine was infact a 'good' expectorant, in comparison to cephaeline; however cephaeline was more efficient as an emetic.[17]

Anti cancer effect of emetine: An effective strategy implimented by scientists is the 'drug repositioning'. Emetine (EMT) have been shown to possess anti-tumor activity.[81] The anti-cancer effect of EMT was first stated forward on malignant human tumors.[82] In course of time, the review showed that EMT exhibits its anti-tumor effect.[83] This was mainly by apoptosis regulation of pro-apoptotic factors. Mechanisms such as protein biosynthesis inhibition, DNA interaction, also causes the anti tumor effect. The EMT structure was derivatized at the N-2' position then selectively delivered as a prodrug. An enzyme, fibroblast activation

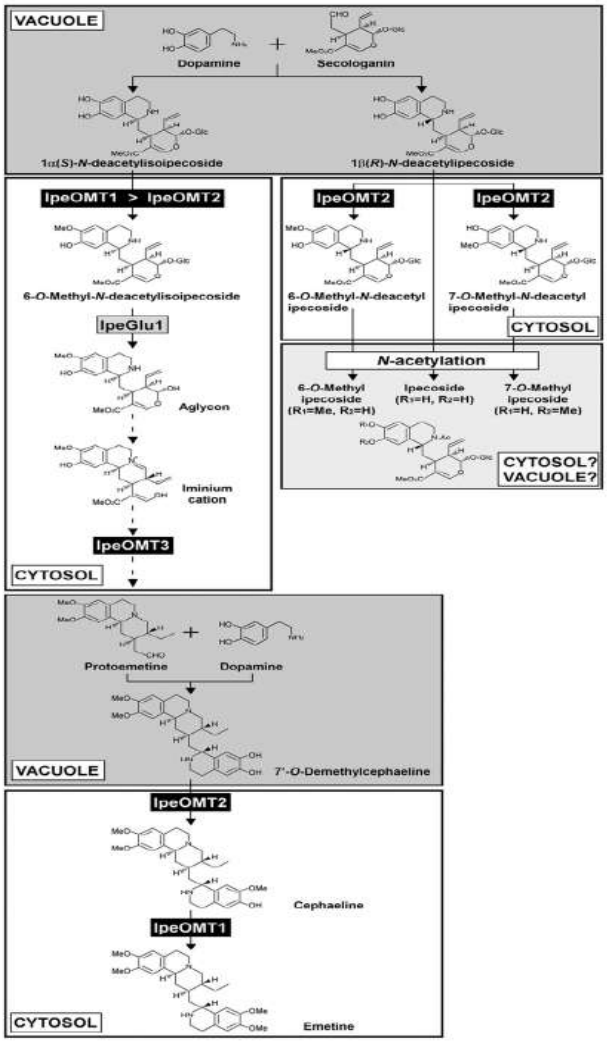

Figure 3: Biosynthetic pathway of Ipecac alkaloids highlighting major catalyzed reactions.

Figure 4: *Carapichea ipecacuanha* (whole plant).

protein (FAP) which is overexpressed in certain cells within the metastatic tumor to cancer cells activates the prodrug.[84] In case of Prostate PC3 cells, cervical C33A cells, breast cancer MCF7 cells as well as MCF7/Adr cells, the alternative splicing of caspase 9 pre mRNA regulatory effects was carried out in response to EMT hydrochloride. It lead to the conclusion that the various splicing patterns of the caspase 9 gene were regulated by EMT and other compounds that acts by resisting or sensitizing the tumors to different cell death inducers.[85]

In Ovarian cancer, administration of cisplatin along with EMT was effective in inducing apoptosis. EMT affects the activation of caspases -3, -7 and -8 and downregulation of bcl-xL leading to apoptosis.[86] EMT checks migration and invasion of human Non-Small-Cell Lung Cancer (NSCLC) cells in cases of lung cancer.[87]

The results of investigations indicated, that Hedgehog (Hh) pathway is usually modulated by EMT and coristatin by binding to vital proteins in regulation of Cancer Stem Cells.[88] Among the first compounds isolated, Emetine sensitizes the pancreatic tumor cells to Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL)-induced apoptosis.[50] Low nano molar concentrations of EMT completely inhibits expression of HIF1α and HIF2α that plays major role in hypoxia signaling and tumor growth progression.[89]

Protein, DNA, RNA synthesis inhibition: The inhibition of ribosomal protein synthesis in mammalian, yeast and plant cells by emetine with respect to concentration- and time-dependence was reported.[90] Emetine also causes inhibiton of protein synthesis irreversibly in HeLa cells by lowering the number of free ribosomes and thereby increasing the polyribosomes.[91] In Chinese Hamster Ovary (CHO) cells, the protein synthesis inhibition is reversible.[92] The mechanism by which protein synthesis inhibition possibly occur are due to the inhibition of the aminoacyl RNA transfer reaction by emetine at 40S ribosomal subunit site.[93] In Exocrine cells and seminal vesicles of pancreas the emetine prevents induced autophagy by stabilizing polyribosomes and blocking of protein synthesis.[94] Emetine has also been reported to inhibit DNA synthesis in sea urchin[95] and mice.[96] Emetine specifically targets and blocks the early S phase of DNA replication.[97]

The potential of this naturally occuring drug to inhibit protein synthesis was used in maintenance of the activity of alcohol dehydrogenase to reduce the pathological alcohol addiction.[98]

Antiparasitic property of emetine: Emetine has been widely utilised in the treatment of amoebiasis and amebic dysentery. The drug inhibits the growth of the causative agent *Entamoeba histolytica*. Emetine inhibits protein synthesis eventually kills the trophozoites of *E. histolytica* by irreversible and noncovalent binding to the peptide-chain elongation site of the 60S subunit of ribosomes.[90] The effect of the drug has been verified as an effective anthelmintic effective against *Protostron gylusrufescens* in infected sheep and goat.[99] It has been tried as trypanocidal agent against *Trypanosoma cruzi* in quest for drug against Chaga's disease.[100] Emetine was effective at *in vitro* anti leishmanial activity against *Leishmania donavani*.[101] Emetine by means of DNA intercalation and inhibition of protein biosynthesis could induce apoptosis in *Trypanosoma brucei*.[102] Emetine and cephaline has potential for production of potent drugs against Leishmaniasis.[103]

Emetine an anti-protozoal agent, potently inhibits both ZIKV and EBOV entry *in vitro* and potent activity *in vivo*. Cephaeline, a desmethyl analog of emetine, also displays a similar efficacy against ZIKV as well as EBOV infections.[104]

Antiviral property of emetine: Emetine has some notable antiviral activities. Emetine was an able antipoxviral agent that blocked vaccinia virus replication at non-cytotoxic.[105] This alkaloid compound was eventually reported to display antiviral activity against four serotypes of Dengue Virus (DENV) and a dosage-dependent reduction of viral infection was observed at a noncytotoxic dose.[106] Emetine inhibits HIV-1 replication by interfering with Reverse Transcriptase Activity.[107]

Infection with Human Cytomegalovirus (HCMV) is a threat for pregnant women and immunocompromised hosts but identification of emetine as HCMV inhibitor have been shown.[108] HCMV inhibition by emetine depended on ribosomal processing S14(RPS14) binding to MDM2, leading to disruption of MDM2-IE2 interactions.

Inhibition of the Nonsense mediated mRNA decay (NMD) Pathway: The mode of action used by cells to check the synthesis of truncated or defective protein is primarily via Nonsense-mediated decay (NMD).[109] Emetine acts as an inhibitor to NMD.[109] Emetine has been subsequently employed in GINI to inhibit NMD in prostate cancer cell lines (DU145, PC3 and LnCaP).[110]

Contraceptive property of Emetine: The potential of emetine as a protein synthesis inhibitor introduced the idea of determining its efficacy as a contraceptive agent when administered locally.[111] An investigation was done in rabbit uterus and the results exhibited the anti implantation effect of emetine dihydrochloride, increased with the rise in concentration.[111] Another study examined the suitability of emetine ditartrate as

an emergency contraceptive.[112] The uterus and early embryos around implantation, mainly the trophoblast and endometrial cells at the attachment site, are the primary target of the action of emetine ditartrate. Emetine ditartrate could be used to terminate human pregnancy in the initial stages.[112]

Toxic effects of emetine: Although emetine is an alkaloid of immense medicinal value. Its current medicinal use has been discouraged because of toxicity. Chronic usage has been reported to induce myopathy.[113] Along with cardiotoxicity, cardiomyopathy as well is an adverse chronic use of emetine.[114] In an experiment performed on protein pharmacology to ligand chemistry, various other targets were discovered for emetine and these lead to some of the side effects of the pharmacological use.[115]

Mechanism and Mode of Action

The major alkaloids of ipecac (emetine and cephaeline) are apparently pharmacologically active and have both local and central activity. Locally causing an irritant effect on gastric mucosa, whereas the central activity leads to the stimulation of the chemoreceptor trigger zone. While occurrence of vomiting, contents from both the stomach and small intestine are expelled.[116] Patients who are hypoxic, dyspneic, not able to swallow, hypovolemic or comatose the effect of emetics are contradicted. Emetics should not be given after ingestion of petrolatum or similar chemical compounds as the chance of subsequent aspiration out competes the potential toxicity. Overdose of ipecac usually leads to cardiotoxicity. In the presence of strychnine intoxication, or with other CNS stimulants, use of emetics might precipitate seizures.[116] Adsorption of ipecac syrup by activated charcoal may occur, therefore these drugs should not be administered simultaneously. In such cases, ipecac syrup should be given first and then administration of activated charcoal only once if vomiting has occurred. The effectiveness of ipecac may be decreased by consumption of dairy products and carbonated beverages. Biologically active emetine, C-1´ have the R configuration and the 2´ position have a secondary amine.[90] The epimer, 2 (isoemetine) with the S configuration at C-1′ is inactive. The activity was absent in case of 3 (O methylpsychotrine) with unsaturation at the 1′ - 2′ positions and 4 (N-methylemetine) indicating that the position must be a secondary amine. The unsaturation at the 2-3 positions to give 5 (dehydroemetine) and the asymmetry is lost at carbons 2 and 3 but this change does not affect protein synthesis inhibition. The tertiary nitrogen is converted into a quaternary ammonium moiety by oxidation to 6 (1,2,3,4,5,11b trisdehydroemetine) which results in loss of activity. In course of time, results obtained confirmed the R configuration at the C-1′ position and methoxy group at C-7′ is a necessary structural requirement for the biological activities of emetine. C-7′.[117]

CONCLUSION

The aim of this review was to showcase the valuable applications of plant species *Carapichea ipecacuanha,* its unique emetic properties and various compounds. Due to its potential toxicity and effect of overdose, ipecac syrup is not recommended. However, chemical compounds such as emetine, cephaline extracted from the plant has multiple function in treating various ailments. Thus, it is important to get familiar with the plant species from a medicinal perspective. The policy makers and health administrators should encourage research works based on medicinal plants that are given utmost priorities.[118] Plant-derived pharmaceuticals are fast growing and becoming the major commercial development in biotechnological industry. They also provide the futuristic opportunity to provide low-cost pharmaceuticals to the developing nations.[119] The recent researches conducted on herbal plants or medicine, have been a significant achievements in the pharmacological evaluation of various plants used for long in traditional systems of medicine. Therefore, plants can be a major source of medicines due to availability of its active compounds that can be added and prescribed through standardized dosages as crude or processed drugs for the betterment of humankind.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

WHO: World Health Organization; **AYUSH:** Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy; **PHC:** Primary Health Care; **SOI:** Surup of Ipecac; **AACT:** Academy of Clinical Toxicology; **EAPCCT:** European Association of Poison Centre, Clinical Toxicologists; **AAP:** Americam Academy of Pediatrics; **NAA:** Napthalene Acetic Acid; **IBA:** Indole

Butyric Acid; **EMT:** Emetine; **NSCLC:** Non-small Cell Lung Cancer; **TRAIL:** Tumor Necrosis Factor Related Apoptosis Inducing Ligand; **DNA:** Deoxyribonucleic Acid; **RNA:** Ribonucleic Acid; **ZIKV:** Zika Virus; **EBOV:** Ebola Virus; DENV: Dengue Virus; **HIV:** Human Immuno Virus; **HCMV:** Human Cytomegalo Virus; **NMD:** Non-sense Mediated mRNA decay; **CNS:** Central Nervous System.

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Leaf architectural studies in *Phyllanthus* L. (Phyllanthaceae) from Arunachal part of Eastern Himalaya in India

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Abstract

Leaf architectural characters of seven species of Phyllanthus L. (Phyllanthaceae) were studied on the basis of foliar morphometric characters. The characters studied include leaf attachment, petiole features, laminar shape, apex and base shape including angles, margin type, blade class vein category etc. Estimation of vein islet number and minor venation details like the areole size, absolute vein islet and vein termination number were recorded and distinct description based on the leaf architectural traits for each species have been done. The quantitative data revealed some distinct variation among the species with some expressing close relatedness. A dichotomous key for the species have been constructed and relationships among the taxa have been represented through a dendrogram.

Keywords: Leaf, architecture, *phyllanthus,* Arunachal, Eastern Himalaya.

Introduction

The family Phyllanthaceae Martynov is a group of flowering plants with maximum distribution along the tropics including the south-temperate zone with sparse distribution towards the north-temperate[1]. The family is predominant in the Paleotropical, Neotropical, Cape and Australian biogeographic regions[2] with habits predominantly of shrubs and small trees and rarely of herbs[3]. Phyllanthaceae is the second largest segregate of Euphorbiaceae *sensu lato* comprising of about 2000 species further grouped into 54 to 60 genera[4]. The genera are further grouped into two monophyletic sub-families and ten distinctly defined monophyletic tribes[3]. The family has been segregated into 10 sub-genera and 50 sections based on nrITS and plastid *mat K*sequence studies[5].

Some major genera under the family include *Cleistanthus* with about 140 species, *Antidesma* (100 spp.), *Aporosa* (90 spp.), *Uapaca* (60 spp.), *Baccaurea* and *Bridelia* with about 50 species each[6].

The genus *Phyllanthus* was first described by Linnaeus[7] and as per Mabberley[8], the genus is represented by 750 – 800 species distributed globally with 51 species under 6 sub-genera found in India out of which 18 species are endemic[9].

In angiosperms, majority of the dicotyledonous taxa has a consistent pattern of leaf architecture and method of describing leaf shape, size, marginal configuration, gland characters, venation patterns etc. is of immense importance for both extinct and extant taxa[10]. Leaves are anatomically much varied organs and shows significant variations both between and within genera and specific occasionally in familial lines[11].

Over the years, foliar architecture study has proven useful in identification of living as well as fossil angiospermic species[12]. The pattern of leaf reticulation is evident to be the result of evolutionary convergence in leaf form[13]. In plants, the diversity in form and function of leaf corresponds to high diversity in geometry of venation network[14]. Taxonomic study involving the study of morphological characters of flower and fruit have been playing key role in identification of plants since long. Compared to floral and fruit characters, leaves are generally given less priority in taxonomic and comparative studies due to detailed characterization of different leaf attributes[15]. Leaves of cretaceous and tertiary angiosperms that were studied[16], proved to be an ecologically significant in understanding the semi-quantitative proxy measurements of plant evolutionary patterns[17].

The present study deals with leaf architecture of seven species of *Phyllanthus* L. Phyllanthaceae, to understand and delineate the foliar micro-morphological characters based on morphometry, venation pattern, marginal ultimate venation, areolation, apex and base shape including angles, leaf texture, appearance of tertiary and quaternary veins, vein angles etc. and thereby draw taxonomic discourses for easy identification at the species level.

Materials and methods

The present work on leaf architecture of some species of *Phyllanthus* L. has been carried out with specimens collected from the North-east Indian state of Arunachal Pradesh located at 26°28'N – 29°36'N Latitudes and 91°30'E – 97°30'E Longitudes (Figure-1).

The seven species under study were *Phyllanthus amarus* Schumach. & Thonn., *Phyllanthus emblica* L., *Phyllanthus fraternus* G.L. Webster*, Phyllanthus myrtifolius* (Wight) Mull. Arg., *Phyllanthus reticulatus* Poir.*, Phyllanthus urinaria* L. and *Phyllanthus virgatus* G. Frost.

For studying the morphometric characters, guidelines prescribed in the *Manual of Leaf Architecture*[18] was followed where laminar area was calculated by measuring the leaf length and width (mm) and thereby dividing the product by ⅔. On the basis of laminar size, the type of blade class was determined with leaf apex and base angles measured using a protractor. Detailed venation architecture was analysed following the method[19].The specimens were immersed in 2.5 % NaOH solution for about 5 – 12 days or until the leaf lamina appeared clear. The leaves were then washed with water to remove the traces of NaOH and were further cleared with soft brush without damaging the leaf tissue. If the leaf remained unclear, it was boiled in lactic acid to attain the desired level of clearing[20]. The leaf skeleton of each specimen was stained with safranin, dehydrated through ethanol grades and mounted in DPX[21].

The processed specimens were observed under Olympus compound microscope followed by detailed observation in Leitz, Stereoscopic Microscope for understanding various qualitative as well as quantitative foliar characters.

Using the stage micrometer and the camera lucida, the area of the total microscopic field was determined. For each species, area of the lamina (A), vein islet number (*vi*) and vein termination (*vt*) were recorded. Three such drawings were made, one from each of the apex, middle and basal portion of the lamina. The numbers of vein islets were then divided by the total area of the microscopic field to determine the number of the vein-islets per sqmm[22]. In case of incomplete vein-islets, two incomplete vein-islets were considered as one for the ease of calculation. Areole size in mm^2 was also determined with the help of ocular micrometer.

The Absolute vein islet number (A_{vi}) and absolute vein termination number (A_{vt}) were calculated[23].

A_{vi}= A x *vi*, where A_{vi} is the absolute vein islet number per area of the lamina. A is laminar area in mm^2 and *vi* is the average vein islet number/mm^2, A_{vt} = A x *vt*, where A_{vt} is the absolute vein islet termination number per area of the lamina. A is laminar area in mm^2 and *vt* is the average vein islet termination number/mm^2.

Dendrogram based on Bray-Curtis cluster analysis was prepared analyzing the leaf architecture characters using PAST *ver.* 3.24.

A dichotomous key was prepared on the basis of diagnostic descriptions made for each taxa under study.

Results and discussion

All the studied tax a showed simple leaf with alternate phyllotaxy and swollen petiole base. The lamina margin was observed to be entire and among the general features exhibited, secondary vein category, tertiary vein angle variability, vein islets count and areolation characteristics proved to be useful in distinguishing them. The macro-morphological characters especially the blade class, lamina ratio, margin type and the differences in the venation pattern at second and third degree category served as important features for taxonomic segregation (Tables-1-4).

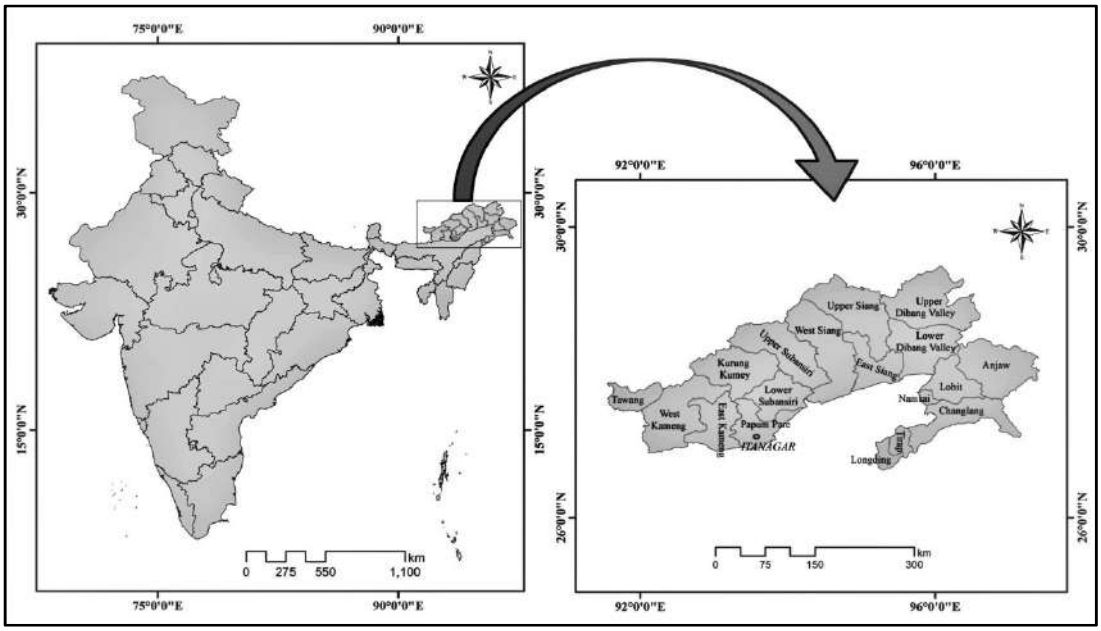

Figure-1: Map showing study area.

Phyllanthus amarus Schumach. and Thonn.: Leaves alternate, blade class leptophyll; petiole with swollen base; lamina oblong, 7 – 9 x 3 – 4.5mm, entire, rounded apex with minute mucro, base rounded, slightly oblique with acute base angle; secondary veins festooned brochidodromous with more than one additional sets of loops outside the main brochidodromous; irregular 2° vein spacing and uniform 2° vein angle; tertiary veins randomly reticulate where tertiaries anastomose with 2° and other 3° veins at random angles; weak intersecondaries, poorly developed areoles and fusion of high order veins with those running close to the margin forming fimbrial veins (Figure-2A).

Indian distribution: Throughout the country mainly in tropical and subtropical region.

Global distribution: Pantropical and sub-tropical regions.

Phyllanthus emblica L.: Leaves alternate, blade class nanophyll; petiole with swollen base; lamina oblong, 14–15 x 3 – 4mm, entire, rounded apex with minute mucro, base rounded with acute angle; secondary veins terminates to a strong vein closely parallel to the leaf margin classified as intra-marginal vein with irregular 2° vein spacing and uniform 2° vein angle; tertiary veins random reticulate where tertiaries anastomose with 2° and other 3° veins at random angles; strong intersecondaries, moderately developed areoles and fusion of high order veins with those runningclose to the margin forming fimbrial veins (Figure-2B).

Indian distribution: Cultivated throughout the country including Andaman Islands in warmer areas. Also common in the wild.

Global distribution: Sri Lanka, Pakistan, Nepal, Bhutan, Bangladesh, Thailand, China, W. Malaysia, Lesser Sunda Islands.

Phyllanthus myrtifolius (Wight) Mull. Arg.: Leaves alternate, blade class nanophyll; petiole with swollen base; lamina elliptic, 10–12 x 3–5mm, entire, apex convex and with acute angle, base cuneate with acute angle; secondary veins festooned brochidodromous with more than one additional sets of loops outside the main brochidodromous; irregular 2° vein spacing and 2° vein angle smoothly decreasing downward; tertiary veins random reticulate where tertiaries anastomose with 2° and other 3° veins at random angles; weak intersecondaries, with moderately developed areoles, marginal ultimate veins recurved to form loop (Figure-2C).

Indian distribution: Commonly grown in gardens as low hedge plant in warm regions.

Global distribution: Widely grown in pantropical regions.

Phyllanthus fraternus Webs.: Leaves simple, alternate, sessile, blade class nanophyll; lamina elliptic, 8–9 x 3–5mm, slightly oblique, entire, apex convex and with broader angle, base rounded, slightly oblique, with acute angle; secondary veins with more than one additional sets of loops outside the main brochidodromous; irregular discontinuity; tertiary veins intersect between secondaries with an abrupt angular discontinuity; intersecondaries absent, poorly developed areoles and fusion of high order veins with those running close to the margin forming fimbrial veins (Figure-2D).

Indian distribution: Throughout the country in warmer places.

Global distribution: Pakistan, Bangladesh, Nepal, West Indies, Africa; a pantropical weed.

Phyllanthus reticulatus Poir.: Leaves simple, alternate, blade class nanophyll; petiole with swollen base; lamina elliptic, 17–19 x 9–10mm, entire, apex rounded-obtuse and with broader angle, base rounded with obtuse angle; secondary veins joined together forming prominent arches, brochidodromous with irregular 2° vein spacing and uniform 2° vein angle; tertiary veins dichotomizing with weak intersecondaries; poorly developed areoles and fusion of high order veins with those running close to the margin forming fimbrial veins (Figure-2E).

Indian distribution: Throughout the country including Andaman & Nicobar Islands in warmer areas.

Global distribution: Pakistan, Nepal, Bhutan, Sri Lanka, S.E. Asia, Tropical China, Malaysia, Australia, Tropical Africa.

Phyllanthus urinaria L.: Leaves simple, alternate; blade class nanophyll; petiole with swollen base; lamina oblong, 13–15x 4–5mm, symmetric, entire, apex convex-rounded and with acute angle, base rounded with acute angle; secondary veins brochidodromous, joined together forming prominent arches; irregular 2° vein angle and spacing abruptly increasing towards the base; Tertiary veins intersect between secondaries with an abrupt angular discontinuity; weak intersecondaries, poorly developed areoles and marginal ultimate vein recurved to form loops (Figure-2G).

Indian distribution: Almost throughout the country including Andaman and Nicobar Islands.

Global distribution: Native of southern Asia but now widespread in tropical and subtropical regions.

Phyllanthus virgatus G. Forst.: Leaves simple, alternate, blade class nanophyll; petiole with swollen base; lamina narrowly ovate-oblong, 15–17 x 4–5 mm, entire, apex convex-obtuse with acute angle and a minute mucro, base rounded with acute angle; secondary veins joined together forming prominent arches, brochidodromous with irregular 2° vein spacing with uniform angle; tertiary veins randomly reticulate where tertiaries anastomose with 2° and other 3° veins at random angles; no intersecondaries, poorly developed areoles and marginal ultimate veins recurved to form loop (Figure-2F).

Indian distribution: Throughout the country including Andaman & Nicobar Islands.

Global distribution: Mainly distributed in Sri Lanka to SE Asia, S. China, Indo-China and Malaysia.

Table-1: Foliar morphometric characters of the studied species.

Species	Leaf attachment	Leaf organization	Petiole features	Mean laminar length (mm)	Mean laminar width (mm)	Mean laminar area (mm^2)
Phyllanthus amarus	Alternate	Simple	Base swollen	8.5	3.75	21.12
Phyllanthus emblica	Alternate	Simple	Base swollen	14.5	3.5	33.6
Phyllanthus fraternus	Alternate	Simple	Sessile	8.8	3.5	29.5
Phyllanthus myrtifolius	Alternate	Simple	Base swollen	10.5	4.0	27.72
Phyllanthus reticulatus	Alternate	Simple	Base swollen	18.0	10.0	118.8
Phyllanthus urinaria	Alternate	Simple	Base swollen	14.0	4.5	40.92
Phyllanthus virgatus	Alternate	Simple	Base swollen	16.0	4.5	47.52

Table-2: Foliar morphometric characters of the studied species.

Species	Petiole length (mm)	Blade class	Laminar Shape	Laminar L:B ratio	Apex shape	Apex angle	Base shape	Base angle	Margin type
Phyllanthus amarus	0.5	Leptophyll	Oblong	2.66:1	Rounded	Acute	Rounded	Acute	Entire
Phyllanthus emblica	1.0	Nanophyll	Oblong	4.14:1	Convex	Acute	Rounded	Acute	Entire
Phyllanthus fraternus	Sessile	Nanophyll	Oblong	2.51:1	Convex	acute	Rounded	Acute	Entire
Phyllanthus myrtifolius	0.1	Nanophyll	Elliptic	2.62:1	Convex	Acute	Cuneate	Acute	Entire
Phyllanthus reticulatus	2.5	Nanophyll	Elliptic	1.8:1	Rounded	Acute	Rounded	Obtuse	Entire
Phyllanthus urinaria	1.0	Nanophyll	Oblong	3.11:1	Convex	Acute	Rounded	Acute	Entire
Phyllanthus virgatus	0.1	Nanophyll	Oblong	3.55:1	Convex	Acute	Rounded	Acute	Entire

Table-3A: Micro-morphological characters of veins from the studied species.

Specimen	1°vein category	2°vein category	2°vein spacing	2°vein angle	Inter 2°vein	3°vein category
Phyllanthus amarus	Pinnate	Festooned brochidodromous	Irregular	Uniform	Weak intersecondaries	Random reticulate
Phyllanthus emblica	Pinnate	Intramarginal vein	Irregular	Uniform	Strong intersecondaries	Random reticulate
Phyllanthus fraternus	Pinnate	Festooned brochidodromous	Irregular	Uniform	Absent intersecondaries	Alternate percurrent
Phyllanthus myrtifolius	Pinnate	Festooned brochidodromous	Irregular	Smoothly decreasing towards base	Weak intersecondaries	Random reticulate
Phyllanthus reticulatus	Pinnate	Brochidodromous	Irregular	Uniform	Weak intersecondaries	Dichotomizing
Phyllanthus urinaria	Pinnate	Brochidodromous	Irregular	Abruptly increasing towards base	Weak intersecondaries	Alternate percurrent
Phyllanthus virgatus	Pinnate	Brochidodromous	Irregular	Uniform	Absent intersecondaries	Random reticulate

Table-3B: Micro-morphological characters of veins from the studied species.

Specimen	3°vein angle	3° vein angle variability	4°vein category	Areolation	Marginal Ultimate Venation
Phyllanthus amarus	Perpendicular	Inconsistent	Absent	Poorly developed	Fimbrial vein
Phyllanthus emblica	Obtuse	Inconsistent	Dichotomizing	Moderately developed	Fimbrial vein
Phyllanthus fraternus	Obtuse	Inconsistent	Absent	Poorly developed	Fimbrial vein
Phyllanthus myrtifolius	Obtuse	Inconsistent	Dichotomizing	Moderately developed	Looped
Phyllanthus reticulatus	Obtuse	Inconsistent	Regular polygonal	Poorly developed	Fimbrial vein
Phyllanthus urinaria	Obtuse	Inconsistent	Absent	Poorly developed	Looped
Phyllanthus virgatus	Obtuse	Inconsistent	Dichotomizing	Poorly developed	Looped

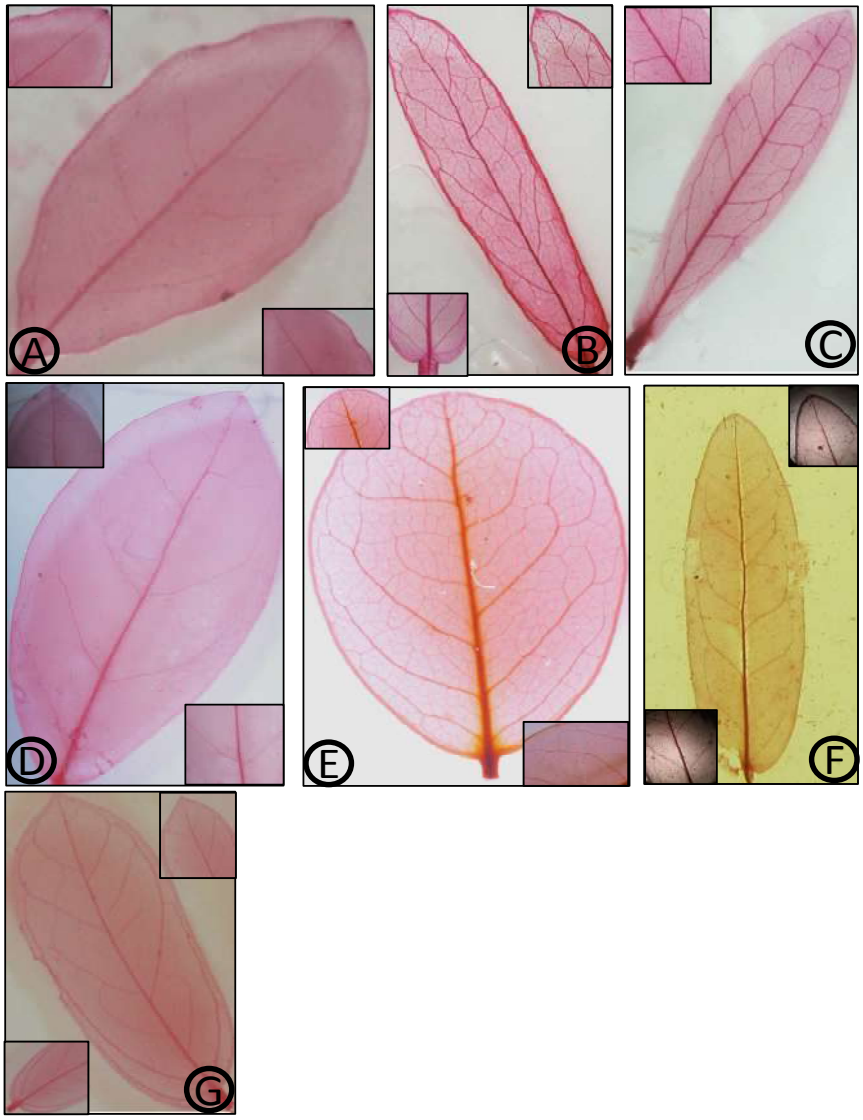

Figure-2: A. *P. amarus;* B. *P. emblica;* C. *P.myrtifolius;* D. *P. fraternus;* E. *P.reticulatus;* F. *P. virgatus;* G. *P. urinaria.*

Table-4: Quantitative characters of leaf venation from the studied species.

Species	Leaf area /mm^2	No of veins on one side	Angle between 1° - 2° veins	Average Vein islets/ mm^2 (A/M/B)	Vein islets termination number	Average size of areole/ mm^2	Absolute vein islets number	Absolute vein islet termination number
Phyllanthus amarus	21.12	10-12	20 – 25	4/5/4.3	2 – 3	0.114 ±0.030	93.77	52.8
Phyllanthus emblica	33.6	16-20	30 – 70	3.33/4.66/3.66	2 – 3	0.065±0.005	130.36	84.0
Phyllanthus fraternus	29.5	10-12	20 – 39	5/6.33/5.66	2 – 3	0.105±0.031	166.97	73.75
Phyllanthus myrtifolius	27.72	18-20	60 – 72	5/6/5.66	3 – 4	0.045±0.007	153.8	97.02
Phyllanthus reticulatus	118.8	12-14	30 – 40	3/4/3.33	2 – 3	0.06±0.002	408.67	297.0
Phyllanthus urinaria	40.92	12-15	43 – 50	3.66/5.66/5	3 – 4	0.097±0.006	195.18	143.22
Phyllanthus virgatus	47.52	12-14	48 – 60	1/3/2.66	2 – 3	0.742±0.120	126.4	118.8

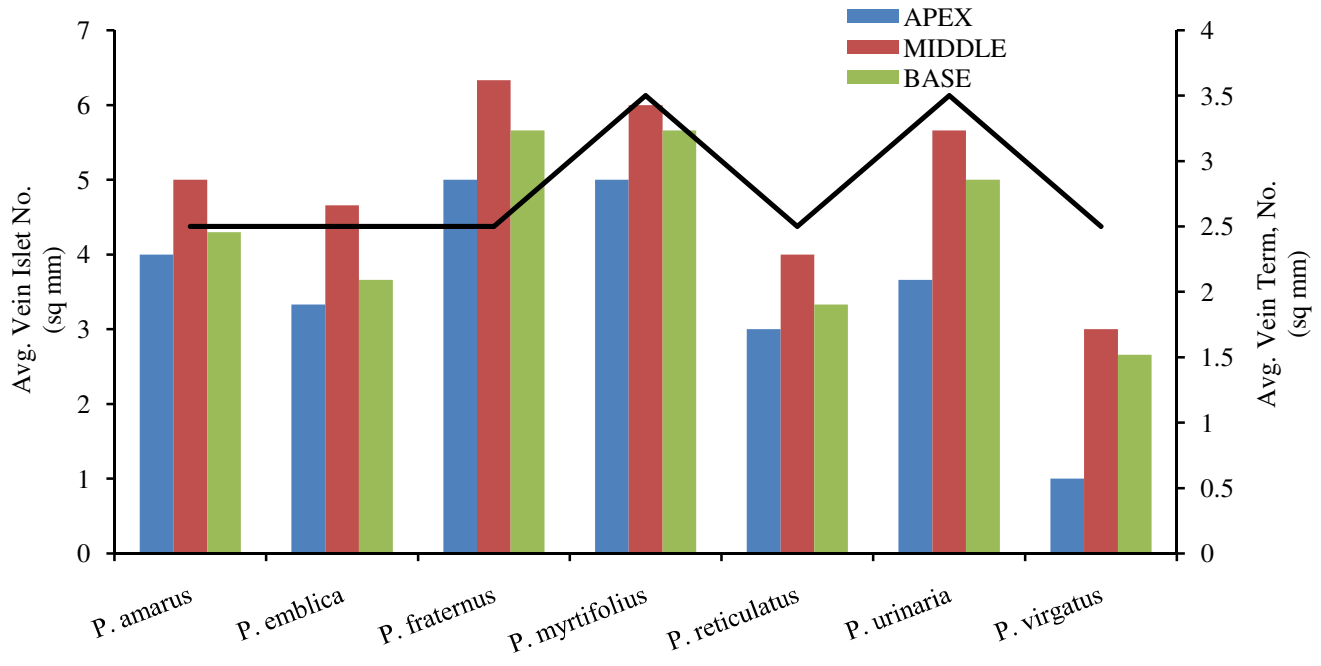

Figure-3: Graph showing average vein islet and vein termination number.

The dendogram based on Bray-Curtis cluster analysis were basically based on the quantitative morphometric and minor venation traits that include mean lamina length, breadth and area, and vein islet number per sq mm of leaf surface, areole size, angle of variation between 1° and 2° veins, absolute vein islet number and absolute vein termination number. On the basis of these quantitative foliar morphometric characters for seven species of *Phyllanthus,* majority of species have common ancestral line with *Phyllanthus emblica* showing more than 90% similarity to *Phyllanthus myrtifolius* and above 85% with *Phyllanthus virgatus.* More than 80% similarity have also been observed between *Phyllanthus amarus* and rest of the species excluding *Phyllanthus reticulatus* which remains out-grouped from the clade showing less than 60% similarity with the other six species under study (Figure-4).

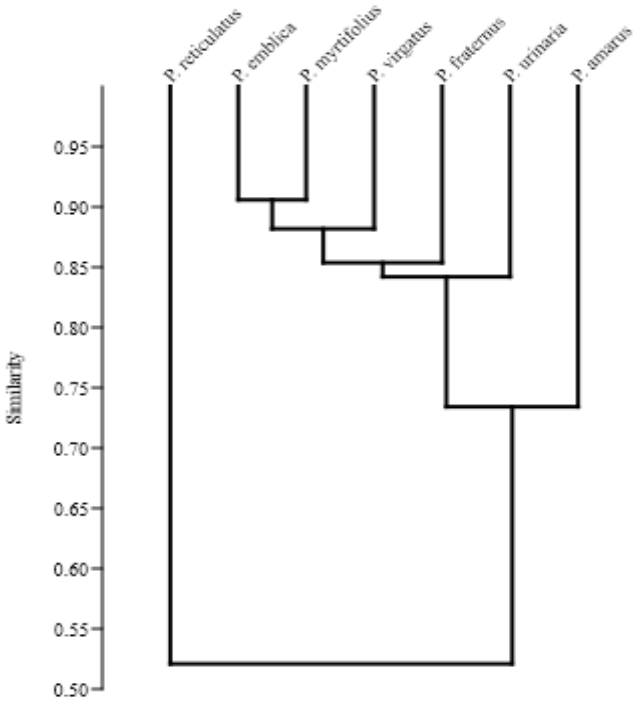

Figure-4: Dendrogram based on cluster analysis obtained from quantitative morphometric characters.

Key to seven species of Phyllanthus L. using leaf architectural characters

1a. Leaf organization simple, lamina elliptic……..…2
1b. Leaf organization simple, lamina oblong……3
2a. Leaf base cuneate, apex convex…… *Phyllanthus myrtifolius*
2b. Leaf base rounded, apex rounded.…*Phyllanthus reticulatus*
3a. Leaf base rounded, apex rounded or convex,1° vein order pinnate, 2° vein order Festooned brochidodromous….....4
3b. Leaf base rounded, apex convex……..5
4a.Leaf apex rounded, 1°vein order pinnate, 2°vein order festooned brochidodromous, 3° vein order random reticulate, weak intersecondaries …….*Phyllanthusamarus*
4b. Leaf apex convex, 1° vein order pinnate, 2° vein order festooned brochidodromous, 3° vein order alternate percurrent, intersecondaries absent.....*Phyllanthusfraternus*
5a. 1° vein order pinnate, 2° vein order intramarginal, stronginter secondaries....*Phyllanthus emblica*
5b. 1° vein order pinnate, 2° vein order brochidodromous, intersecondaries weak or absent……..……6
6a. 2° vein order brochidodromous, intersecondaries weak, 2° vein angle abruptly increasing towards the base…….....*Phyllanthus urinaria*
6b. 2° vein orderbrochidodromous, intersecondaries absent, 2° vein angle uniform ……....*Phyllanthus virgatus*

Discussion: Leaf lamina is functionally significant part of a plant that plays a direct role in carbon assimilation, magnifying the biomass allocation to lamina and diminishing its support investment[24]. As most of the dicotyledonous taxa possesses consistent leaf architectural pattern, a thorough study on foliar morphometry is utmost important to understand both phylogenetic as well as ecological studies[25]. Leaf shape has a major impact on the support investment of common laminar area and laminar mass[26]. Species with larger leaves and with less xylem has an added advantage to flourish well in humid and non-stressful environmental conditions[27]. In the present study, the lamina shapes were mostly oblong in *P. amarus, P. emblica, P. fraternus, P. urinaria* and *P. virgatus,* whereas it was elliptic only in *P. myrtifolius* and *P. reticulatus*. The laminar apex angle was acute for all the species and the apex shape was mostly convex, with *P. amarus* and *P. reticulatus* with rounded apex.

Leaf venation patterns and their shapes are strongly correlated[28]. The characters are also responsible for both mechanical support as well as translocation of water and nutrients to long distance including photo assimilates[29]. The primary vein category in the studied species have been found to be pinnate in all the species whereas the secondary vein category varied from festooned brochidodromous in species like *P. amarus, P. myrtifolius* and *P. fraternus* with brochidodromous in *P. reticulatus*, *P. virgatus* and *P. urinaria.* However, the venation was intramarginal in *P. emblica.* Simple leaves with entire margins and brochidodromous venation reflects the primitive pattern of angiosperms[30]. In extant arborescent, the brochidodromous venation was more typical in tropical floras whereas non-brochidodromous in northern temperate floras[31].

Variation in leaf venation patterns can provide significant data that can be used in group identification[32]. Works on leaf architecture on Brassicaceae showed craspedodromous or pinnate-festooned brochidodromous type of major venation pattern[33]. The major venation patterns observed in the family Acanthaceae were pinnate craspedodromous in *Acanthus ilicifolius* and acrodromousin *Lepidagathis trinervis*[34]. Studies on leaf architecture of *Quercus*[35] and *Quercus* sub-genus *Cyclobalanopsis*[36] represent some species with brochidodromous leaf venation with less specialized venation in subgenus *Cyclobalanopsis.* Based on such work, it was presumed that the brochidodromous venation type is relatively primitive while eucamptodromous venation as advanced. Third degree venation category exhibited reticulate type and reticulate orthogonal formed a distinguishing feature in delineating certain varieties of *Mangifera indica*[37]. The present study showed third degree vein category as random reticulates in *P. amarus*, *P. emblica*, *P. myrtifolius, P. virgatus*; alternate percurrent in *P. fraternus*and *P. urinaria* and dichotomizing in *P. reticulatus*.
Morphologically, the first recognizable vein order is the pro-cambium of midvein (primary vein). In continuity with the midvein, the secondary veins are formed, appearing acropetal, basipetalor divergent patterns depending upon the species[38]. The usual trend of evolution is represented by regular increase in both low and high order venation, as proven by the leaves of fossil angiosperms[39]. A significant aspect of leaf architecture is the minor venation pattern that constitutes the tertiary veins and the next order of finer branches that arises[30]. The angle of divergence of lower order veins in the leaf is valuable for

optimizing the unfolding process with respect to time, energy and geometry[40]. The major function of marginal leaf venation is to avoid desiccation[41]. The development of high fluid pressure has also been illustrated[42]. The marginal vein supplies sufficient water to the margin that with maximum water stress and hence advantageous for both mechanical stability and water supply[43]. The present study showed looped marginal ultimate venation pattern in *P. myrtifolius, P. fraternus*, *P. virgatus* and *P. urinaria* and presence of fimbrial vein in *P. amarus*, *P. emblica*, and *P. reticulatus.*

Leaf areoles are minute tissue areas in leaf that remains surrounded by the fourth and fifth degree veins and can have diverse shapes and arrangements. The formation of incomplete and irregularly shaped with randomly distributed areoles was observed in the Myrtaceae family[44]. Based on the development pattern of areole the species can be differentiated into groups. The study on leaf venation of some medicinal species of *Bauhinia* segregated the species into three main groups[47]. The studied *Phyllanthus* species can be differentiated broadly into two groups based on the areole structure. Moderately developed in *P. emblica, P. myrtifolius* and poorly developed in *P. amarus*, *P. reticulatus*, *P. fraternus, P. virgatus* and almost lacking in *P. urinaria.* The shapes were quadrangular, pentagonal to irregular and the size range varied from maximum in *P. virgatus* followed by *P. amarus*and *P. fraternus*. Smaller areoles were observed in *P. urinaria, P. emblica*, *P. reticulatus* and *P. myrtifolius.*

The vein islet number can be specific for a species and could be significant for understanding diagnostic character[46]. Vein islet and termination values are important parameters in evaluating the pharmacognostic characters of crude drugs from important medicinal plants[47]. The vein-islets number count from apex, middle and base portion of the lamina were estimated for the species. The maximum vein islets number count was observed from middle and basal portion and minimum from apex of the lamina. The average vein islet number per mm^2 was highest in *P. fraternus* and least in *P. virgatus.* However, the maximum count of absolute vein islet number was recorded in *P. reticulates* followed by *P. urinaria* and least count was observed in *P. amarus* (Figure-2).

Conclusion

Among the seven species of *Phyllanthus* L. of Phyllanthaceae studied, it poses some difficulties to segregate these plants solely on the basis of superficial leaf morphology. By observing the detail foliar morphometric characters with the help of simple devices like dissecting and compound light microscope, some valuable data that has been generated absolutely serves as important tool for their recognition at the species level. The differences in vein architecture have been categorized at one to four degree levels. The major and minor venation details have been presented. The result of this study could be immensely significant in plant taxonomy and morpho-systematics, especially in dealing with non-reproductive plant parts and in the overlapping species complex.

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