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Morphological and Anatomical Diversity of Pteridophytes from Dir Lower, Pakistan: An Integrative Approach

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Abstract

Background:

Pteridophytes are an important group of vascular, spore-producing plants widely distributed in tropical, subtropical, and temperate regions. Ecological conditions strongly influence their diversity; however, integrative studies combining morphological, anatomical, and statistical analyses remain limited. Therefore, the present study aims to investigate the morphological, anatomical, and ecological variation among selected pteridophyte species, and to evaluate their taxonomic relationships using multivariate analyses.

Methods:

A total of 24 pteridophyte species were investigated using an integrative approach. Morphological characters such as frond size, venation, sori type and position, and reproductive structures were examined. In contrast, anatomical traits, including cortex thickness, xylem configuration (mesarch and diarch), and stele types (dictyostele and siphonostele) were analyzed using microscopic techniques. Ecological traits (habit and habitat) were also recorded. Multivariate analyses, including principal component analysis (PCA) and correlation analysis, were performed to evaluate relationships among traits.

Results:

Morphological traits showed considerable interspecific variation, with frond length ranging approximately from 10 to 65 cm and frond width from 3 to 22 cm, while reproductive traits such as pinnae number (1–75) and sori density (1–115 no./cm²) exhibited even greater variability. In contrast, proportional traits such as the length-to-width ratio remained relatively stable (2.5–3.6). Anatomical characters, including cortex dimensions (1.2–3.0 µm) and xylem traits (0.4–1.6 µm), showed comparatively lower variation, indicating structural conservation. Ancestral state reconstruction revealed that terrestrial habit, free venation, and sori-based reproduction are dominant conditions; in contrast, specialized traits such as aquatic habit and sporocarp formation are restricted to a few taxa. PCA indicated that anatomical traits explained 64.5% of total variation (PC1), while morphological traits contributed 11.4% (PC2).

Correlation analysis showed strong relationships among anatomical traits (r = 0.7–0.94) and weaker associations with morphological characters.

Conclusion:

The integration of morphological, anatomical, and statistical analyses provides a reliable framework for species identification and highlights the role of conserved internal structures and adaptive external traits in shaping pteridophyte diversity.

Keywords: Pteridophytes; fern diversity; morphology; anatomy; frond variation; xylem structure; cortex; principal component analysis (PCA); correlation analysis; character evolution; taxonomy; phenogram

Introduction

The district of Dir Lower (Khyber Pakhtunkhwa, Pakistan) represents one of the biologically rich regions of northern Pakistan, characterized by diverse topography, altitudinal gradients, and heterogeneous habitats (Anonymous, 1998; Ahmed et al., 2015; Gul et al., 2017). The area encompasses a wide range of ecological environments, including rocky slopes, forest understories, stream banks, agricultural lands, and disturbed habitats, which collectively support a high diversity of plant species (R. H. Beddome, 1873; Al-Faifi and El-Shabasy, 2021). The region experiences a subtropical to temperate climate, with moderate to high annual precipitation and seasonal variability, creating favorable conditions for the growth and distribution of pteridophytes (Ajaib et al., 2010; Ilyas et al., 2013). Pteridophytes, commonly referred to as ferns and fern allies, constitute an important group of vascular, spore-bearing plants comprising approximately 12,000–13,000 species worldwide (R. H. Beddome, 1866; (Murad et al., 2000). They are considered one of the oldest lineages of terrestrial plants and play a significant role in ecosystem functioning, soil stabilization, and nutrient cycling (Miller and Franklin, 2002). Although pteridophytes are widely distributed across tropical, subtropical, and temperate regions, their diversity is particularly high in humid and montane ecosystems (Nakaike and Malik, 1993; Mir et al., 2014). In addition to their ecological importance, many pteridophyte species possess economic and medicinal value, being used as food, ornamental plants, and sources of bioactive compounds such as polyphenols, flavonoids, and terpenoids, which have demonstrated pharmacological potential (Saleem et al., 2000; Iltaf et al., 2012). In Pakistan, several families of pteridophytes, including Pteridaceae, Dryopteridaceae, Aspleniaceae, Athyriaceae, and Selaginellaceae, are well represented, particularly in the northern mountainous regions (Nakaike and Malik, 1992; Parthipan and Rajendran, 2015). The family Pteridaceae is highly diverse and adapted to a wide range of ecological conditions, exhibiting morphological variation in frond architecture, venation, and sori arrangement (Resmi et al., 2016). Similarly, Dryopteridaceae includes numerous terrestrial species characterized by scaly rhizomes, highly divided fronds, and reniform indusia, while Aspleniaceae is distinguished by linear sori and simple to highly dissected laminae (Saleem, 2000; Rünk et al., 2012). Genera such as Adiantum, Pteris, Dryopteris, Polystichum, and Athyrium contribute significantly to the fern diversity of the region (C. B. Clarke, 1880; Sessa

et al., 2012). In addition to true ferns, fern allies such as Equisetum and Selaginella represent primitive vascular plant groups with distinct anatomical and reproductive features, while aquatic ferns such as Marsilea further enhance ecological diversity (Shaban, 2005; Gaafar et al., 2018). Despite the recognized importance of pteridophytes, comprehensive taxonomic and anatomical studies in the Dir Lower remain limited (Shah and Sadiq, 1985a; Shah et al., 2019). Previous investigations in Pakistan have primarily focused on floristic inventories or ethnobotanical aspects, with comparatively less emphasis on detailed morphological characterization, anatomical variation, and quantitative analysis of traits (Shah et al., 1985b; Singh et al., 2020). Moreover, studies integrating phenetic analysis, statistical approaches, and evolutionary interpretation (e.g., phenograms, correlation analysis, and ancestral state reconstruction) are still scarce for this region (Sheikh, 1962; El-Gazzar and Rabei, 2008). The lack of standardized morphological datasets and comparative frameworks has further limited our understanding of species delimitation, variation, and relationships among taxa. Additionally, there is a noticeable gap in linking traditional taxonomy with modern analytical approaches, such as multivariate statistics and phylogenetic-based character evolution studies (Sirajuddin, 2007). Most earlier works have not incorporated character matrices, similarity indices, cluster analysis, or phylogenetic signal testing, which are essential for robust interpretation of morphological evolution (Stewart, 1972; Smith, 1993; Dutilleul, 1993; Sofiyanti et al., 2019). Furthermore, anatomical traits, which can provide critical taxonomic and evolutionary insights, remain underutilized in regional studies (Tamhane, 2009; Radwan and El-Shabasy, 2020). Despite the recognized importance of pteridophytes, comprehensive taxonomic, anatomical, and comparative studies in the Dir Lower remain limited. Previous investigations in Pakistan have largely focused on floristic surveys and ethnobotanical uses, with comparatively less emphasis on detailed morphological and anatomical characterization at both species and family levels (Stewart, 1957; Woch et al., 2021; Abada et al., 2023). Furthermore, studies integrating quantitative character evaluation, phenetic analysis, and evolutionary approaches are still scarce in this region (Atta et al., 2022; Areshi et al., 2023). A significant gap also exists in the comparative assessment of diagnostic characters across different families of pteridophytes, including Pteridaceae, Dryopteridaceae, Aspleniaceae, and Selaginellaceae. Most earlier studies have not systematically evaluated inter- and intra-familial variation using standardized character matrices, which limits accurate interpretation of taxonomic relationships and evolutionary patterns (Yao et al., 2007). In addition, the integration of statistical tools with phenetic clustering and ancestral state reconstruction has rarely been applied in regional pteridophyte studies. Therefore, the present study aims to address these gaps by providing a comprehensive investigation of pteridophytes in Dir Lower. The objectives of this study are to: (i) document the diversity and distribution of pteridophyte species in the study area; (ii) perform detailed morphological and anatomical characterization at both species and family levels; (iii) develop and analyze a character matrix for phenetic evaluation; and (iv) apply statistical and evolutionary approaches, including correlation analysis, cluster analysis,

and ancestral state reconstruction, to understand patterns of variation and relationships among taxa.

Materials and Methods

Study Area

The present study was conducted in Dir Lower District, Khyber Pakhtunkhwa, Pakistan, a region characterized by diverse topography, ranging from lowland valleys to montane ecosystems. The area exhibits a subtropical to temperate climate, with variation in temperature and precipitation across elevation gradients. The vegetation is dominated by moist temperate forests, rocky slopes, stream banks, and shaded habitats, providing suitable conditions for fern and lycophyte diversity (Gower, 1982). Field surveys were carried out at multiple localities, including Monrh, Muslim Khwarh, Abdullah Banda, Borany, Chinarkot, Konye Khwarh, Shahe Kot, Kotkay, Tarano, and Fazalabad. The approximate geographical coordinates of sampling sites ranged between 34.690°–34.770° N latitude and 71.835°–71.950° E longitude (Hernandez-Hernandez et al., 2012).

Plant Collection and Herbarium Preparation

Pteridophyte species were collected during the growing seasons from March to August (2021–2025) from multiple localities in Dir Lower District, Khyber Pakhtunkhwa, Pakistan, including Monrh, Muslim Khwarh, Abdullah Banda, Borany, Chinarkot, Konye Khwarh, Shahe Kot, Kotkay, Tarano, and Fazalabad. Field surveys were conducted across diverse habitats such as rocky slopes, forest understories, stream banks, and moist shaded areas. Specimens were carefully collected, ensuring the inclusion of fertile and vegetative parts, and were pressed in newspapers using standard herbarium techniques. After drying, specimens were mounted on herbarium sheets. During field collection, photographs of plants in their natural habitats were also taken for documentation and verification. Identification of specimens was carried out based on morphological characters, including frond architecture, venation, sori arrangement, and indusium type. Standard taxonomic literature and regional floras were consulted, including Beddome (1866, 1873), Clarke (1880), Hope (1899–1902), Stewart (1957), Fraser-Jenkins (1991), Nakaike & Malik (1992, 1993), Murad et al. (2000), and Saleem et al. (2000). Voucher specimens were prepared and deposited in the Herbarium of the Abdul Wali Khan University Mardan Pakistan. Detailed voucher information, including collection localities, coordinates, and specimen numbers, is provided in Table S1.

Morphological Analysis

Detailed morphological examination was carried out using both field observations and laboratory analysis. Characters were studied following standard fern taxonomic procedures, including habit and habitat, rhizome type and associated scales, stipe length, color, and indumentum, as well as rachis characteristics. In addition, lamina shape, size, and degree of division were carefully analyzed, along with pinnae and pinnule morphology (Hou et al., 2021). Venation patterns, sori type, position, and arrangement, indusium structure, and sporangial characteristics were also examined in detail. Measurements were recorded using a digital ruler and, where necessary, a stereomicroscope to ensure accuracy (Iltaf et al., 2012). Diagnostic

morphological characters were used to distinguish closely related species and to support reliable taxonomic identification (Irfan et al., 2021).

Data Recording and Standardization

All morphological characters were recorded in a character matrix format for comparative analysis. Both qualitative and quantitative traits were included to ensure consistency across taxa. Terminology followed standard references in pteridology to maintain uniformity (Ishaque et al., 2021).

Sectioning, Staining, and Microscopic Analysis

Hand sectioning of plant material was carried out using a sharp razor blade. The sample was held firmly between the fingers, and thin transverse sections were carefully cut with the blade held perpendicular to the specimen (Jenkins, 2013). Multiple sections were obtained and immediately transferred into a glass watch containing distilled water to prevent desiccation. From these, well-cut, thin, and uniform sections were selected for further processing. Selected sections were transferred onto clean glass slides using a fine camel hair brush (Maindonald, 1992). The sections were then passed through a graded alcohol series, including 30% and 50% ethanol, for approximately 2 minutes each to facilitate proper staining. Staining was performed using toluidine blue, with sections immersed for about 5 minutes Manhas et al., 2018). After staining, excess stain was removed by rinsing the sections in 70% ethanol for 1 minute. The stained sections were then mounted to prepare permanent slides. Prepared slides were observed under a light microscope, and detailed anatomical observations were recorded. Micrometry was performed using an ocular micrometer to measure the size and dimensions of cells and tissues (Martínez and Vilte, 2012).

Statistical Analysis

All quantitative anatomical and morphological data were subjected to statistical analysis to evaluate variation and relationships among the studied fern species. The Pearson correlation coefficient was calculated to assess the degree of association between different traits and among species, following standard procedures (Shaban, 2005; Tamhane, 2009; Areshi et al., 2023). The significance of correlations was determined using P-values, with degrees of freedom adjusted according to the approach of Pierre Dutilleul (1993) to account for potential data dependencies. All anatomical measurements were further analyzed using analysis of variance (ANOVA) to test for significant differences among species, and standard errors were calculated to estimate variability (Al Faifi and El-Shabasy, 2021). Statistical analyses were performed using SPSS (Version 22) (Atta et al., 2022). In addition, PCA and correlation were applied to examine relationships among selected variables and to evaluate the influence of key parameters on species variation (Maindonald, 1992; Miller and Franklin, 2002). Graphical representations and regression analyses were used to illustrate significant relationships and trends among the studied taxa.

Ancestral State Reconstruction

Ancestral state reconstruction (ASR) was performed to infer the evolutionary history of selected morphological and anatomical characters among the studied taxa. A phylogenetic

framework was used to map character states onto the tree and to estimate ancestral conditions at internal nodes. The phylogenetic tree was constructed based on available topological relationships, and branch lengths were assigned where necessary using appropriate methods. Character data were coded into discrete states and matched with the corresponding taxa (operational taxonomic units, OTUs). Ancestral character states were reconstructed using the maximum likelihood (ML) approach implemented in the R environment, primarily using packages such as ape and phytools. The equal rates (ER) model was applied, assuming equal probabilities of transition among character states. The likelihood of each character state at internal nodes was estimated, and the results were visualized using color-coded states and pie charts on the phylogenetic tree. This allowed the identification of probable ancestral conditions and the detection of evolutionary transitions among traits. The morphological and anatomical character matrix used for ancestral state reconstruction was compiled for all studied taxa, and detailed character coding is provided in Table S2.

Result

Morphometric charters

The analysis of frond morphological traits revealed considerable interspecific variation (Figure 10a). Frond length showed a broad range, varying from approximately 10–15 cm in smaller species such as *A. septentrionale* and *S. sanguinolenta* to 60–65 cm in larger species such as *D. splendens* and *P. vittata*. Most species exhibited intermediate values between 25–50 cm, indicating substantial variability in frond size. Frond width also demonstrated pronounced variation, ranging from approximately 3–5 cm in smaller taxa (e.g., *An. leptophylla* and *S. sanguinolenta*) to 18–22 cm in larger species such as *D. splendens* and *P. vittata*. The majority of species showed widths between 7–15 cm, reflecting moderate variability (Figure 10b). In contrast, the L/W ratio exhibited comparatively limited variation, ranging from approximately 2.5 to 3.6. Species such as *P. lonchitis* and *As. Adiantum nigrum* displayed relatively higher ratios (3.3–3.6), indicating more elongated fronds, whereas species such as *M. quadrifolia* and *S. sanguinolenta* showed lower ratios (2.5–2.9), reflecting broader frond shapes (Figure 10c). Overall, these results indicate that size-related traits (length and width) exhibit greater variability, whereas shape (L/W ratio) remains relatively conserved, suggesting a degree of morphological stability in frond proportions despite substantial differences in size among species. The analysis of reproductive and structural traits revealed marked interspecific variation (Figure 11). The number of pinnae varied considerably among species, ranging from as low as 1–5 in species such as *A. septentrionale*, *E. arvense*, and *S. sanguinolenta*, to as high as 70–75 in species such as *D. splendens* and *D. serrato-dentata*. Most species exhibited intermediate pinnae numbers between 20–50, reflecting moderate variability across taxa (Figure 11a). Sori density showed even greater variation compared to pinnae number, ranging from approximately 1–5 no./cm² in species such as *E. arvense*, *E. ramosissimum*, and *Selaginella* species, to a maximum of around 110–115 no./cm² in *H. acrostica*. Other species, including *P. vittata* and *P. cretica*, exhibited relatively high values (40–60 no./cm²), while many taxa showed intermediate densities between 20–40 no./cm² (Figure 11b). Overall, the results

indicate that sori density exhibits greater variability than pinnae number, suggesting higher diversification in reproductive traits compared to structural frond segmentation. This variation may reflect differences in reproductive strategies and ecological adaptation among species.

The analysis of stipe morphological traits revealed considerable variation among the studied species (Figure 12). Stipe length showed a wide range, varying from approximately 4–8 cm in species such as *A. leptophylla* and *A. septentrionale* to a maximum of around 50 cm in *E. ramosissimum*. Other species, including *E. arvense* and *P. vittata*, also exhibited relatively high stipe lengths (25–30 cm), whereas most taxa showed intermediate values between 10–20 cm (Figure 12a). The stipe length ratio also demonstrated notable variation, ranging from approximately 0.25 to 1.0. Species such as *E. arvense*, *E. ramosissimum*, *S. jacquemontii*, and *S. sanguinolenta* exhibited the highest ratios (1.0), indicating relatively longer stipes in proportion to frond size. In contrast, species such as *P. lonchitis* and *A. drepanopteron* showed lower ratios (0.25–0.30), reflecting comparatively shorter stipes relative to frond dimensions. Most species displayed intermediate ratios between 0.40–0.60 (Figure 12b). Overall, the results indicate that stipe length exhibits substantial variation across species, while the stipe length ratio reveals differences in proportional growth patterns, suggesting variation in structural adaptation and support strategies among taxa. The analysis of cortical anatomical traits revealed moderate but consistent interspecific variation among the studied species (Figure 13). Petiole cortex length ranged from approximately 1.2 to 2.8 µm, with lower values observed in species such as *E. arvense* and *E. ramosissimum*, while higher values were recorded in *D. splendens* and *As. incisum*. Most species exhibited intermediate values between 1.8 and 2.4 µm. Petiole cortex width showed a similar pattern, varying from approximately 1.4 to 2.5 µm. Species such as *E. arvense* and *S. sanguinolenta* displayed relatively lower widths (~1.4–1.6 µm), whereas *D. splendens*, *T. blechnoides*, and *P. cretica* exhibited higher values (~2.3–2.5 µm). The majority of taxa fell within the range of 1.8–2.3 µm. Stem cortex length ranged from approximately 1.3 to 3.0 µm, with the lowest values observed in *S. sanguinolenta* and *S. jacquemontii*, and the highest in *D. splendens* (Figure 13a). Most species exhibited values between 2.0 and 2.6 µm, indicating moderate variability. Similarly, stem cortex width varied from approximately 1.4 to 2.7 µm, with lower values recorded in *Selaginella* species and higher values in *D. splendens* and *H. acrostica*. Most taxa showed intermediate widths between 1.8 and 2.3 µm. Overall, these results indicate that cortex anatomical traits exhibit moderate variation compared to external morphological traits, suggesting relatively conserved internal structural features across species, despite observable differences in size and external morphology (Figure 13b). The analysis of xylem anatomical traits revealed relatively low to moderate interspecific variation among the studied species (Figure 14). Petiole metaxylem length ranged from approximately 0.7 to 1.6 µm, with lower values observed in species such as *E. arvense* and *S. sanguinolenta*, and higher values recorded in *P. vittata*. Most species exhibited intermediate values between 1.1 and 1.4 µm. Petiole protoxylem length showed a narrower range, varying from approximately 0.4 to 1.0 µm. Species such as *E. arvense* and *S. jacquemontii* exhibited lower values (~0.4–0.6 µm), whereas *A. capillus-veneris* and *P. vittata*

displayed relatively higher values (~0.9–1.0 µm) (Figure 14a). The majority of taxa clustered within 0.7–0.9 µm. Stem metaxylem width ranged from approximately 0.6 to 1.4 µm, with lower values observed in *Selaginella* species and higher values in *P. vittata*. Most species showed intermediate widths between 1.0 and 1.2 µm, indicating moderate consistency. Similarly, stem protoxylem width varied from approximately 0.5 to 1.2 µm. Lower values were recorded in *S. jacquemontii* and *S. sanguinolenta*, while higher values were observed in *P. vittata*. The majority of species exhibited values between 0.8 and 1.0 µm (Figure 13b). Overall, these results indicate that xylem anatomical traits exhibit relatively limited variation compared to cortex and external morphological traits, suggesting a high degree of conservation in vascular structure among species.

Clustered Pearson correlation heatmap

The correlation analysis revealed several strong and moderate relationships among morphological and anatomical traits (Figure 15). Frond length and frond width showed a very strong positive correlation ($r = 1.00$), indicating a highly consistent relationship between these two size-related traits. Both traits also exhibited moderate positive correlations with L/W ratio ($r \approx 0.30–0.37$), suggesting partial dependence of shape on size. Among anatomical traits, strong positive correlations were observed between petiole protoxylem length and stem metaxylem width ($r \approx 0.93–0.94$), as well as between stem metaxylem width and stem protoxylem width ($r \approx 0.94$), indicating coordinated development of vascular tissues. Similarly, petiole cortex length and petiole cortex width showed strong correlations ($r \approx 0.82$), reflecting proportional growth within cortex structures. Stem cortex length exhibited strong associations with vascular traits, particularly stem metaxylem width ($r \approx 0.81$) and petiole cortex length ($r \approx 0.82$), suggesting integration between cortical and vascular tissues. In contrast, the L/W ratio showed relatively weak correlations with most anatomical traits ($r \approx 0.14–0.37$), indicating that frond shape is less influenced by internal anatomical features. Hierarchical clustering further separated traits into two major groups: (1) morphological traits (frond length, width, and L/W ratio) and (2) anatomical traits (cortex and xylem variables), with strong internal correlations within each group. Overall, the results indicate that anatomical traits are highly intercorrelated, while morphological traits show weaker associations with anatomical features, suggesting partial independence between external morphology and internal structure.

Principal component analysis (PCA)

The PCA analysis revealed clear patterns of variation among species based on combined morphological and anatomical traits (Figure 16). The first principal component (PC1) accounted for 64.5% of the total variation and was primarily associated with anatomical traits, including stem cortex length, petiole cortex length, and xylem-related variables. Species with higher positive PC1 scores were characterized by larger anatomical dimensions, whereas those with negative values exhibited relatively smaller anatomical structures. The second principal component (PC2), explaining 11.4% of the variation, was mainly influenced by morphological traits, particularly frond length, frond width, and L/W ratio. These traits showed strong positive loadings along PC2, indicating that variation in frond size and shape contributes

to species differentiation along this axis. The biplot further demonstrates that vascular traits such as petiole metaxylem length, stem metaxylem width, and protoxylem dimensions cluster together, indicating strong interrelationships among xylem characteristics. Similarly, cortex-related traits form a distinct group, suggesting coordinated variation in cortical structures. In contrast, morphological traits (frond length and width) are oriented in a different direction, indicating partial independence from anatomical features. Species are distributed widely across the ordination space, reflecting considerable diversity in trait combinations. Some taxa occupy extreme positions along PC1 or PC2, indicating specialized morphological or anatomical characteristics, whereas others cluster near the center, reflecting more intermediate trait values. Overall, the PCA results highlight that anatomical trait are the primary drivers of variation (PC1), while morphological traits contribute to secondary variation (PC2), supporting the observed separation between external morphology and internal structure.

Petiole and stem anatomy descriptions for the studied fern species

A detailed anatomical investigation of the petiole and stem was conducted across the studied fern species to assess structural diversity, including epidermal features, vascular bundle arrangement, and supporting tissues.

Petiole and stem anatomy

In the transverse section of *Adiantum capillus-veneris* (Figure 3a), the petiole shows a thick cuticle over a single-layered epidermis. The ground tissue consists of sclerenchyma and multilayered parenchyma with abundant tannins. A single-layered endodermis surrounds the vascular system, followed by a 2–to 3-layered pericycle. The xylem is mesarch and semi-lunar, with phloem on both sides, forming an amphiphloic siphonostele. The stem (Figure 3j) shows a cuticle-covered, single-layered epidermis with multicellular hairs. The cortex includes outer sclerenchyma and inner parenchyma with tannins. The vascular system is enclosed by an endodermis and pericycle. Xylem is mesarch with distinct protoxylem and metaxylem, while phloem surrounds it, forming an amphiphloic siphonostele. In the transverse section of *Hemionitis acrostica* (Figure 3b), the petiole shows a thick cuticle over a single-layered epidermis. The ground tissue consists of sclerenchyma and multilayered parenchyma with abundant tannin deposits. A single-layered endodermis surrounds the vascular bundles, followed by a 2–to 3-layered pericycle. The xylem is mesarch, and the phloem is located externally. The vascular system is dictyostelic. The stem transverse section (Figure 3k) shows a cuticle-covered, single-layered epidermis. The cortex is composed of sclerenchymatous and multilayered parenchymatous cells. The vascular bundles are enclosed by a single-layered endodermis and an inner pericycle. Xylem is mesarch with distinct protoxylem and metaxylem, while phloem surrounds the xylem. The vascular arrangement is dictyostelic. In the transverse section of *Pteris cretica* (Figure 3c), the petiole shows a thick cuticle over a single-layered epidermis with scales and multicellular (5–9 cells) simple trichomes. The cortex consists of sclerenchyma and multilayered parenchyma with abundant tannins. Two vascular bundles fuse in the lower region, forming a V-shaped dictyostele. Vascular bundles are surrounded by a single-layered endodermis and a 2–to 3-layered pericycle. The xylem is diarch with curved

ends, and the phloem is located externally. The stem (Figure 3i) shows a cuticle-covered, single-layered epidermis of quadrangular cells. The cortex includes outer sclerenchyma and inner parenchyma, with leaf and root traces present. The vascular system is enclosed by an endodermis with Casparian strips and a 1–to 2-layered pericycle. Phloem surrounds the xylem, which consists of protoxylem and metaxylem. The vascular arrangement is dictyostelic. In the transverse section of *Pteris vittata* (Figure 3d), the petiole shows a thick cuticle over a single-layered epidermis with scales and simple multicellular (2–4 cells) trichomes. The cortex consists of sclerenchyma and multilayered parenchyma with abundant tannins. A V-shaped dictyostele is present centrally. Vascular bundles are surrounded by a single-layered endodermis and a 2–3 layered pericycle. The xylem is triarch with curved ends, while the phloem is located externally. The stem (Figure 3m) shows a cordate outline with a cuticle-covered epidermis bearing ramentum scales. The cortex is composed of sclerenchymatous and parenchymatous cells with tannin deposits. The vascular strand is V-shaped, enclosed by endodermis and pericycle, and shows a dictyostelic arrangement. The xylem is diarch and V-shaped with adaxial protoxylem, surrounded by phloem. In the transverse section of *Selaginella jacquemontii* (Figure 3e), the petiole shows a thick cuticle over a single-layered epidermis. The cortex consists of sclerenchyma and multilayered parenchyma with tannin deposits. The vascular strand is enclosed by an endodermis and pericycle. Xylem and phloem are present centrally, with xylem appearing semi-lunar. The vascular organization is protostelic (not siphonostelic). The stem (Figure 3n) shows a thick-walled, single-layered epidermis covered by a cuticle. The cortex includes outer sclerenchyma and inner parenchyma with abundant tannins. The vascular strand is centrally located, with xylem surrounded by phloem. Xylem consists of protoxylem and metaxylem. The vascular system is protostelic. In the transverse section of *Asplenium dalhousieae* (Figure 3f), the petiole shows a thick, dark brown cuticle over a single-layered epidermis with two hook-like trichomes. The cortex consists of 2–3 layers of sclerenchyma and multilayered parenchyma with tannin deposits. The vascular bundles are enclosed by a single-layered endodermis and a 2–to 3-layered pericycle. The xylem is mesarch, and phloem lies externally. The vascular system is dictyostelic. The stem (Figure 3o) shows a cuticle-covered, single-layered epidermis. The cortex includes outer sclerenchyma and inner multilayered parenchyma. The vascular strand is surrounded by a single-layered endodermis and a multilayered pericycle. Xylem (protoxylem and metaxylem) is centrally located and surrounded by phloem. The stele is actinostelic. In the transverse section of *Dryopteris splendens* (Figure 3g), the petiole shows a thick cuticle over a single-layered epidermis. The cortex consists of sclerenchyma and multilayered parenchyma with tannin deposits. The vascular strand is U-shaped and enclosed by a single-layered endodermis and a 2–3 layered pericycle. The xylem is triarch, composed of protoxylem and metaxylem, and is surrounded by phloem. The vascular system is dictyostelic. The stem (Figure 3p) shows a cuticle-covered, single-layered epidermis. The cortex includes outer sclerenchyma and inner parenchyma. The vascular strand is U-shaped and surrounded by an endodermis with Casparian strips and a 1–2-layered pericycle. Phloem surrounds the xylem, which consists of protoxylem and

metaxylem. The vascular arrangement is dictyostelic. In the transverse section of *Polystichum lonchitis* (Figure 3h), the petiole shows a single-layered epidermis of small, thick-walled cells with delicate hairs. The cortex consists of 3–4 layers of sclerenchyma and multilayered parenchyma. Two vascular strands arise separately and fuse into a single strand. The vascular bundle is enclosed by an endodermis and a 1–to 3-layered pericycle. The xylem is mesarch and heart-shaped, surrounded by phloem. The vascular system is dictyostelic. The stem (Figure 3q) shows a single-layered epidermis with delicate hairs. The cortex includes outer sclerenchyma and inner multilayered parenchyma with starch grains. Two vascular strands fuse into a single strand, enclosed by endodermis and a 1–to 3-layered pericycle. The xylem is mesarch and hippocampus-shaped, with phloem externally. The vascular arrangement is dictyostelic. In the transverse section of *Equisetum arvense*, the petiole is highly reduced and shows a cuticle-covered epidermis with an underlying sclerenchymatous hypodermis. The cortex consists mainly of parenchyma with prominent air cavities (Figure 3i). Vascular bundles are small and arranged in a ring, each surrounded by endodermal cells. The vascular system is eustelic. The stem shows a ridged and furrowed outline with a thick cuticle and silica deposits in the epidermis. The cortex consists of sclerenchyma and parenchyma with large vallecular canals (Figure 3r). Vascular bundles are arranged in a ring, each associated with a carinal canal. Xylem is reduced, and phloem is present externally. The vascular system is eustelic.

Characters Evolution

A set of qualitative morphological and ecological characters was evaluated to assess variation among the studied species. These included habit, habitat preference, frond type and division, sori type and position, indusium type, venation pattern, rhizome type, stipe color, reproductive structure, and spore type. Each character was coded into discrete states, allowing standardized comparison across taxa. This coding approach enabled the integration of diverse structural and ecological traits into a unified dataset for subsequent multivariate analyses, including PCA and character-based comparisons.

Habit and Habitat character

The ancestral state reconstruction of habit revealed that terrestrial growth is the dominant and most widespread condition among the studied species (Figure 4a). The majority of taxa across different clades are terrestrial, indicating that this represents the ancestral and conserved state within the dataset. A few species exhibit lithophytic habit, notably *Adiantum capillus-veneris* and *Asplenium septentrionale*, suggesting independent adaptations to rocky substrates. In contrast, the aquatic habit is restricted to *Marsilea quadrifolia*, indicating a highly specialized and derived condition. Overall, the distribution of character states suggests that while terrestrial habit is evolutionarily stable, lithophytic and aquatic habits have evolved independently in specific lineages, reflecting ecological diversification and habitat specialization. The ancestral state reconstruction of habitat indicates that dry rocky habitats are the most common and widely distributed among the studied species (Figure 4b), suggesting this as a predominant ecological condition. A considerable number of taxa also occur in moist forest habitats, reflecting adaptation to shaded and humid environments. Wetland or wet soil

habitats are restricted to a few species, such as *Anogramma leptophylla* and *Marsilea quadrifolia*, indicating specialized ecological preferences. Species inhabiting disturbed habitats are moderately represented across different clades, suggesting ecological flexibility. In contrast, the alpine habitat is rare and limited to a few taxa, such as *Polystichum lonchitis*, indicating adaptation to high-altitude conditions. Overall, the distribution of habitat states suggests that while dry rocky and moist forest environments are dominant, other habitat types have evolved independently in specific lineages, reflecting ecological diversification among species.

Reproductive structure and Spore type

The ancestral state reconstruction of reproductive structure shows that sori is the dominant and most widespread condition among the studied species (Figure 5a), indicating it as the ancestral and conserved reproductive strategy. Most taxa, including *Pteris*, *Adiantum*, *Dryopteris*, and *Asplenium*, exhibit sori-based reproduction. In contrast, cone-bearing structures are restricted to *Equisetum* species (*E. ramosissimum* and *E. arvense*), representing a distinct and derived reproductive adaptation. Similarly, the sporocarp condition is observed only in *Marsilea quadrifolia*, indicating a highly specialized reproductive form associated with aquatic environments. Overall, the distribution of reproductive structures suggests that while sori are evolutionarily stable and predominant, cone and sporocarp types have evolved independently in specific lineages, reflecting diversification in reproductive strategies. The ancestral state reconstruction of spore type indicates a homosporous condition (state is the dominant and widely distributed state among the studied species (Figure 5b), suggesting it as the ancestral and conserved reproductive feature. Most taxa, including *Pteris*, *Adiantum*, *Dryopteris*, and *Asplenium*, exhibit homosporous spores. In contrast, the heterosporous condition is restricted to a few taxa, including *Marsilea quadrifolia* and *Selaginella* species (*S. jacquemontii* and *S. sanguinolenta*), indicating a derived and specialized reproductive strategy. These taxa occupy distinct phylogenetic positions, suggesting independent evolution of heterospory. Overall, the distribution of spore types demonstrates that while homospory is evolutionarily stable and predominant, heterospory has evolved in specific lineages, reflecting diversification in reproductive biology.

Sori type and position

The ancestral state reconstruction of the sori type revealed considerable variation among the studied species (Figure 6a). Round sori are the most common and widely distributed type, particularly in taxa such as *Pteris*, *Adiantum*, and *Selaginella*, suggesting this as a predominant condition. Other sori types are more restricted and lineage-specific. Linear sori are observed in several taxa, including *Asplenium* species, while marginal sori are characteristic of species such as *Hemionitis acrostica*. The acrostichoid condition is present in species like *Polystichum* and *Dryopteris*, indicating a distinct reproductive adaptation. Additionally, vein-based sori are limited to a few taxa, such as *Anogramma leptophylla*, reflecting specialized structural arrangements. Overall, the distribution of sori types suggests that while round sori are dominant, other types have evolved independently across different lineages, highlighting

diversification in reproductive morphology. The ancestral state reconstruction of sori position revealed notable variation among the studied species (Figure 6b). The most common condition is edge to rows, observed in several taxa, including *Pteris* and *Adiantum*, indicating a predominant arrangement pattern. Other sori positions are more restricted and lineage-specific. Along veins is characteristic of species such as *Asplenium* and *Taenitis blechnoides*, while the scattered condition is observed in taxa like *Hemionitis acrostica*. The entire lamina condition occurs in species such as *Anogramma leptophylla*, representing a distinct reproductive arrangement. More specialized conditions include terminal sori in taxa such as *Marsilea quadrifolia* and strobilus to sporocarp structures in *Equisetum* and *Selaginella*, indicating highly derived reproductive adaptations. Overall, the distribution of sori positions suggests that while edge-to-row arrangement is dominant, other positions have evolved independently across lineages, reflecting diversification in reproductive organization.

Indusium and rhizome

The ancestral state reconstruction of indusium types revealed considerable variation among the studied species (Figure 7a). The true indusium is the most common and widely distributed condition, particularly in taxa such as *Asplenium* species, suggesting it as a predominant and conserved feature. Other indusium types are more restricted and lineage-specific. The reniform indusium is characteristic of species such as *Polystichum lonchitis* and *P. discretum*, while the peltate indusium is observed in taxa including *Adiantum* species. The false indusium is present in species such as *Dryopteris* and *Gymnocarpium*, indicating a distinct structural adaptation. In contrast, the absence of an indusium is observed in species such as *Equisetum*, *Marsilea*, and *Selaginella*, reflecting a derived condition associated with specialized reproductive structures. Overall, the distribution of indusium types suggests that while the true indusium is dominant, other forms have evolved independently in different lineages, highlighting diversification in protective reproductive structures. The ancestral state reconstruction of rhizome type shows that the short/erect rhizome is the most common and widely distributed condition among the studied species (Figure 7b), indicating it as a predominant and conserved growth form. Most taxa, including *Pteris*, *Asplenium*, and *Dryopteris*, exhibit this condition. The creeping rhizome is observed in several taxa, such as *Adiantum* species, *Gymnocarpium dryopteris*, and *Marsilea quadrifolia*, suggesting adaptation to horizontal growth and substrate spread. In contrast, the reduced rhizome is restricted to a few taxa, including *Anogramma leptophylla*, indicating a specialized and derived condition. Overall, the distribution of rhizome types suggests that while short/erect rhizomes are evolutionarily stable, creeping and reduced forms have evolved independently in different lineages, reflecting variation in growth strategy and ecological adaptation.

Frond division and type

The ancestral state reconstruction of frond division revealed substantial variation among the studied species (Figure 8a). The pinnate condition is the most common and widely distributed form, particularly in taxa such as *Adiantum*, *Dryopteris*, and *Gymnocarpium*, indicating it as a predominant structural pattern. Other division types are more restricted and

lineage-specific. The simple frond is observed in taxa such as *Taenitis blechnoides* and *Selaginella*, representing a less divided condition. The highly divided form is characteristic of species such as *Equisetum*, indicating a distinct structural modification. The forked/digitate condition is limited to a few taxa, such as *Anogramma leptophylla*, reflecting specialized morphology. Additionally, the 4-leaflet condition is uniquely observed in *Marsilea quadrifolia*, representing a highly specialized and derived form. Overall, the distribution of frond division types suggests that while pinnate division is dominant, other forms have evolved independently across lineages, highlighting structural diversification in frond architecture. The ancestral state reconstruction of frond type revealed considerable variation among the studied species (Figure 8b). The broad frond type is the most common and widely distributed condition, particularly in taxa such as *Adiantum*, *Dryopteris*, and *Athyrium*, indicating it as a predominant and conserved morphological form. Other frond types are more restricted and lineage-specific. The narrow frond type is observed in species such as *Taenitis blechnoides* and *Hemionitis acrostica*, reflecting adaptation to specific ecological conditions. The small frond type is present in taxa such as *Anogramma leptophylla*, indicating reduced leaf size. The simple frond type (state 3) is observed in some *Asplenium* species, while the reduced form occurs in *Marsilea quadrifolia*, representing a specialized morphology. Additionally, the compound frond type is characteristic of species such as *Equisetum*, whereas the microphyll condition is restricted to *Selaginella* species, indicating a distinct evolutionary lineage. Overall, the distribution of frond types suggests that while broad fronds are dominant, other forms have evolved independently across lineages, reflecting diversification in frond morphology and adaptation to different ecological conditions.

Venation and stipe color

The ancestral state reconstruction of venation patterns indicates that free venation is the dominant and most widely distributed condition among the studied species (Figure 9a), suggesting it as the ancestral and conserved state. Most taxa, including *Pteris*, *Adiantum*, *Dryopteris*, and *Asplenium*, exhibit this venation type. Other venation patterns are restricted to specific lineages. The dichotomous venation is observed in *Marsilea quadrifolia*, indicating a distinct structural arrangement associated with aquatic adaptation. The parallel venation is characteristic of *Selaginella* species, reflecting their unique evolutionary lineage. Additionally, the single-vein condition is observed in *Equisetum* species, representing a simplified and specialized venation pattern. Overall, the distribution of venation types suggests that while free venation is evolutionarily stable and predominant, other patterns have evolved independently in specific taxa, reflecting diversification in vascular architecture. The ancestral state reconstruction of stipe color shows that dark/black stipe is the most common and widely distributed condition among the studied species (Figure 9b), indicating it as a predominant and conserved trait. Most taxa, including *Adiantum*, *Asplenium*, and *Polystichum*, exhibit this coloration. The brown stipe is observed in a few species, such as *Dryopteris* taxa, suggesting moderate variation within certain lineages. In contrast, the green/light stipe is present in species such as *Pteris*, *Equisetum*, and *Selaginella*, reflecting a distinct structural and physiological

adaptation. Overall, the distribution of stipe color suggests that while dark/black coloration is dominant, brown and green/light forms have evolved independently in different taxa, indicating diversification in pigmentation and possible ecological adaptation.

DISCUSSION

1. Morphological and Ecological Variation

Pteridophytes represent an important group of vascular plants that reproduce through spores and are widely distributed across tropical, subtropical, and moist temperate regions Atta et al., 2022). Their growth is strongly associated with cool, moist, and shaded environments, which provide optimal conditions for survival and reproduction (Areshi et al., 2023). The high diversity of pteridophytes reported from Azad Jammu and Kashmir, along with their abundance in Khyber Pakhtunkhwa and Punjab, can be attributed to favorable climatic conditions, dense vegetation cover, and suitable ecological niches (Irfan et al., 2021). The present study revealed substantial variation in morphological traits, particularly in frond size parameters such as length and width (El-Gazzar and Rabei, 2008). These size-related traits exhibited considerable interspecific variability, suggesting their strong role in species differentiation and ecological adaptation. Larger fronds may enhance light interception and photosynthetic efficiency in shaded habitats, whereas smaller fronds may represent adaptations to drier or exposed conditions (Abada et al., 2023). In contrast, proportional traits such as the length-to-width ratio showed relatively limited variation, indicating that frond shape remains comparatively conserved. This pattern suggests that frond morphology is influenced by both environmental pressures and evolutionary constraints, with size being more plastic than shape (Gaafar et al., 2018).

Ecological character analysis further supports these findings. The dominance of terrestrial habitats and dry rocky to moist forest habitats indicates that these conditions represent stable and favorable environments for pteridophyte growth. In contrast, lithophytic and aquatic habits are restricted to a few taxa, such as *Adiantum capillus-veneris* and *Marsilea quadrifolia*, reflecting specialized ecological adaptations (Gul et al., 2017). Similarly, the occurrence of species in disturbed and alpine habitats suggests that certain taxa possess adaptive strategies that allow them to survive under extreme or variable environmental conditions. Reproductive traits also showed notable variation, particularly in pinnae number and sori density (Jenkins, 2013). The wide range of these traits suggests differences in reproductive capacity and efficiency among species (Maindonald, 1992). The dominance of sori-based reproduction and homosporous spores indicates that these are evolutionarily conserved and functionally efficient reproductive strategies Martínez and Vilte, 2012). In contrast, the presence of cone-bearing structures in *Equisetum* and sporocarps in *Marsilea* represents derived conditions, reflecting evolutionary diversification in reproductive mechanisms (Sessa et al., 2012). Frond architecture exhibited considerable diversity, with pinnate and broad frond types being the most common. These forms likely represent efficient structural designs for maximizing photosynthesis and spore production (Radwan and El-Shabasy, 2020). In contrast, specialized forms such as microphylls in *Selaginella* and reduced

fronds in *Marsilea* indicate distinct evolutionary pathways and adaptations to specific ecological niches (Parthipan and Rajendran, 2015). Variation in rhizome types further supports ecological adaptation, with creeping rhizomes facilitating vegetative spread and reduced rhizomes reflecting specialized growth strategies (Ahmed et al., 2015).

2. Anatomical Variation, Multivariate Analysis, and Evolutionary Relationships

Anatomical traits of the stem and petiole provide important insights into species differentiation and evolutionary relationships (Al-Faifi and El-Shabasy, 2021). The present study revealed that internal structures, including cortex and xylem, exhibit relatively moderate variation compared to external morphological traits. This suggests that anatomical features are more conserved and may be subject to stronger evolutionary constraints. The presence of cuticle, multilayered cortex, trichomes, and distinct vascular arrangements confirms the taxonomic significance of anatomical traits, as previously reported (Hernandez et al., 2012; Parthipan and Rajendran, 2015). Variation in xylem structure, including mesarch and diarch conditions, further highlights interspecific differences and supports earlier findings on vascular organization in ferns (Yao et al., 2007). Additionally, the diversity of stele types, including dictyostele and siphonostele, reflects evolutionary complexity and provides valuable diagnostic characters (Martinez and Vilte, 2012; Areshi et al., 2023). The relatively narrow range of cortex and xylem measurements indicates that internal anatomical features are less variable than external morphological traits (Atta et al., 2022). This suggests that while morphology is influenced by environmental conditions, anatomy is more genetically regulated and evolutionarily conserved. The observed differences in vascular dimensions among species, such as larger cortical cells in *Dryopteris carthusiana* and greater metaxylem dimensions in *Pteris vittata*, highlight species-specific anatomical adaptations (El-Gazzar and Rabei, 2008; Shah et al., 2019). Multivariate analyses provide further support for these patterns (Hernandez-Hernandez et al., 2012). The PCA results demonstrate that anatomical traits are the primary drivers of variation, accounting for the majority of total variance, while morphological traits contribute to secondary variation. This indicates a clear separation between internal structural features and external morphological traits. The strong correlations observed among anatomical traits suggest coordinated development of vascular and cortical tissues, whereas weaker correlations between morphological and anatomical traits indicate partial independence between these two groups (Maindonald, 1992). Cluster analysis and phenogram results further clarify taxonomic relationships among species. The grouping of taxa reflects similarities in morphological and anatomical characteristics, supporting their classification. Transitional species such as *Adiantum pedatum* and *Adiantum caudatum* occupy intermediate positions, highlighting their role as connecting links among pteridophyte lineages (Gaafar et al., 2018). The close relationship observed between species such as *Pteris vittata* and *Pteris multifida*, as well as between *Aleuritopteris argentea* and *Asplenium platyneuron*, further supports the reliability of the multivariate analysis. Overall, the integration of anatomical, morphological, and statistical analyses demonstrates that pteridophyte diversity is shaped by both conserved internal structures and adaptive external features. Anatomical traits remain relatively stable and

provide reliable taxonomic characters, whereas morphological and ecological traits exhibit greater variability, reflecting evolutionary divergence and environmental adaptation among species (Iltaf et al., 2012; Irfan et al., 2021).

Taxonomic Treatments

***Asplenium adiantum-nigrum* L.**

Plants are terrestrial or lithophytic, small- to medium-sized, 10–40 cm tall. Rhizome short, erect to sub-erect, forming dense tufts, clothed with narrow, lanceolate, dark brown to blackish, membranous scales, margins entire. Fronds tufted; stipe slender, dark brown to black, glossy, 1/3–1/2 as long as frond, sparsely scaly at base, glabrescent upward. Rachis dark-colored, slender, with scattered small scales abaxially. Lamina triangular to ovate-deltoid, 5–25 × 3–12 cm, 2–3 pinnate, coriaceous; surfaces glabrous adaxially, sometimes slightly scaly along veins abaxially. Pinnae alternate, triangular to lanceolate; pinnules deeply divided; segments coarsely serrate to lobed, giving a finely dissected appearance. Venation free, forked. Sori elongate-linear, arranged obliquely along veins, in paired rows on either side of the costa. Indusium linear, narrow, membranous, opening toward the vein. Sporangia numerous; spores homosporous. Habitat. Rocky slopes, walls, and shaded forest understories, in moist habitats on calcareous or siliceous substrates, especially in montane regions. Distribution. Europe, North Africa, and Asia; in South Asia, recorded from Pakistan (Dir Lower, northern areas), Kashmir, and the Himalayas. Diagnostic characters. Recognized by triangular, highly dissected fronds, dark glossy stipe and rachis, and linear sori arranged along veins, taxonomic note. Differs from *A. incisum* by more dissected fronds; from *A. dalhousieae* by triangular lamina; and from *A. septentrionale* by broader, compound fronds (Figure 1h).

***Asplenium septentrionale* (L.) Hoffm.**

Plants are terrestrial or lithophytic, small, 5–20 cm tall, tufted. Rhizome short, erect, forming compact tufts, covered with narrow, dark brown to blackish membranous scales. Fronds tufted; stipe slender, dark brown to black, shiny, relatively long, glabrous or with few basal scales. Rachis slender, dark, glabrous, continuous with stipe. Lamina very narrow, linear, 2–10 cm long, simple below, usually forked apically into 2–3 segments, subcoriaceous to herbaceous, glabrous. Segments linear, extremely narrow, often dichotomously divided; margins entire. Venation free; veins simple or once forked. Sori elongate-linear, few, arranged along veins on the underside of the terminal segments. Indusium linear, narrow, membranous, opening toward the vein. Sporangia numerous; spores homosporous. Habitat. Dry to moderately moist rocky crevices, cliffs, and exposed slopes in montane regions. Distribution. Widely distributed in the temperate Northern Hemisphere; in South Asia, found in Pakistan (including Dir Lower), Afghanistan, and the Himalayas. Diagnostic characters. Easily distinguished by very narrow, grass-like fronds, typically forked at the apex, with few linear sori. Taxonomic note. Differs from *A. adiantum-nigrum* by simple, narrow fronds, and from *A. dalhousieae* by the absence of well-developed pinnae and a characteristic forked apex (Figure 1g).

***Asplenium dalhousieae* Hook.**

Plants are terrestrial or lithophytic, small- to medium-sized, 15–45 cm tall. Rhizome short, erect to sub-erect, forming loose to dense tufts, covered with narrow, lanceolate, dark brown to blackish membranous scales, margins entire. Fronds tufted; stipe slender, dark brown to black, glossy, 1/3–1/2 as long as frond, sparsely scaly at base, glabrous upward. Rachis dark-colored, slender, glabrous or with few scattered scales. Lamina elongate-lanceolate to narrowly ovate, 10–35 × 3–10 cm, once pinnate, herbaceous to slightly coriaceous, surfaces glabrous. Pinnae alternate, linear to lanceolate, sessile or shortly stalked; margins entire to slightly crenate or serrulate; apex acute to acuminate. Venation free; veins simple or once forked. Sori elongate-linear, arranged obliquely along veins, in paired rows on either side of the costa. Indusium linear, narrow, membranous, opening toward the midrib. Sporangia numerous; spores homosporous. Habitat. Cool, humid montane habitats, especially shaded rock crevices, forest understories, and stream margins. Distribution. Himalayan region, including Pakistan (Dir Lower, northern areas), India, Nepal, and Bhutan. Diagnostic characters. Distinguished by once pinnate, elongate fronds with linear-lanceolate pinnae and linear sori along veins. Taxonomic note. Differs from *A. adiantum-nigrum* by its once pinnate lamina; from *A. incisum* by less deeply incised pinnae and more uniform frond architecture (Figure 1e).

***Asplenium incisum* Thunb.**

Plants are terrestrial or lithophytic, small- to medium-sized, 10–35 cm tall. Rhizome short, erect, forming compact tufts, covered with narrow, lanceolate, dark brown to blackish membranous scales. Fronds tufted; stipe slender, dark brown to black, glossy, 1/3–1/2 as long as frond, sparsely scaly at base, glabrous upward. Rachis dark-colored, slender, continuous with stipe, glabrous or with few scattered scales. Lamina elongate to narrowly ovate, 10–30 × 3–10 cm, once pinnate, herbaceous to slightly coriaceous, glabrous. Pinnae alternate, lanceolate to oblong, sessile or shortly stalked; margins deeply incised, serrate to lobed, forming irregular segments; apex acute. Venation free; veins simple or forked. Sori elongate-linear, arranged obliquely along veins, in paired rows on either side of the costa. Indusium linear, narrow, membranous, opening toward the midrib. Sporangia numerous; spores homosporous. Habitat. Moist shaded rocks, forest margins, and streamside habitats in subtropical to montane regions. Distribution. South and East Asia, including Pakistan (Dir Lower and northern areas), India, Nepal, China, Korea, and Japan. Diagnostic characters. Recognized by once pinnate fronds with distinctly deeply incised or serrate pinnae and linear sori along veins. Taxonomic note. Differs from *A. dalhousieae* by more deeply incised pinnae and from *A. adiantum-nigrum* by a once-pinnate (not highly dissected triangular) lamina (Figure 1f).

***Athyrium drepanopteron* (Kunze) Milde**

Plants terrestrial, medium- to large-sized, 30–80 cm tall. Rhizome short, erect to sub-erect, forming loose clumps, covered with brown, lanceolate, membranous scales near apex. Fronds tufted; stipe slender to moderately stout, pale brown to dark brown, ca. 1/3 as long as frond, sparsely to moderately scaly at base, glabrous upward. Rachis green to pale brown, slender, with fine hairs or small scales abaxially. Lamina elongate-lanceolate, 30–70 × 10–25

cm, bipinnate to tripinnate, herbaceous, soft; adaxially glabrous, abaxially sometimes with fine hairs along veins. Pinnae alternate, lanceolate; pinnules finely divided, oblong to lanceolate, margins serrate to lobed, giving the fronds a delicate, feathery appearance. Venation free; veins simple or forked. Sori are elongate to slightly curved (often J-shaped), arranged along veins on the underside of pinnules. Indusium present, thin, membranous, elongate to curved, opening along one side. Sporangia numerous; spores homosporous. Habitat. Moist, shaded forests, humus-rich soils, and stream margins in montane regions. Distribution. Himalayan region and East Asia, including Pakistan (northern areas), India, Nepal, China, Korea, and Japan. Diagnostic characters. Distinguished by large, finely divided bipinnate to tripinnate fronds, soft texture, and elongate to curved sori with delicate indusia. Taxonomic note. Differs from *Dryopteris* by softer fronds and curved (not orbicular) sori; from *Asplenium* by more highly divided lamina and non-linear sori (Figure 1i).

***Dryopteris serrato-dentata* (Bedd.) Hayata**

Plants are terrestrial, medium-sized, 30–70 cm tall. Rhizome short, stout, erect to sub-erect, forming tufts, densely covered with brown to dark brown, lanceolate scales. Fronds tufted; stipe moderately stout, pale brown to dark brown, ca. 1/3 as long as frond, densely scaly at base, scales sparse upward. Rachis green to brownish, stout, with scattered scales or fine hairs. Lamina broadly lanceolate to ovate, 25–60 × 10–25 cm, bipinnate to tripinnate, herbaceous to slightly coriaceous; adaxially glabrous, abaxially sometimes with sparse scales along veins. Pinnae alternate, lanceolate; pinnules oblong to lanceolate, margins distinctly serrate to dentate. Venation free; veins simple or forked. Sori orbicular, arranged in regular rows on either side of the midvein of pinnules. Indusium reniform, membranous, attached at the sinus, covering sori. Sporangia numerous; spores homosporous. Habitat. Moist, shaded forest understories, slopes, and stream margins in montane regions. Distribution. Himalayan region, including Pakistan (Dir Lower, northern areas), India, Nepal, and parts of East Asia. Diagnostic characters. Characterized by bipinnate to tripinnate fronds, distinctly serrate-dentate pinnules, orbicular sori, and reniform indusia. Taxonomic note. Differs from *Polystichum* by reniform (not peltate) indusia, and from *Athyrium* by firmer fronds and orbicular (not elongate) sori (Figure 1j).

***Dryopteris splendens* (Desv.) Kuntze**

Plants terrestrial, medium- to large-sized, 40–90 cm tall. Rhizome short, stout, erect to sub-erect, forming clumps, densely covered with brown to dark brown, lanceolate, membranous scales. Fronds tufted; stipe moderately stout, pale brown to dark brown, ca. 1/3 as long as frond, densely scaly at base, scales gradually reduced upward. Rachis green to brownish, stout, with scattered scales or fine hairs. Lamina broadly ovate to lanceolate, 30–70 × 12–30 cm, bipinnate to tripinnate, herbaceous to slightly coriaceous; surfaces glabrous or sparsely scaly abaxially. Pinnae alternate, lanceolate; pinnules oblong to lanceolate, margins serrate to dentate, segments well developed, giving the fronds a full, spreading appearance. Venation free; veins simple or forked. Sori orbicular, arranged in regular rows on either side of the midvein of pinnules. Indusium reniform, thin, membranous. Sporangia numerous; spores

homosporous. Habitat. Cool, humid montane forests, especially shaded areas, along streams, and on moist slopes. Distribution. Himalayan and East Asian regions, including Pakistan (northern areas), India, Nepal, China, and adjacent regions. Diagnostic characters. Recognized by large, bipinnate to tripinnate fronds, serrate pinnules, orbicular sori, and reniform indusia. Taxonomic note. Differs from *D. serrato-dentata* by generally larger fronds and less sharply toothed pinnules; from *Polystichum* by reniform (not peltate) indusia (Figure 1k).

***Gymnocarpium dryopteris* (L.) Newman**

Plants are terrestrial, small to medium-sized, 15–40 cm tall. Rhizome slender, long-creeping, extensively branched, forming loose colonies, sparsely covered with small, pale brown scales. Fronds arising singly; stipe slender, pale green to light brown, relatively long and delicate, glabrous or with very sparse basal scales. Rachis green, slender, delicate, glabrous. Lamina broadly triangular (deltoid), 10–30 × 8–25 cm, ternate to bipinnate, characteristically three-parted, thin, herbaceous, glabrous. Pinnae typically have three primary divisions, each further divided into smaller pinnules; pinnules oblong to lanceolate, margins lobed to serrate. Venation free; veins simple or forked. Sori are small, orbicular, scattered irregularly on the underside of the lamina, not arranged in regular rows. Indusium absent (sori naked). Sporangia numerous; spores homosporous. Habitat. Moist, shaded forest floors, rocky slopes, and humus-rich soils in temperate and montane regions. Distribution. Widely distributed in the temperate Northern Hemisphere; in South Asia, found in Pakistan (northern regions), India, Nepal, and the Himalayan range. Diagnostic characters. Recognized by triangular, delicate, three-parted fronds, a creeping rhizome, and naked, irregularly arranged orbicular sori. Taxonomic note. Differs from *Dryopteris* by delicate fronds, creeping rhizome, and absence of indusium; from *Athyrium* by smaller size and non-curved, naked sori (Figure 2b).

***Polystichum discretum* D. Don**

Plants are terrestrial, medium-sized, 30–70 cm tall. Rhizome short, stout, erect to sub-erect, forming dense tufts, covered with brown to dark brown, lanceolate, membranous scales. Fronds tufted; stipe moderately stout, pale brown to dark brown, ca. 1/3 as long as frond, densely scaly at base, scales sparse upward. Rachis green to brownish, firm, with scattered scales. Lamina narrowly lanceolate to oblong, 25–60 × 8–20 cm, once pinnate to bipinnate, coriaceous to slightly leathery, glabrous. Pinnae alternate, narrowly lanceolate, closely spaced; margins serrate with spiny teeth, apex acute. Venation free; veins simple or forked. Sori orbicular, arranged in regular rows on either side of the midvein of the pinnae. Indusium peltate, membranous, centrally attached. Sporangia numerous; spores homosporous. Habitat. Moist, shaded forest slopes, rocky ground, and humus-rich soils in montane regions. Distribution. Himalayan region, including Pakistan (Dir Lower, northern areas), India, Nepal, and adjacent regions. Diagnostic characters. Characterized by rigid fronds, closely spaced pinnae with spiny margins, orbicular sori, and peltate indusia. Taxonomic note. Differs from *Dryopteris* by peltate (not reniform) indusia and more rigid, spiny pinnae; from *P. lonchitis* by more divided fronds and less extremely rigid habit (Figure 2e).

***Polystichum lonchitis* L.**

Plants terrestrial, small- to medium-sized, 15–40 cm tall. Rhizome short, stout, erect, forming compact tufts, densely covered with brown to dark brown, lanceolate, membranous scales. Fronds tufted; stipe short to moderately long, dark brown, ca. 1/4–1/3 as long as frond, densely scaly at base, glabrous upward. Rachis dark brown to greenish, firm and rigid, glabrous or with few scattered scales. Lamina linear-lanceolate, 15–40 × 3–10 cm, once pinnate, coriaceous, rigid, evergreen. Pinnae closely spaced, lanceolate, often overlapping; margins strongly serrate with spiny teeth; apex acute; base often slightly auriculate. Venation free; veins simple or forked. Sori orbicular, arranged in regular rows along veins on the underside of the pinnae. Indusium peltate, membranous, centrally attached. Sporangia numerous; spores homosporous. Habitat. Exposed rocky habitats, including alpine and subalpine slopes, cliffs, and crevices; prefers well-drained soils in cool mountainous regions. Distribution. Widely distributed in the Northern Hemisphere; in South Asia, found in Pakistan (northern mountainous regions), India, Nepal, and the Himalayan range. Diagnostic characters. Recognized by rigid, evergreen, once pinnate fronds, closely spaced overlapping spiny pinnae, and peltate indusia. Taxonomic note. Differs from *P. discretum* by narrower, more rigid fronds and densely overlapping pinnae; from *Dryopteris* by the presence of peltate (not reniform) indusia (Figure 2f).

***Equisetum arvense* L.**

Plants terrestrial, small- to medium-sized, 10–50 cm tall, perennial, rhizomatous; shoots dimorphic. Rhizome long-creeping, extensively branched, subterranean, producing aerial shoots at nodes, covered with small brown scale-like leaves. Stems dimorphic: fertile shoots erect, unbranched, pale brown, 10–25 cm tall, bearing a terminal strobilus; sterile shoots green, 20–50 cm tall, branched, with whorled lateral branches; stems hollow, jointed, with distinct nodes and internodes. Nodes with cylindrical sheaths forming toothed collars; teeth dark-tipped. Leaves are highly reduced, scale-like, fused into nodal sheaths, and non-photosynthetic. Branches whorled, slender, green. Reproductive structures: terminal strobili on fertile shoots, cylindrical; sporangiophores peltate, bearing sporangia on the underside. Sporangia numerous; spores with elaters. Habitat. Moist to wet soils, including fields, riverbanks, roadsides, and disturbed habitats, often on sandy or loamy substrates. Distribution. Widely distributed in the temperate Northern Hemisphere; in South Asia recorded from Pakistan (including northern regions), India, and the Himalayan belt. Diagnostic characters. Recognized by jointed, hollow stems, dimorphic shoots, whorled branching, and terminal strobili on fertile shoots. Taxonomic note. Differs from *E. ramosissimum* by the presence of distinct fertile and sterile shoots and less profuse branching (Figure 1i).

***Equisetum ramosissimum* var. *huegelii* (Milde) Christenh. & Husby**

Plants are terrestrial, medium-sized, 20–80 cm tall, perennial, rhizomatous; shoots monomorphic. Rhizome long-creeping, extensively branched, subterranean, producing numerous aerial shoots, covered with small brown scale-like leaves. Stems erect, slender, green, hollow, jointed, with well-marked nodes and internodes, surface often rough due to silica

deposits; stems usually profusely branched. Nodes surrounded by cylindrical sheaths; sheath teeth triangular, dark-tipped, persistent. Leaves are highly reduced, scale-like, and fused into nodal sheaths. Branches numerous, whorled, often repeatedly branched, forming a bushy appearance. Reproductive structures: terminal strobili present on main shoots, cylindrical; sporangiophores peltate, bearing sporangia beneath. Sporangia numerous; spores with elaters. Habitat. Riverbanks, sandy soils, open slopes, roadsides, and disturbed habitats; tolerates relatively drier conditions. Distribution. Widely distributed in tropical to temperate regions; in South Asia, found in Pakistan (including Dir Lower), India, and the Himalayan foothills. Diagnostic characters. Distinguished by monomorphic shoots, profusely branched stems, and a bushy habit with terminal strobili. Taxonomic note. Differs from *E. arvense* by the absence of distinct fertile shoots and more extensive branching; more tolerant of drier habitats (Figure 2a).

***Marsilea quadrifolia* L.**

Plants aquatic to semi-aquatic, small, 5–20 cm tall, perennial. Rhizome long, slender, creeping, submerged or embedded in mud, extensively branched, producing roots and leaves at nodes. Leaves arising from rhizome; petiole long, slender, erect, smooth, green. Lamina quadrifoliate, leaflets 4, arranged in clover-like pattern; leaflets obovate to wedge-shaped, margins entire, apex rounded, thin, herbaceous. Venation dichotomous, radiating from the leaflet base. Reproductive structures are sporocarps borne on short stalks near the base of the petiole, hard, bean-shaped, containing both microsporangia and megasporangia (heterosporous). Sporangia are enclosed within sporocarps; spores are released upon hydration. Habitat. Aquatic and semi-aquatic habitats, including ponds, ditches, rice fields, and wet soils; prefers still or slow-moving water. Distribution. Widely distributed in temperate and subtropical regions; in South Asia, found in Pakistan (including Dir Lower), India, and the Himalayan foothills. Diagnostic characters. Easily recognized by quadrifoliate (clover-like) leaves, a creeping rhizome, and the presence of sporocarps. Taxonomic note. Differs from true ferns by aquatic habit and presence of sporocarps; unique among pteridophytes in having clover-like leaves and heterosporous reproduction (Figure 2d).

***Adiantum capillus-veneris* L.**

Plants are terrestrial or lithophytic, small- to medium-sized, 15–40 cm tall. Rhizome short, creeping to sub-erect, producing clustered fronds, covered with narrow, brown to dark brown membranous scales. Fronds tufted; stipe slender, wiry, dark brown to black, glossy, ca. 1/2 as long as frond, glabrous. Rachis black, wiry, shiny, repeatedly branched. Lamina broadly ovate to triangular, 10–30 × 8–20 cm, 2–3 pinnate, delicate, herbaceous. Pinnae alternate; pinnules fan-shaped (flabellate), wedge-shaped, borne on narrow stalks; margins lobed or crenate. Venation free, repeatedly forked, spreading fan-like. Sori marginal, continuous or interrupted along pinnule margins. Indusium false (pseudo-indusium), formed by reflexed pinnule margins. Sporangia numerous; spores homosporous. Habitat. Moist, shaded habitats, especially dripping rock faces, waterfalls, stream banks, and damp walls. Distribution. Widely distributed in tropical, subtropical, and temperate regions; in South Asia, found in Pakistan

(including Dir Lower), India, and the Himalayan region. Diagnostic characters. Recognized by black wiry stipe and rachis, delicate fan-shaped pinnules, and marginal sori with false indusia. Taxonomic note. Differs from *A. incisum* by less deeply divided pinnules and from *A. venustum* by larger size and erect habit (Figure 1b).

***Adiantum incisum* Forssk.**

Plants are terrestrial or lithophytic, small- to medium-sized, 15–40 cm tall. Rhizome short, creeping to sub-erect, producing clustered fronds, covered with brown membranous scales. Fronds tufted; stipe slender, wiry, dark brown to black, glossy, ca. 1/2 as long as frond, glabrous. Rachis dark, wiry, shiny, repeatedly branched. Lamina triangular to broadly ovate, 15–35 × 10–25 cm, 2–3 pinnate, delicate, herbaceous. Pinnae alternate; pinnules fan-shaped to wedge-shaped, deeply incised or lobed; margins irregular. Venation free, repeatedly forked. Sori marginal, often segmented due to deep incisions of pinnules. Indusium false (pseudo-indusium), formed by reflexed pinnule margins. Sporangia numerous. Habitat. Moist, shaded habitats, especially along streams, forest margins, and rock crevices. Distribution. South and East Asia, including Pakistan (Dir Lower, northern regions), India, Nepal, China, and Southeast Asia. Diagnostic characters. Distinguished by deeply incised pinnules, triangular fronds, and marginal sori with false indusia. Taxonomic note. Differs from *A. capillus-veneris* by more deeply lobed pinnules and from *A. venustum* by larger size and erect habit (Figure 1b).

***Adiantum venustum* D. Don**

Plants are terrestrial or lithophytic, small, 10–25 cm tall. Rhizome slender, creeping, extensively branched, forming colonies, covered with small brown scales. Fronds arising in clusters; stipe slender, wiry, dark brown to black, glossy, relatively long, glabrous. Rachis thin, wiry, dark, repeatedly branched. Lamina broadly triangular to ovate, 5–20 × 5–15 cm, 2–3 pinnate, delicate, herbaceous. Pinnae alternate; pinnules small, rounded to fan-shaped, shallowly lobed or crenate. Venation free, repeatedly forked. Sori marginal, small, discrete along pinnule edges. Indusium false (pseudo-indusium). Sporangia numerous. Habitat. Cool, moist, shaded forest floors, rock crevices, and humus-rich slopes. Distribution. Himalayan region, including Pakistan (Dir Lower, northern areas), India, Nepal, and adjacent regions. Diagnostic characters. Recognized by small size, creeping habit, delicate triangular fronds, and small, rounded pinnules. Taxonomic note. Differs from *A. capillus-veneris* by smaller size and creeping habit, and from *A. incisum* by less deeply incised pinnules (Figure 1c).

***Anogramma leptophylla* (L.) Link**

Plants terrestrial, very small, 3–15 cm tall, annual. Rhizome extremely short or indistinct, not persistent. Frond's delicate; stipe slender, pale green to light brown, 1/3–1/2 as long as frond, glabrous. Rachis slender, green, glabrous. Lamina ovate to triangular, 3–12 × 2–8 cm, 2–3 pinnate, finely divided, very thin, herbaceous. Pinnae alternate; pinnules small, deeply lobed or dissected, forming a lacy appearance. Venation free; veins simple or forked. Sori small, borne along veins, irregularly distributed, not strictly marginal. Indusium absent (sori naked). Sporangia are numerous; spores are produced rapidly within a short life cycle. Habitat. Moist open habitats, including rock crevices, walls, cultivated fields, and disturbed

soils, are often affected after rainfall. Distribution. Widely distributed in tropical, subtropical, and temperate regions; in South Asia, found in Pakistan (including Dir Lower), India, and the Himalayan foothills. Diagnostic characters. Distinguished by very small size, annual habit, finely divided, delicate fronds, and absence of indusium. Taxonomic note. Differs from *Adiantum* by lacking marginal sori and false indusia, and from most ferns by its annual life cycle (Figure 1d).

***Hemionitis acrostica* (Balb.) Mosyakin**

Plants are terrestrial or lithophytic, small- to medium-sized, 10–30 cm tall, perennial. Rhizome short, erect to sub-erect, forming small tufts, covered with brown to dark brown membranous scales. Fronds clustered; stipe slender, pale brown to dark brown, short to moderate in length, glabrous or sparsely scaly near base. Rachis green to brownish, slender, glabrous. Lamina simple to shallowly lobed, broadly ovate to irregular in outline, 5–20 × 3–15 cm, herbaceous to slightly coriaceous, glabrous or slightly hairy. Lobes broad, with entire to slightly undulate margins. Venation free; veins simple or forked. Sori acrostichoid, covering much or nearly all of the abaxial lamina surface. Indusium absent. Sporangia numerous and densely distributed on the fertile surface. Habitat. Dry to moderately moist rocky slopes, exposed habitats, and crevices, often with seasonal moisture. Distribution. Mediterranean to West and South Asia; in South Asia, recorded from Pakistan (including Dir Lower) and adjacent regions. Diagnostic characters. Distinguished by simple to lobed fronds, acrostichoid sori covering the underside, and adaptation to relatively dry habitats. Taxonomic note. Differs from *Adiantum* and *Pteris* by lacking marginal sori and indusia, and from most other ferns by its acrostichoid sori and reduced frond division (Figure 2c).

***Taenitis blechnoides* (Willd.) Sw.**

Plants are terrestrial or lithophytic, medium-sized, 20–60 cm tall, perennial. Rhizome short, creeping to sub-erect, producing clustered fronds, covered with narrow brown membranous scales. Fronds clustered; stipe slender to moderately stout, pale brown to dark brown, ca. 1/3 as long as frond, sparsely scaly at base, glabrous upward. Rachis green to brownish, slender, glabrous. Lamina simple, strap-shaped, linear-lanceolate, 20–50 × 3–10 cm, entire, herbaceous to slightly coriaceous, glabrous. Pinnae absent; margins entire, occasionally slightly undulate. Venation free to subparallel; veins simple or forked, running longitudinally. Sori elongate-linear, arranged along veins or near margins, parallel to the long axis of the lamina. Indusium absent or very poorly developed. Sporangia numerous. Habitat. Moist, shaded forests, especially on rocky slopes and humus-rich soils in humid environments. Distribution. Tropical and subtropical Asia, including Pakistan (northern regions), India, Nepal, China, and Southeast Asia. Diagnostic characters. Recognized by simple strap-shaped fronds, elongate linear sori, and a lack of well-developed indusia. Taxonomic note. Differs from *Pteris* by having an entire, undivided lamina, and from *Hemionitis* by linear rather than acrostichoid sori (Figure 2i).

***Pteris cretica* L.**

Plants are terrestrial or lithophytic, medium-sized, 20–60 cm tall, perennial. Rhizome short, creeping to sub-erect, producing clustered fronds, covered with brown to dark brown membranous scales. Fronds clustered; stipe slender to moderately stout, pale brown to dark brown, ca. 1/3–1/2 as long as frond, sparsely scaly at base, glabrous above. Rachis green, slender, glabrous, sometimes slightly grooved. Lamina elongate, 20–50 × 7–18 cm, once pinnate, herbaceous to slightly coriaceous, glabrous. Pinnae few to several, opposite or subopposite, linear-lanceolate, widely spaced, margins entire; terminal pinna usually larger and more elongate. Venation free; veins simple or forked. Sori are continuous along the pinna margins, forming linear bands. Indusium false (pseudo-indusium), formed by reflexed pinna margins. Sporangia numerous. Habitat. Moist, shaded habitats, especially rocky slopes, walls, and forest margins. Distribution. Widely distributed in tropical and subtropical regions; in South Asia, present in Pakistan (including Dir Lower), India, and the Himalayan foothills. Diagnostic characters. Recognized by once pinnate fronds with broad linear pinnae and continuous marginal sori. Taxonomic note. Differs from *P. vittata* by broader pinnae and fewer segments, and from *Taenitis* by pinnate rather than simple fronds (Figure 2g).

***Pteris dactylina* Hook.**

Plants are terrestrial or lithophytic, medium-sized, 20–60 cm tall, perennial. Rhizome short, creeping to sub-erect, producing clustered fronds, covered with brown membranous scales. Fronds clustered; stipe slender to moderately stout, pale brown to dark brown, ca. 1/3–1/2 as long as frond, sparsely scaly at base, glabrous above. Rachis green, slender, glabrous, sometimes slightly grooved. Lamina 20–50 × 8–20 cm, deeply divided, digitately or irregularly segmented, herbaceous to slightly coriaceous. Segments narrow, elongated, finger-like, arising from a common point or short rachis; margins entire to slightly undulate. Venation free; veins simple or forked. Sori are continuous along segment margins, forming linear bands. Indusium false (pseudo-indusium), formed by reflexed segment margins. Sporangia numerous. Habitat. Rocky, well-drained habitats, often in open or partially shaded montane regions. Distribution. South and Central Asia, including Pakistan (Dir Lower and northern areas), India, and the adjacent Himalayan regions. Diagnostic characters. Distinguished by deeply divided finger-like frond segments and continuous marginal sori. Taxonomic note. Differs from *P. cretica* by digitately divided fronds and from *P. vittata* by broader, more irregular segments (Figure 2i).

***Pteris vittata* L.**

Plants are terrestrial or lithophytic, medium-sized, 30–90 cm tall, perennial. Rhizome short, creeping to sub-erect, producing clustered fronds, covered with brown membranous scales. Fronds clustered; stipe slender to moderately stout, pale brown to dark brown, ca. 1/3–1/2 as long as frond, sparsely scaly at base, glabrous above. Rachis green, slender, glabrous. Lamina elongate, 30–80 × 10–28 cm, once pinnate, herbaceous to slightly coriaceous, glabrous. Pinnae numerous, very narrow, linear, widely spaced; margins entire; terminal pinna usually longer than lateral pinnae. Venation free; veins simple or forked. Sori are continuous along the pinna margins, forming linear bands. Indusium false (pseudo-indusium), formed by reflexed

pinna margins. Sporangia numerous. Habitat. Open to semi-shaded rocky slopes, walls, roadsides, and disturbed soils; tolerant of dry to moderately moist conditions. Distribution. Widely distributed in tropical, subtropical, and warm temperate regions; in South Asia, present in Pakistan (including Dir Lower), India, and the Himalayan foothills. Diagnostic characters. Easily recognized by long, narrow linear pinnae, a widely spaced arrangement, and continuous marginal sori. Taxonomic note. Differs from *P. cretica* by much narrower and more numerous pinnae, and from *P. dactylina* by lacking digitately divided fronds.

***Selaginella jacquemontii* Spring**

Plants are lycophytic, small, creeping to ascending, 5–20 cm long, perennial, forming loose mats or patches. Stems slender, green, prostrate to ascending, dichotomously branched, producing roots at nodes. Rhizome not differentiated as in ferns, stems are creeping and rooting at nodes. Leaves microphyllous, small, scale-like, arranged in four rows, with two larger lateral rows and two smaller median rows; lateral leaves ovate to lanceolate, median leaves smaller, margins entire to slightly serrulate, texture thin. Ligule present at the base of each leaf on the adaxial side. Venation represented by a single unbranched vein in each microphyll. Strobili terminal, compact, cylindrical, borne at branch tips. Sporophylls similar to vegetative leaves, slightly modified, bearing sporangia in axils. Sporangia heterosporous, producing microsporangia and megasporangia. Habitat. Moist, shaded rocks, forest floors, and slopes in subtropical to montane regions. Distribution. South and Central Asia, including Pakistan (Dir Lower and northern regions), India, Nepal, and the adjacent Himalayan areas. Diagnostic characters. Recognized by creeping mat-forming habit, four-rowed microphylls, and terminal strobili. Taxonomic note. Differs from true ferns by microphyllous leaves and heterosporous reproduction; differs from *S. sanguinolenta* by looser growth form and less compact branching (Figure 2j).

***Selaginella sanguinolenta* (L.) Spring**

Plants are lycophytic, small, 5–15 cm long, perennial, forming dense, compact mats. Stems prostrate to ascending, densely dichotomously branched, rooting at nodes, often cushion-like in habit. Leaves microphyllous, small, scale-like, arranged in four rows; lateral leaves ovate to lanceolate, closely overlapping, median leaves smaller, margins entire or slightly serrulate, often appressed to the stem. Ligule present at the base of each leaf. Venation represented by a single unbranched vein. Strobili terminal, short, compact. Sporophylls are similar to vegetative leaves but more closely arranged. Sporangia heterosporous, producing microspores and megaspores. Habitat. Dry to moderately moist rocky slopes, open ground, and well-drained soils; more xeric than related species. Distribution. Widely distributed in Asia and Europe; in South Asia, found in Pakistan (including Dir Lower), India, and the Himalayan region. Diagnostic characters. Distinguished by dense compact mat-forming habit, closely appressed leaves, and tolerance of drier environments. Taxonomic note. Differs from *S. jacquemontii* by more compact growth, denser branching, and adaptation to drier habitats (Figure 2k).

Integrated Taxonomic Key to the Studied Species

(Based on morphological, reproductive, and anatomical characters)

1a. Plants heterosporous; sporocarps or strobili present → 2

1b. Plants homosporous; spores produced in sori → 4

2a. Leaves microphyllous; sporangia in strobili → *Selaginella* spp.

2b. Leaves compound; sporocarps present → *Marsilea quadrifolia*

4a. Reproductive structures in cones (strobili) → *Equisetum* spp.

4b. Reproductive structures as sori on fronds → 5

5a. Fronds simple or nearly entire → 6

5b. Fronds pinnate or highly divided → 7

6a. Sori covering entire lamina (acrostichoid) → *Hemionitis acrostica*

6b. Sori marginal or vein-based → *Taenitis blechnoides*

7a. Venation free → 8

7b. Venation parallel, dichotomous, or single → 10

8a. Indusium absent → 9

8b. Indusium present → 11

9a. Sori marginal; delicate fronds → *Adiantum* spp.

9b. Sori scattered or along veins → *Anogramma leptophylla*

10a. Venation parallel; microphyllous leaves → *Selaginella* spp.

10b. Single vein; segmented stems → *Equisetum* spp.

11a. Sori linear along veins → *Asplenium* spp.

11b. Sori round or irregular → 12

12a. Fronds robust; sori round → *Dryopteris* spp.

12b. Sori arranged in rows → *Pteris* spp.

Anatomical Key (Petiole and Stem)

13a. Stem receiving 1–2 vascular strands → 14

14a. Stem receives two vascular strands at base, later fused → 15

14b. Stem receives a single vascular strand → 16

15a. Xylem mesarch → *Christella dentata*

15b. Xylem diarch or triarch → *Pteris multifida*

16a. Ground tissue differentiated into outer sclerenchyma and inner parenchyma → *Aleuritopteris argentea*

16b. Ground tissue multilayered (sclerenchyma + parenchyma) → 17

17a. Xylem V-shaped → 18

17b. Xylem U-shaped → *Dryopteris carthusiana*

18a. Xylem with curved ends (petiole) → *Pteris vittata*

18b. Xylem with straight ends → 19

19a. Xylem not triarch → *Pteris multifida*

19b. Xylem diarch → 20

19c. Xylem mesarch → 21

20a. Dictyostele present → *Asplenium platyneuron*

21a. Xylem semi-lunar shaped → *Adiantum pedatum*

21b. Amphiphloic siphonostele present → 22

22a. Semi-lunar vascular strand → *Adiantum caudatum*

22b. Mesarch xylem → 23

23a. Xylem heart-shaped (petiole) → *Christella dentata*

23b. Xylem hippocampus-shaped (stem) → *Christella dentata*

Key to the Families of Studied Pteridophytes

1a. Fronds monomorphic, rarely with fertile fronds taller and more erect → Aspleniaceae

1b. Fronds not as above; plants creeping or with basal scales → 2

2a. Fronds remote from one another; segments anadromously arranged → Dryopteridaceae

2b. Fronds reduced, scale-like or whorled → 3

3a. Rhizome creeping and branched with distinct nodes; stems jointed → Equisetaceae

3b. Rhizome slender, covered with short hairs or glabrous → 4

4a. Spores enclosed inside bean-shaped sporocarps attached to the stipe by a short peduncle; sporocarp containing 2–30 sori → Marsileaceae

4b. Sporophylls ovate to lanceolate; sporangia single per sporophyll → 5

5a. Plants epilithic or occasionally epiphytic; rhizome erect → Selaginellaceae

5b. Plants terrestrial on rocks; rhizomes stout with dictyostele → 6

6a. Lamina herbaceous or papery; hairs unicellular, acicular or absent → Thelypteridaceae

6b. Lamina variously dissected; surfaces scaly; hairs unicellular or multicellular → 7

7a. Sori linear, J-shaped, or horseshoe-shaped; fronds orbicular or reniform → Athyriaceae

7b. Sori mostly confluent along veins or marginal commissures; fronds usually scaly → Pteridaceae

Conclusion

The present study of 24 pteridophyte species revealed substantial variation in morphological, ecological, and reproductive traits, while anatomical features such as cortex thickness, xylem configuration (mesarch/diarch), and stele type (dictyostele and siphonostele) showed comparatively lower variation. Terrestrial habit, sori-based reproduction, and free venation were identified as dominant and stable characters, whereas specialized traits such as aquatic habit, sporocarps, and cone-bearing structures were restricted to a few taxa. PCA results indicated that anatomical traits explained 64.5% of total variation (PC1), while morphological traits contributed 11.4% (PC2). Overall, the integration of morphological, anatomical, and statistical analyses provides a reliable framework for species identification and enhances understanding of evolutionary relationships among pteridophytes.

Author Contributions

Shakir Ullah conceived and designed the study, conducted fieldwork, performed morphological and anatomical analyses, carried out statistical analyses (including PCA and correlation), and drafted the manuscript. Lubna Shakir contributed to data analysis and manuscript writing. Ghani Subhan and Rahid Khan assisted in field sampling, data collection, and preliminary data processing. Nazli Rahid, Mohammad Sohail, Falak Naz, Azmat Noreen, Sajid Ali, and Hasnain Khan contributed to laboratory work, data organization, and literature review. All authors read, reviewed, and approved the final manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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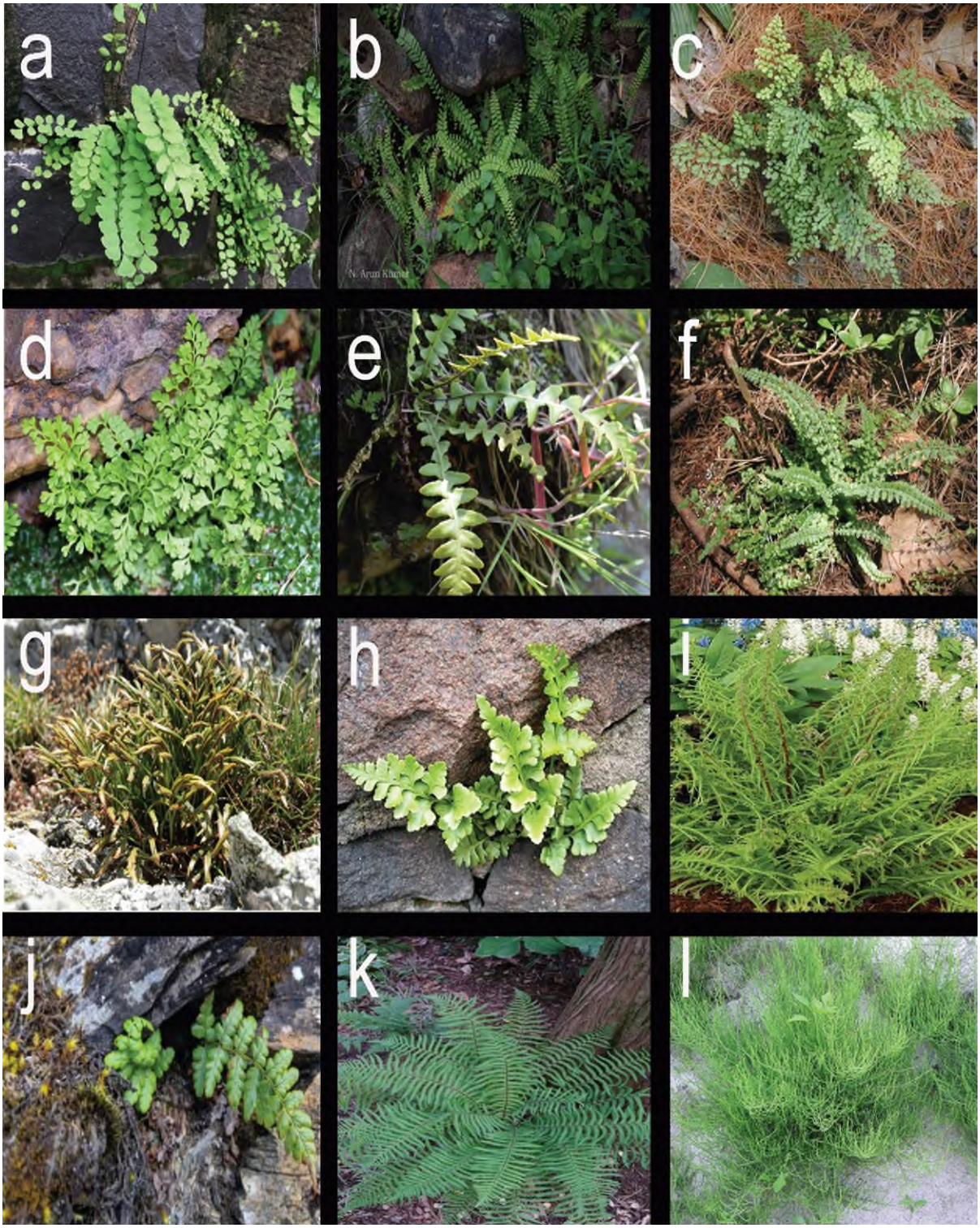


Figure 1. Pteridophytes of Dir Lower (Maidan), Khyber Pakhtunkhwa, Pakistan. (a) *Adiantum capillus-veneris* L., (b) *Adiantum incisum* Forssk., (c) *Adiantum venustum* D. Don, (d) *Anogramma leptophylla* (L.) Link, (e) *Asplenium dalhousieae* Hook., (f) *Asplenium incisum* Thunb., (g) *Asplenium septentrionale* (L.) Hoffm., (h) *Asplenium adiantum-nigrum* L., (i) *Athyrium drepanopteron* (Kunze) Milde, (j) *Dryopteris serrato-dentata* (Bedd.) Hayata, (k) *Dryopteris splendens* (Desv.) Kuntze, (l) *Equisetum arvense* L. Photographs illustrate variation in frond morphology and reproductive structures. Photographs by Mohammad Sohail.

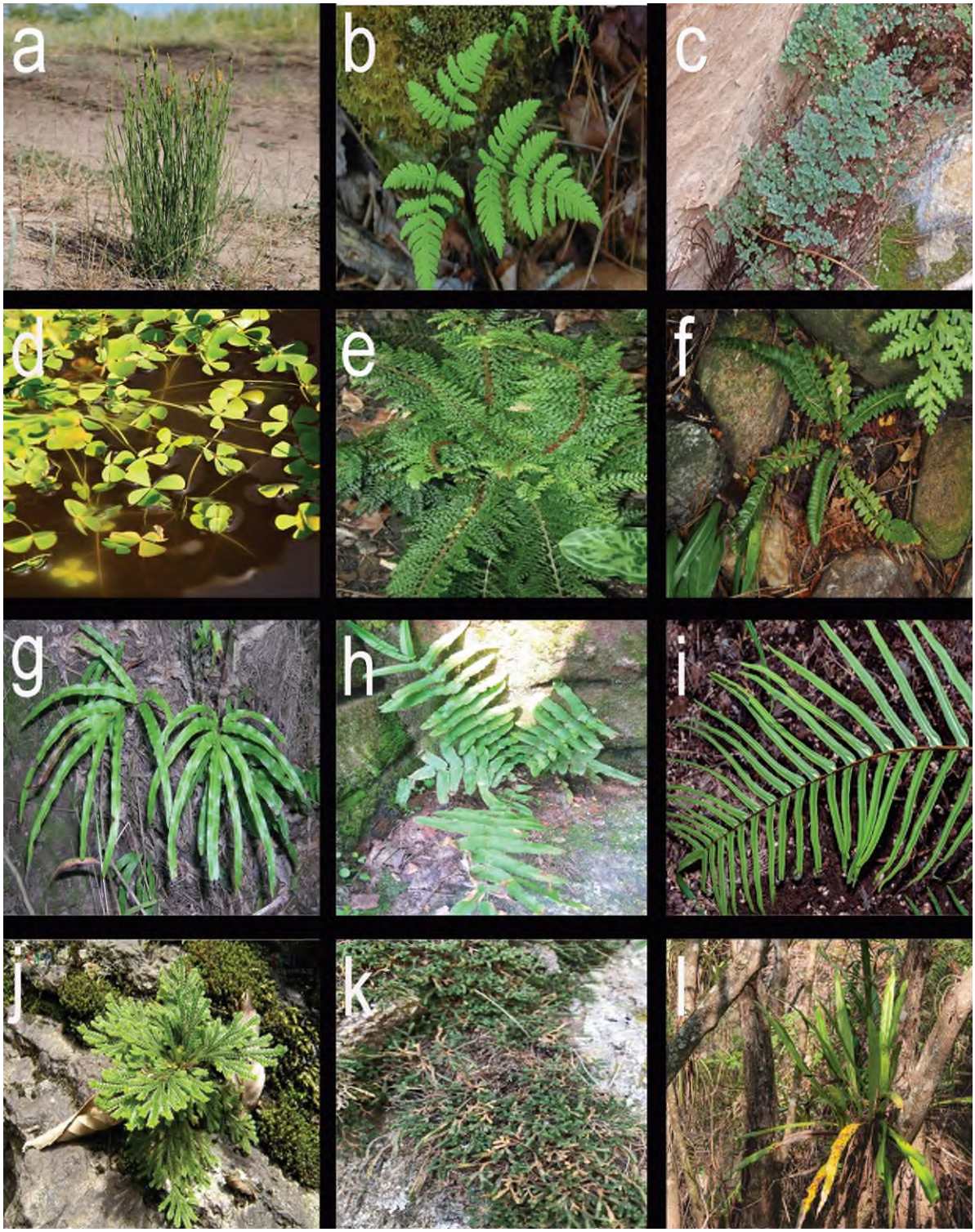


Figure 2. Pteridophytes of Dir Lower (Maidan), Khyber Pakhtunkhwa, Pakistan. (a) *Equisetum ramosissimum var. huegelii* (Milde) Christenh. & Husby, (b) *Gymnocarpium dryopteris* (L.) Newman, (c) *Hemionitis acrostica* (Balb.) Mosyakin, (d) *Marsilea quadrifolia* L., (e) *Polystichum discretum* D. Don, (f) *Polystichum lonchitis* L., (g) *Pteris cretica* L., (h) *Pteris dactylina* Hook., (i) *Pteris vittata* L., (j) *Selaginella jacquemontii* Spring, (k) *Selaginella sanguinolenta* (L.) Spring, (l) *Taenitis blechnoides* (Willd.) Sw. Photographs illustrate variation in frond morphology, reproductive structures (sori, strobili, and sporocarps), and ecological adaptations. Photographs by Mohammad Sohail.

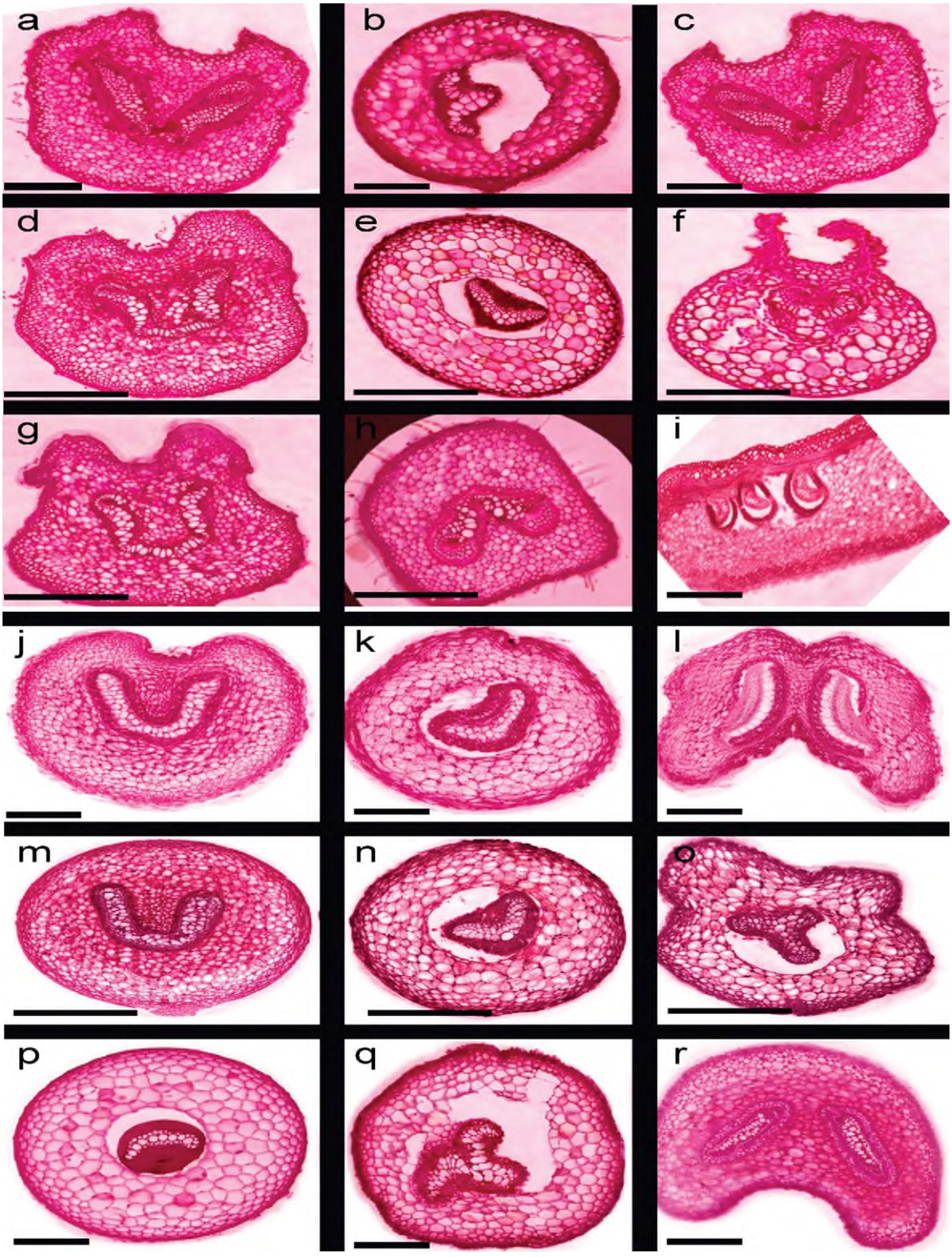


Figure 3: Transverse section of petioles and stem of representative species (a) Adiantum capillus-veneris, (b) Hemionitis acrostica, (c) Pteris cretica, (d) Pteris vittata, (e) Selaginella jacquemontii, (f) Asplenium dalhousieae, (g) Dryopteris splendens, (h) Polystichum lonchitis, (i) Equisetum arvense. Stem (j) Adiantum capillus-veneris, (k) Hemionitis acrostica, (l) Pteris cretica, (m) Pteris vittata, (n) Selaginella jacquemontii, (o) Asplenium dalhousieae, (p) Dryopteris splendens, (q) Polystichum lonchitis, (r) Equisetum arvense. (Scale bar = 1mm)

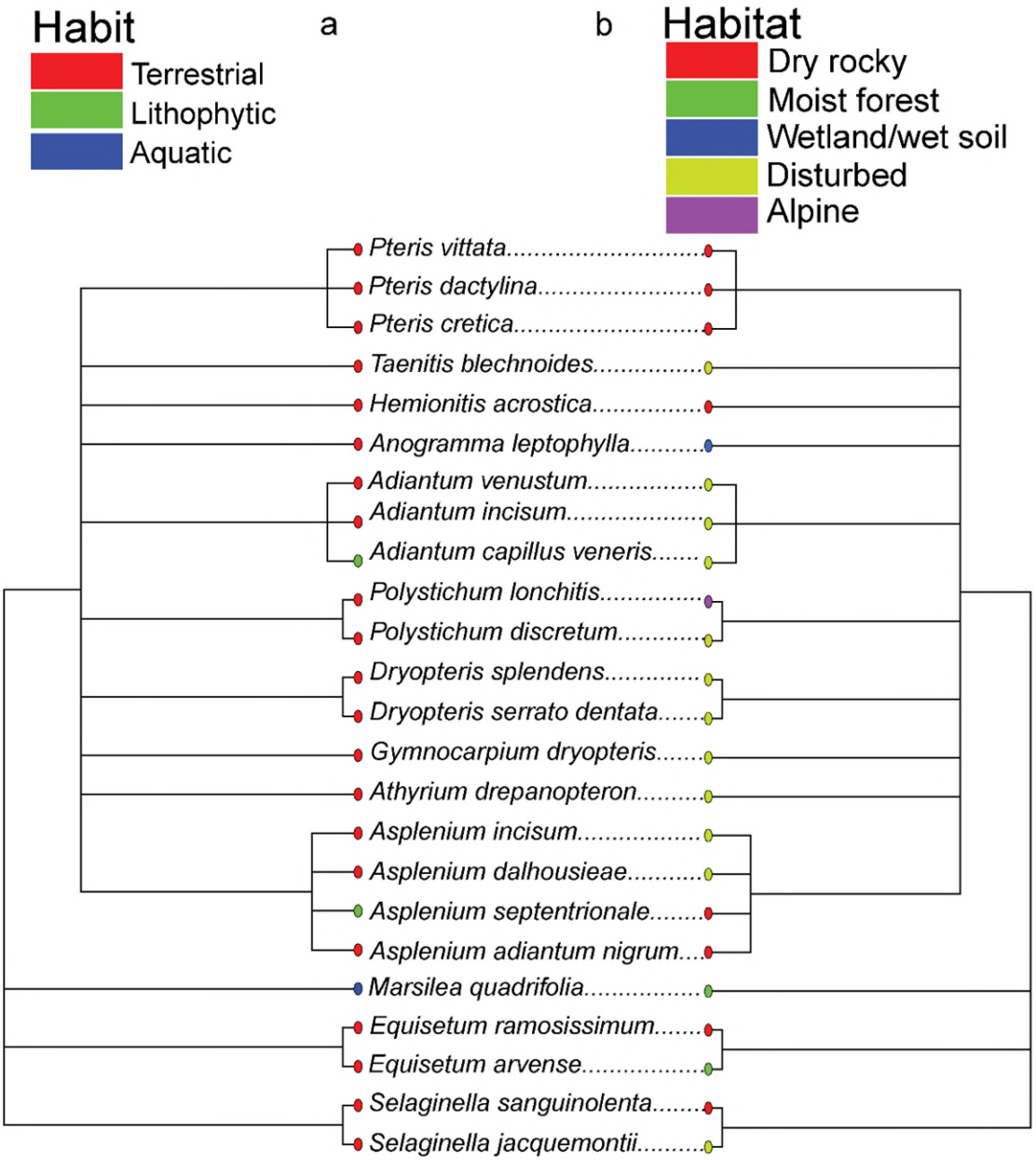


Figure 4. Phylogenetic tree of pteridophytes from Dir Lower (Maidan), Pakistan, showing ancestral states of (a) habit and (b) habitat inferred using maximum likelihood.

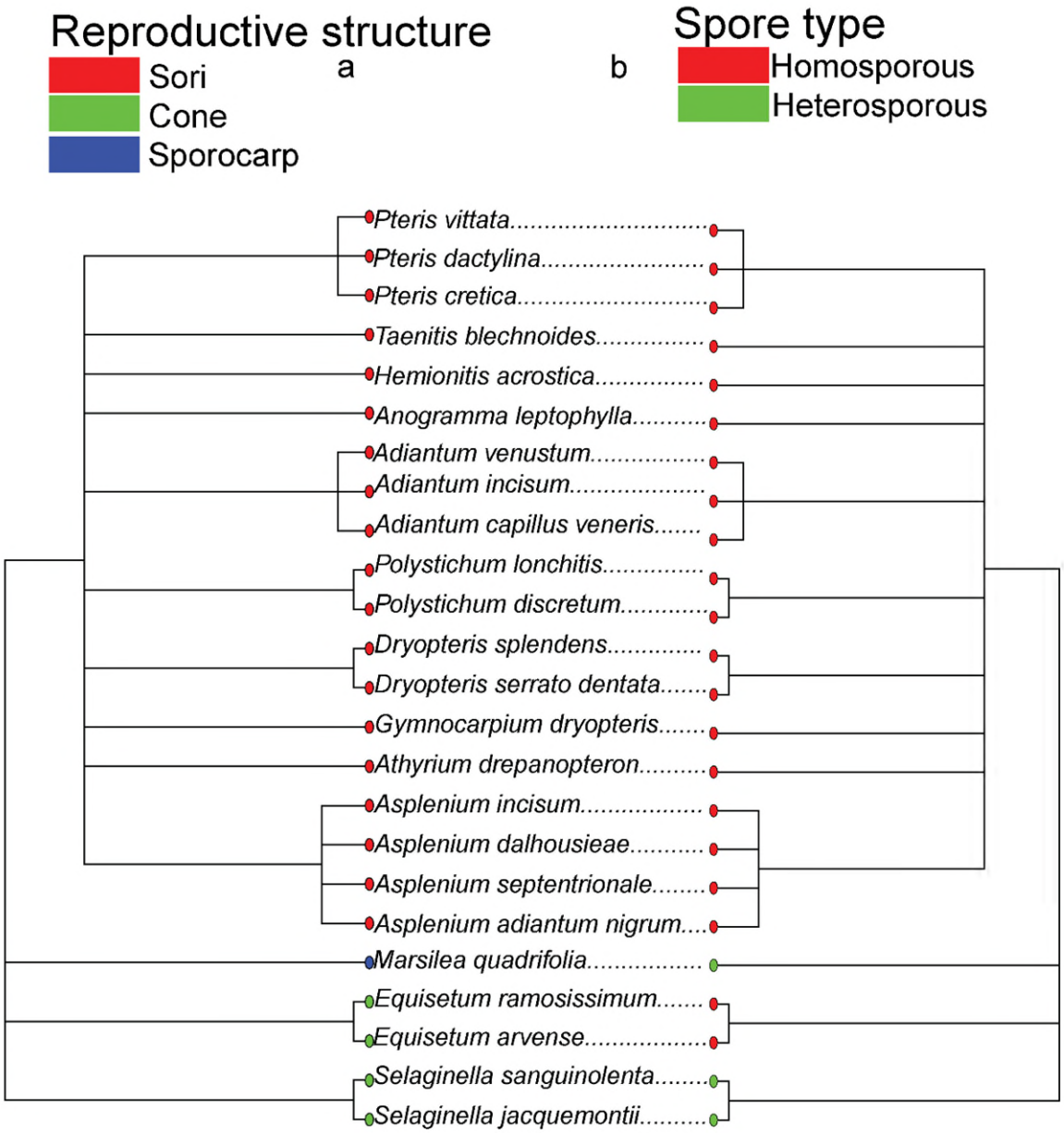


Figure 5. Phylogenetic tree of pteridophytes from Dir Lower (Maidan), Pakistan, showing ancestral states of (a) reproductive structure and (b) spore types inferred using maximum likelihood.

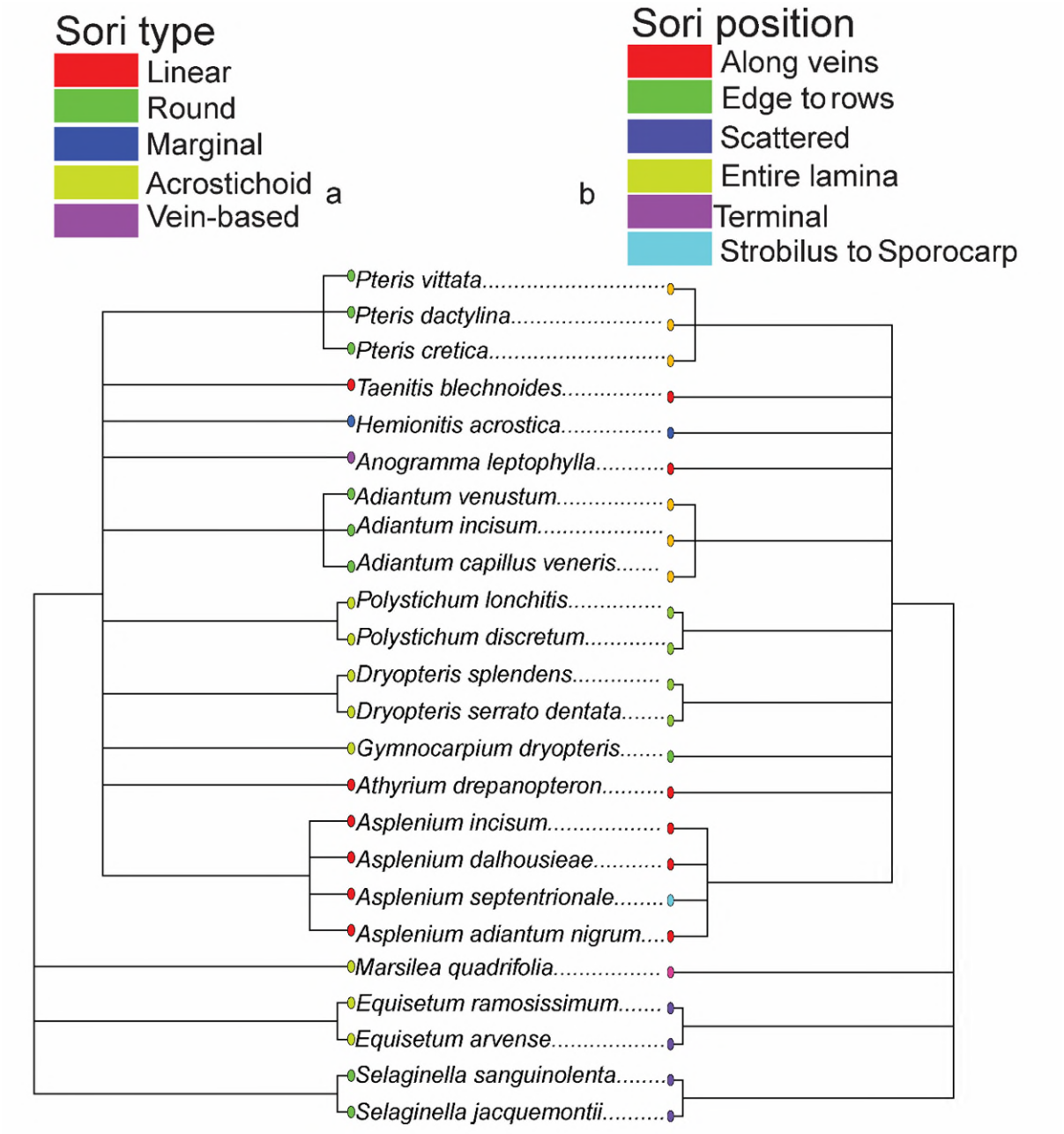


Figure 6. Phylogenetic tree of pteridophytes from Dir Lower (Maidan), Pakistan, showing ancestral states of (a) sori type and (b) sori position inferred using maximum likelihood.

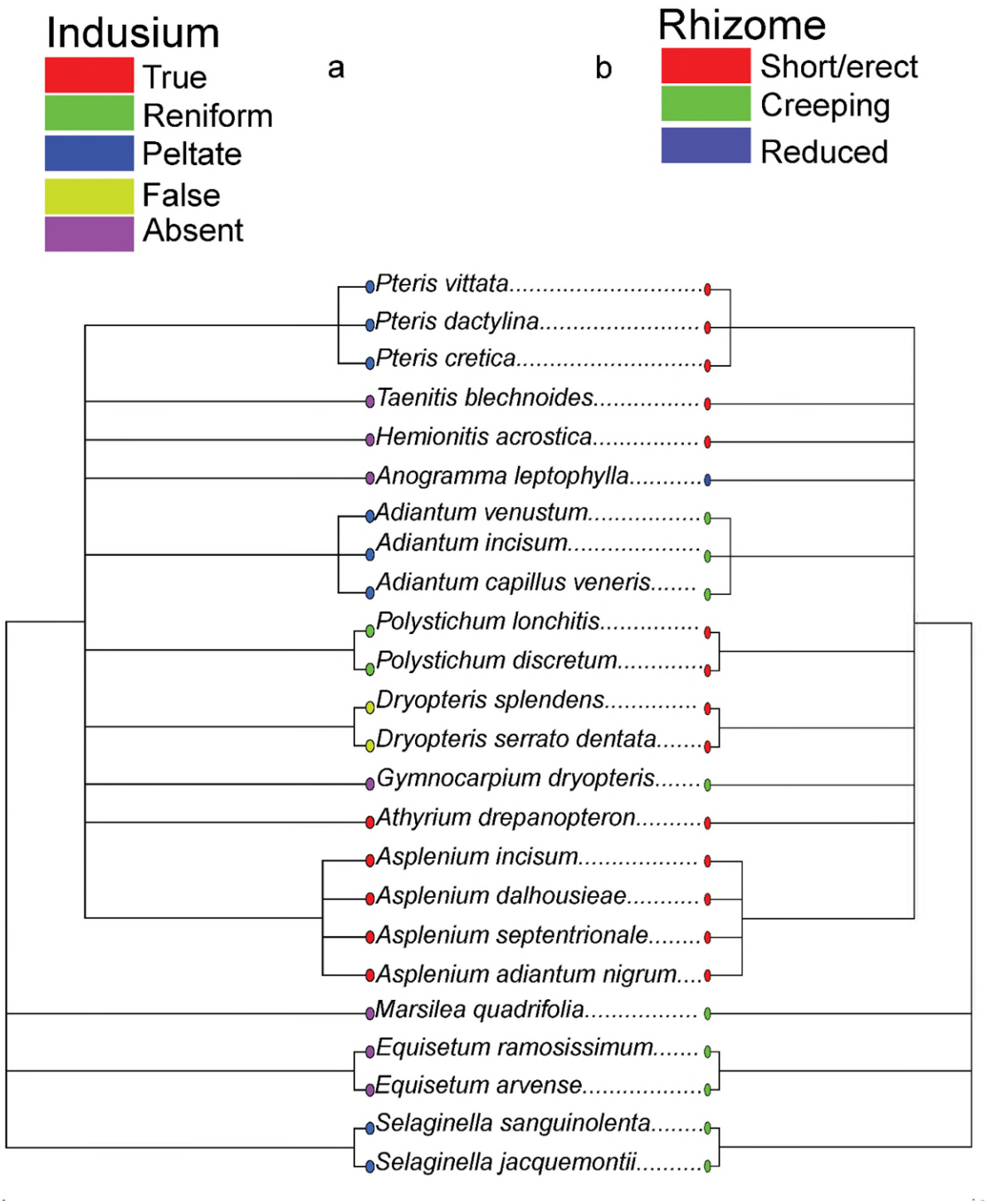


Figure 7. Phylogenetic tree of pteridophytes from Dir Lower (Maidan), Pakistan, showing ancestral states of (a) indusium type and (b) rhizome inferred using maximum likelihood.

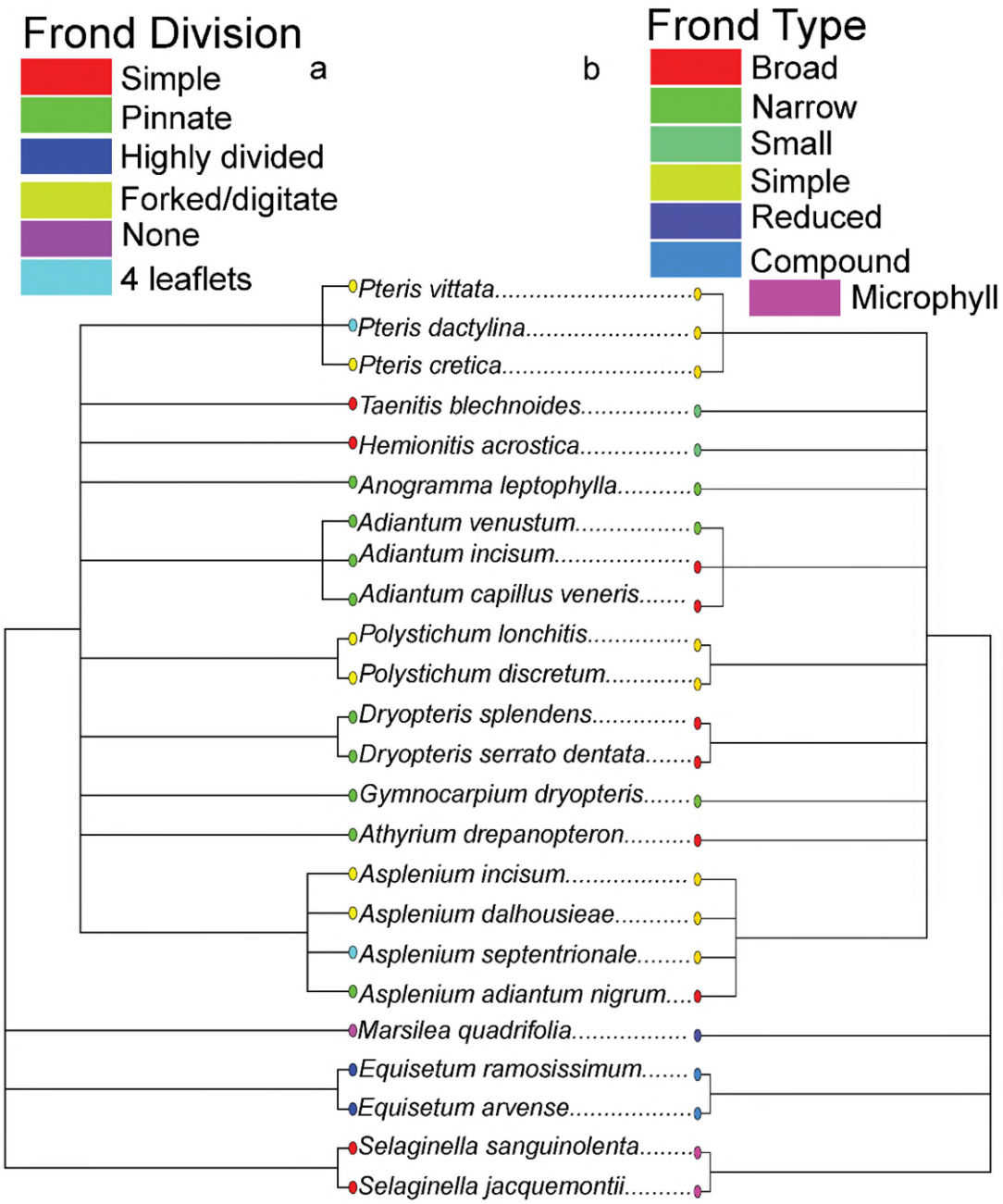


Figure 8. Phylogenetic tree of pteridophytes from Dir Lower (Maidan), Pakistan, showing ancestral states of (a) frond Type and (b) frond Division inferred using maximum likelihood.

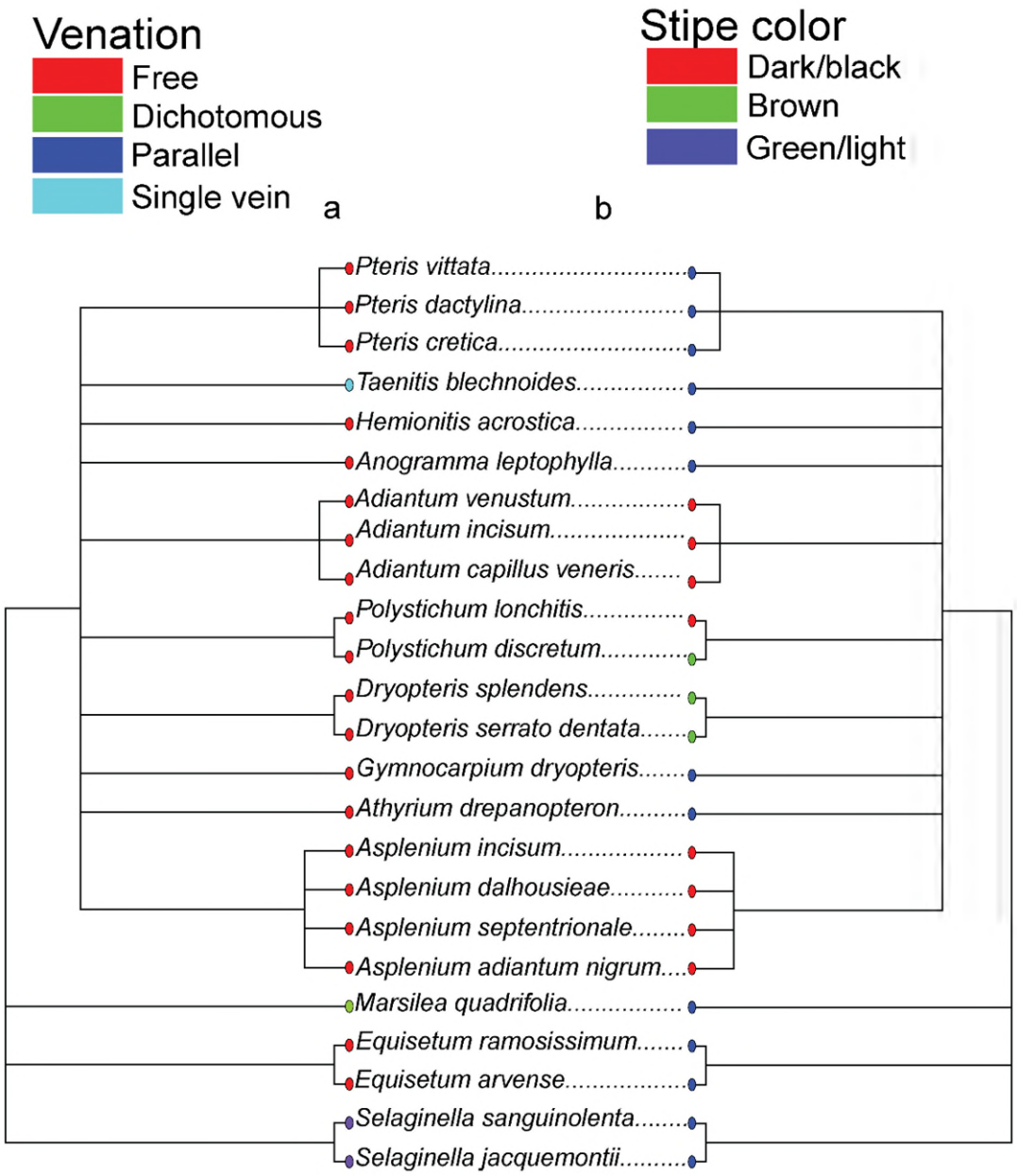


Figure 9. Phylogenetic tree of pteridophytes from Dir Lower (Maidan), Pakistan, showing ancestral states of (a) venations and (b) stipe color inferred using maximum likelihood.

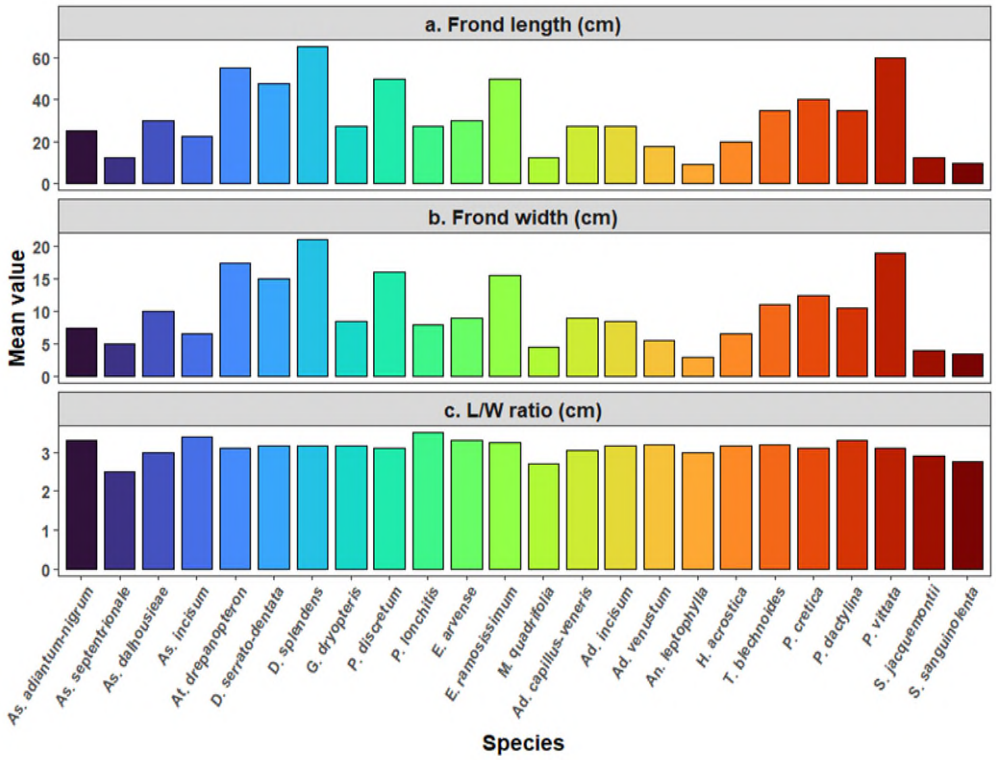


Figure 10: Bar plots showing **a** frond length (cm), **b** frond width (cm), and **c** length/width ratio of the studied pteridophyte species. Values represent the mean of recorded ranges.

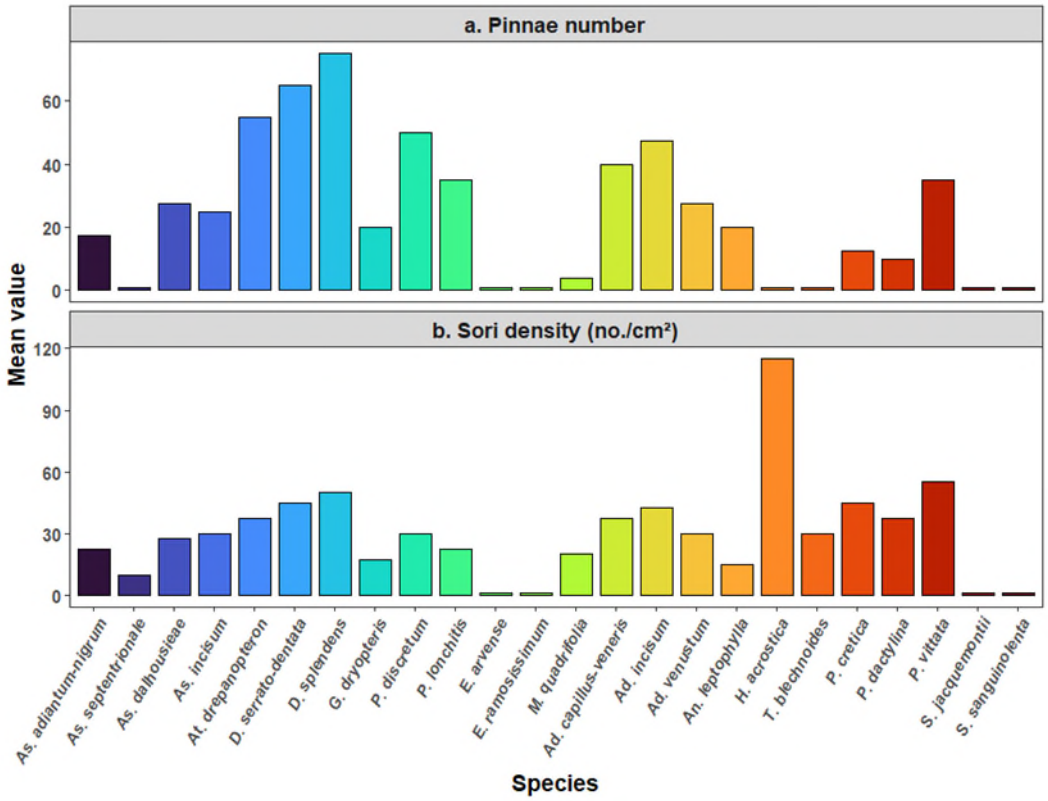


Figure 11: Bar plots showing **a** pinnae number and **b** sori density (no./cm²) of the studied pteridophyte species. Values represent the mean of recorded ranges.

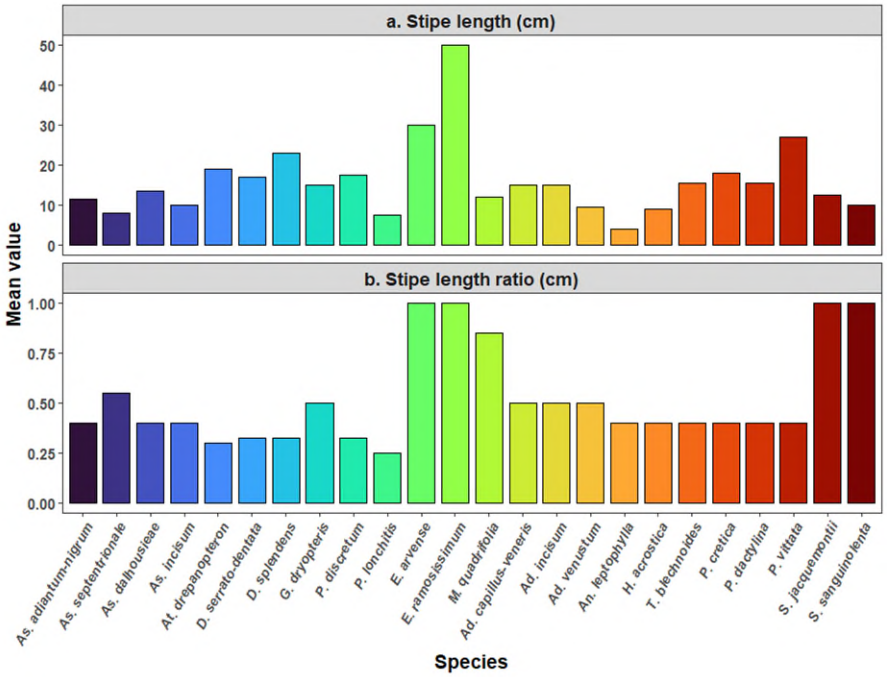


Figure 12: Bar plots showing a stipe length (cm) and b stipe length ratio (cm) of the studied pteridophyte species. Values represent the mean of recorded ranges.

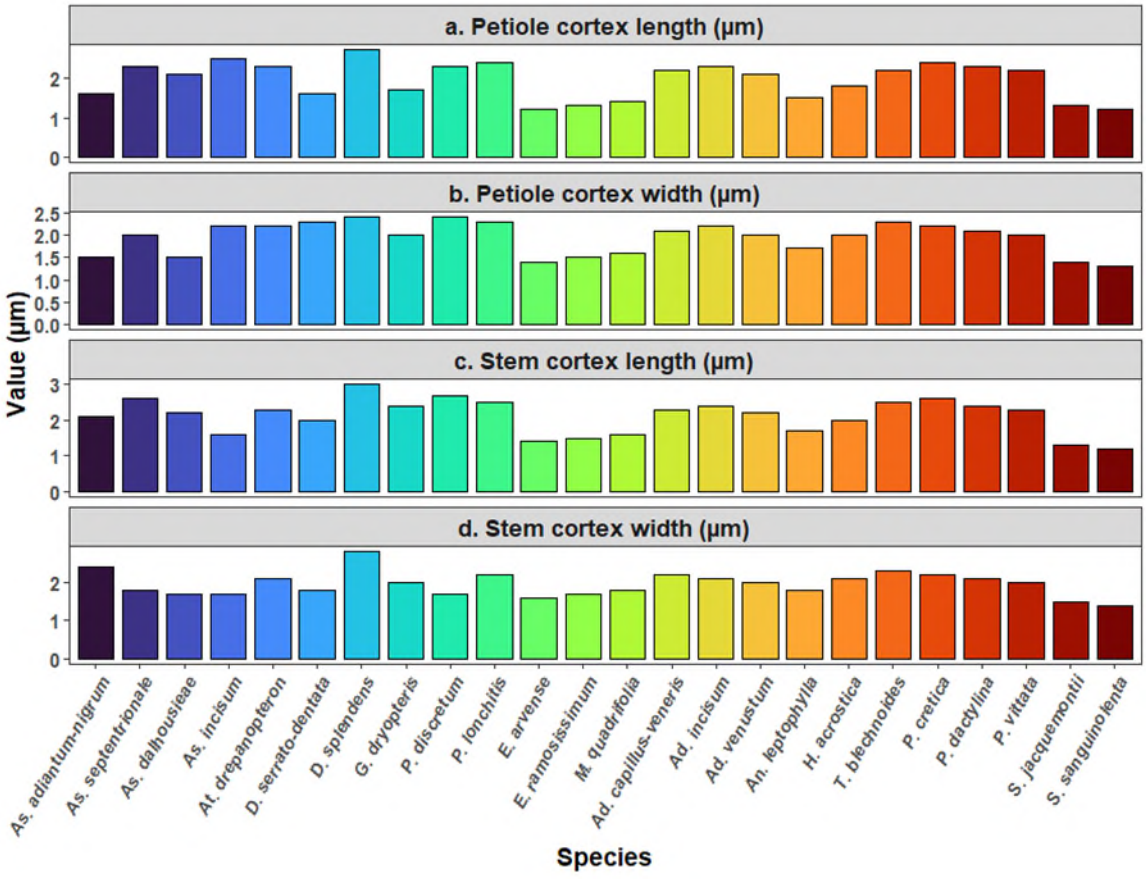


Figure 13: Bar plots showing **a** petiole cortex length (µm), **b** petiole width (µm), **c** stem cortex length (µm), and **d** stem width (µm) of the studied pteridophyte species.

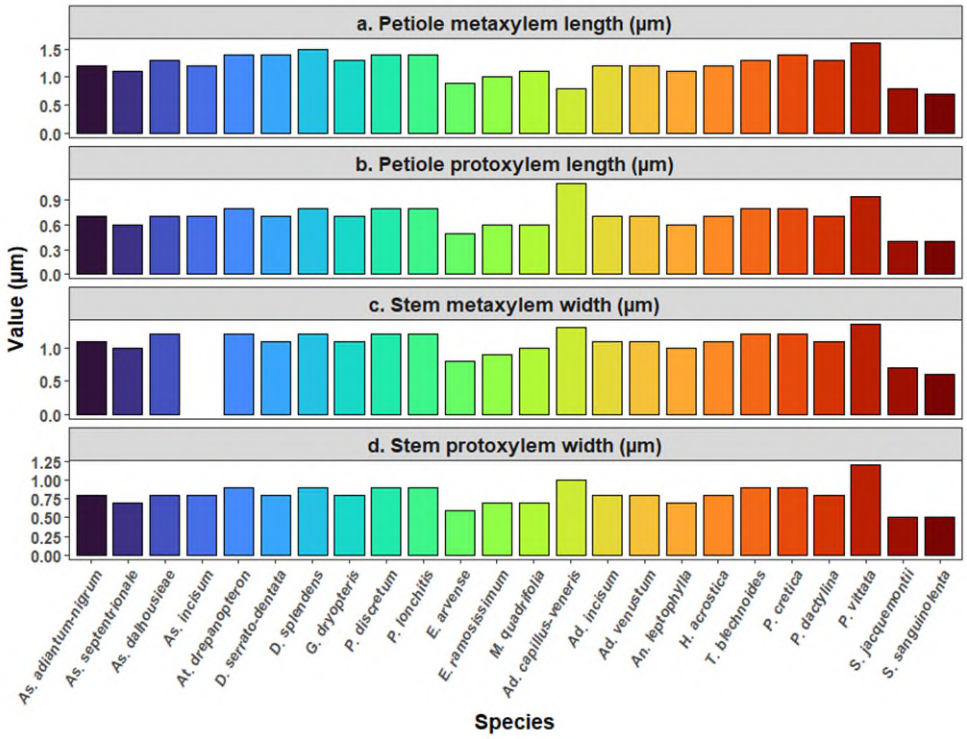


Figure 14: Bar plots showing **a** petiole metaxylem length (µm), **b** petiole protoxylem length (µm), **c** stem metaxylem width (µm), and **d** stem protoxylem width (µm) of the studied pteridophyte species.

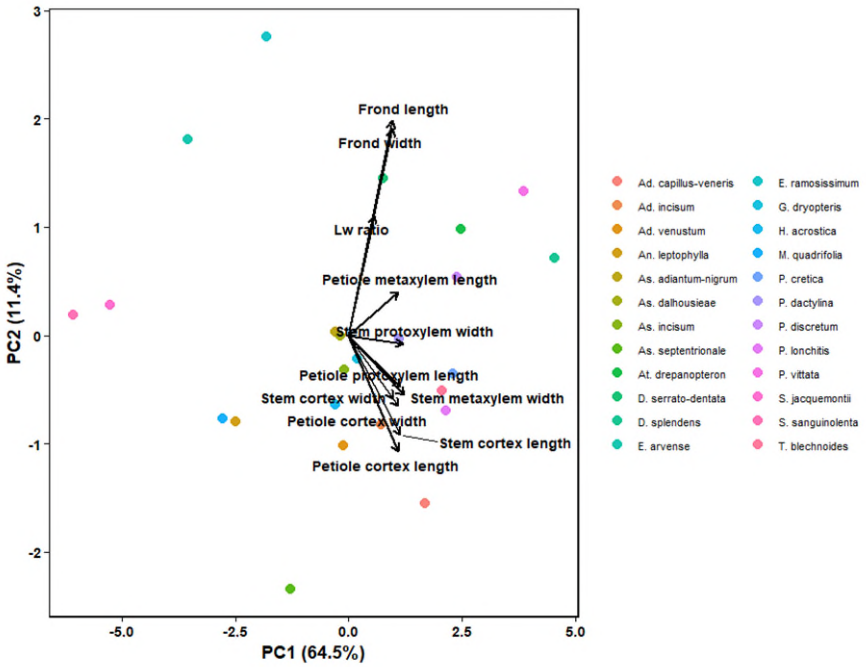


Figure 15. Principal component analysis (PCA) biplot showing the distribution of species and the contribution of morphological and anatomical traits. Points represent species, while arrows indicate the direction and magnitude of trait loadings. The first two principal components explain 64.5% (PC1) and 11.4% (PC2) of the total variation, respectively.

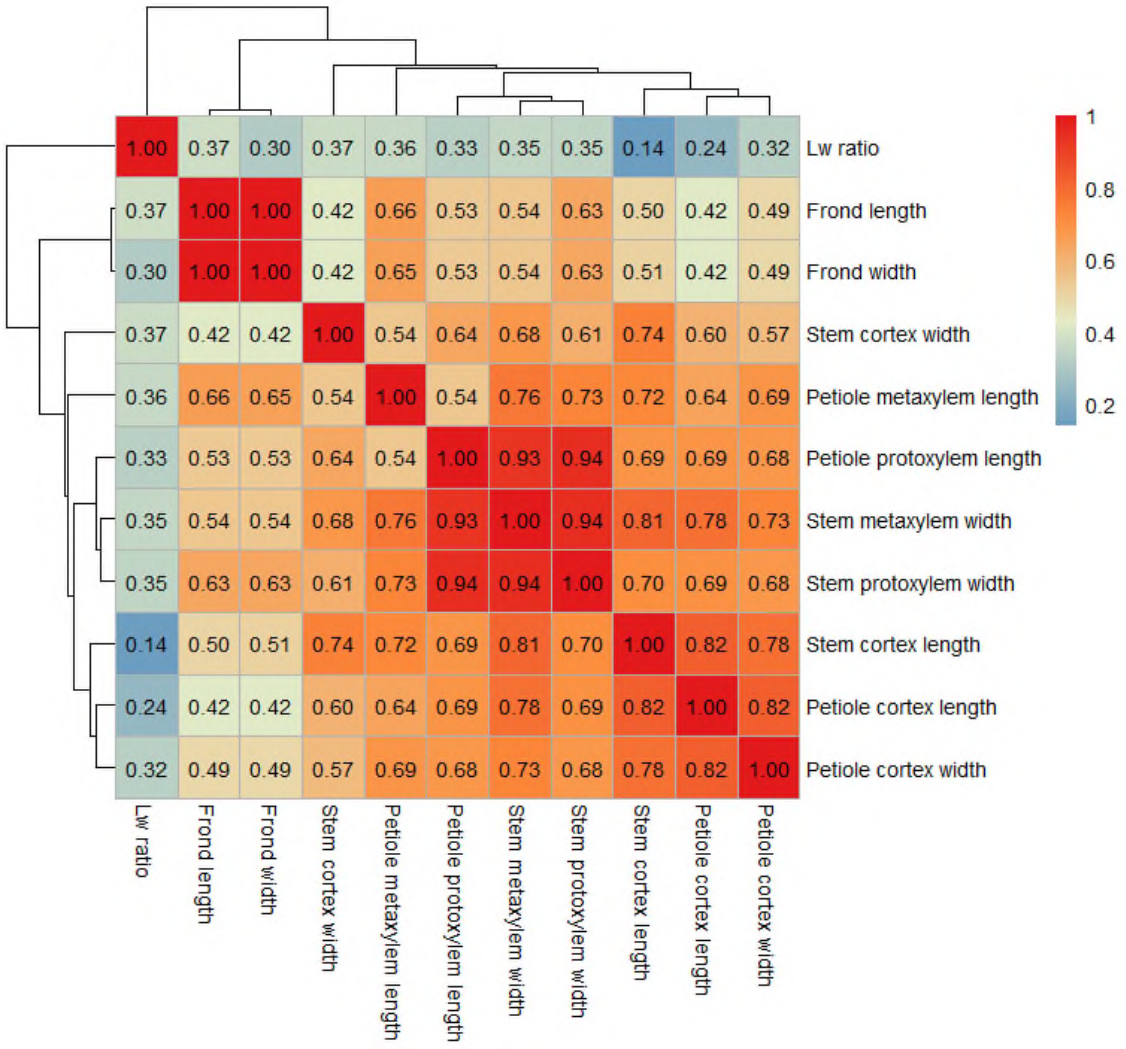


Figure 16: Clustered Pearson correlation heatmap of morphological and anatomical traits. Colors represent correlation strength (blue = negative, red = positive), and values indicate correlation coefficients. Dendrograms show hierarchical clustering of traits.

Supplementary Files

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- SupplementaryTable20260312.xlsx