Revelation and Science
Special Issue: Plant Science 3 (1447H/2026) 23-32

Leaf Anatomical Structure of *Acrostichum aureum* L. and *Acrostichum speciosum* Wild.

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ABSTRACT
Acrostichum L. are common species of fern that can be found in mangrove forests in Malaysia. Mangroves are a naturally tense environment with high salinity, high temperatures, and hypoxic soil that necessitated adaptation in plant structure such as leaf anatomy. The leaf functions as a vital organ for assimilation in plants, and the anatomical characteristics of leaves can provide understanding into the plant's adaptability to the environment to some degree. Thus, the objective of this study is to identify the leaf anatomical characteristics of *Acrostichum aureum* and *Acrostichum speciosum*. The leaf samples were collected from mangrove forests located in Kuantan and Pekan, Pahang. The procedures involved such as cross section using sliding microtome on the petiole, lamina, midribs and marginal and observation under light microscope. The findings have demonstrated the presence of both similarities and variability in the anatomical properties of leaves. The observed anatomical features encompass the outlines of the midrib and margin, the arrangement of vascular bundles within the midrib, the existence of hypodermis layers in the lamina, the presence of bundle sheath extensions, and the presence of a spongy parenchyma. In summary, the findings of this study indicate that anatomical traits possess taxonomic value, particularly in the context of classification, with a particular emphasis on the species level.

Keywords: Acrostichum, leaf anatomy, mangrove fern

Introduction
Ferns are the second largest group of vascular plants in the world, with more than 10,000 species found in a number of habitat of habitats including rainforests, temperate forests, aquatic habitats, semi-arid deserts, savannas, high alpine mountains, and mangroves (Schneider e*t al.*, 2004; de Arruda *et al.*, 2021). Consequently, these plants have a variety of morphological, anatomical, and physiological adaptations (Page, 2002). *Acrostichum* Linnaeus, a rhizomatous fern belonging to the Pteridaceae family, holds significant ecological value as a constituent species within the mangrove community. According to Tomlinson (1986) as cited in Moreno 2016, *Acrostichum*, also known as the "mangrove fern," is a prominent species of fern that exhibits vigorous growth inside mangrove habitats.

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On the other hand, it predominantly occupies the understory of mangrove backwaters and possesses the unique ability to flourish in brackish water, setting it apart from other fern varieties. Mangrove plant growth is influenced by a diverse range of biotic and abiotic variables. They grow in the unstable marine intertidal zone, which has harsh plant growth conditions such as high salinity, tidal fluctuations, and long-term climate changes (Ernesto *et al.,* 1990; Naskar *et al.,* 2015). Plants that live in high saline environments have evolved to minimize water loss to avoid dehydration and to exclude salts that are harmful to their metabolism (Naskar *et al.,* 2015).

Acrostichum is tolerant of a broad spectrum of soil salinities and requires full sun exposure for rapid and optimal development (Ernesto *et al.,* 1990). Therefore, *Acrostichum* is able to effectively colonise disturbed regions devoid of trees or with limited tree cover due to its ability to thrive in locations with ample sunlight

exposure (Moreno *et al.,* 2016). *Acrostichum* preferred habitat is inshore marsh areas that receive some saline water from high tides and some fresh water from inflowing streams (Arnold *et al.,* 1963). *Acrostichum* genus consist of three distinct species, namely. *A. danaeifolium* Langsd. & Fisch., *A. aureum* L., and *A. speciosum* Willd (Adams *et al.,* 1979; Mehltreter *et al.,* 2003; *Zhang et al.,* 2016). Based on previous study, *A. danaeifolium* and *A. speciosum* are restricted to the Atlantic east-Pacific and Indo West-Pacific areas, while *A. aureum* is the only mangrove species found in both areas (Zhang *et al.,* 2016; Duke 1992.).

The Malaysian mangrove forest is home to two common species of mangrove fern namely *A. aureum* and *A. speciosum. A. aureum*, locally known as '*piai raya'*, is a large species of mangrove fern (Arnold *et al.,* 1963; Shin *et al.* 2015). Its large frond can reach 4 meters in length (Piggott, 1988). Young fronds are crimson red in color. The first 5-8 pairs of leaflets near the tips are sterile, with the underside covered in red-brown spores (Adams *et al.,* 1979; Shin *et al.,* 2015). The sterile leaflets have rounded tips, with a small sharp point on occasion (Andrews, 1990). *A. speciosum*, also known as *'piai lasu'*, is a 1.5-meter-tall fern. During young, the frond can grow to be 0.75 to 1.5 m tall and is brownish green. The first few pairs near the tip are fertile leaflets, with dark brown spores covering the underside. The sterile leaflets gradually narrow to a point (Shin *et al*., 2015).

The *Acrostichum* species is widely recognised as a prominent mangrove fern. Nevertheless, there is a lack of comprehensive measurements or detailed documentation regarding its anatomical characteristics, particularly those pertaining to its leaves. A comprehensive understanding of leaf anatomy is crucial for evaluating the adaptations of a species to a certain environment. Hence, the objective of this research is to evaluate the taxonomic importance of the leaf anatomy of two *Acrosthichum* species, with the aim of contributing valuable supplementary information for the identification, classification, and adaptation of mangrove ferns in response to abiotic stress.

Methodology

The research was conducted on two species of mangrove fern, *A. aureum* and *A. speciosum*. Fresh leaf samples were collected from Kuantan and Pekan mangrove forests in Pahang. The specimens of leaves were compressed and dried in an oven at 55^{o} C for two weeks. The voucher specimens were kept at the International Islamic University of Malaysia's Kuantan Herbarium. Fresh leaf samples have been preserved in AA solutions in a 3:1 ratio (70% alcohol: 30% acetic acid). Using a sliding microtome, sections of petiole, midribs, leaf lamina, and marginal were sectioned at range 30 – 35 µm and stained with Safranin and Alcian blue. After dehydration for 2 minutes with 50%, 70%, 95% and 100% alcohol, the slides were mounted in Euparal. A video camera attached to a Leica microscope was used to capture anatomical images.

Taxonomic Treatment

1. *Acrostichum aureum* L. (Figure 1)

Plant: Fern, 1 – 4 m tall. **Rhizome:** Stout, erect, base of stalk covered with large scales. **Stem:** They are thickened towards the base, dark brown with a broad, pale, thin margin, mixed with narrow, thin scales. **Frond:** Large (1-3 m long), pinnae many (not more than 30 leaflets), 20 – 23 cm long, 3.0 cm wide, alternate, entire margin, slightly wavy, narrowly oblong, apex rounded, base cuneate, adaxial dark green, abaxial pale green, young frond reddish in colour, fertile pinnae near the tip, stipe about 0.3 m. The leaflets may be spaced far apart and are often irregularly distributed. The lowest leaflets are always widely spaced, with long stalks that measure up to 3 cm. The tops of fertile leaves are

rusty-brown coloured, later turning dark brown. The undersides of these 10-20 topmost leaflets are uniformly covered with large sporangia. Tips of the longer, sterile leaflets are abruptly rounded or blunt, with a short tip. **Leaf venation** is net-like. The broad scales, up to 1 cm long, are restricted to the base of the fronds; they have a thickened middle, an entire edge and leave no prominent scar. **Spores** are large and have a tetrahedral shape.

(a)

(b)

Figure 1: *Acrostichum aureum. a) Habit b) Leaf apex*

2. Acrostichum speciosum Wild. (Figure 2)

Plant: Fern, 1 – 4 m tall. **Rhizome:** stout, erect, base of stipes covered with dark brown scale. Scales on the rhizome are up to about 8 mm long. **Leaf** frond large, 27 – 33 cm long, 3.0 – 4.0 cm wide, pinnae many, entire margin, pinnately compound, narrowly elliptic, apex acute, base cuneate, adaxial dark green, abaxial pale green, young frond brownish-green in colour, fertile pinnae near the tip, form dark brown sporangia, smaller than sterile pinnae, stipe about 0.3 m. These are uniformly covered with large sporangia. Leaflets measure about 28 by 10 cm, and the tips of the smaller, sterile leaflets are narrowly pointed. **Leaf venation** is net-like. The broad scales, up to 1 cm long, are restricted to the base of the leaves. Scales have a thickened middle and an entire margin. **Spores** are large and tetrahedral in shape.

(a)

(b)

Figure 2: *Acrostichum speciosum. a) Habit b) Leaf apex*

Results and Discussion

Findings in this study have shown some commons characteristic and variations of leaf anatomical characteristics that can be used in the identification of *Acrostichum* species.

Based on the observation, it was noted that the petiole of *A. aureum* (Figure 3a) exhibited a small concavity on the adaxial side and a convex shape on the abaxial side. In contrast, the petiole of *A. speciosum* (Figure 3b) displayed a flat surface on the adaxial side and a U-shaped contour on the abaxial side. Besides, there is also a presence of trichome at the epidermis in *A. aureum* (Figure 3a).

Both species of *Acrosthichum* exhibited variations in the size of their vascular bundles, which were dispersed within the ground tissue. Notably, the largest vascular bundle was found in the upper central region of the ground tissue. The vascular bundles next to the hypodermis have a reduced size compared to those located in the central region. According to the research conducted by Martinez *et al.* (2012) and Palacios-Rios *et al.* (2019), it has been proven that the configuration of vascular bundles in the petiole and the morphology of xylem can serve as distinguishing factors among species within the *Pteris* genus.

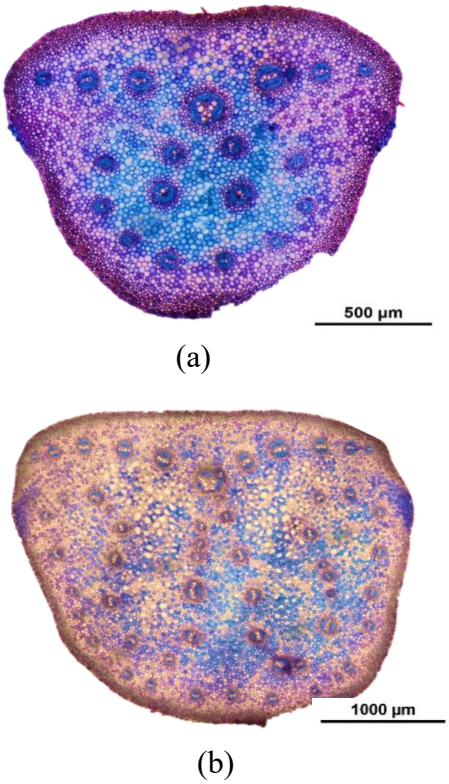


Figure 3: *a) Petiole of A. aureum. b) Petiole of A. speciosum.*

The findings of the present study also demonstrated a distinct variation in the midrib outline between the two species (Figure 4). The outlines of the midrib were described by considering the adaxial and abaxial outlines. The adaxial surface of *A. aureum* (Figure 4a) exhibits a flat line, but the adaxial surface of *A. speciosum* (Figure 4b) displays a U-shape. Conversely, the abaxial surface of both species is concave in shape.

Mantovani *et al.* (2009) suggest that the midrib outline can serve as a diagnostic characteristic for taxonomic purposes within the *Anthurium,* section *Urospadix* subsection Flavescentiviridia of the Araceae family. In addition, Adeniran *et al.* (2020) have also documented the importance of midrib outline in the taxonomic classification of taxa belonging to the genera *Isolona* and *Monodora* within the Annonaceae family. The vascular bundle in the midrib for both species are also scattered. The vascular bundle arrangement was corresponding

with the studied by Rashid *et al.* (2020). Similar to another fern (Bercu. 2007; Sârbu *et al*., 2017; Popovoci *et al.,* 2018), the vascular bundle within the midrib exhibited a hadrocentric pattern, same goes with petiole, characterised by the positioning of the xylem cells encircled by the phloem.

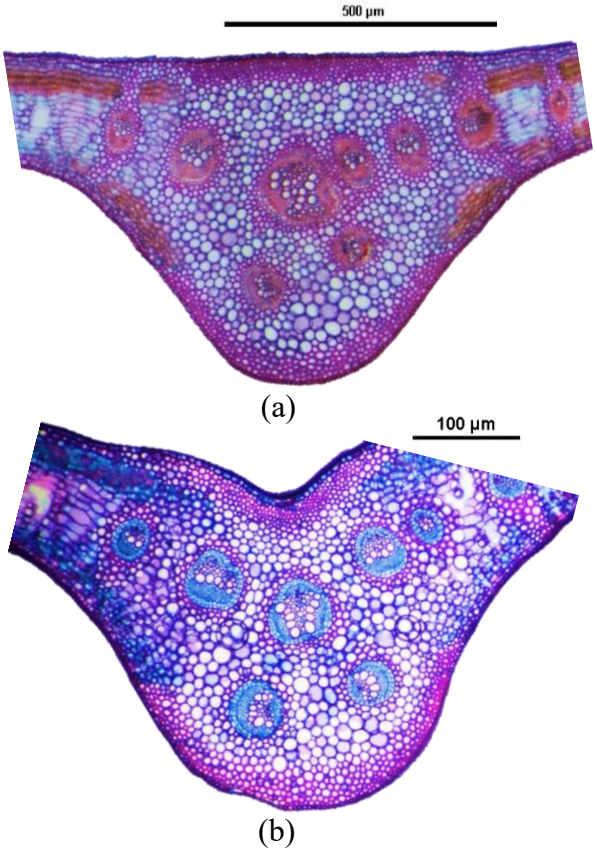


Figure 4: *a) Midrib of A. aureum. b) Midrib of A. speciosum.*

The transverse section of the leaf margin exhibits a downward outline in both *Acrostichum* species. However, *A. aureum* (Figure 5a) displays a rounded end, whereas *A. speciosum* (Figure 5b) exhibits a pointy end. According to Noraini *et al*. (2009), the margin of *Parashorea* species showed same shape, obtuse and rounded, for all studied species. Furthermore, the use of margin outline in classifying *Mangifera* species has resulted in a taxonomy comprising only two major groupings (Norfaizal et al., 2013; Tipmontiane et al., 2018). Result of this study showed more layers of collenchyma cells present near the tip of the margin of *A. speciosum* compared to *A. aureum* (Figure 5a, 5b).

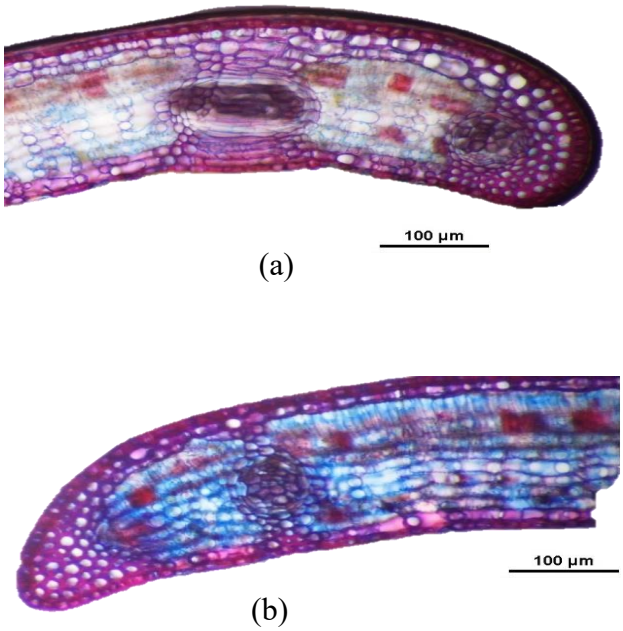
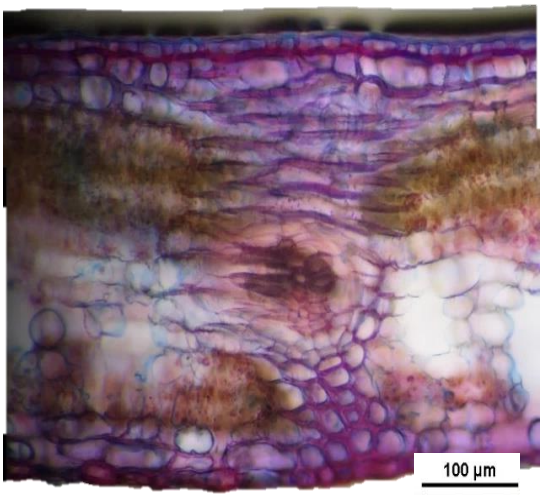
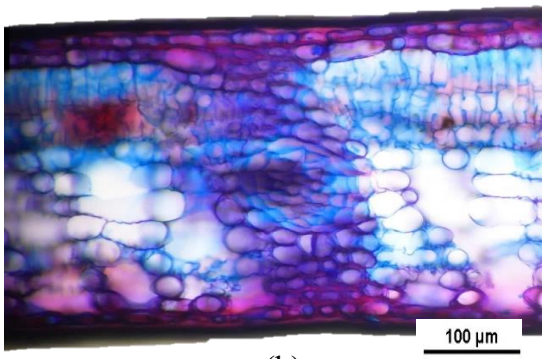


Figure 5: *a) Margin of A. aureum. b) Margin of A. speciosum.*

The lamina of *A. aureum* (Figure 6a) and *A. speciosum* (Figure 6b) show a uniseriate epidermis covered by the cuticle on adaxial and abaxial surface. According to Lucena *et al*. (2011), leaves with a uniseriate epidermis absorbed more light energy, which is required for photosynthesis. Cuticle thickness is believed to perform an important role in water conservation, protection against photochemical damage from UV radiation, and mechanical resistance to diverse forces such as winds and high tides (Lucena *et al.*, 2011; de Arruda *et al.*, 2021).



(a)



(b)

Figure 6: *a) Lamina of A. aureum. b) Lamina of A. speciosum.*

In addition, the results also indicate the presence of a double palisade layer beneath the epidermis and spongy parenchyma in the central region of the lamina for both *Acrostichum* species. Intercellular gaps inside the spongy parenchyma of *Acrostichum* have been seen in other mangrove angiosperms, such as *Bruguiera gymnorrhiza, Excoecaria agallocha, Heritiera fomes, Phoenix paludosa*, and *Xylocarpus granatum* (Nandy *et al.,* 2007).

Numerous vascular bundles arranged in parallel series were found in lamina. The leaves of both *Acrostichum* species were classed as heterobaric due to the presence of bundle sheath extensions (BSEs) formed by sclerenchyma cells surrounding the vascular bundle and extending towards both epidermises (Kenzo *et al.,* 2007). The leaf species exhibiting heterobaric leaf structure and exhibiting bundle sheath extensions were predominantly observed in habitats characterised by elevated levels of solar radiation and limited water availability, such as the upper canopy, emergent layers, and forest clearings (Kenzo et al., 2007). According to Inoue *et al.* (2015), the presence of heterobaric leaves can enhance water transport efficiency and simultaneously enhance photosynthetic performance in the presence of intense light.

Key to the species based on leaf anatomical structure

1a. Margin downwards, rounded end. Midrib flat line adaxial, concave adaxial. Petiole small concavity on adaxial, concave abaxial, presence of trichome on epidermis, anomocytic and paracytic stomata……..……. *A. aureum*

1b. Margin downwards, pointy end. Midrib U-shaped adaxial, concave abaxial. Petiole flat surface adaxial, U-shaped contour abaxial, absence of trichome, anomocytic stomata.…….…… *A. speciosum*

Conclusion

In summary, the anatomical characteristics of leaves provide significant value, particularly in the context of identifying and classifying *Acrosthicum* species. The findings of this study reveal certain variations in anatomical characteristics within both *Acrostichum* species, which possess systematic significance, particularly in their adaptation to the environment. These variations include the presence of a thick cuticle at the epidermis surface, the presence of a bundle sheath, and the presence of spongy parenchyma at lamina. In addition, it is noteworthy that both species exhibited distinct variations such as the outlines of the petiole, midrib and margin.

Acknowledgement

This research was funded by Al-Sultan Abdullah History & Civilisation Research Centre (AL-

ASAR) grant number (SPG22-058-0058). We acknowledge and express our gratitude to Jabatan Perhutanan Negeri Pahang (JPNP) and Majlis Biodiversity Negeri Pahang (MBNP) for their invaluable approval and support throughout this study.

References

Adams, D.C. and Tomlinson, P.B., 1979. Acrostichum in Florida. *American Fern Journal*, *69*(2), pp.42-46.

Adeniran, S.A., Kadiri, A.B. and Olowokudejo, J.D., 2020. Comparative midrib anatomy of Monodora Dunal. and Isolona Engl.(Annonaceae) from West-central Africa. *Bangladesh Journal of Plant Taxonomy*, *27*(2), p.283.

Arnold, C.A. and Daugherty, L.H., 1963. The fern genus Acrostichum in the Eocene Clarno formation of Oregon.

Andrews, S.B., 1990. *Ferns of Queensland-a handbook to the ferns and fern allies*. Department of Primary Industries. Pp 427

Bercu, R., 2007. On The Anatomy of The Endangered Plant Species Aspleniumlepidum (Aspleniaceae). *Studia botanica*, *38*, pp.125-131.

de Arruda, E.C.P., da Costa Lima, G. and de Paiva Farias, R., 2021. Leaf anatomical traits and their ecological significance for Acrostichum aureum (Pteridaceae), a remarkable fern species growing in mangroves. *Aquatic Botany*, *171*, p.103379.

Duke, N. C. 1992. Mangrove Floristics and Biogeography. In Robertson, A. I. & Alongi, D. M. (Eds.), *Tropical Mangrove Ecosytems*. American Geophysical Union. Pp. 63 – 100.

Inoue, Y., Kenzo, T., Tanaka-Oda, A., Yoneyama, A. and Ichie, T., 2015. Leaf water use in heterobaric and homobaric leafed canopy tree species in a Malaysian tropical rain forest. *Photosynthetica*, *53*, pp.177-186.

Kathiresan, K. and Bingham, B.L., 2001. Biology of mangroves and mangrove ecosystems. Advance in Marine Biology. Pp 81-25

Kenzo, T., Ichie, T., Watanabe, Y. and Hiromi, T., 2007. Ecological distribution of homobaric and heterobaric leaves in tree species of Malaysian lowland tropical rainforest. *American Journal of Botany*, *94*(5), pp.764-775.

Lucena, I., Maciel, V.E.D.O., Silva, J.B., Galvíncio, J.D. and Pimentel, R.M.D.M., 2011. Leaf structure of mangrove species to understand the spectral responses. *Journal of Hyperspectral Remote Sensing*, *2*, pp.19-31.

Mantovani, A., Pereira, T.E. and Coelho, M.A.N., 2009. Leaf midrib outline as a diagnostic character for taxonomy in Anthurium section Urospadix subsection Flavescentiviridia (Araceae). *Hoehnea*, *36*, pp.269-277.

Martínez, O.G. and Vilte, I., 2012. The structure of petioles in Pteris (Pteridaceae). *American Fern Journal*, *102*(1), pp.1-10.

Medina, E., Cuevas, E., Popp, M. and Lugo, A.E., 1990. Soil salinity, sun exposure, and growth of Acrostichum aureum, the mangrove fern. *Botanical Gazette*, *151*(1), pp.41-49.

Mehltreter, K. and Palacios-Rios, M., 2003. Phenological studies of Acrostichum danaeifolium (Pteridaceae, Pteridophyta) at a mangrove site on the Gulf of Mexico. *Journal of tropical Ecology*, *19*(2), pp.155-162.

Moreno-Domínguez, R., Cascales-Miñana, B., Ferrer, J. and Diez, J.B., 2016. Acrostichum, a pioneering fern of floodplain areas from the Late Oligocene Sariñena Formation of the Iberian Peninsula. *PLoS One*, *11*(9), p.e0162334.

Nandy, P., Das, S., Ghose, M. and Spooner-Hart, R., 2007. Effects of salinity on photosynthesis, leaf anatomy, ion accumulation and photosynthetic nitrogen use efficiency in five Indian mangroves. *Wetlands Ecology and Management*, *15*, pp.347-357.

Naskar, S. and Palit, P.K., 2015. Anatomical and physiological adaptations of mangroves. *Wetlands ecology and management*, *23*(3), pp.357-370.

Noraini, T. and Cutler, D.F., 2009. Leaf anatomical and micromorphological characters of some Malaysian Parashorea (Dipterocarpaceae). *Journal of Tropical Forest Science*, pp.156-167.

Norfaizal, G.M. and Latiff, A., 2013, November. Leaf anatomical characteristics of Bouea, Mangifera and Spondias (Anacardiaceae) in Malaysia. In *AIP Conference Proceedings* (Vol. 1571, No. 1, pp. 394-403). American Institute of Physics.

Page, C.N., 2002. Ecological strategies in fern evolution: a neopteridological overview. *Review of palaeobotany and palynology*, *119*(1-2), pp.1-33.

Palacios-Rios, M., Galan, J.M.G.Y., Prada, C. and Rico-Gray, V., 2019. Structure of the petioles and costae of Mexican and Central American species of Pteris (Polypodiopsida,Pteridaceae). *Phytotaxa*, *401* (2), pp.101-106.

Piggott A. G. 1988. Fern on Malaysia in colour. Tropical Press Sdn. Bhd. Pp 458

Popovici, P.C., Ancuceanu, V.R., Olaru, T.O., Stoicescu, C.S. and Dinu, M., 2018. Toxicity Assessment of (L.) Schott, Fam. Nephrolepidaceae. *Acta Biologica Marisiensis*, *1*(1), pp.27-36.

Rashid, P., Shethi, K.J. and Ahmed, A., 2020. Leaf anatomical adaptation of eighteen mangrove plant species from the Sundarbans in Bangladesh. *Bangladesh Journal of Botany*, *49*(4), pp.903-911.

Sandilyan, S. and Kathiresan, K., 2012. Mangrove conservation: a global perspective. *Biodiversity and Conservation*, *21*, pp.3523-3542.

Sârbu, A., Smarandache, D., Marinescu, A.T., Paraschiv, A.M., Mihai, C. and Velicu, A.M., 2017. Anatomical-histological observations conducted on aquatic ferns In the Danube Delta. *Journal of Plant Development*, *24*.

Schneider, H., Schuettpelz, E., Pryer, K.M., Cranfill, R., Magallón, S. and Lupia, R., 2004. Ferns diversified in the shadow of angiosperms. *Nature*, *428*(6982), pp.553-557.

Shin, L.S., Aziah, M. and Tong, J., 2015. Mangrove guidebook for Malaysia. *Wetlands International, Kuala Lumpur*. Pp 7-10

Tipmontiane, K., srinual, A. and kesonbua, W., 2018. Systematic significance of leaf anatomical characteristics in some species of Mangifera L.(Anacardiaceae) in Thailand. *Tropical Natural History*, *18*(2), pp.68-83.

Tomlinson, P.B., 1986. the Botany of Mangroves Cambridge University Press London.

Zhang, Z., He, Z., Xu, S., Li, X., Guo, W., Yang, Y., Zhong, C., Zhou, R. and Shi, S., 2016. Transcriptome analyses provide insights into the phylogeny and adaptive evolution of the mangrove fern genus Acrostichum. *Scientific Reports*, *6*(1), p.35634.

Article History
Received: 17 October 2025
***Accepted*:** 28 January 2026