

RESEARCH ARTICLE

Comparative Study on Foliar and Petiole Anatomy of the Genus *Bruguiera* L. in Mangrove Forest of Kerala

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Abstract

Leaf and petiole anatomical profiling of three species of *Bruguiera* L. occurring in the mangrove forest of Kerala was investigated in this study. The study aims to search for the stable features marking out *Bruguiera* species. Anatomical characters in all three taxa showed similarities because of its generic closeness. Three species were distinguished based on the difference in the number of vascular bundles in midrib and petiole, number of palisade layer and type of stomata. Nevertheless the three *Bruguiera* species can be grouped anatomically based on the type and pattern of sclereids. Brachy sclereids present in all three taxa but the astero sclereids were found only in *B. gymnorrhiza*. Through this anatomical study, we concluded that all the species have some unique features to withstand extreme environmental habitat.

Keywords: Anatomical characters, anticlinal walls, astero sclereids, *Bruguiera*, environmental habitat.

Introduction

Bruguiera is the largest genus in the Rhizophoraceae (Hou, 1958; Tomlinson, 1986; Hogarth, 1999; Saenger, 2002; Sheue, 2003) and all six described *Bruguiera* species belong to the "Indo-Malayan" group of mangroves, which extend from East Africa to Australia and the West Pacific. Based on flower size and the pollinating agent, various authors (Tomlinson, 1986; Noske, 1993) generally divided *Bruguiera* into two groups. *Bruguiera* species with large, recurved flowers (*B. gymnorrhiza*, *B. sexangula*, *B. exaristata* and *B. hainesii*) are considered to be bird-pollinated, while the remaining two species (*B. cylindrica* and *B. parviflora*) with comparatively smaller and erect flowers are probably insect-pollinated. As discussed by various botanists (Watson, 1928; Symington, 1940; Wyatt-Smith, 1953), it was difficult to identify the different *Bruguiera* species solely on the basis of vegetative structures: for e.g., between *B. gymnorrhiza* and *B. sexangula*. The leaves of *B. sexangula* are usually more yellow-green and with shorter petioles than those of *B. gymnorrhiza*. But such generalizations are invalid when there are other dominant prevailing ecological factors (e.g. sun versus shade leaves, nutrient limitations etc.) present in the habitats affecting foliar development and morphology. In Kerala, out of four species belonging to the genus *Bruguiera*, *B. cylindrica* has relatively wide distribution; however, it is not recorded from Kottayam district. *Bruguiera gymnorrhiza* is a rare species which is not represented in Malapuram, Kozhikode, Kannur and Kasargode. *Bruguiera sexangula* is one of the endangered species, which is represented only in two districts namely Kollam and Ernakulam.

Bruguiera parviflora has wide distribution in the northern parts of Kerala which is not present in Trivandrum, Kollam, Alappuzha and Kottayam (Mohandas, 2012). Considering the above facts in view, leaf and petiole anatomical profiling of three species of *Bruguiera* L. occurring in the mangrove forest of Kerala was investigated in this study.

Materials and methods

Collection and identification of plant: Leaf samples were collected from the intertidal zones of Kumbalam (9° 5' N: 76° 12' E) of Ernakulam district in Kerala and identified by Botanical survey of India (BSI), Coimbatore. One of the healthy plants was selected and the mature leaves from fifth and sixth node were taken for anatomical studies. Sections were made at a position approximately half way between the base and apex of a sector from one side of the lamina, stained with Toluidine blue O and mounted in 50% glycerin. The slides analyzed by trilocular compound microscope (10093409) and imaged by using the camera Olympus E-PL3. Measurements were taken using Magnus Pro software. The Scanning Electron Microscopic images of leaf sample were taken using Zeiss ultra 55. For the SEM studies, abaxial side of leaf was sputter-coated with gold and micrographs were taken using Zeiss ultra 55.

Result

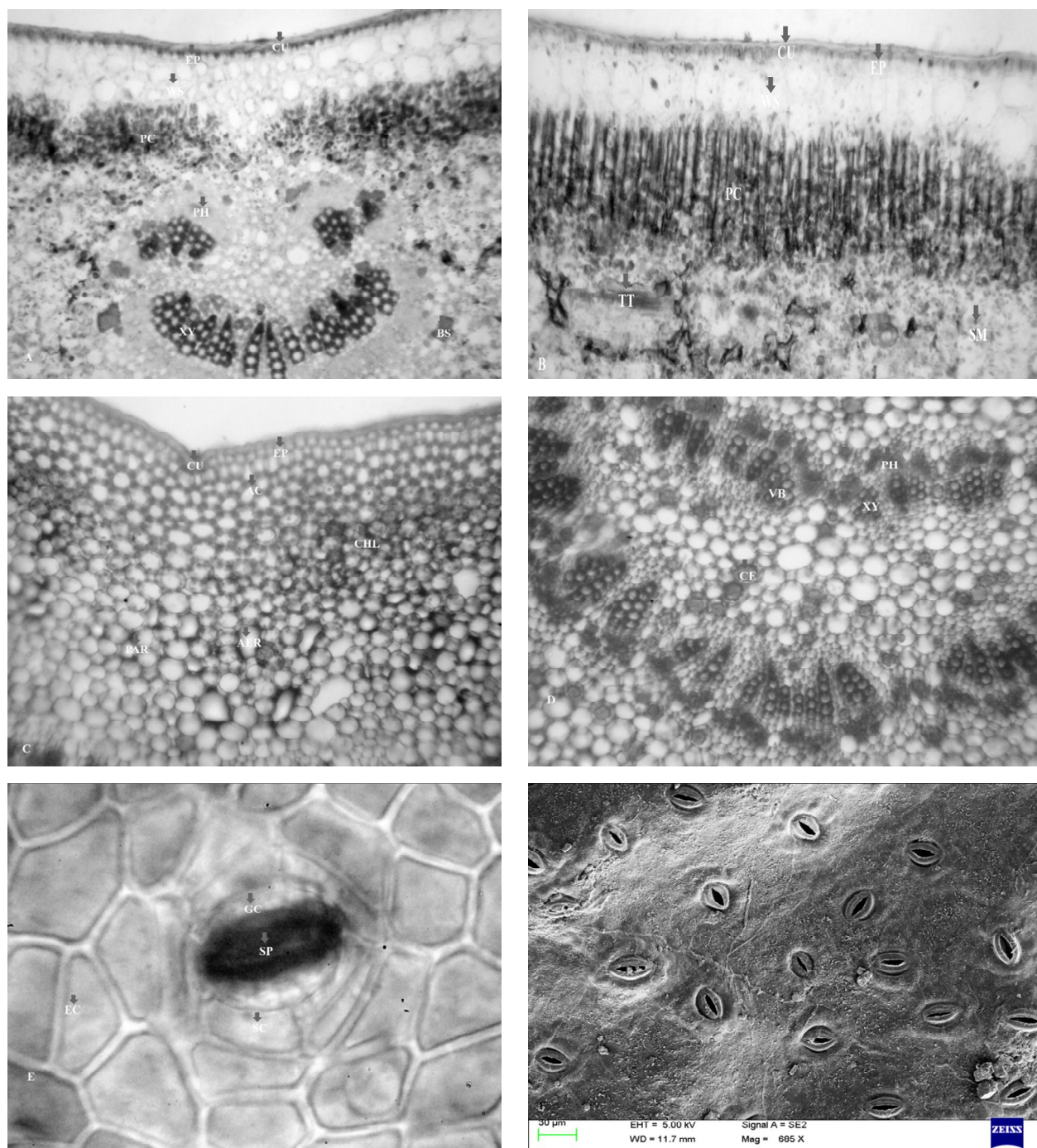
***Bruguiera cylindrica*:** Leaf epidermis single layered with thick waxy cuticle, epidermal cells are not cutinized wholly, stomata confined to the lower epidermis. Hypodermis one layer thick both inner to upper and lower epidermis.

Table 1. Laminar characters of some mangroves.

Species	UCT (μm)	LCT (μm)	UET (μm)	LET (μm)	UHT (μm)	LHT (μm)	PL (μm)	PW (μm)	LT (μm)	SL (μm)	SW (μm)
<i>Bruguiera cylindrica</i>	0.99	0.21	2.31	1.78	14.84	10.33	9.58	2.00	83.04	13.70	2.65
<i>Bruguiera gymnorhiza</i>	1.11	0.31	2.84	1.71	12.54	10.97	12.10	2.00	84.99	13.32	1.36
<i>Bruguiera sexangula</i>	0.94	0.21	1.58	1.89	11.89	12.96	11.18	1.89	81.69	11.73	2.07

UCT-Upper cuticle thickness, LCT-Lower cuticle thickness, UET-Upper epidermal thickness, LET-Lower epidermal thickness, UHT-Upper hypodermal thickness, LHT-Lower hypodermal thickness, LT-Lamina thickness, PL-Palisade length, PW-Palisade width, SL-Stomatal length, SW- Stomatal width.

Fig. 1. *Bruguiera cylindrica* A&B-Midrib and lamina portion of leaf (10X), C&D- Anatomy of petiole(10X), E-Abaxial surface of leaf with stomata (100X), F-SEM of abaxial side of leaf (3000X). CU-Cuticle, CH-Chlorenchyma, EP-Epidermis, GC-Guard cells, PC-Palisade cell, CF-Crystalliferous cell, PAR-Parenchyma, PH-Phloem, BS-Brachy sclereids, SC-Subsidiary cells, SP-Stomatal pore, SP-Spongy mesophyll, VB-Vascular bundle, TT-Terminal tracheids, WS-Water storage tissue, XY-Xylem.



Water storing tissue is uniseriate. The palisade tissue below the upper epidermis is one to two layer in thickness. Spongy tissue was multilayered. Mesophyll contains crystalliferous cells. Short tracheids present at vein endings. Stomata were deeply sunken and confined to the lower epidermis. Large air cavities present in the midrib and between the mesophyll. Vascular bundle collateral. Stomata were accompanied on either side by one or more subsidiary cells, they lie parallel to the long axis of pore and guard cells; rubiaceous type. But ranunculaceous type stomata also found. In this type, stomata surrounded by limited of epidermal cells, which do not show any difference from other epidermal cells. Microanatomy of lower epidermis showed ridges and furrow like appearance with unbranched hairs. Stomata were deeply sunken. Epidermal cells polygonal in shape with crystalliferous cells present. Petiole arc shaped in outline. The adaxial outline is concave; the abaxial surface convex. Epidermis uniseriate and polygonal in shape with thick cuticle. Hypoderm 7-8 layered with angular collenchyma cells and inner parenchyma cells. Inner portion consists of numerous air cavities. Crystalliferous cells found in cortex. Vascular bundles collateral arranged in ring and 30-32 in number (Fig. 1 and Table 1).

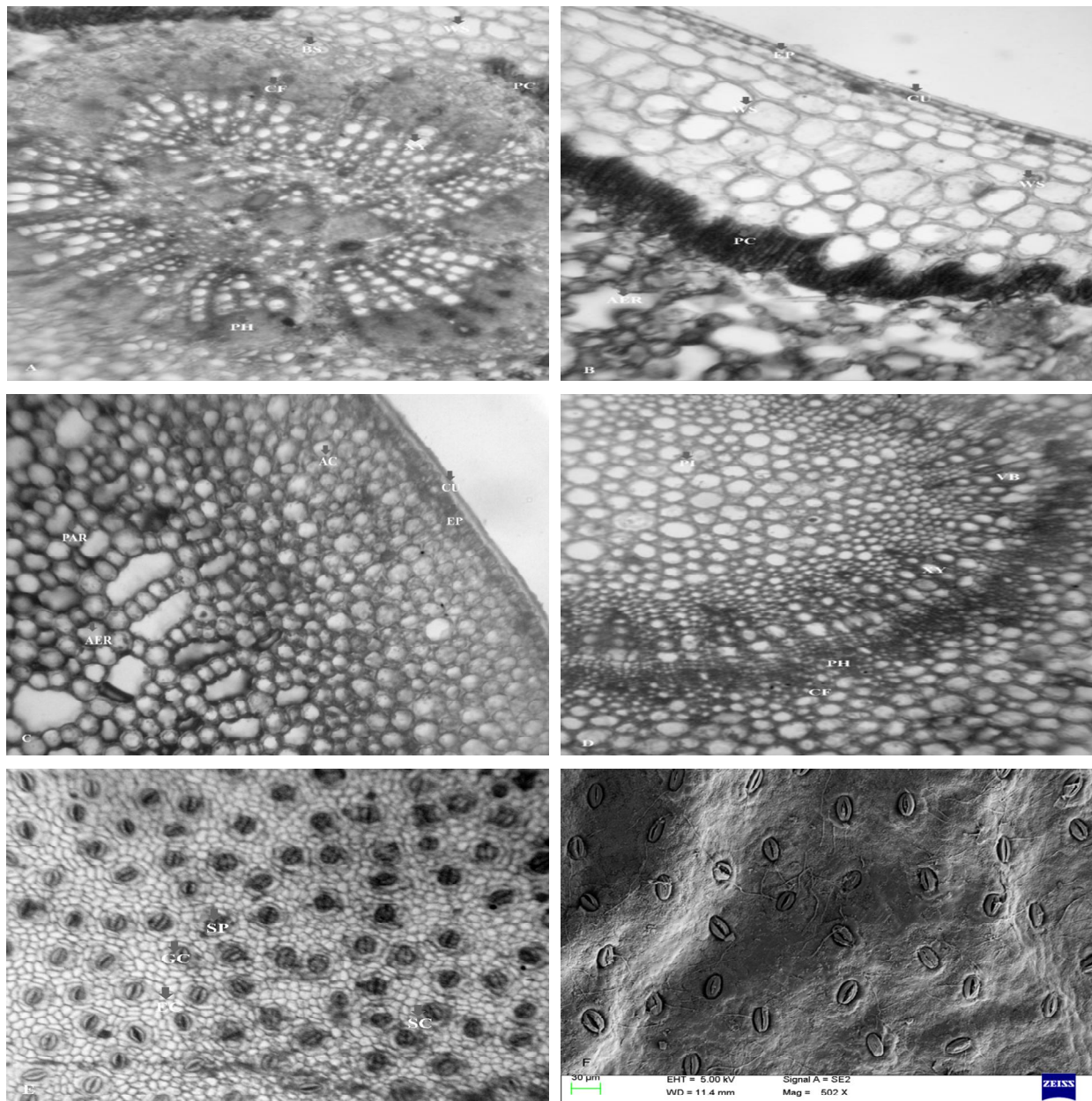
Bruguiera gymnorhiza: Leaf dorsiventral and petiolate, with thick smooth cuticle. Epidermis single layered in both surfaces. The epidermis is always cutinized wholly. The outer wall of the epidermal cells was thicker than the rest usually straight and mostly rectangular. The adaxial epidermal cells were often larger than those of the abaxial cells. The hypodermis of adaxial side composed of six to seven layers of colourless cells with polygonal shape. The colourless cells function as water storage tissue. The mesophyll cells composed of thin walled chlorenchyma. These cells can be differentiated single layer of adaxial, anticlinally extended palisade and spongy parenchyma cells, were loosely arranged and contains large number of air spaces. Astero sclereids were scatterly arranged in the abaxial cells of epidermis. Patches of brachy sclereids found in the hypodermal region and vascular cylinder. Crystalliferous cells were found in hypodermis and mesophyll region, stomata hypostomatic, and surrounded by limited of epidermal cells, which do not show any difference from other epidermal cells; ranunculaceous type. Micro anatomy of the lower epidermis showed ridges and furrow like appearance with unbranched hairs. Stomata were deeply sunken. Epidermal cells polygonal in shape. Petiole showed thick cuticle. The adaxial outline was more or less flattened; the abaxial surface convex. Epidermis and hypodermis uniseriate. Epidermal cells polygonal. Cortex wide and made of outer collenchyma, 8-9 layers and inner parenchyma cells. Inner portion consists of numerous air cavities. Vascular bundles were collateral arranged in ring 20-21 in number (Fig. 2 and Table 1).

Bruguiera sexangula: Leaves as dorsiventral in nature, hypostomatic and marked dissimilarity between the upper and lower halves. This species have thick cuticle over the epidermis and smooth in this taxa with one layer of colourless tissue represented adaxial hypodermis. The adaxial epidermal cells are larger in size compare to those of abaxial cells. Epidermal cells polygonal shape and cutinized wholly. Crystalliferous cells were found in hypodermis and mesophyll region. Mesophyll is composed of thin walled chlorenchyma. The cells can be differentiated in to one layer of adaxial, anticlinally extended palisade cells. The spongy parenchyma cells were loosely arranged and contain large number of air spaces. Sclereids were present in vascular region. Stomata were hypostomatic, and surrounded by limited of epidermal cells, which do not show any difference from other epidermal cells; ranunculaceous type. Microanatomy of lower epidermis showed outer surface showing ridges and furrow like appearance with unbranched hairs. Stomata were deeply sunken. Epidermal cells polygonal in shape. Petiole arc shaped in outline. The cuticle thick. The adaxial outline concave; the abaxial surface convex. Epidermis and hypoderm uniseriate. Epidermal cells polygonal. Cortex wide and made of outer collenchyma, 8-9 layers and inner parenchyma cells. Inner portion consists of numerous air cavities. Vascular bundles were collateral arranged in ring 28-30 in number (Fig. 3 and Table 1).

Discussion

Rhizophoraceae well known as the richest mangrove family, having four exclusively mangrove taxa (*Bruguiera*, *Ceriops*, *Kandelia* and *Rhizophora*) (Tomlinson, 1986). Present study discussed species comprising the genera *Bruguiera* and three species *B. cylindrica*, *B. gymnorhiza* and *B. sexangula*. All species leaves dorsi-ventral in nature, petiolate and hypostomatic. Three taxa consist of leaves succulent and high lamina thickness. Cuticle very thick in all species and epidermal cells polygonal in shape. The three species of *Bruguiera* showed polygonal epidermal cells, anticlinal walls were straight and thick on the adaxial surface of *B. gymnorhiza* and *B. sexangula* but straight and slightly curved in *B. cylindrica*. Water storage tissues bilayered in *B. sexangula* and *B. cylindrica*. In *B. gymnorhiza*, it is six to seven layered. Poompozhi and Kumarasamy (2014) reported that it is unilayered in *B. cylindrica*. All three species were hypostomatic. Ranunculaceous stomata were found in *B. gymnorhiza*, (Samadder and Jayakumar, 2015), Das and Ghose (1993) also reported similar findings. In *B. cylindrica*, rubiaceous type were reported by Poompozhi and Kumarasamy (2014), Samadder and Jayakumar (2015) observed cruciferous and rubiaceous type. Present study observed rubiaceous and ranunculaceous type stomata. Investigation has noticed that large distribution of oxalate calcium crystals found in all species mesophyll which might be helping for mechanical support.

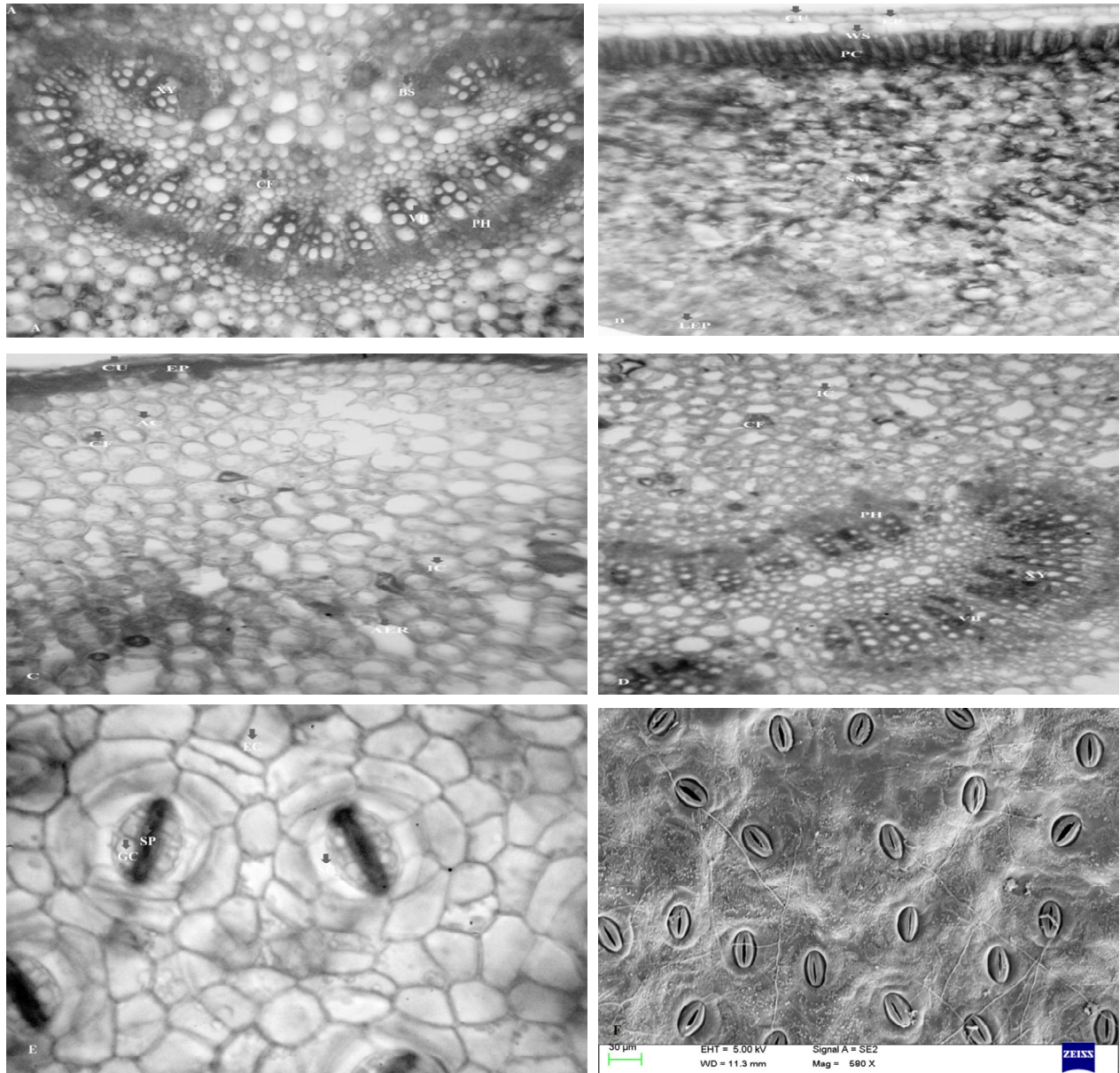
Fig. 2. *Bruguiera gymnorrhiza*; A&B-Midrib and lamina portion of leaf (10X), C&D-Anatomy of petiole (10X), E-Abaxial surface of leaf with stomata (10X), F-SEM of abaxial surface of leaf (3000 X). CU-Cuticle, AC-Angular collenchyma, EP-Epidermis, AER-Aerenchyma, GC-Guard cells, PC-Palisade cell, CF-Crystalliferous cell, PAR-Parenchyma, PH-Phloem, BS-Brachy sclereids, SC-Subsidiary cells, SP-Stomatal pore, SP-Spongy mesophyll, VB-Vascular bundle, TT-Terminal tracheids, WS-Water storage tissue, XY-Xylem.



Palisade tissues unilayered thickness in *B. gymnorrhiza* and bilayered in *B. sexangula* and *B. cylindrica*. Zimmermann (1983) reported that both sclereids and tracheids are involved in capillary water storage. Tomlinson (1986) suggested that in addition to water storage, sclereids might also provide mechanical support to leaves with diminished turgor or discourage herbivores. The coriaceous nature of leaves is due to the presence of sclereids. The spongy tissues possess large intercellular spaces in all the taxa. In the midrib, all species were studied exhibited conjoint, collateral and closed bundles surrounded by bundle sheath.

Brachy sclereids found in all three taxa but astero sclereids were present only in *B. gymnorrhiza*. Microanatomy showed that all the species have stomata deeply sunken and unbranched hairs present. Crystalliferous cells (Calcium oxalate crystals) were found in abaxial side. The number of vascular bundles in the midrib of *B. sexangula* is 28-30, in *B. cylindrica* 30-32, *B. gymnorrhiza* 20-21. Nurnida and Noraini (2014) reported petiole anatomy of *B. sexangula* adapted to a dynamically ever changing marshy habitat with regular tidal influence in coastal zone.

Fig. 3. *Bruguiera sexangula*: A&B-Midrib and lamina portion of leaf (10X), C&D-Anatomy of petiole (10X), E-Abaxial surface of leaf with stomata (100X), F-SEM of abaxial surface of leaf (3000X). CU-Cuticle, AC-Angular collenchyma, EP-Epidermis, AER-Aerenchyma, GC-Guard cells, PC-Palisade cell, CF-Crystalliferous cell, IC-Inner cortex, PAR-Parenchyma, PH-Phloem, BS- Brachy sclereids, SC-Subsidiary cells, SP-Stomatal pore, SP-Spongy mesophyll, VB-Vascular bundle, TT-Terminal tracheids, WS-Water storage tissue, XY-Xylem.



Conclusion

All three taxa showing similarities because of its generic closeness. Regardless, anatomical characters help for classification and identification. Three species can also be distinguished based on the number of vascular bundles in the mid rib and petiole, number of palisade layer, type of stomata and type of sclereids. Brachy sclereids present all three taxa but astero sclereids found only *B. gymnorrhiza*. Through this anatomical study we concluded that all the species have some unique features to withstand extreme environmental habitat.

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