Delineation of Halophytic Mangroves and Associated Species Using Morphological and Anatomical Characteristics

Enrico L. Replan* and Pastor L. Malabrigo, Jr.

***Abstract*—True mangroves and mangrove associates were studied based on morphological and anatomical characteristics to taxonomically delineate species according to their correct ecological group including controversial species. Specimens of mangrove species were collected at three (3) different sites of known mangrove formation namely, Batangas, Aurora and Palawan. Specifically, the study aimed to (1) determine morphological and anatomical differences between true mangroves and mangrove associates, (2) provide clear basis on the difference in adaptations in terms of presence in certain areas or restrictions in habitat. Mangrove forest were surveyed using a 20m x 100m plot covering the seaward, middle zone and the landward zone. The study recorded 20 morpho-species, 18 genera belonging to 14 families of which 10 species are established true mangroves, 6 are questionable species (e.g. controversial species) and 4 are established mangrove associates. A key matrix comprising the set parameters was developed to examine all species according to their morphological features (e.g. leaf traits, reproductive mechanism, etc.) and anatomical features (e.g. specialized cells, laminar characteristics, etc.). Additionally, field observations whether the species is restricted to estuarine or non-estuarine zone is noted and accounted. Results shows that among the questionable species, *A. ebracteatus*, *P. acidula*, *X. granatum* and *E. aggalocha*, including *D. spathacea* fell into the range of a true mangrove based on the set parameters. True mangroves are true halophytes and mangrove associates are glycophytes with certain salt tolerance. Salinity factor also limits the distribution of species per zones. All species are tabulated together with their suggested classification.**

***Keywords*— halophytes, true mangroves, mangroves associates, glycophytes.**

I. Introduction

Mangroves are trees and shrubs that grow in saline coastal habitats in the tropics and subtropics. It is one of the most productive ecosystems and a natural renewable resource [1]. Ecologically, they are well known to withstand different catastrophes and their uniqueness as a mangrove ecosystem, thus, its biota is constantly under physiological stress caused by extreme environmental conditions [2]. Hence, mangroves are distinguished by their unique morphology and anatomy.

On the other hand, mangroves fall into two groups according to their habitats in nature: true mangroves (TM) and mangrove associates (MA) [3]. Further, these plant communities depend on terrestrial and tidal waters for nutrients and minerals from upland erosion as substrate for support [4]. As of today, many ecologist and researches are on debate as to the exact composition of a mangroves forest particularly in terms of taxonomically and ecologically correct grouping of species. There has been a continuous debate on the species aspect of mangrove ecosystems that eventually produced the so-called "controversial mangrove species." These controversial species were regarded by some authors as either true mangroves or mangrove associates based on their observations.

In the Philippines, studies on the classification of mangroves in terms of the aforementioned classification are lacking and many data are based widely on observations in the field and findings of other mangrove authors abroad. The study focuses on the taxonomic delineation of mangrove species as being true mangroves and mangrove associates to help researchers on the basis of identification and classification. Further, combination of set parameters such as morphological as well as anatomical served as basis for the study of mangrove species including the controversial ones. Additionally, the study was conducted to gain proper knowledge and understanding of the characteristics of mangrove species and their adaptive mechanisms. It may further help in preparing or revising species lists of different mangroves, which in turn can support development of policy towards mangrove conservation in Philippines.

II. Methodology

A. Collection and Preparation of Samples

Site surveys, data collection and gathering were all done to complete the required samples for the study. Leaf collection, photographs of the site were all done to satisfy morphological and anatomical requirements. Additionally, from the site

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survey, observations of the presence of the species to estuarine and non-estuarine areas are noted

B. Microscopy and Analysis

The histological slides were process from a rotary microtome and some are hand-made from cross sections in the middle portion of the leaf blade with the aid of a specialized steel blade. The samples were fixed in FAA (70% ethanol, 10% formalin, 5% acetic acid (90: 5: 5). Leaf specimens were further processed using high concentrations of specific alcohols and chemicals. The sections was mounted on slides after being dehydrated through a series of alcohol 50%, 70%, 95 and 100%. After dihydrations, specimens were fixed with sodium hypochlorite (10%). The epidermal tissue analysis used fragments of 1 cm^2 from leaves immersed in sodium hypochlorite 20% until dissociation. The period of immersion in this solution was variable for some species with exchange at intervals over 24 hours [5]. Immersion was variable for some mangrove species because of their relative leaf hardness.

The dihydrated cross sections and epidermal fragments were stained with Safranin 1% and Fast green 1% (1:1), and mounted in aqueous glycerin 50% (Sass, 1951). Digital images were obtained under a Nikon Olympus 3X Optical microscope equipped with advanced micron scaling. The anatomical structures were measured using image analysis program Image Tool [6] under different magnification levels. The anatomical features classification followed [7]. Images were enhanced using MS Microsoft Outlook 2010.

III. Results And Discussion

A 2000-m^2 plot were laid out to mangrove sites namely: (1) Batangas (Nasugbu), (2) Palawan (Calauit) and (3) Aurora (Baler). In Site 1 (Batangas), fragmented mangrove forest was encountered and small patches of mangrove species. A total of 12 species, 10 genera belonging to 9 families was present in the plot. From the first plot (B1Z1), seaward zone is dominated by *Sonneratia alba*, followed by two species of the genus Avicennia namely, *Avicennia marina* and *Avicennia marina* var. *rumphiana*. In the transition zone going to the middle zone (B1Z2), *Rhizophora apiculata* and *Rhizophora mucronata* along with *Aegiceras corniculatum* was observed. Occurrence of *Acanthus ebracteatus* and *Xylocarpus granatum* was also observed. On the landward zone (B1Z3), *Excoecaria aggalocha*, *Acrostichum aureum*, *Glochidion littorale* and *Heritiera littoralis* are present. Slightly flooded areas going to greater inland portion characterized by muddy to sandy substrate was dominated by *Sesuvium portulacastrum*.

In Site 2 (Palawan), a total of 10 species, 9 genera belonging to 8 families present in the plot. The seaward zone (P1Z1) is dominated by *A. marina* that form a dense line of vegetation along the stretch of the marine ecotone followed by *R. stylosa*. Further, some individuals of *Lumnitzera littoreus* was observed as fringe species. On the middle zone (P1Z2), *Bruguiera parviflora*, *Sonneratia alba* and *Xylocarpus granatum* were present. In the transition of middle to landward zone (P1Z3), species of *E. Aggalocha* and *A. ebracteatus* dominates the backdrop where freshwater coming from a Caiban river merge with the seawater.

Similar with the first two sites, Site 3 (Aurora) forms a set of mangroves species per zone. *A. marina* and *S. alba* that dominates the seaward zone (A1Z1) and on the same stretch, a dense line of *R. apiculata*, followed by *R. mucronata* species that also dominate the fringe zone. Middle zone (A1Z2) was dominated by some individuals of *Bruguiera gymnorrhiza* along with *A. corniculatum*. On the other side of the zone, a dense stand of *Kandelia candel* (including juvenile stage seedlings) forms an aggregate vegetation along with *A. ebractreatus*. In the landward zone (A1Z3), species of *E. aggalocha* followed by *H. littoralis* and *A. aureum* dominates the backdrop where freshwater coming from the Ditinagian River merge with the seawater. Patches of *Nypa fruticans* was observed forming a dense stand of rhizome roots.

A. Morphological Characteristics

Morphological characters was examined for all the species studied. Parameters like root system (e.g. aerial roots, stilt roots, pneumatophores, etc.), reproductive mechanism (e.g. cryptovivipary and vivipary) and leaf traits (e.g. leaf characters, succulence, etc.) were all examined. Table 1 presents the different species exhibiting the types of reproductive mechanism.

TABLE I: Mangroves and their types of germination.

SPECIES	TYPE OF GERMINATION	
	Cryptovivipary	Vivipary
B. gymnorrhiza	-	✓
R. stylosa	-	✓
R. apiculata		✓
R. mucronata	-	✓
A.marina	✓	-
X. granatum	-	-
S. alba	-	✓
A.ebracteatus	-	-
E.aggalocha	Seeds*	-
A.corniculatum	✓	-
N. fruticans	✓	-
A.aureum	Spores**	-
S. portulacastrum	Seeds*	-
L.littoreus	-	-
D.spathacea	Seeds*	-
H. littoralis	Seeds*	-
K. candel		✓
G. littorale	Seeds*	-
P. acidula	Diaspores*	

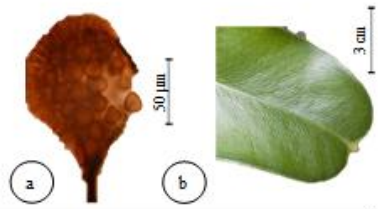
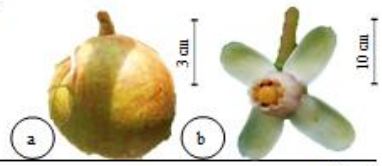
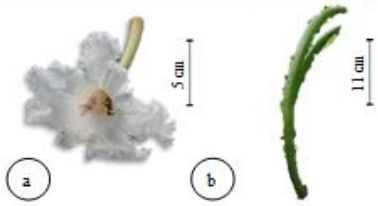
Legend: (*) *not found/observed during the survey*.

A. Fruits, Seeds and Flowers

In the case of defining species for its adaptive morphology, true mangroves has relatively more adaptive reproductive features than associates [8]. Morphological characteristics such as the shape and structure of flowers, fruits, leaves and stems, and sometimes on ecological affinities. Spores are observed to species of fern, *A. aureum*, while majority of the species that

does not exhibit vivipary or cryptovivipary has seeds as means of reproductive dispersal unit. The generic term 'propagule' is generally used because the main dispersal unit is not always a seed [9]. In fact, mangroves have a wide variety of dispersal units, and only a few species are dispersed as seeds; these include *Excoecaria*, *Dolichandrone*, and *Xylocarpus*. Table 2 shows some of the observed mangrove species that exhibits reproductive types and their morphometric characterizations.

TABLE II: MORPHOMETRIC CHARACTERIZATION OF REPRODUCTIVE PARTS OF SOME OF THE MANGROVE SPECIES.

SPECIES	DESCRIPTION	REPRODUCTIVE PARTS (ILLUSTRATION)
Acrostichum aureum	▪ **Flowers/Fruits**. No flowers and seeds, reproduced by means of spores(Pteridophyta); (a) Spores; (b) Spores on the leaf (abaxial surface)	
Xylocarpus granatum	▪ **Flower**. Inflorescences 3-6 cm long; flowers bisexual. ▪ **Fruit/Seeds**. Fruits large, globose, 10-15 cm diameter with many, closelypacked, large tetrahedral seeds.	
Dolichandrone spathacea	▪ **Flower**. Raceme 3-7-flowered, 3-5 cm long; calyx pathaceous; corolla funnel-shaped, 12-17 cm long, color white ▪ **Fruit/Seeds**. Capsule linearcompressed, with numerous thick, corky, lateral-winged seeds.	

B. Root system

As what as [10] had emphasized, TM possess root system such as stilt roots, cable roots, pneumatophores and knee roots to combat saline areas, while MA possess minor root system such as wiry roots, some aerial roots, and a major tap root system as an effect of freshwater influx. As observed in the three sites, majority of species have two major root system common for fringe species namely, stilt roots and pnuematophores. The observed roots system is in connection with the salt tolerances of TM and MA. The results complied with the conclusion of [11] that there are three (3) general strategies that a mangrove tree can use to manage salt – avoid by excluding it from the water taken in by the roots, eliminate or dispose of it once it gets inside the plant, or store it somewhere where it can do no harm. Apart from stilt roots, it is also observed that in most species surveyed, the main root system composed of many wiry and extensive woody cable roots that is subterranean in nature, and the aerial roots are formed either by the periodic upwards and then downwards growth of the underground roots (e.g. *B. gymnorrihiza* and *C. tagal*). The vertical extension of the upper surface of the underground roots (e.g. Xylocarpus and Heritiera) or, as in the case of pneumatophores in *A. marina* and *S. alba*, as vertical lateral branches of the main root.

TABLE III: OCCURRENCE OF DIFFERENT ROOT TYPES OF MANGROVES AS OBSERVED IN THE STUDY SITES.

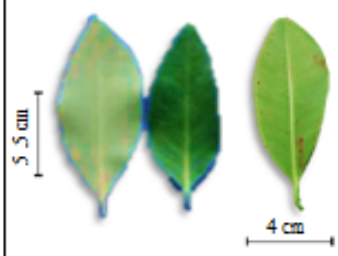
SPECIES	ROOT SYSTEM	SITE
Acanthus ebracteatus	Surface cable, aerial roots and stilt roots	1, 2, 3
Aegiceras corniculatum	Surface cable roots	1,2
Avicennia marina	Surface cable roots, Pneumatophores, Knee roots	1, 2, 3
Bruguiera gymnorrhiza	Pneumatophores, knee roots, stilt roots, butress and aerial roots	1,3
Ceriops tagal	Knee roots, stilt and butress	1,2
Excoecaria agallocha	none	1,2
Heritiera littoralis	Butress	1,3
Lumnitzera littoreus	Knee roots	2,3
Nypa fruticans	Surface cable roots	1, 2, 3
Rhizophora mucronata	Stilt roots, aerial roots	1, 2, 3
Sonneratia alba	Surface cable, pneumatophores, butress	1, 2, 3
Xylocarpus granatum	Surface cable roots, pneumatophores	1, 2
Kandelia candel	Stilt roots	2
Rhizophora stylosa	Stilt roots	2, 3
Acrosthicum aureum	none	1, 2
Sesuvium portulacastrum	none	1
Pemphis acidula	None	(4)

C. Leaf traits

According to [12], leaf traits such as leathery leaves and succulence as brought about by the enlarged cell layers of

hypodermis and mesophyll (as discussed in Anatomical Characterization) that is observed on mangrove species contribute to the increased in such traits. [13] also mentioned that salt is a chief factor that contribute to the increase of succulence of leaves and may be attributed on the important photosynthetic process. Table 4 shows the different leaf traits of some of the mangroves in the study.

TABLE IV: OCCURRENCE OF DIFFERENT ROOT TYPES OF MANGROVES AS OBSERVED IN THE STUDY SITES.

SPECIES	LEAF TRAITS	ILLUSTRATION (LEAF)
Avicennia marina	Leaves are leathery, Medium size leaves. Opposite. Ovate shape, Lamina. Elliptic oblong. Somewhat rounded apex. Upper surface is glabrous. Midrib is prominent. Small petiole with exstipulate; greyish-white tomentose beneath, usually turning black when dry.	
Acanthus ebracteatus	Leaf margins usually deeply toothed, and tipped with rigid sharp spines. Fleshy, leathery leaves. Opposite. Glabrous surface. Oblong shape. Dentate margin with spines. Small petiole with exstipulate.	
Bruguiera gymnorrhiza	Leathery leaves. Medium size. Opposite. Ovate shape. Lamina elliptical oblong. Acute apex. Upper surface glabrous. Prominent midrib. Exstipulate.	
Bruguiera parviflora	Leaves greenish-yellow. Simple. Thick leathery leaves. Medium size. Opposite. Ovate shape. Lamina elliptical oblong. Acute apex. Prominent midrib. Exstipulate.	

D. Anatomical Characteristics

Structural anatomy of the leaf was examined and laminar characteristics such as cuticular layer, thickness of upper and lower epidermis, hypodermal layer, palisade/spongy ratio, thickness of palisade layer, spongy layer and water storage tissues in all species varied significantly. Two (2) species showed isobilateral structures namely, *S. alba* and *K. candel*, while the others are dorsiventral or isobilateral in tissue arrangement. In the former, the two palisade layers below upper and lower epidermis enclose the middle layers of spongy parenchyma. On the other hand, *R. mucronata* and *R. stylosa* have similar internal structures with regards to tissue arrangements following fundamental cells of epidermis (epidermal tissue layer) ranging from square to circular, on both sides. On the other hand, *A. marina* is dorsiventral and shows characteristics of thick water storage tissues, and adaptations for conservation of water. The thickness of leaves presently studied varies from 17.83 µm (*A. marina*) to 26.30µm (*K. candel*). Most of the species investigated have moderately thick leaves with about equal numbers in the two groups. Average measurements of the tissues for the two groups are shown in Table 5.

TABLE V: OCCURRENCE OF DIFFERENT ROOT TYPES OF MANGROVES AS OBSERVED IN THE STUDY SITES.

TISSUE LAYERS	TRUE MANGROVES		MANGROVE ASSOCIATES	
	AVERAGE MEASUREMENTS (mm)	PERCENTAGE (%)	AVERAGE MEASUREMENTS (mm)	PERCENTAGE (%)
Cuticle	3.43	3.23	2.31	4.48
Upper epidermis	12.67	11.94	8.59	16.68
Hypo-epidermis	22.47	21.18	8.91	17.30
Palisade layer	31.36	29.56	7.97	15.47
Spongy mesophyll(including lower hypodermis or palisade layer when present)	19.12	18.02	16.02	31.11
Lower epidermis	11.79	11.11	5.00	9.71
Cuticle	5.34	5.03	2.7	5.24
Leaf thickness	106.1	100	51.5	100

E. Controversial species

Results of microscopic characterization shows that the adaxial surface of the leaf is more compact than abaxial. Values for epidermal cells and other tissues are listed in Appendix Table 1. On the other hand, epidermal cells on both surfaces of pinnae are sinuous walled) and cutinized. Epidermal cells are elongated near veins and midrib portion with an average size of 9.09 ± 1.25 µm. The adaxial epidermal cells (5.90 ± 3.33 µm) were smaller than abaxial epidermal cells (6.8 ± 0.0 µm). Moreover, the abaxial cells width was greater than that of epidermal cells of adaxial surfaces. Consequently, adaxial epidermal cells exhibited a greater density than abaxial surfaces (Fig 1).

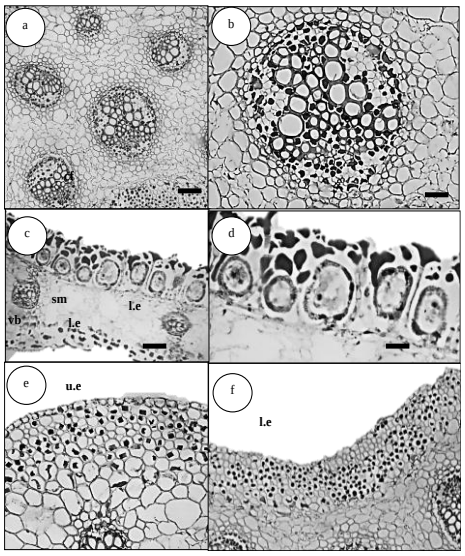


Fig.1 Transverse section of sectional view of leaf lamina of *A. aureum* showing (a) midrib (b) vascular bundle showing endodermis with casparian thickenings, (c) Bundle sheath extension in the laminar region, (d) sporangia with spores (e) lignified hypodermis in the midrib.

On the other hand, *G. littorale* has high value laminar tissues with an average measurement of 8.53± 2.3 µm. The transverse section of leaf shows thick cuticle (Fig. 2), and has thick line of palisade mesophyll and even distribution of water storage tissues in the laminar portion. Moreover, the hypodermal layer was absent and a single line of palisade tissues was observed. Vascular bundle at the midribal section resembles a U-shape with small vascular sheath extensions. The cells of upper epidermis possess circular shaped parenchyma cells ranging an average size of 6.32 ± 0.30 µm. Palisade tissue consists of 1 to 2 layers. Spongy tissue possesses large intercellular air spaces (Fig. 2). Aqueous tissues (spongy tissues) are also abundant and present beneath the lower epidermis (Fig. 2).

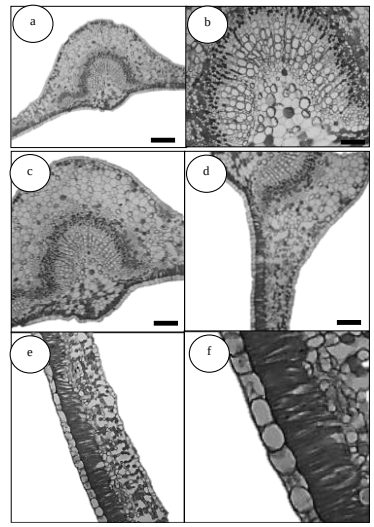


Fig 2. Transverse section of sectional view of leaf lamina of *G. littorale* showing (a) Palisade layers (b) lateral leaf, mesophyll tissues, and epidermal tissues with bridges (c) parenchyma cells from water storage tissue (d) enlarged transcurrent vascular bundles (w/ bridges) in the lateral leaf . Bar: (a,c,d,e) 100 µm; (f) 10 µm; (b) 50 µm.

Other species like *D. spathacea*, another species classified as mangrove associate has also the highest value for the laminar tissues second to *S. portulacastrum* with an average measurement of 13.45 ± 2.1 µm. The transverse section of leaf shows thick cuticle (Fig.3) and vascular bundle sheath with lignified cell structures.

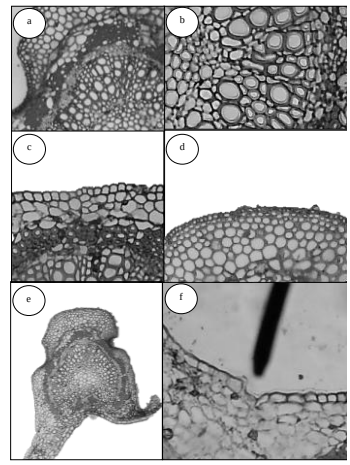


Fig. 3. Transverse section of sectional view of leaf lamina of *D. spathacea* showing (a) vascular bundle sheath (b) lignified cells at the vascular section (c) line of epidermal tissues including the hypoderm (d) collenchyma cells at midrib (e) cuticular layer at enlarge section (f). Salt glands sunken at epidermis.

A lining of sclerenchymaotus cells surrounding the vascular bundle are also evident. Moreover, the hypodermal layer was present and projects a thin line of cells and a whole single line of epidermal tissues. The parenchyma cells and some vessel like structures are near to the vascular formation near the middle portion. The cells of upper epidermis possess circular shaped parenchyma cells ranging an average size of 2.6 ± 0.63µm. Palisade tissue consists of 3 layers. Collenchyma cells are also present from the midribal section.

IV. Conclusion

A. Suggested Classification of the Mangrove Species

Comparison on characteristics of species groups between TM and MA was practically lacking or inadequate. However, with the help of parameters (e.g. anatomical and morphological) and field observations considering other factors could help us to draw some conclusions, and into much greater extent suggest classification of the controversial species. The following controversial mangrove species namely, *H. littoralis*, *P. acidula*, *E. aggalocha*, *X. granatum* and *A. aureum* and their suggested classsification are discussed as follows: Overall, Rhizophoraceae species possesed all the characteristics from the parameters. Based from the generated matrix, anatomical examination shows *B. gymnorrhiza* and *B. parviflora* having accumulating cells (e.g. Astroscleried cells via cork warts) which was found on the laminar section of a mature leaf. As to morphological characters, it has knee roots which is major root system of true mangroves [14] and a viviparous germination. It is also observed to present in the estuarine or middle zone. The species was suggested to be a true mangroves. *R. apiculata*, *C. tagal*, *R. mucronata* and *R. stylosa* similarly exhibits all the set parameters having a secretory cells via cork warts, high values for laminar characteristics and major root system (e.g. stilt roots). They also occurred at the estuarine to middle ward. Overall, the species are suggested and widely known to be true mangroves. Further, Lythraceae species such as *S. alba* and *P. acidula* are classified differently. Although *P. acidula* occurs at beach shorelines occasionally inundated by tides and stagnant sea water, it has no salt glands that can manipulate certain salt levels, though it has high leaf succulence (e.g. mesophyllar tissues and air spaces). All parameters fell into the range of a mangrove associate.

In the case of, *A. ebracteatus* which is initially recorded as mangrove associates, it was suggested to be a true mangrove since it possess salt excretion glands that can manipulate salinity and as it observed to be occupying fringe zone and the middle zone. *A. ebracteatus* as a controversial species is a true mangrove.

X. granatum on the other hand is another controversial species. Based from both anatomical and morphological characterization, it has accumulating cells on the roots and high values of laminar characters, P/S ratio and reproductive mechanism; succulence, respectively. Therefore, *X. granatum* is a true mangrove. In *Aegiceras corniculatum*, high values of laminar characters and presence of scleried cells (e.g. druse cystals) and salt glands were observed. It has viviparous type of germination and a major root system of stilt roots. It occupies the seaward zone which all contributed to a classification of a true mangroves.

In the case of the mangrove fern, *A. aureum* occurs in the landward zone and grow well at freshwater zone [15]. Anatomically, it has no specialized adaptive cells on the leaf but shows distinct structure of aggregate parenchyma cells and transcurrent vascular bundles with bridges. It has spores in terms of reproductive mechanism but does not suit the saline environment for dispersal [16]. Result of anatomical and morphological examination showed that A. aureum is classified as a mangrove associate.

In *E. aggalocha*, it was observed that the species occupies the landward zone from the three study sites. It has salt accumulation abilites but relative upon absorption from the roots only. Laminar characterization shows high values (e.g. abundant water storage tissues), and leaf traits are comparable to other known fringe species. It has buttress root system (as observed in some areas). All the parameters fell into the ranges of true mangroves. The study classified *E. agallocha* as a true mangrove.

H. littoralis is commonly found at the most landward fringe of the mangroves, which is flooded by spring or fluctuating high tides [17]. It has buttress roots that developed into the lower part of the main trunk. However, anatomically, it has no specialized cells that can manipulate certain salinity levels. It has a low laminar characterization values which is comparative to many terrestrial plants [18]. The above mentioned indicated that *H. littoralis* possess the characteristics of glycophytes with lower salt tolerance. In conclusion, the study classified it as a mangrove associate.

D. spathacea is known to be a landward species, but based from site surveys it occurs primarily in the middle zone particularly Batangas and Aurora site. Anatomically, it has salt secretion cells abundant on the adaxial surface of the leaf. It has high values for laminar characteristics particularly thickness of water storage tissues. Morphologically, it has aerial to near buttress roots systems. All parameters fell in the range of true mangroves. The study classified it as a true mangroves.

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