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Comparative Leaves Anatomical Studies of *Bouea, Mangifera* and *Spondias* (Anacardiaceae) in Malaysia

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Abstract: Leaves anatomy of two species of *Bouea*, 11 species of *Mangifera* and two species of *Spondias* were studied in order to see the differences in stomata type, petiole, midrib and lamina anatomy and leaf venation. This study aims to use anatomical characters for species and genus identification. Common characters observed were the absence of trichomes, closed vascular bundles, uniseriate epidermal layers, resin canal in parenchyma cells, anticline wall patterns and druses crystals in leaf lamina transverse sections. All species displayed closed vascular bundles except *Mangifera pajang* which showed a combination of medullary vascular bundles. Uniseriate epidermal layer was observed in all species. All the species showed straight-wavy anticlinal walls. Druses crystals were found in the parenchyma cells of all the species. Four types of stomata were observed namely anomocytic, anisocytic, staurocytic and diacytic. Anomocytic, anisocytic and staurocytic stomata were observed in *Mangifera*, diacytic in *Bouea* and anomocytic in *Spondias*.

Key words: Leaf anatomy, *Bouea, Mangifera, Spondias,* Anacardiaceae, Malaysia.

1. Introduction

Tropical rare fruits are generally neglected because they have not been exploited commercially and there is a lack of improved varieties [1]. It has been estimated that there are about 400 edible indigenous fruit species in the country [2]. These fruit species also experience alternate or irregular fruiting seasons and have restricted habitats. In spite of these constraints, many of these species can be promoted or introduced to local consumers and overseas markets. As some of these indigenous species are vulnerable to loss of genetic diversity, conservation of these species is a priority.

Bouea, Mangifera and *Spondias* are classified under Anacardiaceae family in the order Sapindales. The Anacardiaceae consists of trees, rarely shrubs or lianas, and often with turpentine smelling sap. There are about 70 genera with about 600 species, distributed evenly throughout the tropics and subtropics. Malesia is the richest major tropical area for this family [3].

There are 16 genera and 74 species indigenous to Malaysia, found from the lowlands including swamps, to mountain forests. Certain species such as *Gluta curtisii* and *Swintonia* spp. appear to grow gregariously in the hill and upper dipterocarp forests.

Bouea is a small genus, consisting of only two species, *Bouea macrophylla* and *Bouea oppositifolia.* Both species can be found growing in the home gardens or orchards as well as striving wild in the forest [2]. This genus is native to Asia and Malaysia, extending from north-eastern India to the Moluccas [4]. The centre of diversity is in western Malesia.

The genus *Mangifera* consists of about 40 species

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distributed from India and Sri Lanka in the west, throughout Malaysia and to the Philippines and Papua New Guinea in the east [3, 5]. Thirty species occur in Malaysia with 15 species recorded in Peninsular Malaysia and 16 species in Sabah and Sarawak [3, 5]. Of these, three species namely *M. gracillipes, M. khoonmengiana* and *M. pajang* are endemic to Sabah and Sarawak. Of the 12 species reported to be cultivated, eight are still found in the wild [5-6].

Spondias comprises 10 species occurring in tropical America and in India, Myanmar, Indo-China, southern China, Thailand, throughout the Malesian region and the Solomon Islands. Five species are present within Malesia. Four of them, *S. cytherea, S. mombin, S. pinnata* and *S. purpurea,* are occasionally cultivated throughout the tropics, mainly for their edible fruits [7].

Many species of these genera are native to Malaysia and can be found thriving in the wild and also planted in the orchards. The natural habitat of these species is the tropical rainforest where they are widely distributed from the lowland to lower montane forest at 1800 m above sea level [5]. *Mangifera indica*, the common mango, is widely cultivated and extensively researched on. Unfortunately, the information on the distribution and diversity of its wild relatives are much less comprehensive. In view of this, surveys on the diversity of its wild relatives were carried out in the home gardens and orchards throughout the country. The areas surveyed and specimens collected are listed in Table 1.

Traditionally, the classification of plants is mainly based on morphological and anatomical aspects. The application of anatomical characters in plant identification was first used by Bureau [8], who for the first time to use anatomical features for the determination of taxa in Bignoniaceae. Leaf anatomical studies have been proven to be useful for species grouping and identification [9-10] and it has been of great taxonomic significance [11-12]. Petiolar anatomy of *Thottea* has also been used for interpretation of numerical taxonomic methods [11]. This study aimed to identify the characteristic anatomical features of leaves on 11 taxa of *Mangifera, Bouea* and *Spondias*.

2. Materials and Methods

The materials used in this study were obtained from different sites in Peninsular Malaysia, Sabah and Sarawak (Table 1). The taxa studied were *Mangifera indica, M. foetida, M. pentandra, M. torquenda, M. odorata, M. quadrifida* var. *quadrifida*, *M. quadrifida* var. *longipetiolata, M. pajang, M. caesia, M. griffithii* and *M. lagenifera*. Leaf specimens were fixed in formalin (40%): acetic acid: ethyl alcohol (70%) in a ratio of 5:5:90 (v/v) FAA. Fixed samples were dehydrated in a graded series (20, 35, 55, 75, 95 and 100%) of TBA (tertiary-butyl-alcohol) and embedded in paraffin wax [13]. Single staining with toluidine blue and double staining with safranin and fast green [14] were done for the cross-section. Serial sections of the petioles for each species were observed and microphotographed to visualize the anatomical structures of the species.

Each section was made from the middle part of the petioles and midribs from matured leaves. Epidermal peels were prepared by treatment with Jeffery's fluid (10% nitric acid and 10% chromic acid, 1:1) and stained in Safranin solution. Leaf clearings were prepared by treating the specimens with 1% basic fuchsin in 10% KOH at 60 ºC. The cleaned specimens were washed in 50% alcohol then transferred to 70% alcohol and acidified with hydrochloric acid (HCl). All slides were mounted in Euparal after the final dehydration stages. Three to five matured leaves were sectioned from each specimen.

3. Results and Discussion

3.1 Generic Leaf Anatomical Descriptions

Through observations under light compound Olympus SZH microscope, the cell walls on abaxial and adaxial surfaces of *Mangifera* varied from wavy

Table 1 List of taxa and their localities in Peninsular Malaysia, Sabah and Sarawak.

Taxa	Collection location's, Collector's name, Collection number
Mangifera indica	Melaka, Masjid Tanah, Kg. Londang, Mohd. Norfaizal, G. (MNFG 1001) Negeri Sembilan, Kuala Pilah, Kg. Mampas, Mohd. Norfaizal, G. (MNFG 1002), Kedah, Yan, Kg. Hilir, Mohd. Norfaizal, G. (MNFG1003)
M. caesia	Kedah, Kg. Batu, Baling, Mohd. Norfaizal, G. (MNFG 1004) Melaka, Kg. Durian Daun, Alor Gajah, Mohd. Norfaizal, G (MNFG1005) Negeri Sembilan, Kg. Jeleng, Jelebu, Mohd. Norfaizal, G. (MNFG1006)
M. foetida	Selangor, Kg. Pangsoon, Hulu Langat, Mohd. Norfaizal, G. (MNFG 1007), Negeri Sembilan, Kg. Tg. Kala, Kuala Pilah, Mohd. Norfaizal, G. (MNFG1008), Selangor, Kg. Sungai Lalang, Semenyih, Mohd. Norfaizal, G. (MNFG1009)
M.quadrifida var. *quadrifida*	Kedah, Kg. Ruat, Yan, Mohd. Norfaizal, G. (MNFG1010) Johor, Kg. Tg. Gemok, Mersing, Mohd. Norfaizal, G. (MNFG 1011)
M. torquenda	Kelantan, Kg. Chetok, Tanah Merah, Kelantan, Mohd. Norfaizal, G. (MNFG1012), Sarawak, Kg. Kakeng, Serian, Mohd. Norfaizal, G. (MNFG1013)
M. pajang	Sarawak, Kg. SorakMelayu, Serian, Mohd. Norfaizal, G. (MNFG1014) Sarawak, Kg. Bungai, Sibuti, Mohd. Norfaizal, G. (MNFG1015)
M. odorata	Kelantan, Kg. Paloh, PasirPuteh, Mohd. Norfaizal, G. (MNFG1016) Selangor, Kg. Bt. 18, Hulu Langat, Mohd. Norfaizal, G. (MNFG1017) Terengganu, Kijal, Kemaman, Mohd. Norfaizal, G. (MNFG1018)
M. griffithii	Kedah, Kg. Raga, Yan, Mohd. Norfaizal, G (MNFG1019) Perak, Kg. Bersia, Gerik, Mohd. Norfaizal, G. (MNFG 1020)
M. quadrifida var. *longipetiolata*	Kelantan, Tumpat, Mohd. Norfaizal, G. (MNFG1021) Kedah, Baling, Mohd. Norfaizal, G. (MNFG1022)
M. laurina	Sabah, Kg. Bungaliu, Papar, Mohd. Norfaizal, G. (MNFG1023) Perak, Kg. Tapah Road, Tapah, Mohd. Norfaizal, G. (MNFG1024)
M. pentandra	Perak, Kg. Lama, Kuala Kangsar, Mohd. Norfaizal, G. (MNFG1025) Kedah, Kg. Kota Kuala Muda, Merbok, Mohd. Norfaizal, G. (MNFG1026)
Spondias pinnata	Kelantan, Pasir Puteh (MNFG 1027) Pahang, Jerantut (MNFG1028)
S. cytherea	Johor, Muar (MNFG 1029) Melaka, Jasin (MNFG 1030)
Bouea macrophylla	Negeri Sembilan, Kuala Pilah (MNFG 1031) Kedah, Yan (MNFG 1032) Perak, Gerik (MNFG 1033)
B. oppositifolia	Selangor, Bukit Cherakah (MNFG 1034) Perak, Kuala Kangsar (MNFG 1035) Kelantan, Bachok (MNFG 1036)

to sinuous (Fig. 4a and Table 3). *Mangifera* has anomocytic, anisocytic and staurocytic types stomata (Fig. 4b). Trichomes were absent in all specimens thus supporting Metcalfe and Chalk [9] and Alona [15] observations on the *Mangifera*. Druses were present in mesophyll and parenchyma tissues in petioles transverse section of all studied species. For *Bouea macrophylla* and *B. oppositifolia*, straight-wavy anticlinal walls were observed. *Spondias pinnata* and *S. cytherea* also showed straight-wavy anticlinal walls.

3.2 Midrib Transverse Section

3.2.1 Outline Shape

Adaxial: convex, abaxial: curved down (Fig. 2c). Vascular tissue closed system, except for *M. caesia* that showed adaxial with pointed shape and curved abaxial part (Fig. 2g). Vascular tissue: closed system for all specimens. *Bouea macrophylla* showed rounded shape on the adaxial and slightly curved downward for abaxial part (Fig. 2l). *Bouea oppositifolia* has curved adaxial part and rounded abaxial part. *Spondias cytherea* displayed smaller size of midrib transverse section with curved adaxial part and rounded abaxial part (Fig. 2n) while *S. pinnata* showed slightly convex curved shape for the adaxial and rounded abaxial part (Fig. 2o). Bundle sheaths: Sclerenchymatous; strands of fibres incompletely encircling midrib complex. Crystals: druses present in the parenchyma cells.

3.3 Petiole Transverse Section

The earliest recognition on the variation pattern of the vascular bundle in a cross-section of a petiole can be traced back to 1675. Petiole continues to be one of the most important sources of taxonomic evidence in the field of systematics, at least at the higher level [15]. The outline of the transverse section of the petioles in *Mangifera* can be grouped into four distinct shapes: circular, semi-circular, wing-shaped and boat shaped. All species exhibit a general circular shape except for *M. caesia* (boat shaped) (Fig. 1e), *M. foetida* (semi-circular shaped) (Fig. 1d), *M. pajang* (broad oval) (Fig. 1j). Outline shape: circular shape "O" for *Mangifera pentandra, M. odorata, M. quadrifida* var. *quadrifida, M. laurina* and *M. quadrifida* var. *longipetiolata*. Oval shape in *M. foetida, M. caesia* and *M. indica*. Broad oval shape observed only in *M. pajang* (Fig. 1j). Rounded shape for *B. macrophylla* (Fig. 1l); slightly rounded and curved upward for *B. oppositifolia* (Fig. 1m). Oval for *Spondias cytherea* and *S. pinnata* (Fig. 2n and 2o). Vascular tissue: the presence of ring-shaped bundle sheaths is one of the diagnostic characters for *Mangifera*, although not very useful at the species level. Closed system is found in all species except *M. pajang* which showed medullary vascular system (Fig. 2j). Size of vascular bundle in the studied *Mangifera* specimens varies from small (0.8-1.25 mm in diameter) in *M. quadrifida* var. *longipetiolata* (Fig. 1i) and *M. indica* (Fig. 1k) to as large (2.5-3 mm) as in *Mangifera quadrifida* var. *quadrifida* (Fig. 2c). Vascular bundle in all specimens showed completely enclosed by fibrous cap of sclerenchyma cells except for *M. pajang* (Fig. 2j). While *M. grifithii* (Fig. 2h) showed bundle sheaths extending towards and come into contact with the epidermis on either or both sides of the leaf surfaces. Metcalfe and Chalk [9] described this type as vertically translucent. Resin canal: observed in all species, at the centre of the petiole, scattered at the centre and some part of the margin, except for *Spondias* and this can be used as a diagnostic anatomical characters. Trichome: Nil. Druses: present in the parenchyma cells.

3.4 Leaf Margin Transverse Section

Shape Outline: straight and rounded at the end: *M. pentandra, M. odorata* and *M. laurina* (Fig. 3i-k). Curved downward and rounded at the end: *M. quadrifida* var. *quadrifida, M. foetida*, *M. caesia, M. torquenda, M. griffithii, M. quadrifida* var. *longipetiolata, M. pajang* and *M. indica* (Fig. 3a-h). Trichome: Nil. Druses: present at the parenchyma cells.

4. Discussion

There is considerable anatomical variation among the 11 species of *Mangifera*, two species of *Bouea* and two species of *Spondias* studied. However, some characters are present in all of them such as the closed system of vascular tissue at the centre of the petioles, except *M. pajang* that showed medullary vascular bundle (Fig. 1j). Another characteristic is the presence of single layer of palisade cells of same height (or 1:1, 1:2, 1:3). Druses are present in enlarged cells in the palisade layer of all species.

In the vascular tissue, druses are usually present in the parenchyma cells of the midrib of the leaves and petioles as well as in the phloem cells. Anomocytic, anisocytic and staurocytic and diacytic stomata (Fig. 4 (a-c), Table 2) were observed for all species. The anomocytic type characteristic is the common characters for the Anacardiaceae [9] and forms the basic type for the family. Transverse sections of midribs do not reveal variations in the shape of adaxial and abaxial surfaces. *Mangifera caesia* (Fig. 2g) showed different shape for the abaxial and adaxial parts of the midrib compared to the other *Mangifera* species. The adaxial surface varies from curve to slightly convex and ridged. The abaxial surface shows slight variations among these species.

The UPGMA clustering method was used to construct a dendrogram using the anatomical data (Fig. 5). Intraspecific genetic similarity ranged from 0.75

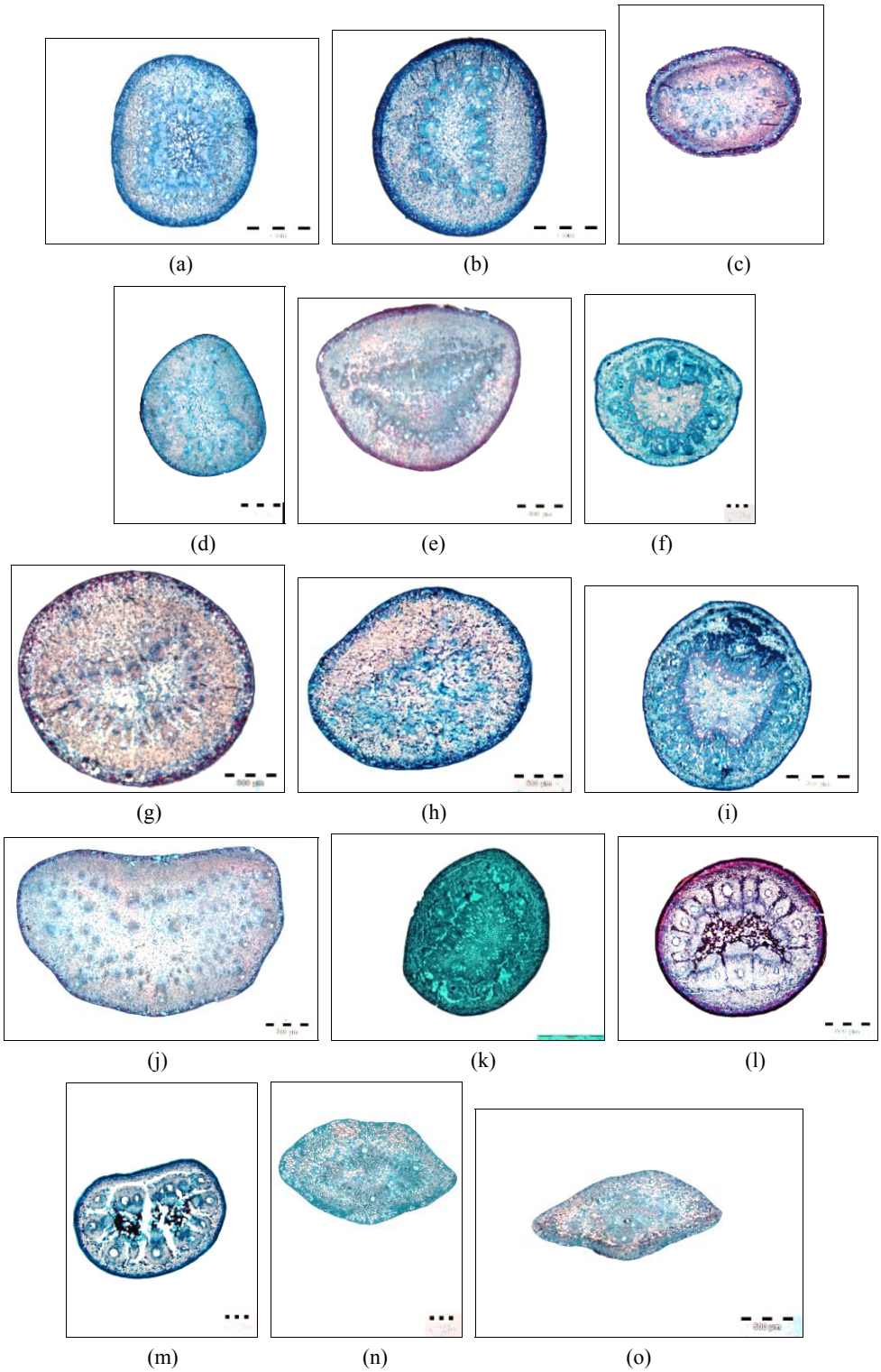

Fig. 1 Petiole trasverse section. (a): *M. pentandra*, (b): *M. odorata*, (c): *M. quadrifida* var. *quadrifida*, (d): *M. foetida*, (e): *M. caesia*, (f): *M. torquenda*, (g): *M. laurina*, (h): *M. griffithii*, (i): *M. quadrifida* var. *longipetiolata*, (j): *M. pajang*, (k): *M. indica*, (l): *Bouea macrophylla*, (m): *Bouea oppositifolia*, (n): *Spondias cytherea* and (o): *Spondias pinnata*.

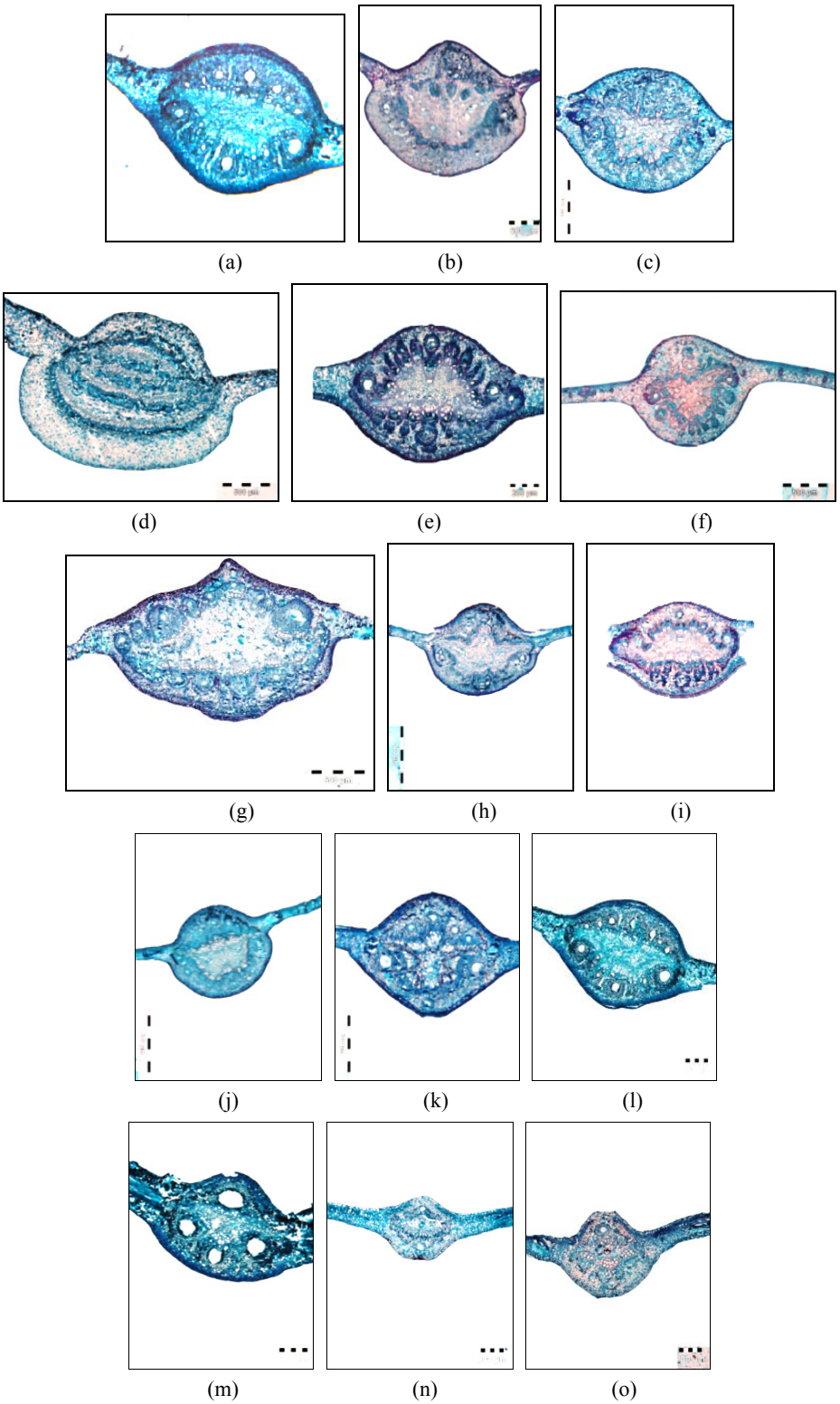

Fig. 2 Midrib transverse section. (a): *Mangifera indica*, (b): *M. pajang*, (c): *M. quadrifida* var. *quadrifida*, (d): *M. griffithii*, (e): *M. torquenda*, (f): *M. caesia*, (g): *M. foetida*, (h): *M. quadrifida* var. *longipetiolata*, (i): *M. odorata*, (j): *M. pentandra*, (k): *M. laurina*, (l): *Bouea macrophylla*, (m): *Bouea oppositifolia*, (n): *Spondias cytherea* and (o): *Spondias pinnata*.

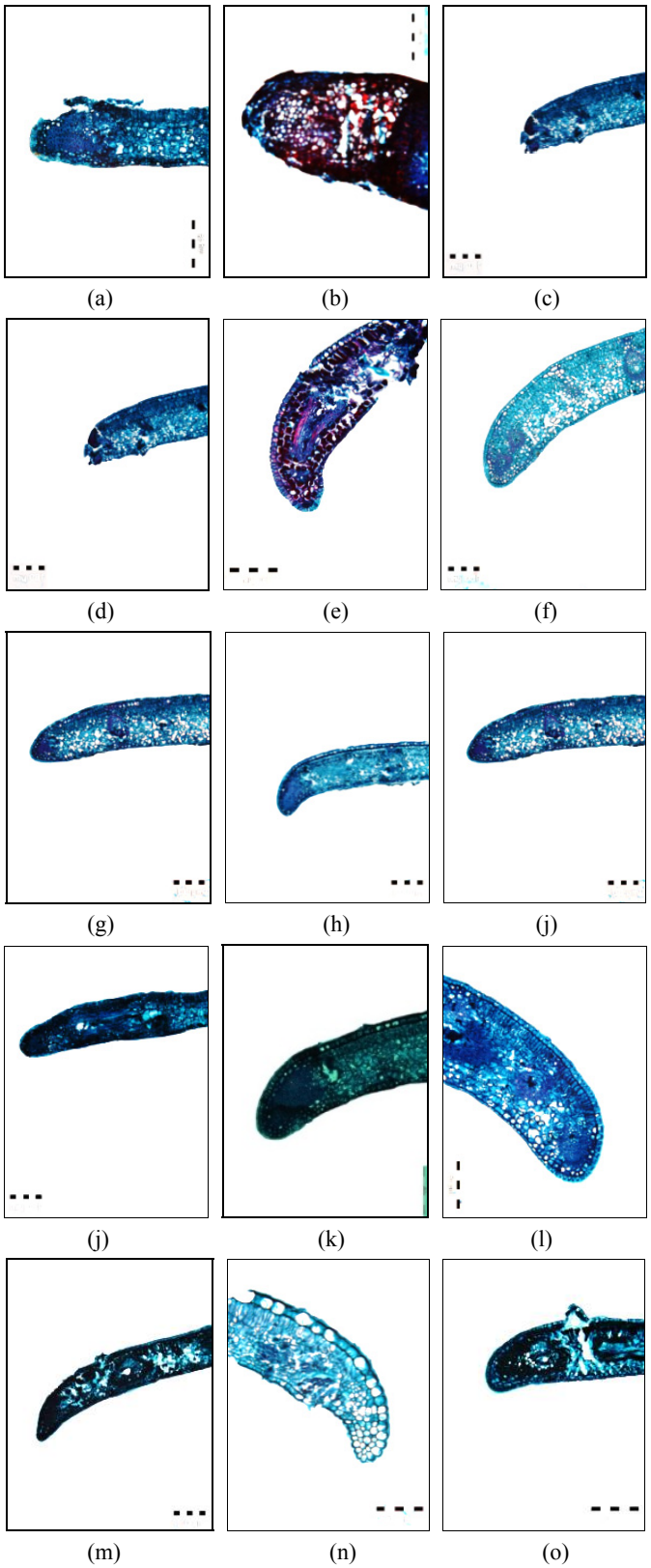

Fig. 3 Leaves margin transverse section. (a): *Mangifera indica*, (b): *M. pajang*, (c): *M. quadrifida* var. *quadrifida*, (d): *M. griffithii*, (e): *M. torquenda*, (f): *M. caesia*, (g): *M. foetida*, (h): *M. quadrifida* var. *longipetiolata*, (i): *M. odorata*, (j): *M. pentandra*, (k): *M. laurina*, (l): *Bouea macrophylla*, (m): *Bouea oppositifolia*, (n): *Spondias cytherea* and (o): *Spondias pinnata*.

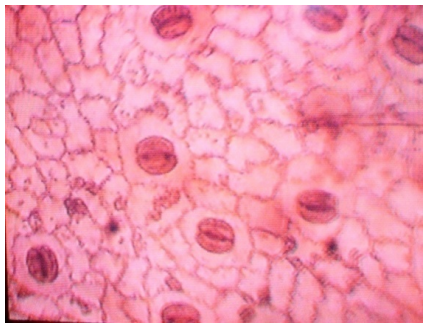

Fig. 4a Anisocytic stomata.

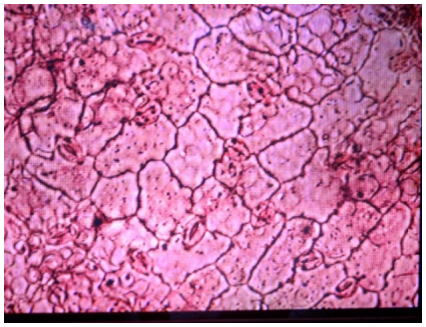

Fig. 4b Anomocytic stomata.

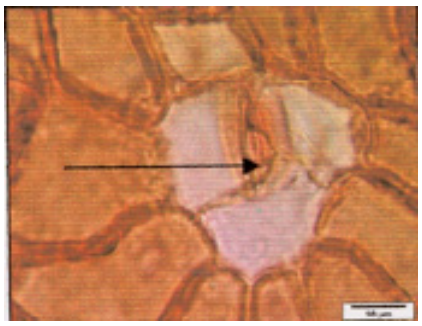

Fig. 4c Staurocytic stomata.

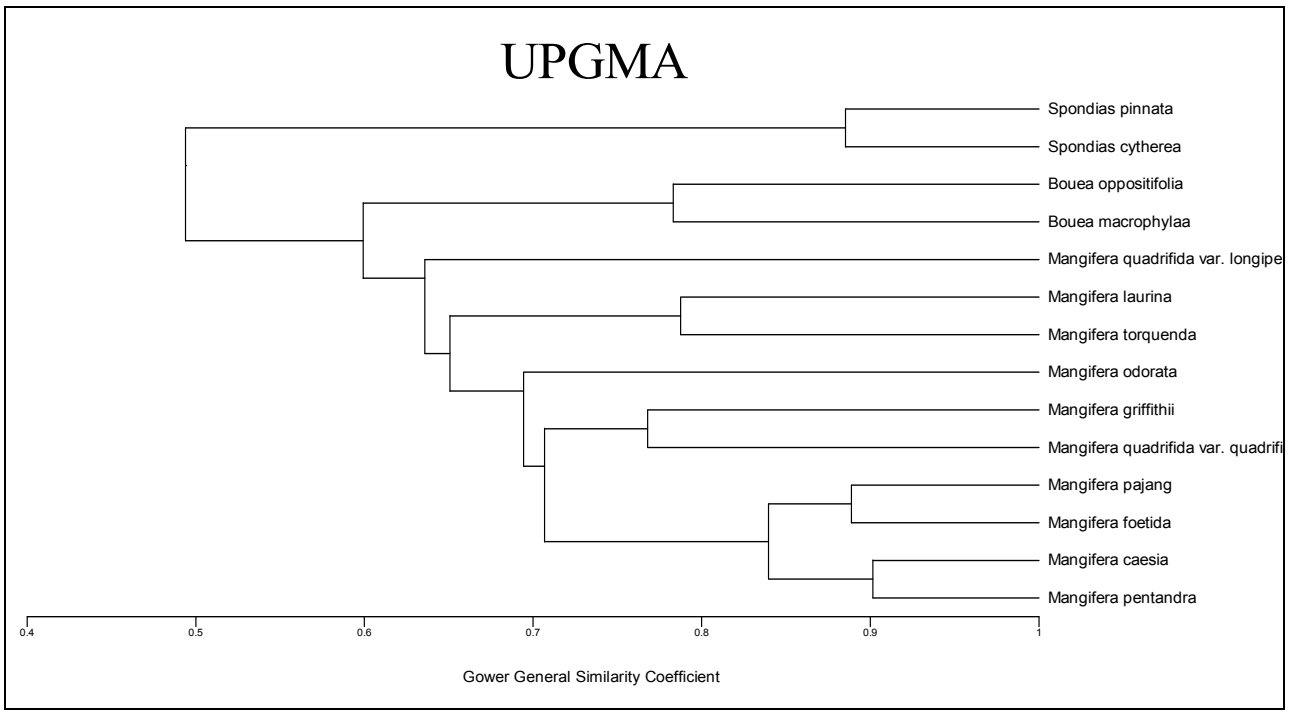

Fig. 5 Cluster analysis for *Bouea, Mangifera* and *Spondias* taxa based on anatomical characters.

Table 2 Stomata type studied taxa.

Species	Stomata type
Mangifera indica	Anomocytic
M. odorata	Anisocyitic
M. caesia	Anisocytic
M. pajang	Anomocytic
M. laurina	Anomocytic
M. indica	Anomocytic
M. griffithii	Staurocytic
M. quadrifida var. *quadrifida*	Anisocytic
M. quadrifida var. *longipetiolata*	Anisocytic
M. torquenda	Staurocytic
M. pentandra	Anisocytic
Bouea macrophylla	Diacytic
B. oppositifolia	Diacytic
Spondias cytherea	Anomocytic
S. pinnata	Anomocytic

(*M. quadrifida* var. *quadrifida* and *M. pajang*) to 0.94 (*M. odorata* and *M. pentandra*). According to Kostermans and Bompard [6], *M. pentandra* was classified under section Euantherae and *M. odorata* was classified under section Limus. Based on the anatomical characters, the similarities among these two species were based on the petioles, leaf venation, midribs, stomata, and leaf margin while the classification by Kostermans and Bompard [6] was based on the morphological characters alone. Although the underlying reason for this mismatch is unclear, this finding might reflect the difference; the current classification of *Mangifera* species based on the specific and related traits is imbalanced genetically.

All the species were much more diverse and easily discriminated between each other. Within the *Mangifera* species studied using anatomical characters, *M. odorata* and *M. pentandra* were the most diverse (94%), followed by *M. pajang* (87%), *M. torquenda* and *M. caesia* (78%), *M. quadrifida* var. *quadifida* (75%), *M. quadrifida* var. *longipetiolata* (74%), *M. foetida* (71%), *M. indica* and *M. griffithii* (86%) and lastly *M. laurina* (83%). Two groups were formed in this similarity study in which the similarity was 70% for *Mangifera laurina, M. indica, M. griffithii, M. quadrifida* var. *quadrifida, M. torquenda* and *M. caesia*. The other group formed with similarity of 71% consists of *M. foetida, M. quadrifida* var. *longipetiolata, M. pajang, M. odorata* and *M. pentandra*. Percentage of similarity among all the *Mangifera* species based on anatomical characters was 65%. In conclusion, anatomical characters are useful and can be used as supporting evidence in determining the genetic relationships among the studied species.

For the anatomical characteristics analysis using UPGMA, two groups were formed namely Group A consisting *Spondias pinnata* and *S. cytherea* (50%), and Group B consisting six subgroups namely B1 (*Bouea macrophylla* and *B. oppositifolia*), B2 (*M. quadrifida* var. *longipetiolata*) B3 (*M. laurina* and *M. torquenda*), B4 (*M. odorata*), B5 (*M. pajang* and *M. foetida*) and B6 (*M. caesia* and *M. pentandra*). From this analysis, we can relate that *Bouea* species are related to the *Mangifera* taxa with relationship with value of 60%, while *Spondias* showed distant relationship at 50% when analysed using anatomical characters.

5. Taxonomic Implication of the Characters

These anatomical characters when used in combination are quite useful in the identification of *Mangifera* species. The results, however, did not show any clustering of characters which could be used to classify the genera into broader groups.

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