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Systematic Significance of Leaf Anatomical Characteristics in Some Species of *Mangifera* L. (Anacardiaceae) in Thailand

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ABSTRACT.– The leaf anatomical comparison of some species in genus *Mangifera* L. from Thailand, such as *M. caloneura* Kurz, *M. camptosperma* Pierre, *M. duperreana* Pierre, *M. foetida* Lour., *M. indica* L., *M. odorata* Griff., *M. pentandra* Hook.f. and *M. qaudrifida* Jack in Roxb. was provided. The specimens were investigated by peeling method, clearing method and transverse sections of lamina and petiole from each species of plant, and conducted an examination under light microscope to determine their systematic significance in species delimitation and identification. The consistent anatomical characteristics in all species are i) the typical cyclocytic and staurocytic stomata in adaxial and abaxial surfaces respectively; ii) the amphistomatic leaves; iii) the jigsaw shape with deeply undulate cell wall in adaxial epidermal cell; iv) the presence of sunken peltate trichomes on lamina and midrib; v) the presence of extension in bundle sheath to both surfaces; vi) the presence of fiber at the apex of leaf margin, midrib and petiole; vii) the presence of resin ducts; vii) the presence of mucilaginous cells in epidermis and midrib; and viii) the presence of prismatic crystals in mesophyll, midrib and petiole. In addition, the significantly anatomical features were useful for species delimitation which are as follows; i) the shape of epidermal cell; ii) the outline of leaf margin, midrib and petiole; iii) the shape of epidermal cell at the apex of leaf margin; iv) the layer of hypodermis; v) the number of palisade cell layers in lamina; vi) the presence of grouped fiber below sunken trichome; vii) the distribution of peltate trichome; viii) the presence of sclereid with ramiform-pitted in petiole; ix) the inclusion in each organ *e.g.* mucilaginous cell, crystal and starch grain; and x) the number of resin ducts in midrib and petiole.

KEY WORDS: Leaf Anatomy, *Mangifera* L., Anacardiaceae, Thailand

INTRODUCTION

Mangifera L. is belonging to Anacardiaceae. There are approximately 40 species in tropical Asia, widely distributed throughout the tropical, subtropical and temperate regions of the world (Tianlu and Barfod, 2008; Chandra and Mukherjee, 2014). In Thailand, *Mangifera* consists of 17 species (Chayamarit, 2010). The habit of *Mangifera* is trees in which resin ducts present in leaves, stems and fruits (Metcalfe and Chalk, 1957; Chayamarit, 2010).

Nowadays, *Mangifera* species are exploited in many ways because their organs can be used for nutrition, ethnomedicine and pharmacology. For instance, fruits can be consumed and are cultivated on large scales as a commercial crop in many tropical and subtropical countries. Leaves of mango (*Mangifera indica* L.) can be utilized to treat several human ailments including hyperdipsia, hemorrhages, diarrhea, ulcers and pharyngopathy. The smoke and ash of burnt leaves have been utilized for the treatment

of scalds and throat diseases (Parvez, 2016). Moreover, the leaf extracts of *M. indica* have been proven to be useful in the development of antidiabetic, anti-tetanus and antibacterial remedy (Aderibigbe et al., 2001; Perpeteuo et al., 2003; Bbosa et al., 2007; Shabani and Sayadi, 2014).

The identification and delimitation of *Mangifera*, in classical classification, were generally based on their floral features (Wannan and Quinn, 1991; Chayamarit, 2010; Chandra and Mukherjee; 2014). Occasionally, it had been difficult to identify accurately the genus in non-breeding periods because of the morphological resemblances between the various species (Yonemori et al., 2002). As a result, the other informative aspects have been imperative in distinguishing *Mangifera; e.g.* molecular phylogenetic analysis, palynology, chemotaxonomy and wood anatomy (Chayamarit, 1997; Wannan, 2006; Hidayat et al., 2011). Through the implementation of these viewpoints, the significant anatomical information has been attained for species identification because of the constant characteristics being found in each species. In addition, the anatomical features can be also applied for species identification with the lesser distinguishable portions of the plant - for example: timbers, twigs and small pieces of plant matter - which lack the discernible properties of the flowers and fruits.

Sharma et al. (2012); Norfaizal and Latiff (2013) compared the epidermis, lamina and petiolar transverse sections on the Indian and Malaysian *Bouea*, *Spondias* and *Mangifera*, the anatomical characteristics which were proved to be useful for identification were stomatal type, layer of hypodermal cell, shape of leaf margin, midrib and petiole, shape of vascular strand, total visual count of vessel and number of resin canals. Furthermore, *Mangifera* species in Thailand are studied insufficiently - for instance; the types of axial parenchyma, types of inclusion and the number of procumbent and upright ray cells were provided for identification in three *Mangifera* species (Phongkrathung and Kermanee, 2013). Likewise, Ganogpichayagrai et al. (2016) applied the stomatal types, shape of epidermal cell, stomatal numbers and palisade ratio for classification among *M. indica* varieties. The anatomical characteristics identified in the work mentioned above suggested that features of leaf epidermis and transverse sections can be addressed to distinguish *Mangifera* species precisely. As a consequence, microscopic anatomical data has proven invaluable in supporting the systematization and discrimination of this genus.

To reiterate earlier point, the anatomical leaf features were the significant taxonomic characteristics that should be utilized to accomplish the species identification and classification (Sharma et al., 2012). Although the earlier anatomical researches had been studied on some *Mangifera* species, they only contribute to a partial examination in Thai *Mangifera* species. Furthermore, the documents of Thai *Mangifera* identification based on anatomical characteristics were insufficient. Hence, the leaf anatomy of *Mangifera* species in Thailand should be studied in order to be database for identification, solving the identification in plant without floral structure and species identification with the lesser distinguishable portions of the plant. The objective of this research aimed to study the leaf anatomy and construct a key based on leaf anatomical characteristics on eight taxa of *Mangifera* in Thailand for species identification and delimitation.

TABLE 1. Locality and habitat of examined specimens

Species	Locality	Habitat	Collector Number
M. caloneura	Mueng District, Surat Thani	Evergreen forest	A. Srinual & K. Tipmontiane 008
M. camptosperma	Koh Yai District, Songkhla	Riverside in evergreen forest	A. Srinual & K. Tipmontiane 005
M. duperreana	Mae Rim District, Chiang Mai	Deciduous forest	A. Srinual & K. Tipmontiane 003
M. foetida	Koh Yai District, Songkhla	Evergreen forest	A. Srinual & K. Tipmontiane 004
M. indica	Mueng District, Surin	Deciduous forest	A. Srinual & K. Tipmontiane 012
M. odorata	Kham Khuean Kaeo District, Yasothon	Evergreen forest	A. Srinual & K. Tipmontiane 018
M. pentandra	Kham Khuean Kaeo District, Yasothon	Evergreen forest	A. Srinual & K. Tipmontiane 017
M. quadrifida	Koh Yai District, Songkhla	Riverside in evergreen forest	A. Srinual & K. Tipmontiane 006

MATERIALS AND METHODS

1. Sampling and Identification

Ten replicates of leaves in each species were collected from different sites in Thailand (Table 1). The leaves were organized to voucher specimens which were deposited at Department of Biology, Faculty of Science, Srinakharinwirot University. The others were preserved in 70% of ethanol for leaf anatomical studies. The studies taxa were *M. caloneura*, *M. camptosperma*, *M. duperreana*, *M. foetida*, *M. indica*, *M. ordorata*, *M. pentandra* and *M. quadrifida*. Identification of studied specimens were provided by Flora of Thailand vol. 10 part 3 (Chayamarit, 2010).

2. Microscopic study

2.1 Peeling and Clearing methods

The middle parts of mature lamina in leaf margin, midrib and the region between leaf margin and midrib were selected and peeled the epidermis by razor. The epidermal peels were stained with safranin, then dehydrated in ethanol series, and finally mounted with DePeX mounting media (Johansen, 1940). Another method for leaf anatomical study was clearing the leaf section by 10% of NaOH. The specimens were stained by safranin, and then dehydrated in ethanol series (Lersten and Curtis, 2001).

2.2 Paraffin Method

The middle portions of petiole and mature leaf blades - which were leaf margin, midrib and the region between leaf margin and midrib - were dehydrated in grade series of TBA (tertiary butyl alcohol). After that, they were infiltrated by paraffin oil and pure paraffin. The infiltrated specimens were embedded and cut by sliding microtome and then double stained with safranin and fast green, before mounting with DePeX mounting media (Johansen, 1940). Permanent slides were kept at the Department of Biology, Faculty of Science, Srinakharinwirot University and ten specimens were provided for each species.

3. Description and Construction a key

Permanent slides of each species were observed and photomicrographs were taken with LEICA MC170HD. The observed anatomical characteristics were described and constructed a key for species identification.

RESULTS

1. Lamina

1.1 Epidermal features

The outlines of epidermal cell, as seen under light microscope, represent a jigsaw shape with deeply undulate cell wall in

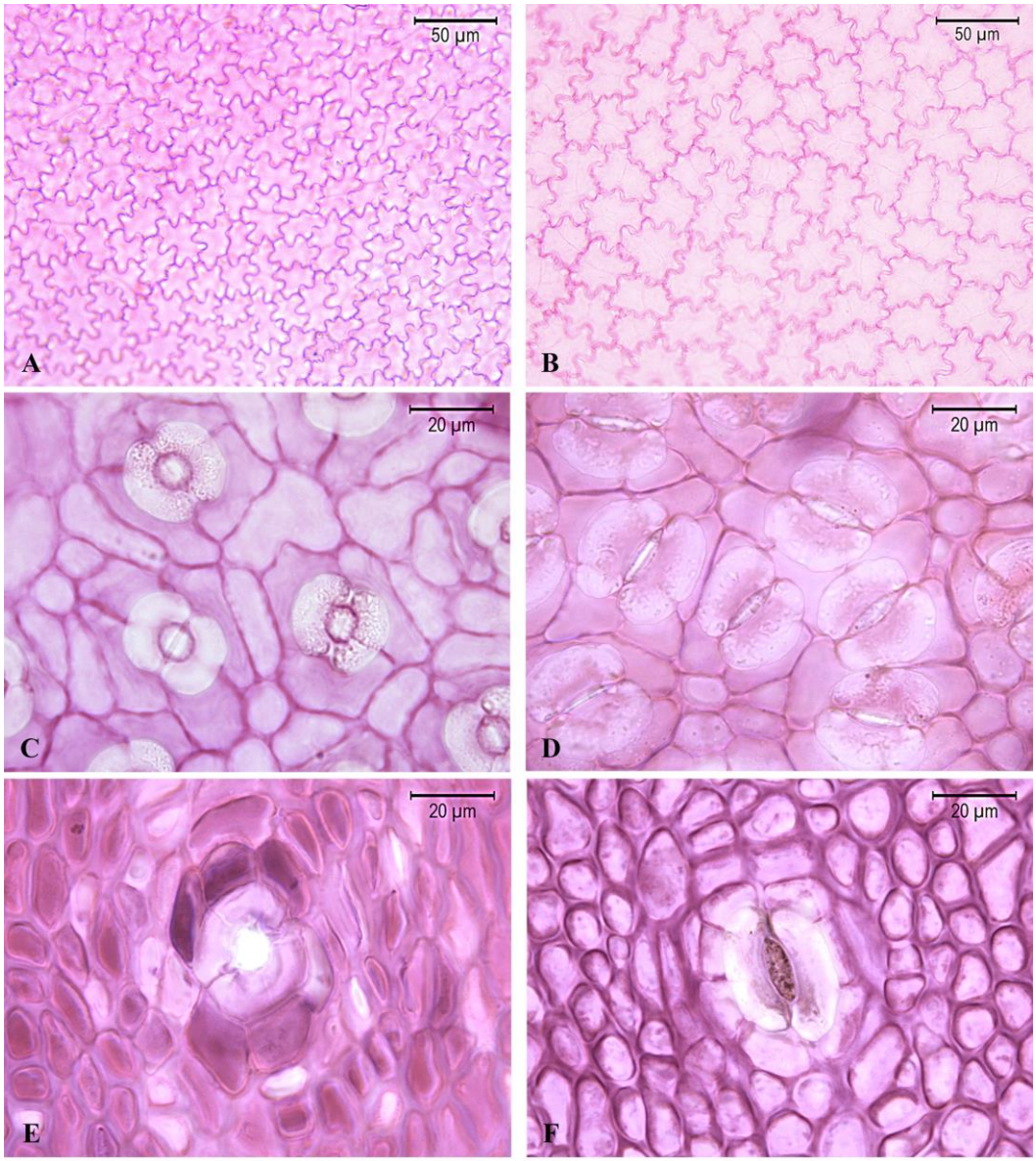

FIGURE 1. Epidermal cell shapes and stomatal types in leaves of *Mangifera* species: **A and B**. Jigsaw shapes with deeply undulate cell wall in adaxial surface (*M. duperreana* (A) and *M. camptosperma* (B)). **C**. Staurocytic stomata with slightly undulate cell wall in abaxial surface (*M. camptosperma*). **D.** Staurocytic stomata with straight cell wall in abaxial surface (*M. qaudrifida*). and **E and F**. Cyclocytic stomata in adaxial surface (*M. indica* (E) and *M. pentandra* (F)).

adaxial epidermis (Fig. 1A, B), and an irregular shape with slightly undulate cell wall in abaxial surface, except in *M. odorata* and *M. quadrifida*, which present straight cell wall (Fig. 1C, D). Amphistomatic leaves are commonly found throughout the genus. They possess cyclocytic and staurocytic stomata on adaxial and abaxial

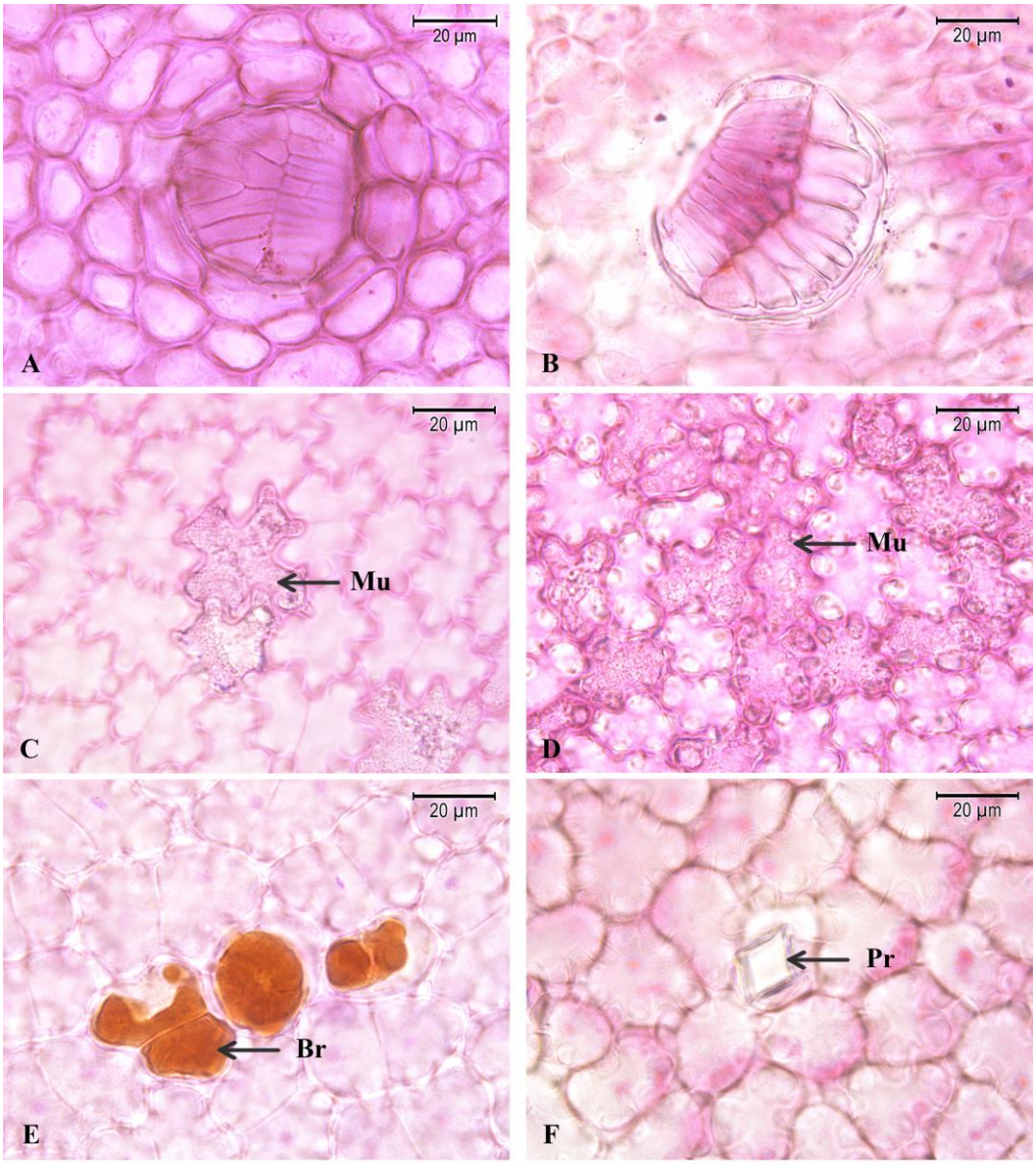

FIGURE 2. Characteristics of trichomes and inclusions in epidermis of *Mangifera* species: **A and B**. Peltate trichomes (*M. pentandra* (A) and *M. caloneura* (B)). **C and D**. Mucilaginous cells (*M. duperreana* (C) and *M. indica* (D)). **E.** Brown inclusions (*M. odorata*). and **F.** Prismatic crystals (*M. caloneura*). (Br = Brown inclusion, Mu = Mucilaginous cell, and Pr = Prismatic crystal)

epidermis respectively (Fig. 1C-F), and peltate trichomes demonstrate on both surfaces of all species (Fig. 2A, B). Inclusions are observed: (i) mucilaginous cells on particular adaxial surfaces, except in *M. caloneura* and *M. pentandra* where the occurrence of mucilage is on both epidermis (Fig. 2C, D); (ii) brown inclusion

TABLE 2. Anatomical characteristics of epidermis of *Mangifera* species

Species	Epidermal cell				Stomatal type		Trichomes		Inclusion and secretory structure					
	Shape		Cell wall						Upper			Lower		
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Mucilage cell	Brown	Prismatic	Mucilage cell	Brown	Prismatic
M. caloneura	Js	Is	Du	Su	Cs	Ss	Pt	Pt	+	-	+	+	-	-
M. camptosperma	Js	Is	Du	Su	Cs	Ss	Pt	Pt	+	-	-	-	-	-
M. duperreana	Js	Is	Du	Su	Cs	Ss	Pt	Pt	+	-	+	-	+	-
M. foetida	Js	Is	Du	Su	Cs	Ss	Pt	Pt	+	+	+	-	-	-
M. indica	Js	Is	Du	Su	Cs	Ss	Pt	Pt	+	-	-	-	-	-
M. odorata	Js	Is	Du	Sr	Cs	Ss	Pt	Pt	+	+	-	-	-	-
M. pentandra	Js	Is	Du	Su	Cs	Ss	Pt	Pt	+	-	+	+	-	-
M. quadrifida	Js	Is	Du	Sr	Cs	Ss	Pt	Pt	+	-	-	-	-	-

Note: - = absent, + = present; **Shape of epidermal cell**: Js = Jigsaw shape, Is = Irregular shape; **Feature of cell wall in epidermal cell**: Du = deeply undulate cell wall, Su = slightly undulate cell wall, Sr = Straight cell wall; **Stomatal types**: Cs = Cyclocytic stomata, Ss = Staurocytic stomata; **Types of trichomes**: Pt = Peltate trichome

on adaxial surface of *M. foetida* and *M. odorata* and abaxial surface of *M. duperreana*, (Fig. 2E); (iii) prismatic crystal on adaxial epidermis of *M. caloneura*, *M. duperreana*, *M. foetida* and *M. pentandra* (Fig. 2F). These are detailed in table 2.

1.2 Leaf margin transverse section

Leaf margin transverse section outlines are straight in *M. pentandra* and *M. quadrifida*, whereas the others represent downward outlines (Fig. 3A, B). The cuticular thickness is distinct at the apex of margin in most specimens, except *M. foetida*. Jigsaw is normal outlines on both surfaces but appearance of epidermal cell on the apex of margin are defined in square and vertical rectangular shapes. The former appearance shows in *M. camptosperma*, *M. duperreana*, *M. foetida* and *M. pentandra*, while another shape indicates in the others species. On the leaf margin, the group of fiber is commonly found (Fig. 3A, B). Besides, sunken peltate trichomes, resin ducts and prismatic crystals locate in leaf margin of all species, except *M. foetida* which absent characteristic of resin duct, nevertheless, mucilaginous cells typically restrict in spongy parenchyma cells of *M. caloneura* and *M. duperreana* (Table 3.).

1.3 The region between leaf margin and midrib transverse section

A single layer of jigsaw shapes on both surfaces is dominant characteristic of all studied species (Fig. 3C). In *M. foetida*, a single hypodermal layer exhibits below the adaxial surface of the lamina (Fig. 3D). All of the species are bifacial leaves. Palisade parenchyma is comprised of a single cell layer on the adaxial surface, excluding *M. odorata* and *M. quadrifida*, which contain two to three layers, with spongy parenchyma zone occur prominently beneath them. The

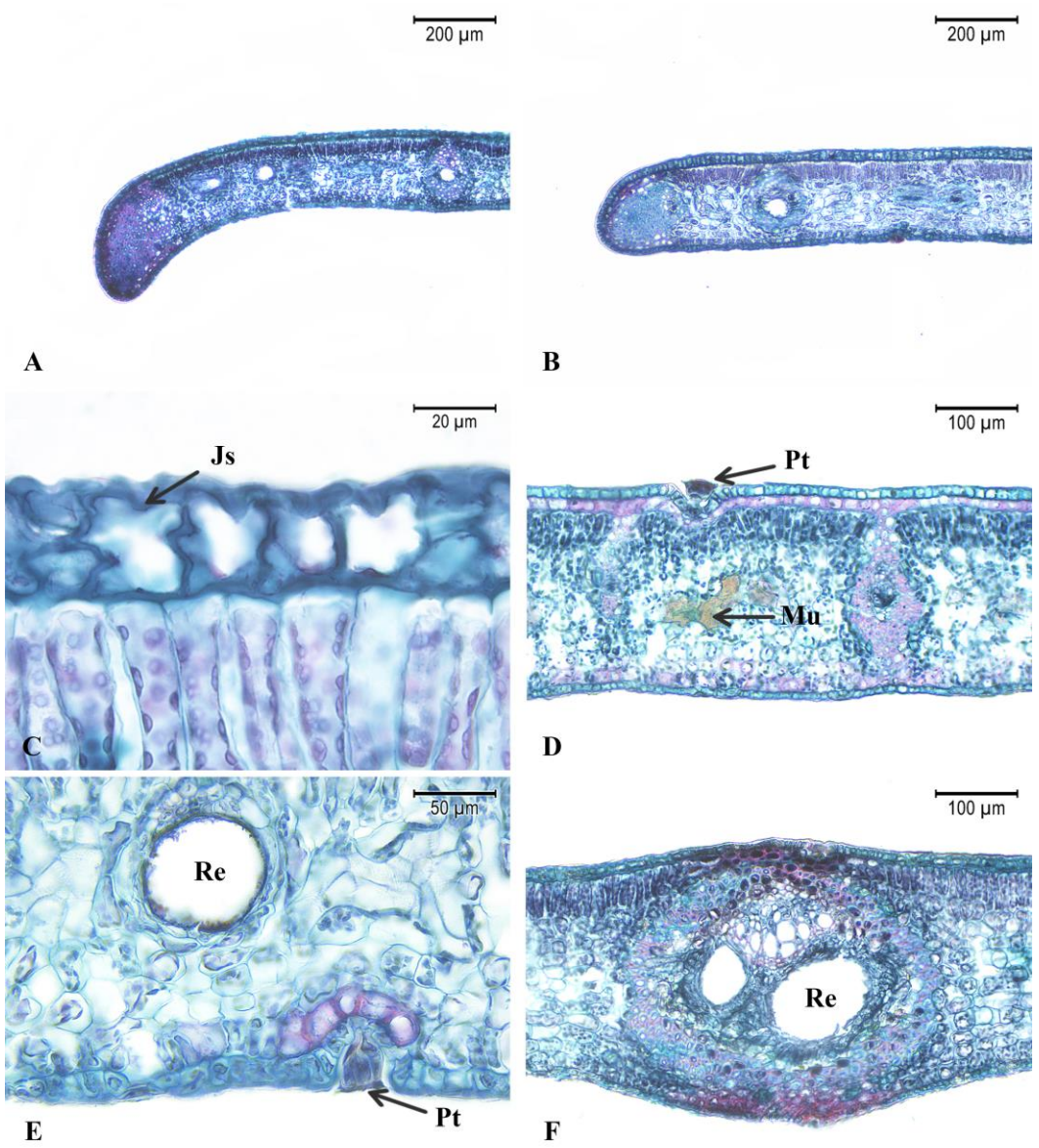

FIGURE 3. Lamina transverse sections: **A.** Downward outline of leaf margin (*M. indica*). **B.** Straight outline of leaf margin (*M. pentandra*). **C.** Jigsaw shape of epidermal cell (*M. pentandra*). **D.** Bundle sheath extension with hypodermis (*M. foetida*). **E.** Grouped fiber beneath sunken peltate trichome (*M. quadrifida*). and **F.** Collateral bundle associated with resin ducts (*M. duperreana*). (Js = Jigsaw shape, Mu = Mucilaginous cell, Pt = Peltate trichome and Re = Resin duct)

peltate trichomes sink down on both epidermis (Fig. 3D, E). On their abaxial leaf surfaces, *M. camptosperm*, *M. odorata*, *M. quadrifida* and *M. foetida* demonstrate grouped fibers beneath the sunken trichomes (Fig. 3E). The vascular bundles are

TABLE 3. Anatomical characteristics of lamina transverse section of studied *Mangifera* species

Species	Leaf margin		The region between midrib and leaf margin									Midrib				
			Mesophyll							Outline						
	Outline	Shape of epidermal cell	Type of leaf	Palisade layers	Hypodermis	Fiber below trichomes	Mucilage cell	Brown inclusion	Crystal	Adaxial	Abaxial	Resin duct	Mucilage cell	Brown inclusion	Crystal	Starch grain
M. caloneura	Dw	Js, Vr	Bf	1	-	-	+	+	Pr	Cv	Cv	5-10	+	+	Pr	+
M. camptosperma	Dw	Js, Sq	Bf	1	-	+	+	+	Pr	Cv	Cv	8	+	+	Pr, Dr	-
M. duperreana	Dw	Js, Sq	Bf	1	-	-	+	+	Pr,	Cv	Cv	6-8	+	+	Pr, Dr	+
M. foetida	Dw	Js, Sq	Bf	1	+	+	+	-	Pr, Dr	Cv	Cv	21-31	+	-	Pr, Dr	-
M. indica	Dw	Js, Vr	Bf	1	-	-	+	-	Pr	Cv	Cv	12-14	+	+	Pr, Dr	-
M. odorata	Dw	Js, Vr	Bf	2-3	-	+	-	-	Pr	Cv	Sc	7	+	+	Pr	-
M. pentandra	St	Js, Sq	Bf	1	-	-	+	+	Pr, Dr	Cv	Cv	10-12	+	+	Pr, Dr	-
M. quadrifida	St	Js, Vr	Bf	2-3	-	+	-	-	Pr, Dr	Cv	Cv	3-6	+	-	Pr	+

Note: - = absent, + = present; **Outline of leaf margin**: Dw = Downward outline, St = Straight outline; **Shape of epidermal cell in leaf margin**: Js = Jigsaw shape, Sq = Square shape, Vr = Vertical rectangular shape; **Type of leaf**: Bf = Bifacial leaf; **Types of crystal**: Dr = Druse crystal, Pr = Prismatic crystal; **Outline of Midrib**: Cv = Convex outline, Sc = Slightly convex outline

collateral bundles (Fig. 3F). The bundle sheath extends to both surfaces which nearly appear at the major vein in place of the minor vein (Fig. 3D). Resin ducts usually present in mesophyll or intercalate with the vascular bundle (Fig. 3E, F). On the other hand, prismatic crystal generally shows in mesophyll cells. Brown inclusion and mucilaginous cells are noted in some species. These are detailed in table 3.

1.4 Midrib transverse section

The abaxial surfaces of *M. odorata* is slightly convex, nonetheless, the regular shape in both surfaces is convex outline (Fig. 4A, B). All studied taxa exhibit sunken peltate trichome (Fig. 4C). Parenchyma and ramiform-pitted sclereid in ground tissue appear beneath epidermis (Fig. 4D). The vascular bundles are collateral bundles which display a complex structure with distal end as a closed ring and surround central pith with intercalated accessory bundle and fibers enclose as the cap of phloem tissue.

Resin ducts usually insert in the outer part of phloem (Fig. 4E), and some species also illustrate these ducts in pith of midrib, *e.g. M. foetida*, *M. indica* and *M. pentandra*. The number of resin ducts varies in each species. Mucilaginous cell and brown inclusion are observed in most of studied species (Fig. 4F), except the absence of brown inclusion in *M. foetida* and *M. quadrifida*. Parenchyma accumulate starch

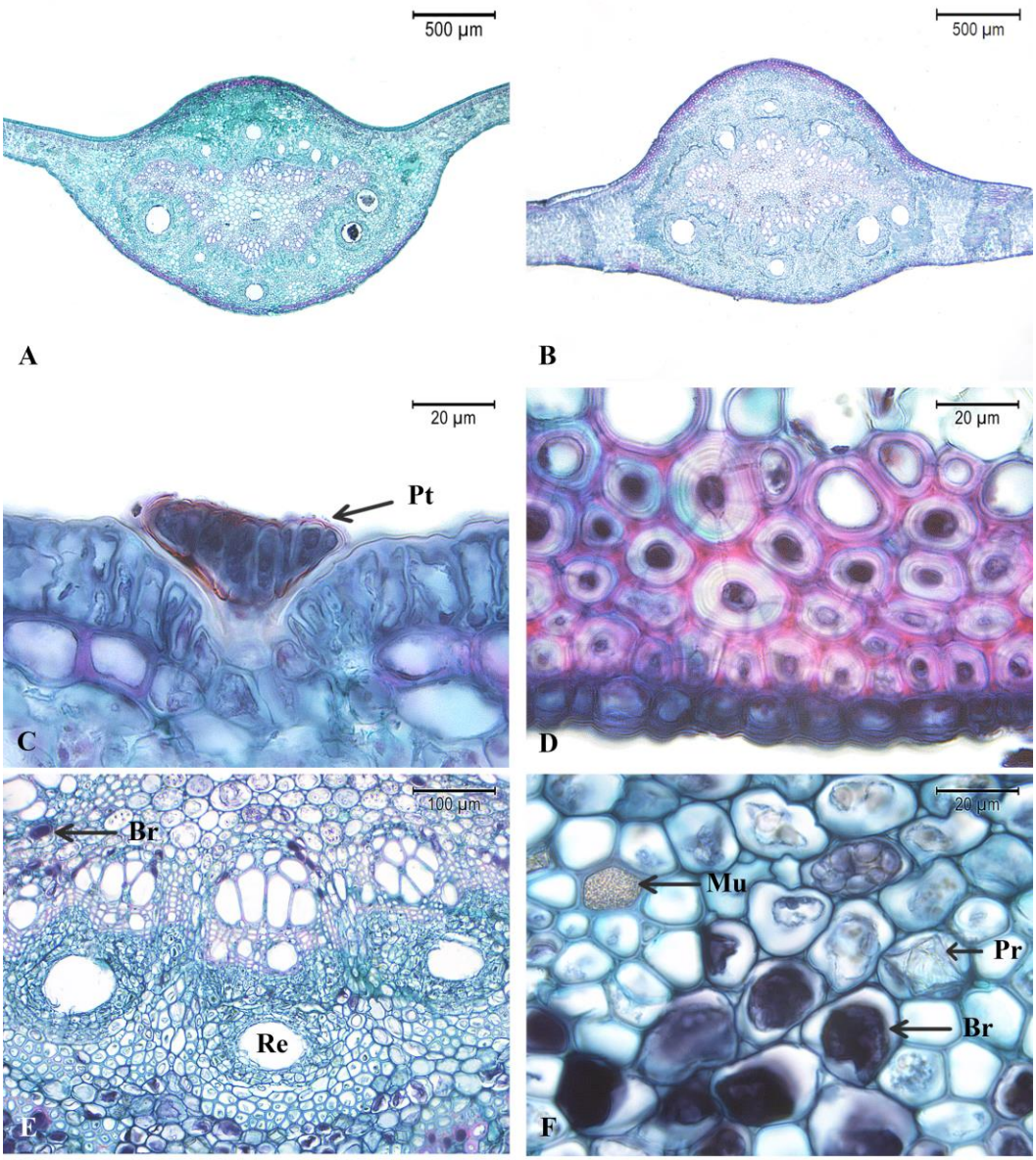

FIGURE 4. Midrib transverse sections: **A**. Convex outline in both surfaces (*M. indica*). **B**. Slightly convex in abaxial surface (*M. odorata*). **C**. Sunken peltate trichome (*M. pentandra*). **D.** Ramiform-pitted sclereid (*M. foetida*). **E.** Association of resin duct in vascular tissues (*M. caloneura*). and **F**. Inclusions (*M. duperreana*). (Br = Brown inclusion, Mu = Mucilaginous cell, Pr = Prismatic crystal, Pt = Peltate trichome and Re = Resin ducts)

as grain forms in *M. caloneura*, *M. duperreana* and *M. quadrifida*. Prismatic and druse crystals exhibit in most species, while *M. caloneura*, *M. odorata* and *M. quadrifida* show only prismatic crystal in their parenchyma cells (Table 3., Fig. 4F).

TABLE 4. Anatomical characteristics of petiolar transverse section of *Mangifera* species

Species	Shape	Trichome	Types of cells	Vascular bundle		Inclusion and Secretory structures						Number of resin ducts
				Number	Shape	Resin duct	Mucilage cell	Brown inclusion	Prismatic crystal	Druse crystal	Starch grain	
M. caloneura	Ci	Pt	Pa, Fi, Sc	1	Ci	+	+	+	+	+	+	11-18
M. camptosperma	Ci	Pt	Pa, Fi, Sc	1	Ci	+	+	+	+	+	+	17-20
M. duperreana	Ci	Pt	Pa, Fi	1	Ci	+	-	+	+	+	-	15-21
M. foetida	Ci	Pt	Pa, Fi, Sc	1	Ci	+	-	-	+	+	+	45-63
M. indica	Ci	Pt	Pa, Fi, Sc	1	Ci	+	+	+	+	+	+	19-23
M. odorata	Pl	Pt	Pa, Fi, Sc	1	Pl	+	+	+	+	+	+	17-25
M. pentandra	Ci	-	Pa, Fi, Sc	1	Ci	+	-	+	+	+	-	17-21
M. quadrifida	Ci	Pt	Pa, Fi, Sc	1	Ci	+	+	+	+	+	+	17-21

Note: - = absent, + = present; **Outline of petiole**: Ci = Circular shape, Pl = Planoconvex in adaxial surface; **Type of trichome**: Pt = Peltate truchome; **Types of cells**: Fi = fiber cell, Pa = Parenchyma cell, Sc = Sclereid cell; **Shape of vascular bundle**: Ci = Circular shape, Pl = Planoconvex outline in adaxial surface

2. Petiole

The outlines of petiolar transverse section are circular in practically species, whilst *M. odorata* indicates planoconvex on adaxial part (Fig. 5A, B). On single-layered epidermis, most studied species occur the sunken peltate trichome (Fig. 5C) but this characteristic absent in *M. pentandra*. Most species, apart from *M. duperreana*, represent parenchyma and ramiform-pitted sclereids in ground tissue (Fig. 5D). Vascular tissues are circular shaped, excluding planoconvex shape in adaxial surface of *M. odorata*. They demonstrate complex structures with distal end as a closed ring and surrounded central pith with intercalated accessory bundle (Fig. 5A, B), where fiber group as cap above phloem tissue.

Resin canals insert in phloem of vascular bundle and ground tissue of petiole in almost studied species (Fig. 5E), especially in *M. foetida*. The mucilaginous cell and brown inclusion show in most species, but some species exhibit only brown inclusion in petiole; *e.g. M. duperreana, M. pentandra* and *M. foetida*. Prismatic and druse crystals can clearly observe in the parenchyma of all studied species. Starch grains illustrate in parenchyma (Fig. 5F), except petiole of *M. dupperreana* and *M. pentandra.* These are detailed in table 4.

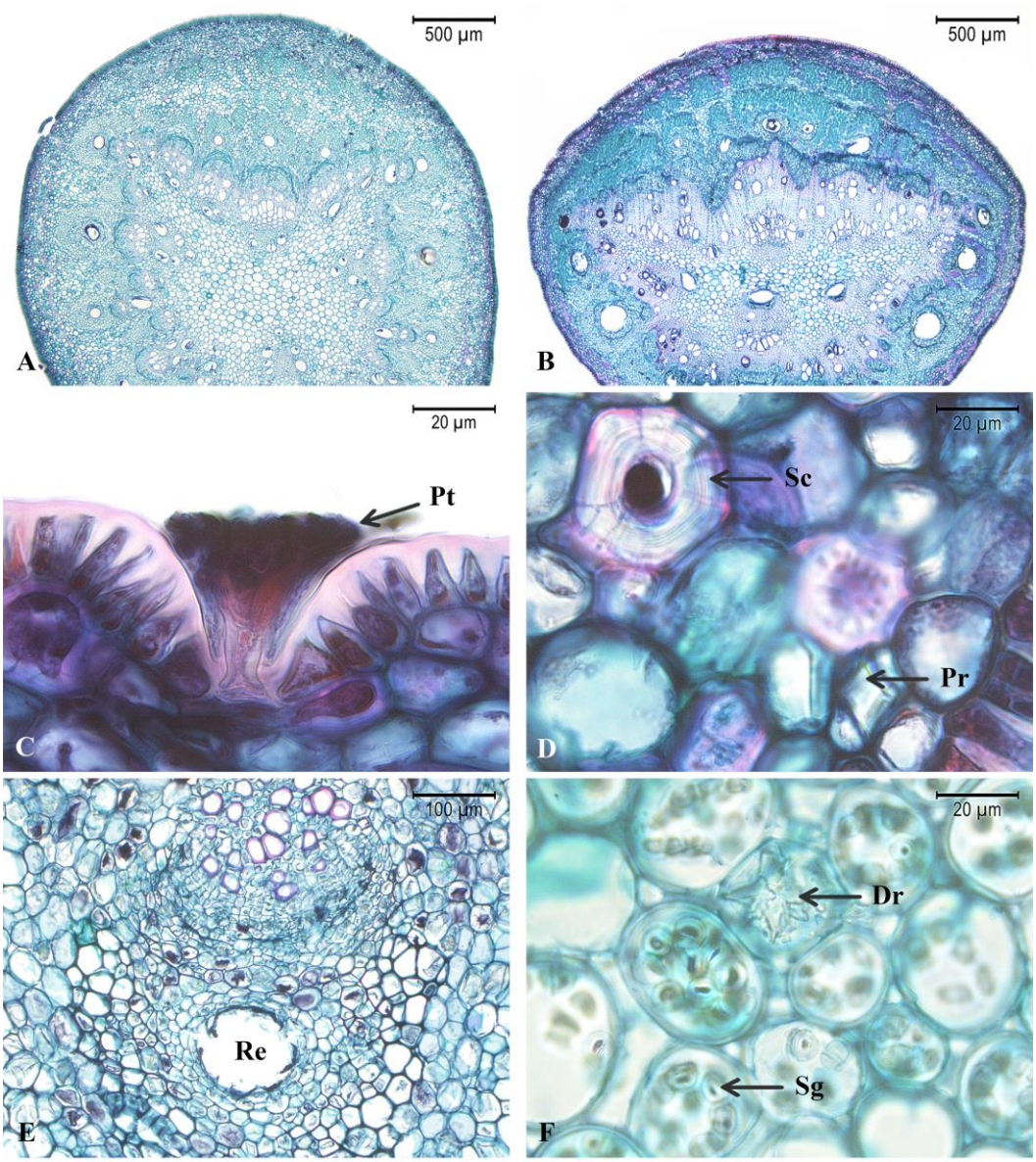

FIGURE 5. Petiolar transverse sections: **A**. Circular shape of petiole (*M. foetida*). **B**. Planoconvex outline in adaxial surface (*M. odorata*). **C**. Sunken peltate trichome (*M. camptosperma*). **D**. Sclereid cell with ramiform pitted (*M. quadrifida*). **E**. Insertion of resin duct in vascular tissue (*M. duperreana*). and **F**. Starch grain and druse crystal (*M. caloneura*). (Dr = Druse crystal, Pr = Prismatic crystal, Pt = Peltate trichome, Re = Resin duct, Sc = Sclereid cell and Sg = Starch grain)

Key to species of *Mangifera* L. for identification based on lamina and petiole anatomical features

1a. Grouped fiber present below the sunken peltate trichome…….…….….. 2.
1b. Grouped fiber absent below the sunken peltate trichome……..……..…….. 5.
2a. Hypodermis present……..…..…… *M. foetida*
2b. Hypodermis absent……...…… 3.
3a. The cell walls of abaxial epidermal cell are slightly undulate.…… *M. camptosperma*
3b. The cell walls of abaxial epidermal cell are straight……...…….. 4.
4a. Brown inclusions present in midrib and adaxial epidermal cell, starch grain absent in petiole…….....…. *M. ordorata*
4b. Brown inclusions absent in midrib and adaxial epidermal cell, starch grain present in petiole.....….. *M. quadrifida*
5a. Ramiform-pitted sclereids absent in petiole, brown inclusions present on abaxial surface ..... *M. duperreana*
5b. Ramiform-pitted sclereids present in petiole, brown inclusions absent on abaxial surface…… 6.
6a. Peltate trichomes absent in petiole……..……. *M. pentandra*
6b. Peltate trichomes present in petiole…….…….……. 7.
7a. Mucilaginous cells present on both surfaces of epidermis, prismatic crystals present only in adaxial epidermis, the number of resin ducts in midrib less than 10…..... *M. caloneura*
7b. Mucilaginous cells present on only adaxial surfaces of epidermis, prismatic crystals absent in epidermis, the number of resin ducts in midrib more than 10…… *M. indica*

DISCUSSION AND CONCLUSIONS

Based on anatomy, leaf epidermal characteristics are informative for species identification, as well as, the features of lamina and petiolar transverse sections are the important data for differentiation in eight studied species; like the appearance of epidermal cell wall, the outline of petiole and the inclusion in the internal structure.

The similar anatomical characteristics among *Mangifera* species are useful for division of genera in Anacardiaceae. The regularly anatomical characteristics of all *Mangifera* are as follows: i) the typical cyclocytic and staurocytic stomata in adaxial and abaxial surfaces, respectively; ii) the amphistomatic leaves; iii) the jigsaw shape with deeply undulate cell wall in adaxial epidermal cell; iv) the presence of sunken peltate trichomes on lamina and midrib; v) the presence of bundle sheath extension to both surfaces; vi) the presence of fiber at the apex of leaf margin, midrib and petiole; vii) the presence of resin ducts; vii) the presence of mucilaginous cells in epidermis and midrib; viii) the presence of prismatic crystals in mesophyll, midrib and petiole. Nevertheless, there are the others characteristics which are helpful for distinguish the studied species.

The deeply undulate cell walls of jigsaw shape and the irregular shape with slightly undulate and straight cell wall are found on adaxial and abaxial surfaces, respectively. Although this finding corresponded partially to investigations by Norfaizal and Latiff

(2013), the oval and round shapes of epidermal cells in Ganogpichayagrai et al. (2016) disagree with epidermal features of this study.

The pattern of stomatal apparatus varies in each plant group, so these characteristics enhance the species identification (Cotthem, 1970). *M. indica* generally had anomocytic stomata (Metcalfe and Chalk, 1957; Norfaizal and Latiff, 2013; Ganogpichayagrai et al., 2016) and recent research notified the actinocytic stomata in same species (Cahyanto et al., 2017). The results from this study disagree with the earlier researches. Moreover, the cyclocytic stomata on adaxial surface and staurocytic stomata on abaxial surface are difference from the anisocytic stomata of *M. odorata*, *M. pentandra* and *M. quadrifida* in Norfaizal and Latiff (2013).

Trichomes occur in epidermis of plants' organs for necessary roles in prevention of losing water, protection by secrete the special substance and attraction the pollinator (Metcalfe and Chalk, 1957). The prior investigation has been instructed that the presence or absence of trichomes, size, color, distribution pattern and type of trichomes can be used as taxonomic characteristics for plant classification (Cooper, 1932; Navarro and Oualidi, 2000; Shaheen et al., 2009). The sunken peltate trichomes are normally remarked on lamina, midrib and petiole of eight studied taxa. Finding peltate trichomes are in agreement with the previous study by Metcalfe and Chalk (1957). As the possession of trichomes, this is contrast with the work of Norfaizal and Latiff (2013) which notified the disappearance of trichomes in *Mangifera* epidermis. The features of sunken peltate trichome in almost studied taxa are considered significant characteristics and may be involved to ecological adaptation (Johnson, 1975; Bibi et al., 2014). Regularly, the limited supply of water induces to modify the anatomy of plant, such as, more cuticular thickness, more trichome density and presence the groove for trichome (Ganong, 1895; Bibi et al., 2014). Therefore, this can assume that the presence or absence of sunken peltate trichome may vary by the plant environment.

Two outlines of leaf margin transverse sections are reported; straight and curve down (Norfaizal and Latiff, 2013). Obtained features from this research of *M. foetida*, *M. indica* and *M. pentandra* correspond to the prior study, nonetheless, the margins of *M. odorata* and *M. qaudrifida* transverse sections differ from the referred research. In this finding, the indication of hypodermal layer represents only in *M. foetida* which disagrees with the earlier investigations by Metcalfe and Chalk (1957) and Norfaizal and Latiff (2013). The attribution of shape and observed location of crystal are useful characteristic in systematics and taxonomy (Franceschi and Hornor, 1980; Franceschi and Nakata, 2005). Prismatic crystals are commonly noted in mesophyll of *Mangifera* in this work. Although the corresponding prismatic crystals of petiolar in *M. indica* of Cahyanto et al. (2017) are indicated, Norfaizal and Latiff (2013) which reported only druse crystals disagrees with this research. As the formation of crystals, plants form the various size and shape of calcium oxalate crystal from derived calcium and synthesized oxalate in environment, besides, the fluctuation of calcium oxalate in the plant environment and physiological process of plant determine the variation of plant crystal (Nakata, 2003; Franceschi and Nakata, 2005).

Characteristics from the midrib transverse sections in this study are in

acceptance with previous study (Norfaizal and Latiff, 2013) that shown convex outlines in almost *Mangifera*, excluding the slightly convex in abaxial surface of *M. odorata* from this study. Identically, closed vascular system and prismatic crystals illustrate in midrib of all studied taxa.

According to Sharma et al. (2012) and Cahyanto et al. (2017), the petiolar transverse sections of *M. indica* varieties appeared the circular shape, 1-layered epidermis, parenchymatous cortex, existence of stone cells, closed vascular system, prismatic and druse crystals and resin ducts that corresponded with present research. As the function of resin canal, the complex mixtures of substance in resin duct resist the herbivorous pest, bacterial invasion and wounding (Mckay et al., 2003; Ferrenberg, 2014). Besides, the complex structures with distal end as a closed ring and surrounded a central pith with intercalated accessory bundle in petiole agree with the results from Metcalfe and Chalk (1957). Noticeably, starch grains in parenchyma cell of *M. indica* petiolar sections have not been reported in the other works.

In conclusion, the classification of *Mangifera* normally based on the morphological or genetic data, however, this information cannot identify *Mangifera* species clearly. Thus, the leaf anatomical characteristics of *Mangifera* species from this work are the valuably informative data for assisting *Mangifera* classification. Although this research does not completely investigate *Mangifera* in Thailand, it gradually increases the fundamentally anatomical perception in this genus and direct the idea for further study. The present study demonstrates the available anatomical feature for delimitation that are as follows: i) the shape of epidermal cell; ii) the outline of leaf margin midrib and petiole; iii) the shape of epidermal cell at the apex of leaf margin; iv) the layer of hypodermis; v) the number of palisade cell layers in lamina; vi) the presence of grouped fiber below sunken trichome; vii) the distribution of peltate trichome; viii) the presence of sclereid with ramiform-pitted in petiole; ix) the inclusion in each organ *e.g.* mucilaginous cell, crystal, starch; x) the number of resin ducts in midrib and petiole. In addition, the number of resin duct are the quatitative data which does not the taxonomic characteristics, so this feature dose not the first priority for construct a key. Nevertheless, the number of resin ducts can support the discrimination between similar *Mangifera* species. In order to illustrate the explicit identification based on the anatomical aspect of *Mangifera* in Thailand, the numerical anatomical analysis and qualitative anatomical data of *Mangifera* that apart from the studied taxa in this work should be applied in the further investigation.

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LITERATURE CITED

Aderibigbe, A.O., Emudianughe, T.S. and Lawal, B.A.S. 2001. Evaluation of the Antidiabetic Action *of Mangifera indica* in Mice. Phytotherapy Research, 15: 456-458.

Bbosa, G.S., Lubega, A., Musisi, N., Kyegombe, D.B., Waako, P.,Ogwal-Okeng, J and Odyek, O.

2007. The Activity of *Mangifera indica* L. Leaf Extracts against the Tetanus causing Bacterium, *Closterium tetani*. African Journal of Ecology, 45(3): 54-58.

Bibi, H., Afzal, M., Muhammad, A., Kamal, M., Ullah, I. and Khan, W. 2014. Morphological and Anatomical Studies on Selected Dicot Xerophytes of District Karak, Pakistan. American-Eutansian Journal of Agricultural & Environmental Sciences, 14(11): 1201-1212.

Cahyanto, T., Sopian, A., Efendi, M. and Kinasih, I. 2017. The Diversity of *Mangifera indica* Cultivars in Sabang West Java Based on Morphological and Anatomical Characteristics. Biosaintifika, 9(1): 156-167.

Chandra, D. and Mukherjee, S.K. 2014. Flora of India: Anacardiaceae. Retrieved July 31, 2017, from http://efloraindia.nic.in/efloraindia/taxonList.action?id=673&type=2.

Chayamarit, K. 2010. Anacardiaceae. In: Flora of Thailand, vol 10 part 3, (K. Chayamarit, eds.). The Forest Herbarium. Bangkok.

Chayamarit, K. 1997. Molecular Phylogenetic Analysis of Anacardiaceae in Thailand. Thai Forest Bulletin (Botany), 25: 1-13.

Cooper, D. C. 1932. The Development of the Peltate hairs of *Shpepherdia Canadensis*. American Journal of Botany, 9(5): 423-428.

Cotthem, W.R.J.V. 1970. A Classification of Stomatal Types. Botanical Journal of the Linnean Society, 63: 235-246.

Ferrenberg, S., Kane, J.M. and Milton, J.B. 2014. Resin Duct Characteristics Associated with Tree Resistance to Bark Beetles Across Lodgepole and Limber Pines. Oecologia, 174: 1283-1292.

Franceschi, V.R. and Horner, H.T. 1980. Calcium Oxalate Crystals in Plants. Botanical Review, 46(4): 361-427.

Franceschi, V.R. and Nakata, P.A. 2005. Calcium Oxalate in Plants: Formation and Function. Annual Review of Plant Biology, 56: 41-71.

Ganogpichayagrai, A., Rungsihirunrat, K., Palanuvej, C. and Ruangrungsi, N. 2016. Characterization of *Mangifera indica* Cultivars in Thailand Based on Macroscopic, Microscopic, and Genetic Characters. Journal of Advanced Pharmaceutical Technology & Reasearch, 7(4): 127-133.

Ganong, W.F. 1895. Present Problems in the Anatomy, Morphology, and Biology of the Cactaceae. Botanical Gazatte, 20(4): 129-138.

Hidayat, T., Pancoro, A. and Kusumawaty, D. 2011. Utility of *matK* Gene to Assess Evolutionary Relationship of Genus *Mangifera* (Anacardiaceae) in Indonesia and Thailand. Biotropia, 18(2): 74-80.

Johansen, D.A. 1940. Plant Microtechnique. The Maple Company, New York and London.

Johnson, H. B. 1975. Plant Pubescence: an Ecological Perspective. The New York Botanical Garden, 41(3): 233-258.

Lersten, N.R. and Curtis, J.D. (2001). Idioblast and Other Unusual Internal Foliar Secretory Structures in Scorphulariaceae. Plant Systematics and Evolution, 227: 63-73.

Mckay, S.A.B., Hunter, W.L., Godard, K., Wang, S.X., Martin, D.M., Bohlmann, J. and Plant, A.L. 2003. Insect Attack and Wounding Including Traumatic Resin Duct Development and Gene Expression of (-)-Pinene Synthase in Sitka Spruce. Plant Physiology, 133: 368-378.

Metcalfe, C.R. and Chalk, L. 1957. Anatomy of the Dicotyledons, vol 2. Oxford University Press, London.

Nakata, P.A. 2003. Advances in our Understanding of Calcium Oxalate Crystal formation and Function in Plants. Plant Science 164: 901-909.

Navarro, T. and El Oualidi, J. 2000. Trichome Morphology in *Teucrium* L. (Labiatae). a Taxonomic Review. Anales del Jardin Botanico de Madrid, 57(2): 277-297.

Norfaizal, M. and Latiff, A. 2013. Leaf Anatomical Characteristics of *Bouea*, *Mangifera* and *Spondias* (Anacardiaceae) in Malaysia. Journal of Life Sciences, 8(9): 758-767.

Parvez, G.M. 2016. Pharmacological Activities of Mango (*Mangifera indica*): A Review. Journal of Pharmacognosy and Phytochemistry, 5(3): 1-7.

Perpeteuo, G.F. and Salgado, J. M. 2003. Effect of Mango (*Mangifera indica* L.) ingestion on blood glucose levels of normal and diabetics rats. Plants Foods for Human Nutrition, 58: 1-12.

Phongkrathung, R. and Kermanee, P. 2013. Anatomy and Some Properties of Woods in *Mangifera indica* L., *M. foetida* Lour. And *M. caloneura* Lour. Thai Journal of Botany, 5(Special Issue): 133-141.

Shabani, Z. and Sayadi, A. 2014. The Antimicrobial in Vitro Effects of Different Concentrations of some Plant Extracts including Tamarisk, March, Acetone and Mango Kernel. Journal of Applied Pharmacoceutical Science, 4(5): 75-79.

Shaheen, N., Ajab, M., Yasmin, G. and Hayat, M.Q. 2009. Diversity of Foliar Trichomes and their Systematics Relevance in the Genus *Hibiscus* (Malvaceae). International Journal of Agriculture & Biology, 11: 279-284.

Sharma, B.G., Albert, S. and Dhaduk, H.K. 2012. Petiolar Anatomy as an Aid to the Identification of *Mangifera indica* L. Varieties. Notulae Scientia Biologicae, 4(1): 44-47.

Tianlu, M. and Barfod, A. 2008. Flora of China: Anacardiaceae. Retrieved July 31, 2017, from http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=10038

Wannan, B.S. 2006. Analysis of Generic Relationships in Anacardiceae. BLUMEA, 51: 165-195.

Wannan, B.S. and Quinn, C. J. 1991. Floral Structure and Evolution in the Anacardiaceae. Batanical Journal of the Linnean Society, 107: 349-385.

Yonemori, K., Honsho, C., Kanzaki, S., Eidthong, W. and Sugiura, A. 2002. Phylogenetic Relationships of Mangifera Species Revealed by ITS Sequences of Nuclear Ribisomal DNA and a Possibility of their Hybrid origin. Plant Systematics and Evolution, 231: 59-75.