TAXONOMIC VALUES OF TRICHOMES TYPES IN MALVACEAE SUBFAMILY BOMBACOIDEAE AND HELICTEROIDEAE

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Abstract. A study was conducted on 24 selected species of *Bombax, Coelostegia, Durio, Kostermansia, Ochroma* and *Pachira* from the Malvaceae subfamily Bombacoideae and Helicteroideae. Objective of this study was to identify leaf micromorphological characteristics which can be used as secondary data for scientific research especially in species, genus and family classification. Observation was conducted under electron scanning microscope on adaxial and abaxial epidermal surface of gold coated dried leaves with various magnification. Results showed there was some significant variation in the types of trichomes of all species studied. These trichome characteristics have taxonomic value and can be used for classifications up to species and genus level. Lepidote trichome was seen as a common character in *Coelostegia, Durio, Kostermansia, Ochroma* and *Pachira* but not in *Bombax*. Capitate glandular trichomes with multiseriate terminal (spathulate terminal lobes) could only be found on adaxial epidermal of *B. anceps* and dendritic trichomes could only be found in *D. affinis*. Trichome diversity in *Pachira* was even more unique as seen in three-armed trichomes (swollen in the middle arm) and non-cushioned stellate trichomes (short armed, thin wall, swollen in the middle and at the end). In conclusion, trichomes have taxonomic value in Malvaceae subfamily Bombacoideae and Helicteroideae.

Keywords: *Bombax, durio, kostermansia*, leaf micromorphology, bombacaceae

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Introduction

Order Malvales consisted of Malvaceae, Tiliaceae, Bombacaceae and Sterculiaceae which share common morphological characteristics [1]. Malvales order had five families, namely Malvaceae, Sterculiaceae, Tiliaceae, Elaeocarpaceae and Bombacaceae [2,3]. Bombacaceae was placed under Malvales along with five other families, Malvaceae, Sterculiaceae, Tiliaceae, Elaeocarpaceae and Scytopetalaceae which included over 3500 species with Malvaceae having the largest number of species, over 1500 species [4]. There were only four families Malvaceae, Tiliaceae, Bombacaceae and Sterculiaceae were in the order Malvales [5,6]. Malvales consisted of eight families, Scytopetalaceae, Elaeocarpaceae, Tiliaceae, Sterculiaceae, Bombacaceae, Malvaceae, Sphaerosepalaceae and Sarcolaenaceae [7]. Whereas Malvales consists of six families consist of Sterculiaceae, Elaeocarpaceae, Tiliaceae, Sphaerosepalaceae, Malvaceae and Bombacaceae [8].

A molecular study using the plastid DNA sequences atpB and rbcL showed Sterculiaceae, Tiliaceae, Bombacaceae and Malvaceae were classified accordingly under the order Malvales and divided these four families into nine subfamilies based on molecular, morphological and biogeographic data. *Durio, Neesia, Coelostegia, Kostermansia, Cullenia* and *Boschia* have been placed under the tribe Durioneae which belongs to the subfamily Helicteroideae. Meanwhile, the traditional family Bombacaceae has been reclassified into Bombacoideae and the genus *Bombax, Ochroma* and *Pachira* were placed under this subfamily [9]. *Boschia, Coelostegia, Cullenia, Durio, Kostermansia* and *Neesia* were placed under the Durionaceae Cheek fam. nov. *Bombax, Ochroma* and *Pachira* still remain in the Bombacaceae [10].

Malvales were divided into Bombacaceae, Byttneriaceae, Brownlowiaceae, Durionaceae, Helicteraceae, Malvaceae, Pentapetaceae, Sparrmanniaceae, Sterculiaceae and Tiliaceae. *Boschia, Coelostegia, Cullenia, Kostermansia, Neesia* and *Durio* are now placed under a new family namely Durionaceae which includes the 'durian' family found in Southeast Asia meanwhile other genera still remain in Bombacaceae (subfamily Durioneae) [11]. A study on wood anatomy of the Malvaceae subfamilies Bombacoideae and Helicteroideae also supported a classification based on DNA molecules that isolates *Bombax* (Bombacoideae) from the subfamily Helicteroideae [12].

Bombacaceae has bright flowers and woody fruit, small seed, silky or cotton-like fibers. However, it is different for the fruit of *Durio zibethinus* L. which has large seeds coated with fleshy aryl [13,14]. *Coelostegia, Durio* and *Neesia* had very similar morphology and suggested *Coelostegia* and *Neesia* should be combined [15]. Combination of the three genera was disagreed because there were clear differences in the characteristics of leaves, trichomes, flowers, fruits and seeds [16]. Bombacaceae was once placed as a tribe of Bombaceae in the Malvaceae [2]. Earlier Bombaceae consisted of three subtribes (including Durioneae) but then formed to Bombacaceae by changing the status of the three subtribes (Adansonieae, Matisieae, Durioneae) to tribes. This division was supported by differences in leaf morphological features for these three tribes. Adansonieae have palmate compound leaves, whereas Matisieae and Durioneae have simple leaves, where the Matisieae have leaves with palmate venation while Durioneae have secondary venation rise from primary venation [17,18].

A study conducted on three genera of the Malvaceae showed that evolutionarily and phyletically, the presence of scaly peltate trichomes would indicate the plant to be the most primitive. Scaly peltate trichomes evolved for adaptation to their environment by undergoing massive changes in morphology, cell elongation or several arms arising from base forming stellate trichomes or clustered trichomes [19]. Flat-walled glandular trichomes, three-armed trichomes (swollen arm in the middle), non-cushioned stellate trichomes (swollen arm at base and ends) and cushioned three-armed trichomes were only found in *Pachira*. Thin-walled glandular trichomes, unicellular short trichomes and 2-8-armed trichomes were only found in *Ochroma*. Multicellular capitate glandular trichomes, capitate glandular trichomes, dendritic trichomes, stellate trichomes, rotate stellate trichomes, lepidote stellate trichomes and dentate trichomes were only found in *Durio*. Multicellular terminal glandular trichomes (spathulate-like terminals) were only observed to be present on the adaxial surface of the *Bombax*'s midrib.

Trichomes serve as one of the mechanical defense systems in plants against insect attacks [20]. Trichomes in *Corchorus* (Tiliaceae) were far different from trichomes observed in Tiliaceae, while for *Muntingia* (Muntingiaceae), the type of trichomes were more similar to the type of trichomes in Tiliaceae [21]. Malvales showed that there were eight common types of trichomes shared between Malvaceae, Tiliaceae and Sterculiaceae while only five types of trichomes were found in Bombacaceae. It was therefore proven that trichome types have taxonomic value and can be used for identification at the genus level [22].

Materials and Methods

Fresh and dried leaf samples were obtained from the FRIM Herbarium in Kepong (KEP), the MARDI Herbarium (MDI), and several forest reserves in Peninsular Malaysia (Table 1). Voucher specimens were kept at the Universiti Kebangsaan Malaysia Herbarium (UKMB) in Bangi. Fresh leaf specimens collected were fixed in AA (a ratio of 1:3 of 70 % ethanol to 30 % Acetic Acid). Whereas dried leaves were boiled and also kept in an AA solution [23,24]. The studied leaf sections (lamina) were cut to a size of 1 cm x 1 cm and soaked in Jeffrey solution for a few hours until the tissues were peeled to obtain a thin layer of leaf epidermal. The epidermal layer was then rinsed with distilled water 2-3 times and then soaked in Safranin and Alcian Blue solution for 24 hours. After that, the sample sections were rinsed with water and dehydrated in a series of alcohol concentrations: 50 %, 70 %, 95 %, and finally 100 %. Finally, the samples were mounted on a microscope slide using Euparal as a permanent medium. Then it was kept in a drying oven at 40 ^{o}C for two weeks. The methods used in the micromorphology study were modified by Barthlott [25]. A 1 cm x 1 cm piece of adaxial and abaxial lamina was cut from the leaf and then fixed onto a stub by double-sided tape in a specimen basket. These small pieces of both parts of the lamina were then coated with a thin layer of gold using a plating machine (Model SC 500) to improve their electrical conducting properties. The gold coating step was performed using a sputter coater and examined under a variable pressure scanning electron microscope (Philips XL Series XL 30).

Table 1: List of Bombacaceae species studied

Species	Code	Locality	Collector	Date	Sources & notes
Bombax anceps	FRI 12941	Pulau Langkawi, Kedah	T.C.Whitmore	19.12.69	Herbarium specimen FRIM, Kepong.
	VB 2201	Pulau Bumbun Besar, Langkawi	Dr. Van Balgooy	16.12.74	Herbarium specimen UKMB, UKM, Bangi
	AZ 5233	Hutan Simpan Sarawak, tepi jalan	A. Zainidun, Hamid Salleh, Zulkifli Mohamad	11.08.94	
Coelostegia borneensis	KEP 94890	Compt 11, Sarawak , Bako	Dr. Majer dan S.F.C. Yong	30.05.62	Herbarium specimen FRIM, Kepong.
	S 24320	National Park, Kuching, Sarawak	P.S. Ashton	14.07.66	
	SK 527	Telok Asam, Bako National Park, Kuching Sarawak	S.K. Yap	23.02.74	
Coelostegia chartacea	SAN 96767	Sg. Bole, Taliwas, Sabah,	Y.F. Lee	06.05.83	Herbarium specimen FRIM, Kepong.
	S 35997	Gunung Santubong, Telok Berlian, Sarawak	Illias dan Jugah	25.04.78	
Coelostegia griffithii	FMS 24445	H. Simpan Sungai Buloh,	Symington	02.10.30	Herbarium specimen FRIM, Kepong.
	FRI 6502	Selangor Hutan Kemabang Kelantan, Kelantan	S. Chelliah	28.06.68	
	SFN 37112	Mandai Road, Singapura	E.J.H. Corner	22.07.40	
Durio affinis	S 31597	Borneo, Sarawak,	Ilias Paie	04.07.72	Herbarium specimen FRIM, Kepong.
	KEP 80086	Kuching. Borneo, Brunei		22.07.54	
Durio carinatus	NT 204	Hutan Nenasi, Pekan, Pahang	Noraini Talip, Mohd Ruzi, Noor Solihani	10.11.07	Fresh specimen
	AZ 1077	Selangor, Genting Sempah, Ridge Trail	A. Zainudin	01.11.83	Herbarium specimen UKMB, UKM, Bangi
Durio dulcis	*NSS 24*	Stesen penyelidikan MARDI,	Noor Solihani	01.06.09	Fresh specimen

		Serdang, Selangor			
Durio excelsus	Kostermans 13513	Borneo, Kalimantan	A. Kostermans	15.08.56	Herbarium specimen FRIM, Kepong.
	Kostermans 12554	Borneo, Kalimantan, Mt. Maranga	A. Kostermans	28.07.56	Herbarium specimen FRIM, Kepong.
Durio grandiflorus	SAN 86886	Borneo, Sabah, Sandakan	WSP, Aban et al.	22.07.77	Herbarium specimen FRIM, Kepong.
	S 24654	Borneo, Sarawak, Kuching, Semenggoh Arboretum	Jusah, Banyeng dan Rosli	16.09.65	Herbarium specimen FRIM, Kepong.
Durio graveolens	*AZ 3668*	Kuala Lumpur, Universiti Malaya, Rimba Ilmu	A. Zainudin	09.07.91	Herbarium specimen UKMB, UKM, Bangi
	NSS 25 *NSS 28*	Stesen penyelidikan MARDI, Serdang, Selangor	Noor Solihani & Nur Shuhada	01.06.09	Fresh specimen
Durio griffithii	*A. LATIF F 4125*	Kelantan, Kuala Krai, Taman Negara	Abdul Latiff, A. Zainudin, Bedul, Shauzi	28.03.95	Herbarium specimen UKMB, UKM, Bangi
	NSS 18	Denai Regenerasi, Pasoh, Negeri Sembilan	Noor Solihani, Abu Hussin, Nurhanim, Che Nurul Aini, Agus	15.10.08	Fresh specimen
	NT 92	Arboretum FRIM, Kepong, Selangor	Noraini Talip, Noor Solihani	27.07.07	Fresh specimen
Durio kinabaluensis	JHB 7044	Sabah, Daerah Keningau: Banjaran Crocker, 19 km barat Keningau, 13.5 batu ke jalan Kimanis	John H. Beaman, Reed S. Beaman, Teofila E. Beaman	23.09.83	Herbarium specimen UKMB, UKM, Bangi
Durio kutejensis	*AZ 3672*	Kuala Lumpur, Universiti	A. Zainudin & Mustaffa	9.11.91	Herbarium specimen UKMB, UKM, Bangi

		Malaya, Rimba Ilmu			
	NSS 22	Stesen penyelidikan MARDI, Serdang, Sel	Noor Solihani, Nur Shuhada	01.06.09	Fresh specimen
Durio lowianus	NSS 25	Stesen penyelidikan MARDI, Serdang, Selangor	Noor Solihani, Nur Shuhada	01.06.09	Fresh specimen
	NSS 43 NSS 44	Jabatan Pertanian Stesen Pembangunan Komoditi Hulu Paka, Dungun, Terengganu	Noor Solihani, Izlamira, Sani Miran	23.02.2010	Fresh specimen
Durio malaccensis	*NSS 004* *NSS 38*	Sungai Tekala, Selangor, Bkt. Lagong, Kompartmen 18, Hutan Lipur Orang Asli, Kepong, Selangor	Noor Solihani Mohamad Ruzi Noor Solihani, Abu Hussin, Fitri	01.08.09 28.10.09	Fresh specimen
Durio macrophyllus	NT 192	Trek PPTC, Tasik Chini, Pahang	Noraini Talip, Noor Solihani dan Mohamad Ruzi	08.11.07	Fresh specimen
	AZ 3903	Terengganu, Ulu Dungun	A. Zainudin, M. Rosli, S. Muzmi	21.10.91	Herbarium specimen UKMB, UKM, Bangi
Durio oxleyanus	NT 134 NT 137	Hutan Simpan Bangi, Selangor	Noraini Talip, Noor Solihani dan Mohamad Ruzi	19.08.07	Fresh specimen
	NSS 23 *NSS 29*	Stesen penyelidikan MARDI, Serdang, Selangor	Noor Solihani & Nur Shuhada	01.06.09	Fresh specimen
Durio pinangianus	NSS 30 NSS 31 NSS 32	Hutan Lipur Bukit Hijau, Kulim, Kedah Sungai Sedim, Kedah	Noor Solihani, Mohamad Ruzi, Samiah, Sani	23.11.08 24.11.08	Fresh specimen

	NSS 36				
Durio singaporensis	*NSS 16*	Petak 392628, Hutan Simpan Pasoh, Negeri Sembilan, Denai Regenerasi,	Noor Solihani, Abu Hussin, Nurhanim, Che Nurul	14.10.08	Fresh specimen
	NSS 17	Pasoh, Negeri Sembilan	Aini, Agus	15.10.08	Fresh specimen
Durio singaporensis	*AZ 3764*	Johor, Bandar Tenggara, Sg. Projek Empangan Linggi, Hutan Dipterocarpa Tanah rendah	A. Zainudin & Hamid Salleh	26.07.91	Herbarium specimen UKMB, UKM, Bangi
Durio testudinarum	*NSS 27*	Stesen Penyelidikan MARDI, Serdang, Selangor	Noor Solihani & Nur Shuhada	01.06.09	Fresh specimen
Durio zibethinus	*AZ 3679*	Selangor, UKM, Taman Pantun	A. Zainudin	16.7.91	Herbarium specimen UKMB,
	AZ 3674	Negeri Sembilan, Juaseh, Jalan ke Bahau, tepi jalan		14.7.91	UKM, Bangi
	NSS 21	Stesen penyelidikan MARDI, Serdang, Selangor	Noor Solihani	01.06.09	Fresh specimen
Kostermansia malayana	FMS 47303	Hutan Simpan Bikam, Perak	C. F. Symington	18.10.38	Herbarium specimen FRIM, Kepong.
	FMS 50651	Kelantan	Salim	11.05.40	Herbarium specimen FRIM, Kepong.
	FRI 12252	Layang-layang, Johor	Wong Yew Kwan	01.05.69	Herbarium specimen FRIM, Kepong.
Ochroma lagopus	KEP7 0403	Selangor, Kepng, FRIM, Field 14	F. G. Moten	25.02.52	Herbarium specimen FRIM, Kepong.
	KEP2 6730	Sandakan, Sepilok FR, Comp. 13	Mat Asri Ngah Sanah Kasir	20.03.97 28.12.48	
	A 983				Herbarium specimen FRIM, Kepong.

Pachira aquatica	FRI26 734 PW 124	Selangor, FRI	Mat Asri Ngah Sanah	25.08.99 09.02.01	Herbarium specimen FRIM, Kepong.

Results and Discussion

There were more than 30 types of trichomes found by observation under the scanning electron microscope as shown in Table 2. The types of trichomes found in *Bombax, Coelostegia, Durio, Kostermansia, Ochroma* and *Pachira* were glandular trichomes, simple trichomes, armed trichomes (2-3 armed), four-armed trichomes, stellate trichomes, rotate stellate, lepidote stellate, dentate, lepidote and dendritic trichomes. Almost all *Durio* species studied had five layers of trichomes on the abaxial epidermis. The first layer consisted of glandular trichomes. The second and third layers were stellate trichome, rotate stellate, lepidote stellate and dentate trichomes. The fourth and fifth layers consisted of lepidote trichomes. The types of trichomes presence in *Coelostegia* and *Ceiba* (Bombacaceae) were found useful to distinguish both genera [26]. Variation, presence and types of trichomes present on the adaxial and abaxial epidermal leaf surfaces of *Durio* were very useful for distinguishing between *Durio* species [27]. Overall, complex trichomes described in this study were determined based on Webster et al. [28]. For example the description of a stellate trichome is according to the percentage of the radius of the trichome. Radial adhesion of 15–30 % is for rotate stellate trichomes, 30–50 % adhesion is for lepidote stellate trichomes, 50–80 % adhesion is for dentate trichomes and 80–100 % radial adhesion is for lepidote trichomes [28]. The presence and type of trichomes are also diagnostic features for some species of Acanthaceae and are very useful for identification up to the species level [29].

A micromorphological study on several *Melastoma* species found the presence of non glandular trichomes was significant for species differentiation with a combination of other features [30]. *Salvia smyrnea* (Lamiaceae) had glandular trichomes containing essential oils scattered on the vegetative surface as well as on the reproductive organs [31]. Glandular trichomes have stalks with glandular or non glandular heads that undergo elongation which contained protein compounds or secreted material for numerous functions [32]. Two types of glandular trichomes were found in this study peltate glandular trichomes and capitate glandular trichomes. Result showed, *B. anceps* had capitate glandular trichomes (multicellular stalks and terminals) (Figure 2(m)) and capitate glandular trichomes with multiseriate terminals (spathulate-shaped lobes terminals) (Figure 2(n)) was present at the adaxial of the midrib and proved by observation under light microscope (Figure 2(w)) but were not observed in other species or genera studied. Whereas, *C. griffithii* has multicellular terminal peltate glandular trichomes. The presence of glandular trichomes such as a swollen knob shape is an important feature for anatomical classification [32]. This glandular trichome refers to the peltate glandular trichome (Figures 2(o) to (s) and (x) to (z)) found in all *Durio* species except *D. lowianus*. Peltate glandular trichomes are diagnostic characteristics found in *Plecnomia olivacea* and are useful for identification at species level [33]. Multicellular capitate glandular trichomes (Figures 2(g) to (j), (t) and (u)) were found in all *Durio* species except in *D. excelsus* and *D. kinabaluensis*. For *O. lagopus* and *P. aquatica* capitate glandular trichomes were found, with *O. lagopus* having stalked glandular trichomes (Figure 2(k)) while *P. aquatica* had capitate glandular trichomes (Figure 2(l)) as well as multicellular terminal peltate glandular trichomes.

Table 2: Types of trichomes observed on the surface of leaf epidermis

Types of trichomes	Spesies	*B. anceps*	*C. borneensis*	*C. chartacea*	*C.griffithii*	*D. affinis*	*D. carinatus*	*D. dulcis*	*D. excelsus*	*D. grandiflorus*	*D. graveolens*	*D. griffithii*	*D.kinabaluensis*	*D. kutejensis*	*D. lowianus*	*D. macrophyllus*	*D. malaccensis*	*D. oxleyanus*	*D. pinangianus*	*D. singaporensis*	*D. testudinarum*	*D. zibethinus*	*K. malayana*	*O. lagopus*	*P. aquatica*
KPTM					/	/	/	/	/	/	/	/	/	/		/	/	/	/	/	/	/			/
KKM						/	/	/		/	/	/		/	/	/	/	/	/	/	/	/			
KKTa TeM		/				/																	/	/	/
KKTa TeM (DPi)																									/
KKTe Mul		/																							
Simple								/						/										/	/
two-armed								/			/			/			/		/					/	
three-armed	(LPaDN)																							/	
four-armed	LPeMe TB (LB)						/																		/
	TB (LPeDT)												/												/
	B (LPaDN)																			/					
	B (LPeDN)													/							/				
	B (LPeDT)						/										/					/			
Stellate	STB(Lpe BDN)						/																	/	
	STB (LPeDT)						/									/				/					/
	STB(LPe MeDT)																								/
	STB (LpeDN)								/	/															
	SB (LPeDT)					/			/			/			/	/	/	/	/			/		/	/
	SB (LPaDN)										/														
	SB(LPeB DN)												/				/				/				
	SB (LPeDN)													/											
Stellate rotate							/	/	/	/	/	/	/	/	/	/	/	/		/		/			
	SRTB(LP eBDT)											/													
	SRB(LPe BDN)																				/				
Lepidote stellate						/		/	/	/			/	/	/	/	/	/	/		/	/			
	SLB(LPe DP)												/												
	SLB(LPe BDN)																				/				
Dentate						/			/	/		/	/		/	/	/	/	/		/	/		/	
Lepidote			/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/		
Dendritic	De (LPDB)					/																			
	De (LM)					/																			

Key:
LPaDN – three-armed (long armed, thin wall); LPeMe – three-armed (short armed, swollen in the middle); KPTM – multicellular terminal peltate glandular; KKM – multicellular capitate glandular; KKTaTeM – capitate glandular trichome (multicellular stalks and terminals); KKTaTeM (DPi)- capitate glandular trichome (multicellular stalks and terminals, flat wall); KKTeMul- capitate glandular trichome with multiseriate terminals (spatula-shaped lobes terminals); TB(LB) - Non-cushioned four-armed trichome (grooved armeds); TB(LPeDT) - non-cushioned four-armed trichome (short armed, thick wall); B(LPaDN) - cushioned four-armed trichome (long armed, thin wall); (LPeDN) - cushioned four-armed trichome (lengan pendek, dindng nipis); B(LPeDT) - cushioned four-armed trichome (short armed, thick wall); STB (LPeBDN) - Non-cushioned stellate (short grooved armed, thin wall); STB (LPeDN) - Non-cushioned stellate (short armed, thin wall); STB (LPeDT) - Non-cushioned stellate (short armed, thick wall); STB(LPeMeDT)- Non-cushioned stellate (swollen short armed, thick wall); SB (LPeDT) - Cushioned stellate (short armed, thick wall); SB (LPaDN) - Cushioned stellate (long armed, thin wall); SB (LPeBDN) – Cushioned stellate (short grooved armed, thin wall); SB (LPeDN) - Cushioned stellate (short armed, thin wall); SRTB(LPeBDT) - Non-cushioned rotate stellate (short grooved armed, thick wall); SRB (LPeBDN) - Cushioned rotate stellate (short grooved armed, thin wall); SLB (LPeDP) – Cushioned lepidote stellate (short armed, thin wall); SLB (LPeBDN) – Cushioned lepidote stellate (short grooved armed, thin wall); De (LPDB) – Dendritic (long armed, grooved wall); De (LM)- Dendritic (swollen armed).

A study on the trichome type in *Durio* reported that the density of glandular trichomes could not be used in species identification but scaly peltate trichome was found very useful for distinguishing *D. malaccensis* from *D. Macrophyllus* [27]. Unique unicellular simple trichomes (Figures 1(s) and (t)) present in *D. dulcis, D. kutejensis, O. lagopus* and *P. aquatica*, could be used in classification.

Two-armed trichomes (Figure 1(u)) were observed in *D. dulcis, D. graveolens, D. kutejensis, D. malaccensis, D. pinangianus* and *O. lagopus*. Three-armed trichomes (short armed, swollen in the middle) (Figure 1(w)) were observed in *P. aquatica*. Three-armed trichomes (long armed, thin wall) were observed on the leaf epidermis of *O. lagopus* (Figure 1(v)). The four-armed trichomes were divided into two types, cushioned and non-cushioned . Non-cushioned four-armed trichomes (grooved arm) (Figure 2(a)) were observed in *D. carinatus*, and cushioned four-armed trichomes (short armed, thick wall) were observed on the abaxial epidermis of *D. carinatus, D. malaccensis* and *D. zibethinus* (Figure 2(b)). Non-cushioned four-armed trichomes (short armed, thick wall) (Figure 2(d)) were observed in *D. kinabaluensis* and *P. aquatica*, while cushioned four-armed trichomes (long armed, thin wall) were found in *D. singaporensis* (Figure 2(c)). Four-armed cushioned trichomes (short armed, thin wall) were observed in *D. kutejensis* and *D. testudinarum*. The armed trichomes were determined to be either cushioned or non-cushioned through observation under scanning electron microscope. Armed trichomes were also observed under the light microscope because their determination requires a 3-dimensional view.

Various forms of stellate trichomes were observed in almost all species studied except in *B. anceps, Coelostegia* and *Kostermansia*. Cushioned stellate trichomes (short armed, thick wall) (Figure 1(n)) were observed in *D. affinis, D. excelsus, D. griffithii, D. lowianus, D. macrophyllus, D. oxleyanus, D. pinangianus, D. zibethinus, O. lagopus* and *P. aquatica*. Cushioned stellate trichomes (long armed, thin wall) (Figure 1(m)) were observed in *D. graveolens*, while cushioned stellate trichomes (short armed, thin wall) were observed in *D. kutejensis*. Cushioned stellate trichomes (short grooved armed, thin wall) were observed in *D. kinabaluensis, D. malaccensis* and *D. testudimarum*. Non-cushioned stellate trichomes (short grooved arm, thin wall) (Figure 1(o)) were observed in *D. carinatus* and *O. lagopus* as well as non-cushioned stellate trichomes (short arm swollen in the middle and at the ends, thick wall) were observed only in *P aquatica* (Figure 1(q)).

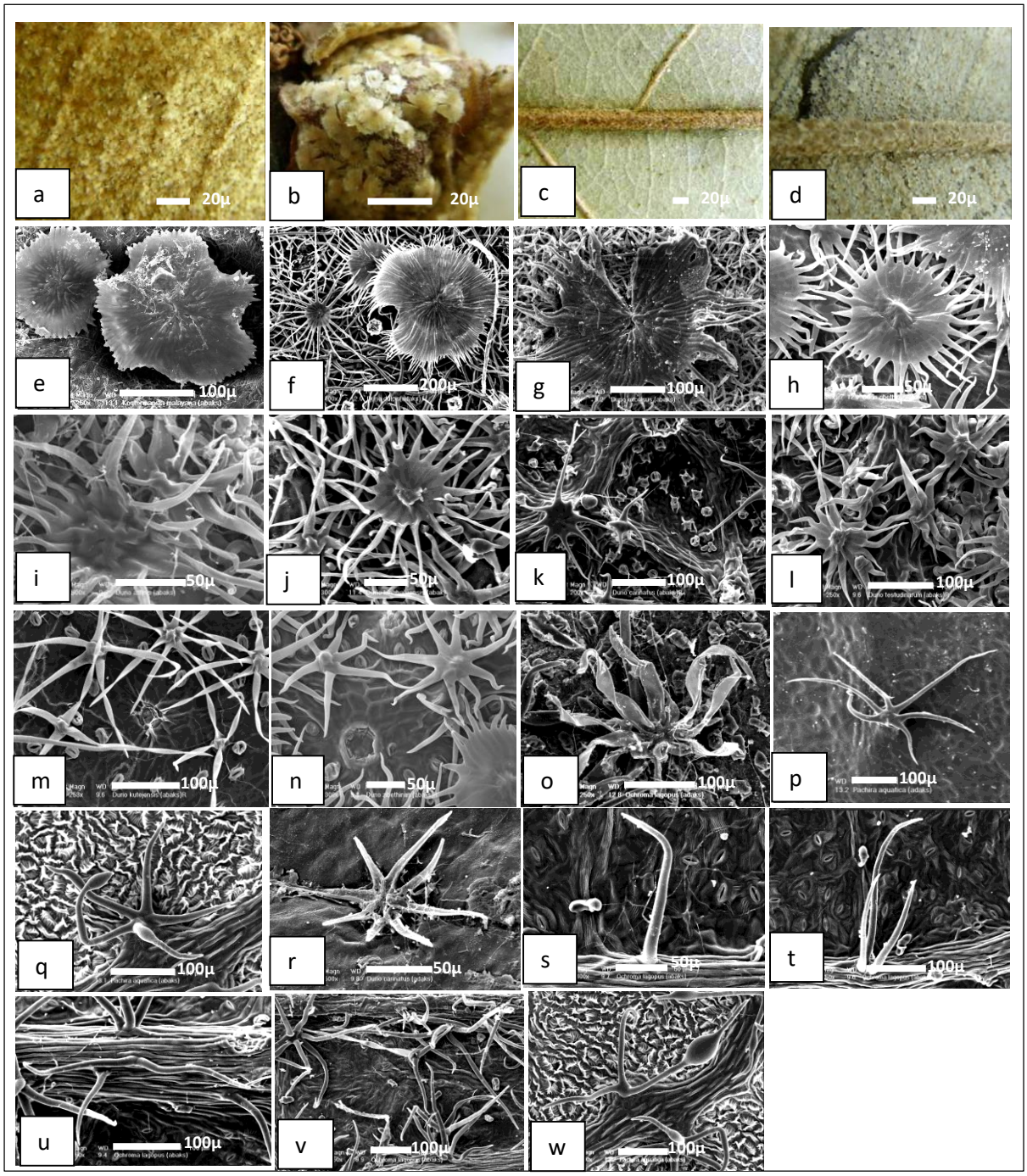

Figure 1: Micrograph images of species studied, examined under LM and SEM. **(**a-b) Golden lepidote trichome, (c-d) Coppery lepidote trichome, (e-f) Lepidote trichome, (g-h) Dentate trichome, (i-j) Stellate lepidote trichome, (k-l) Stellate rotate trichome, (m) Cushioned stellate trichome (long armed, thin wall), (n) Cushioned stellate trichome (short armed, thick wall), (o) Non-cushioned stellate trichome (short grooved armed, flat wall), (p) Non-cushioned stellate trichome (short armed, thick wall), (q) Non-cushioned stellate trichome (short armed, thick wall, swollen at the end and middle), (r) Cushioned stellate trichome (short grooved armed, thick wall), (s-t) Simple trichome, (u) Two-armed trichome, (v) Three-armed trichome (long armed, thin wall) and (w) Three-armed trichome (short armed, swollen in the middle). Scale: (a-d) = 20 µm, (e), (k), (l), (m), (o), (p), (q), (t), (u), (v), (w) = 100 µm, (f) 200 µm, (g) 100 µm and (h), (i), (j), (n), (r), (s) = 50 µm

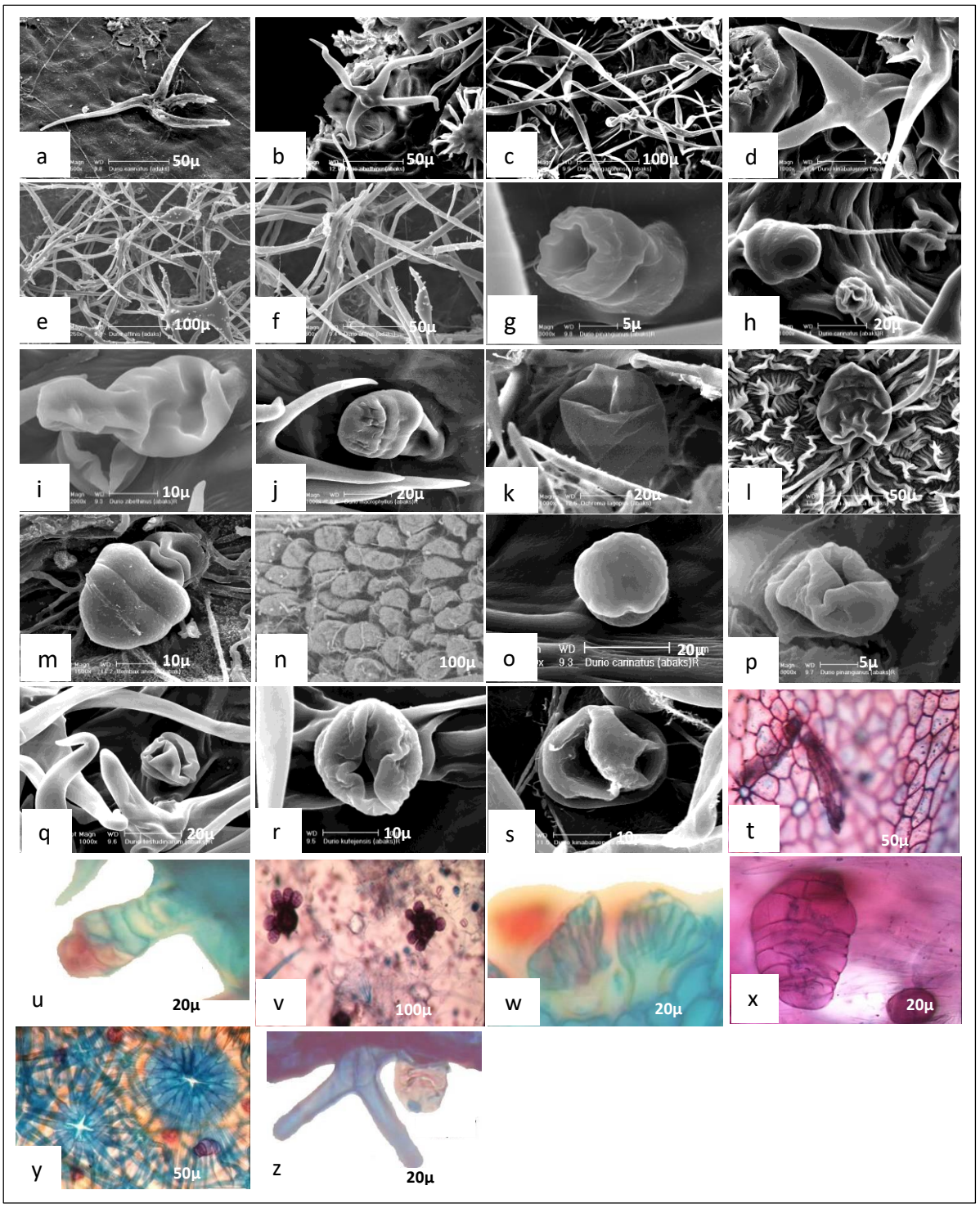

Figure 2: Micrograph images of species studied, examined under LM and SEM. **(**a) Non-cushioned four-armed trichome (short grooved armed, thick wall), (b) Cushioned four-armed trichome (short armed, thick wall), (c) Cushioned four-armed trichome (short grooved armed, thin wall), (d) Non-cushioned four-armed trichome (short armed, thick wall), (e-f) Trichome dendritic, (g-l) Capitate glandular trichome (multicellular stalks and terminals), (m) Capitate glandular trichome (multicellular stalks and terminals) (thin), (n) Capitate glandular trichome (multicellular stalks and terminals) (flat), (o-p) Capitate glandular trichome (multicellular stalks and terminals), (q) Capitate glandular trichome multiseriate terminals, (r) Capitate glandular trichome with multiseriate terminal (spatula-shaped lobes terminal), (s-t) Peltate glandular trichome, (u-y) Peltate glandular trichome multicelluar terminal and (z) Two-armed trichome with peltate glandular trichome (light microscope). Scale: (a), (b), (f), (j), (n), (x) = 50 µm, (c), (e), (p), (q) = 100 µm, (d), (h), (k), (l), (m), (r), (s), (t), (v), (z) = 20 µm, (g), (u) = 5 µm and (i), (o), (w), (y) = 10 µm

Non-cushioned stellate trichomes (short armed, thick wall) (Figure 1(p)) were found in *D. carinatus, D. macrophyllus, D. singaporensis* and *P. aquatica*. Whereas non-cushioned stellate trichomes (short armed, thin wall) were present in *D. excelsus* and *D. grandiflorus*. Various types of stellate trichomes were observed to be present in some of the *Schoutenia* species studied. For example the flat cushioned stellate trichomes (long armed, thin wall) were observed to be present and were an important feature of *S. accrescens* subsp. *Borneensis* [34].

In addition, rotate stellate trichomes were also observed in 13 species (Figure 1(k)) (*D. carinatus, D. dulcis, D. excelsus, D. grandiflorus, D. graveolens, D. griffithii, D. kinabaluensis, D. kutejensis, D. lowianus, D. macrophyllus, D. malaccensis, D. oxleyanus, D. singaporensis, D. zibethinus*), non-cushioned rotate stellate trichomes (grooved short sleeves, thin wall) (*D. griffithii*), cushioned rotate stellate (grooved short sleeves, thin wall) , thin wall) (*D. testudinarum*) (Figure 1(l)), lepidote stellate (Figure 1(i)) (*D. affinis, D. dulcis, D. excelsus, D. grandiflorus, D. grandiflorus, D. kinabaluensis, D. kutejensis, D lowianus, D. macrophyllus, D. malaccensis, D. oxleyanus, D. pinangianus, D. testudinarum* and *D. zibethinus*), cushioned lepidote stellate (short armed, flat wall) (*D. kinabaluensis*) (Figure 1(j)) and cushioned lepidote stellate (short grooved arm, thin wall (*D. testudinarum*). A study recorded the presence of two types of trichomes in the genus *Croton* namely stellate type trichomes (free radius) and lepidote type trichomes (fused radius) [28]. These stellate and lepidote trichomes were also observed in all genus studied except *Bombax*.

Trichomes in the outermost layer were dentate trichomes and lepidote trichomes. Dentate trichomes (Figures 1(g) and (h)) were found in 12 species of *Durio* (*D. affinis, D. excelsus, D. grandiflorus, D. griffithii, D. kinabaluensis, D. lowianus, D. macrophyllus, D. oxleyanus, D pinangianus, D. testudinarum, D. zibethinus, D. kinabaluensis, D. lowianus, D. malaccensis, D. testudinarum*) and also on *O. lagopus*. 10 *Durio* species that showed the presence of dentate trichomes [35].

Lepidote trichomes (Figures 1(e) and (f)) were found in all species except *B. anceps, O. lagopus* and *P. aquatica*. Trichomes not only can be observed on abaxial epidermal but some present on both surface of the adaxial and abaxial epidermal [36]. The silver-coloured composite of lepidote trichome affects the colour of the leaf and is seen on the abaxial epidermis of *Durio* leaves which appeared golden (Figures 1(a) and (b)) or coppery (Figures 1(c) and (d)).

Multicellular armed dentate trichomes were found in *D. griffithii*, whereas multicellular armed lepidote trichomes were found in *D. dulcis*. Lepidote trichomes are scaly peltate trichomes [27,37-39]. This scaly peltate or lepidote trichome was indeed very common in previous studies of species from Bombacaceae. Peltate trichome scales consist of 25-35 cells and undergo cell division into small stalks and peltate heads. The stalk is embedded in the epidermis while the head or terminals are nearly round or uneven, fibrous or crenular at the ends and these peltate trichome scales were present very densely on the immature leaves acted as a microcanopy on the plant surface [19]. In addition, two types of dendritic trichomes were observed through observation under a scanning electron microscope on the adaxial epidermis of *D. affinis*. These dendritic trichomes were either long armed with grooved walls or dendritics with swollen arms (Figures 2(e) and (f)). A dendritic trichome was a trichome protruding from one source of a stalk forming treelike with multiple lateral branches [28,40].

Figure 3 shows a phenogram constructed from UPGMA analysis using MVSP (Multi Variate Statistical Package) Version 3.22 software by Kovach Technology. Based on the

phylogenetic tree constructed B. anceps stood out from other species groups with a Similarity Index of 0.02 (2 % similarity). Based on the characteristics of the trichomes it is clear that this species has multiseriate terminal capitation glandular trichomes (spatula-shaped lobes terminal) which is not present in any other species studied.

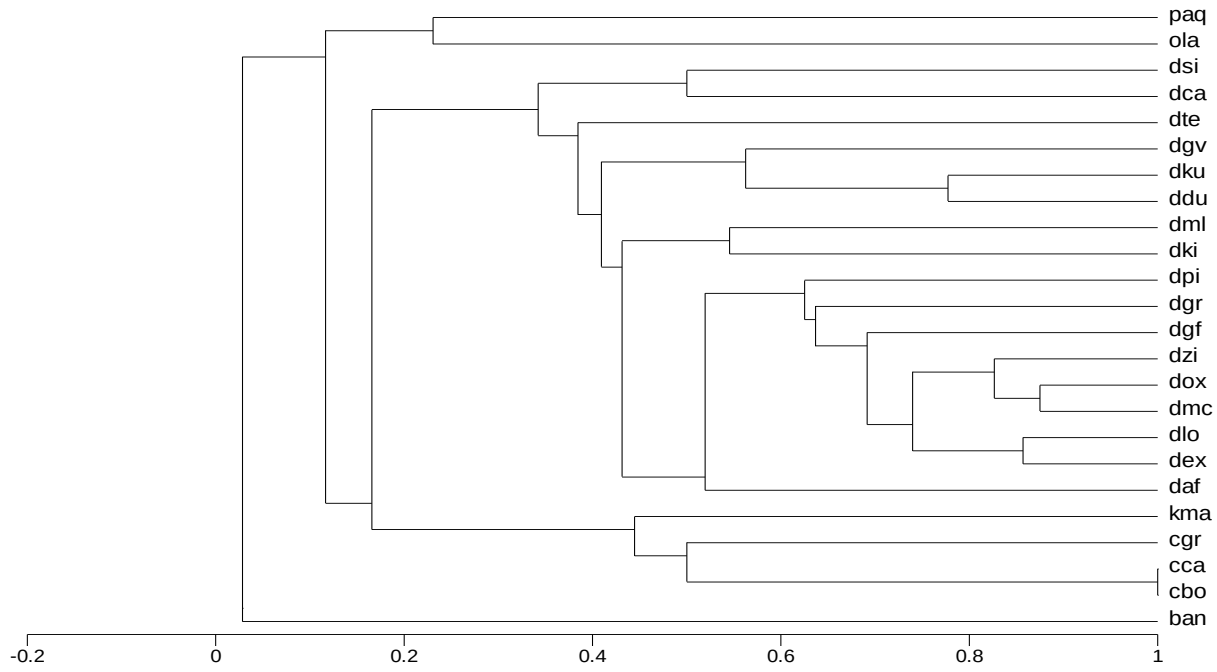

Figure 3: Gower similarity coefficient

Pachira and *Ochroma* have low similarity value of 0.12 (12 % similarity) with *Durio, Kostermansia* and Coelostegia. This was supported by the differences in trichome type. *Pachira* and *Ochroma* shared simple trichomes and three-armed trichomes, while *Durio, Kostermansia* and *Coelostegia* shared lepidote and glandular trichome types.

The phylogenetic tree also shows clear genus grouping between *Durio, Kostermansia* and *Coelostegia*. Six species namely *D. kutejensis, D. zibethinus, D. oxleyanus, D. macrophyllus, D. lowianus* and *D. excelsus* have high similarity values of more than 0.8 (> 80 % similarity). This is because these five species share similar trichome types, namely multicellular capillary glandular cushioned stellate, rotate stellate, lepidote stellate and lepidote trichomes.

While *C. chartacea* and *C. borneensis* showed similarity of 1 (100 % similarity), the trichomes feature alone could not distinguish between these two species and required a combination of several other features to obtain a clearer phylogenetic tree. These two species share the same type of trichomes i.e. lepidote trichome.

The presence of trichomes can be used as a diagnostic feature (Table 3) for the identification of *Bombax anceps, Durio affinis, D. singaporensis, D. graveolens, D. kutejensis, D. griffithii, D. testudinarum, D. kinabaluensis, Ochroma lagopus* and *Pachira aquatica.*

Table 3: List of diagnostic trichomes features

Species	Diagnostic characteristics
Bombax anceps	capitate glandular trichome with multiseriate terminals (spatula-shaped lobes terminals)
Durio affinis	dendritic trichome (long armed, grooved wall), trichome dendritic (swollen armed)
D. carinatus	non-cushioned four-armed trichome (grooved armed)
D. singaporensis	cushioned four-armed trichome (long armed, thin wall)
D. graveolens	cushioned stellatetrichome (long armed, thin wall)
D. kutejensis	cushioned stellatetrichome (short armed, thin wall), lepidote stellate trichome (short grooved armed, thin wall)
D. griffithii	rotate stellate non-cushioned rotate stellate trichome (short grooved armed, thick wall)
D. testudinarum	rotate stellate cushioned rotate stellate trichome (short grooved armed, thin wall)
D. kinabaluensis	lepidote stellate cushioned lepidote stellate trichome (short armed, flat wall)
Ochroma lagopus	three-armed trichome (long armed, thin wall)
Pachira aquatica	capitate glandular trichome (multicellular stalks and terminals) (flat wall), three-armed trichome (swollen armed), cushioned stellatetrichome (swollen short armed, thick wall)

Conclusions

Trichomes have high taxonomic value in Malvaceae subfamilies of Bombacoideae and Helicteroideae. The presence and types of trichomes can be used for species differentiation and identification as well as for grouping genera using UPGMA analysis. However, *Coelostegia, Durio, Kostermansia, Ochroma* and *Pachira* have similarities in the presence of stellate and lepidote trichomes which are not found in *Bombax*, while capitate glandular trichomes with multiseriate terminal (spatula-shaped lobes terminals) found in *Bombax* are very unique and can be a diagnostic characteristic for species identification. In conclusion the anatomical features of trichome types has taxonomic value and can be used for identification up to the species level and as additional data in classification. Trichomes can be used as alternative characteristics for species identification involving sterile specimens especially when specimens do not have sufficient flower, fruit and morphological information.

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Author Contributions

All authors conceived and designed the study. All authors contributed to manuscript revisions. Noor Solihani Binti Shamsuddin conducted the experiments, analysed the data and

wrote the paper. Noraini Talip led the research and helped to draft the manuscript. Mohammad Ruzi Abdul Rahman helped to performed UPGMA analysis. Mohd Norfaizal Ghazalli as provision of resources. Nabilah Mohammad helped edit the manuscript with helpful suggestions. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

Disclosure of Conflict of Interest

No conflict of interest exists.

Compliance with Ethical Standards

Whole processes from beginning to the end were compliance with ethical standards.

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