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Taxonomic significance of petal and sepal micromorphological characteristics in some *Justicia* Nees (Acanthaceae) species in Peninsular Malaysia

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Running title: Petal and sepal micromorphological characteristics

Abstrak: *Acanthaceae* merupakan famili tumbuhan berbunga di bawah order Lamiales dan terdiri daripada sekurang-kurangnya 4000 spesies di kawasan tropika dan juga kawasan subtropika dengan genus *Justicia* merupakan genus terbesar. Kajian mikromorfologi petal dan sepal telah dijalankan ke atas ke atas enam spesies iaitu *J. betonica* L., *Justicia carnea* Lindl., *J. comata* (L.) Lam., *J. gendarussa* Burm.f., *J. procumbens* L. and *J. ptychostoma* Nees. Kaedah kajian termasuklah penetapan bunga di dalam larutan penetap, siri dehidrasi, titik pengeringan genting dan cerapan di bawah mikroskop imbasan elektron. Hasil kajian menunjukkan kesemua enam spesies mempunyai variasi dan perbezaan mikromorfologi pada permukaan epidermis petal dan sepal. Kutikel lilin boleh ditemui pada kedua-dua permukaan epidermis petal dan sepal kesemua spesies yang dikaji. Kutikel lilin butir merupakan satu-satunya jenis kutikel lilin yang ditemui pada kesemua permukaan petal dan sepal spesies kajian. Terdapat lapan jenis trikoma ditemui pada permukaan epidermis sepal dan juga lapan jenis trikoma pada permukaan epidermis petal. *Justicia betonica* boleh dibezakan secara langsung dengan kehadiran stomata amfidiasitik pada permukaan epidermis petal dan stomata tidak hadir pada permukaan petal spesies yang lain. Kesimpulannya, hasil kajian ini membuktikan ciri mikromorfologi petal dan sepal mempunyai kesignifikan sistematik dan boleh digunakan sebagai data sokongan bagi pembezaan dan pengenalanpastian spesies dalam genus *Justicia*.

Kata kunci: mikromorfologi sepal dan petal, *Justicia*, Acanthaceae

Abstract: *Acanthaceae* is a flowering plants family under the order Lamiales and comprises at least 4000 species regardless of tropical and subtropical species with *Justicia* being the largest genus under this family. The study on the flower's petal and sepal micromorphology have been done on six *Justicia* species, namely as *J. betonica* L., *Justicia carnea* Lindl., *J. comata* (L.) Lam., *J. gendarussa* Burm.f., *J. procumbens* L. and *J. ptychostoma* Nees. Methodology used involves fixation, dehydration series, critical point drying technique and observation under scanning electron microscope. Results have shown that all six species have differences in micromorphological characteristics in the sepals and petals epidermal surfaces. Cuticular wax can be found on both abaxial and adaxial epidermal surfaces of petal and sepal for all species studied. Granular cuticular wax is the only type of cuticular wax was found present on petal and sepal of all species studied. There are also eight types of trichomes present on the petal epidermal surface and eight types of trichomes present on the sepal epidermal surface. *J. betonica* can be recognized directly by having amphidiacytic stomata on petal epidermal surface while stomata were absent on the petal epidermal surface of other species. As a conclusion, findings in this study have proven that petal and sepal micromorphological characteristics have systematic significant and can be used as supporting data for species differentiation and identification in the genus *Justicia*.

Keywords: sepal and petal micromorphology, *Justicia*, Acanthaceae

INTRODUCTION

Acanthaceae is a flowering plant family under the order of Lamiales and comprises at least 4000 species regardless of tropical and subtropical species (Borg 2008). According to Hu *et al.* (2011) there are about 220 genera and 4000 species in the pantropical and subtropical region with few species can be found in the temperate region. About 35 genera are native in Peninsular Malaysia and few have been brought to Peninsular Malaysia as ornamental plants. Acanthaceae is also the third largest tropical plants after the Myrtaceae and Melastomataceae (Grant 1995).

Acanthaceae comprises of three subfamilies which are Acanthoideae, Thunbergioideae and Nelsonioideae (Melchior 1964; Keng 1986; Borg & Schonenberger 2011). Then, Scotland and Vollesen (2000) classified Acanthaceae into three subfamilies, Nelsonioideae, Acanthoideae and Thunbergioideae. While McDade *et al.* (2008) consider the family consists of four subfamilies, Acanthoideae, Nelsonioideae, Thunbergioideae and Andrographideae. But later, Vollesen (2008) elevated the Tribe Ruellieae to subfamily Ruellioideae. New findings by Schwarzbach and McDade (2002) and Borg (2008) have suggested that *Avicennia* has a sister relationship with Acanthaceae but still questionable and unclear in Acanthaceae lineages. The species from the Acanthaceae can be found in many habitat, having lots of morphological characters and biogeographical distribution. Taxonomic studies of Asian *Justicia* have been done by few researchers such as Clarke (1885), Benoist (1935), Hansen (1989), Cramer (1998) and Ruengsawang *et al.* (2012), but the taxonomical study on the *Justicia* in Malaysia is still scarce with only researches by some researchers such as Ridley (1923) and Ruth (2009) been known. In 2017, a study on current morphology-based classification of "justicioid" lineage with focus on Old World taxa by Kiel *et al.* (2017) confirmed a strong support of monophyly of New World justicioids including all New World species of *Justicia*. They also added that even though some genera of justicioids are monophyletic, *Justicia* is grossly polyphyletic. The results were obtained by the combinations of diagnostic utility of morphological characters for major clades focusing on the inflorescences, androecia, seeds and pollen.

Justicia is the largest genus in Acanthaceae with about 600 species and distributed in the tropical and subtropical as well in both hemisphere (Wasshaushen 1989). Mabberley (2008) stated that, *Justicia* L. is one of the largest and most complex genera in Acanthaceae. According to McDade *et al.* (2008), even though Acanthaceae is an important component in the tropical and subtropical all over the world, taxonomists are still having scarce information about the family especially in part of petal and sepal micromorphology.

In this study, the investigation focused just at the micromorphological characteristics of petal and sepal epidermal surfaces on some *Justicia* species in Peninsular Malaysia in order to understand more on the micromorphological characteristics of its' flower (especially on the petals and sepals).

MATERIALS AND METHODS

Six species were chosen (*J. betonica* T. Anderson, *J. carnea* Hook. ex Nees, *J. comata* Vellozo ex Nees, *J. gendarussa* J. Macrae ex Nees, *J. procumbens* T. Anderson ex Nees, and *J. ptychostoma* Nees). Fresh samples of flower were used in this study and were collected from several forests reserved as shown in Table 1 and Figure 1. Observation under scanning electron microscope follows Barthlott *et al.* (1998) and Noraini (2006) with few modifications. Small piece of petal and sepal were fixed in 4% glutaraldehyde for 12-24 hours at 4°C prior to the dehydration in alcohol series (35%, 50%, 75%, 95%, 100%, 100% % 100%) followed by critical point drying using drying machine model Polaron CPD 7501, were then been attached to the aluminium stubs with double sided adhesive tape and then sputter-coated with a thin film of gold using Gold Coater model BioRad SC500 to improve the electrical conducting properties of the leaf surface. Samples then were examined under a scanning electron microscope model Philips XL Series XL 30 with magnification up to 10000x. The

description of petal and sepal micromorphology follows Radford *et al.* (1974) and Singh and Jain (1975).

RESULTS

Petal

Justicia comata

Wax: present in form of film layer and granular on both abaxial and adaxial epidermal. **Cuticular striation on abaxial epidermal:** obscure, periclinal and anticlinal wall cannot be differentiated. **Cuticular striation on adaxial epidermal:** clearly define, with periclinal wall merged at certain area, forming conical shape papillae, anticlinal wall sunken, tiny striae present at certain area of epidermal surface and present almost parallel towards the peak of papillae, no striae present at the peak of papillae. **Stomata:** absent. **Trichome:** simple multicellular or simple uniseriate (short to long, with echinate ornamentation) sparsely scattered on the abaxial epidermal and peltate glandular trichome (multicellular terminal – 2 cells) sparsely scattered on the adaxial epidermal.

Justicia betonica

Wax: present in form of film layer and flakes cuticular waxes on the abaxial epidermal, film and granular cuticular waxes present on the adaxial epidermal. **Cuticular striation on abaxial epidermal:** clear, periclinal wall merged and anticlinal epidermal sunken. **Cuticular striation on adaxial epidermal:** clear, periclinal walls sunken, striae present at certain area forming parallel line heading to the middle part of periclinal wall. **Stomata:** amphistomatic, parafacial, present sparsely scattered, subsidiary and epidermal cell can be differentiated, three subsidiary cells, anisocytic stomata, elliptical in shape, epidermal cells/ subsidiary cells do not over arching the stomata, stomata size 12.66 – 16.32 μm long, 9.38 – 12.43 μm width. **Trichome:** peltate glandular (terminal multicellular, 4-cells), sparsely scattered on the abaxial epidermal and simple multicellular or simple uniseriate (short to long, echinate ornamentation) present densely scattered especially on the venation area and sparsely scattered on adaxial epidermal of petal.

Justicia procumbens

Wax: present in form of film layer and granule cuticular wax on both abaxial and adaxial epidermal. **Cuticular striation on abaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken. **Cuticular striation on adaxial epidermal:** clear, with periclinal wall merged forming conical shape with wide base papillae, striae present on the entire surface of periclinal wall almost parallel towards the peak of papillae, strips present on the peak of papillae, anticline wall sunken. **Stomata:** absent. **Trichome:** simple multicellular or simple uniseriate (short to long, echinate ornamentation) present less dense on the surface of abaxial epidermal of petal, capitate glandular (multicellular short stalk – multicellular terminal) sparsely scattered on the adaxial epidermal surface.

Justicia carnea

Wax: in form of film layer and granule cuticular wax present on both abaxial and adaxial epidermal. **Cuticular striation on abaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken, fine parallel cuticle strips present on the abaxial epidermal surface. **Cuticular striation on adaxial epidermal:** clear, with periclinal wall merged forming lobe-shaped papillae, anticlinal wall sunken, striae with rugulate pattern present randomly on the surface of periclinal wall. **Stomata:** absent. **Trichome:** capitate glandular (long stalk, short middle cell and swollen long base cell – multicellular

terminal), simple unicellular (short, conical shape) and simple multicellular or simple uniseriate (short to long, echinate ornamentation) sparsely scattered on the abaxial epidermal surface of petal.

Justicia ptychostoma

Wax: crustose and granule cuticular wax present on the abaxial epidermal surface, film layer and granule cuticular wax present on the adaxial epidermal surface of petal. **Cuticular striation on abaxial epidermal:** unclear, periclinal wall merged and anticlinal wall sunken at certain area, anticlinal wall merged and periclinal wall sunken at certain area. **Cuticular striation on adaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken, striae present and randomly arranged in the epidermal surface. **Stomata:** absent. **Trichome:** simple multicellular or simple uniseriate (short to long, echinate ornamentation) and simple unicellular (short, echinate ornamentation) sparsely scattered on the abaxial epidermal surface of petal.

Justicia gendarussa

Wax: present in form of film layer and granule cuticular wax on both abaxial and adaxial epidermal surface. **Cuticular striation on abaxial epidermal:** clear, periclinal wall merged forming lobe-shaped papillae, anticlinal wall sunken, striate pattern striae present on the surface of periclinal wall. **Cuticular striation on adaxial epidermal:** clear, periclinal wall sunken and anticlinal wall merged forming crest. **Stomata:** absent. **Trichome:** peltate glandular (multicellular terminal – 2 cells) and peltate glandular (multicellular terminal – 4 cells) sparsely scattered on the abaxial epidermal surface, simple multicellular or simple uniseriate (short, conical shape) very sparsely scattered on the adaxial epidermal surface.

Sepal

Justicia comata

Wax: in form of film layer, crustose and granule cuticular wax present on the abaxial epidermal, Film layer and granule cuticular wax present on the adaxial epidermal surface. **Cuticular striation on abaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken. **Cuticular striation on adaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken. **Stomata:** superficial, hypostomatic (present only on the abaxial epidermal surface), present less dense, randomly distributed, clear differences between subsidiary cells and epidermal cells, 3 subsidiary cells, anisocytic stomata, elliptical shape, epidermal cells/subsidiary cells do not surround the stomata, stomata size 15.0 – 16.5 µm long x 10.2 – 11.9 µm width. **Trichome:** peltate glandular (multicellular terminal – 4 cells) present dense on both abaxial and adaxial epidermal surfaces of sepal (arrow), simple unicellular (short to long, echinate ornamentation) present less dense on the abaxial epidermal surface, simple unicellular (short to long, without echinate ornamentation) sparsely scattered on both abaxial and adaxial epidermal surface.

Justicia betonica

Wax: in form of film layer and granule cuticular wax present on both abaxial and adaxial epidermal surface. **Cuticular striation on abaxial epidermal:** unclear, periclinal wall sunken and anticlinal wall merged forming crest, fine strips of cuticle present parallel on the anticlinal and periclinal wall. **Cuticular striation on adaxial epidermal:** unclear, with periclinal wall sunken and anticlinal wall merged forming crest (thin at some part and thick at some part), papillae present, fine unparallelled strips present on the papillae. **Stomata:** absent. **Trichome:** simple unicellular (short to long, echinate ornamentation) and simple multicellular or simple uniseriate (short to long, echinate ornamentation) densely scattered on the abaxial epidermal surface.

Justicia procumbens

Wax: in form of film layer, granule and flakes cuticular wax present on both abaxial and adaxial epidermal surface. **Cuticular striation on abaxial epidermal:** clear, periclinal wall merged and anticline wall sunken. **Cuticular striation on adaxial epidermal:** clear, periclinal wall merged and anticline wall sunken. **Stomata:** parafacial, amphistomatic, present on abaxial and adaxial epidermal surface densely scattered, subsidiary cells and epidermal cells can be differentiated, two subsidiary cells, diacytic stomata, elliptical, epidermal cells/subsidiary cells do not encircle the stomata, stomata size 11.5 – 16.9 μm long x 7.2– 11.9 μm width. **Trichome:** peltate glandular (multicellular terminal – 8 cells), capitate glandular (short stalk – unicellular terminal) sparsely scattered on the abaxial epidermal surface, simple multicellular or simple uniseriate (short to long, echinate ornamentation) and capitate glandular (multicellular long stalk – lobes unicellular terminal) present randomly and very densely scattered on the abaxial epidermal surface, simple multicellular or simple uniseriate (short to long, echinate ornamentation) and peltate glandular (multicellular terminal – 4 cells) present on the adaxial epidermal surface.

Justicia carnea

Wax: in form of film layer, granules and flakes present on both abaxial and adaxial epidermal surface. **Cuticular striation on abaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken. **Cuticular striation on adaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken. **Stomata:** superficial, hypostomatic (present only on the abaxial epidermal surface), randomly and sparsely scattered, clear differences between subsidiary cells and epidermal cells, 2 subsidiary cells, anisocytic stomata, elliptical, epidermal cells/subsidiary cells do not encircle the stomata, stomata size 13.6 – 15.7 μm long x 7.9 – 8.3 μm width. **Trichome:** simple multicellular or simple uniseriate (short to long, echinate ornamentation) and peltate glandular (multicellular terminal – 8 cells) sparsely scattered on both abaxial and adaxial epidermal surface.

Justicia ptychostoma

Wax: in form of film layer, wax block, flakes and granule cuticular present on abaxial epidermal surface. Film layer, flakes and granule cuticular wax present on adaxial epidermal surface. **Cuticular striation on abaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken. **Cuticular striation on adaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken. **Stomata:** superficial, hypostomatic (present only on the abaxial epidermal surface), randomly and sparsely scattered, clear differences between subsidiary cells and epidermal cells, 2 subsidiary cells, parasitic stomata, elliptical, epidermal cells/subsidiary cells do not surround stomata, stomata size 17.4 – 20.0 μm long x 9.7 – 12.7 μm width. **Trichome:** simple unicellular (short to long, without echinate ornamentation) densely scattered on both abaxial and adaxial epidermal surface, simple unicellular (short to long, echinate ornamentation) very sparsely scattered on abaxial and adaxial epidermal surface, and peltate glandular (multicellular terminal – 4 cells) sparsely scattered on the abaxial and adaxial epidermal surface of sepal.

Justicia gendarussa

Wax: film layer, granules and flakes present on abaxial and adaxial epidermal surface. **Cuticular striation on abaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken. **Cuticular striation on adaxial epidermal:** clear, periclinal wall merged and anticlinal wall sunken. **Stomata:** parafacial, amphistomatic, sparsely scattered on abaxial and adaxial epidermal surface, randomly distributed, clear differences between subsidiary cells and epidermal cells, 3 subsidiary cells, anisocytic stomata, elliptical, epidermal cells/subsidiary cells do not encircle the stomata, stomata size 28.1 – 35.2 μm long x 13.7 – 16.2 μm width. **Trichome:** simple unicellular (short to long, echinate ornamentation) and peltate glandular (multicellular terminal – 8 cells) present on abaxial epidermal surface, capitate glandular and capitate glandular (multicellular short stalk – multicellular terminal, 2 cells) present only on adaxial epidermal surface.

DISCUSSION

Common Micromorphological Characteristics

Cuticular waxes can be found on both abaxial and adaxial epidermal surfaces of petal and sepal in all species studied. Granular cuticular wax is the only type of wax that is present on petal and sepal of all species studied. Thus, these characters could be used to group all species studied under the same genus *Justicia*.

Interspecies Variation

According to Inamdar (1967), trichomes can be used for species delimitation. Trichomes are physical resistivity structure that are present on plants (Levin 1929), and having characters that can be used for plant taxonomical study (Metcalf & Chalk 1979). There are eight types of trichomes present in the petal and eight types present in the sepal. Trichomes present in the petals; simple unicellular, simple multicellular (simple uniseriate), capitate and peltate glandular. Simple and glandular trichomes present in all *Justicia* species studied but vary in detail micromorphological structures. Results have shown that some *Justicia* species can be differentiated and identified based on the types of trichomes. Types of trichomes present are shown in Figure 2.

Presence of cuticular wax on both abaxial and adaxial epidermal surfaces of petal and sepal in all species were recorded. Types of cuticular wax present on petal and sepal epidermal surfaces; film layer, granules, flakes, block, and crustose. The occurrence of cuticular waxes is very useful for species identification and this proven in this study. Crustose cuticular wax is only found in the petal of *J. ptychostoma*. Grouping and species differentiation in the genus can be done using the types of cuticular wax present. According to Barthlott and Frolich (1983), the cuticular wax occurrence on epidermal does have a taxonomical value and can be used in species identification. Types of cuticular waxes present can be seen in Figure 3.

According to Watson (1962), the morphology of stomata has long been looked upon as a one of the useful taxonomic criteria. Stomata occurrence and types are also very useful in the species recognition in *Justicia*. For instances *J. betonica* can be recognized directly by having amphidiacytic stomata on petal epidermal surface while stomata were absent in the petal epidermal surface of other species. *Justicia procumbens* and *J. gendarussa* can also be differentiated from other species by the presence of amphistomatic stomata on its sepal. *Justicia comata*, *J. carnea* and *J. ptychostoma* do have stomata but only on the abaxial epidermal surface of sepal (hypostomatic). Therefore, the occurrence of stomata is very useful for taxonomic study of *Justicia* (Acanthaceae). Study on flowers anatomy by O'Neill (2010) in *J. brandegeana* have shown that stomata were only present on the abaxial epidermal surface (hypostomatic). However, in the study by Patil and Patil (2011), the results showed that *J. trinervia* and *J. wynaddensis* were having amphistomatic and hypostomatic stomata respectively. Figure 4 shows the types of stomata occurrence in the species studied.

The variation can also be seen in the petal and sepal epidermal cuticle ornamentation. Different types of cuticular striation present undoubtedly indicate that each species can be differentiated from others. According to Tewary and Sarkar (1985) in their study on *Vatica* (Dipterocarpaceae) in India, cuticle ornamentation does have high taxonomical value and can be used in for species identification. Cuticle ornamentation variation of the species studied can be seen in Figure 5.

CONCLUSION

As a conclusion, the study on the petal and sepal micromorphological characteristics can be used as supporting data for species differentiation and identification in *Justicia*.

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Figure 1. Species used in this study. A) *J. betonica*, B) *J. carnea*, C) *J. comata*, D) *J. gendarussa*, E) *J. procumbens*, F) *J. ptychostoma*.

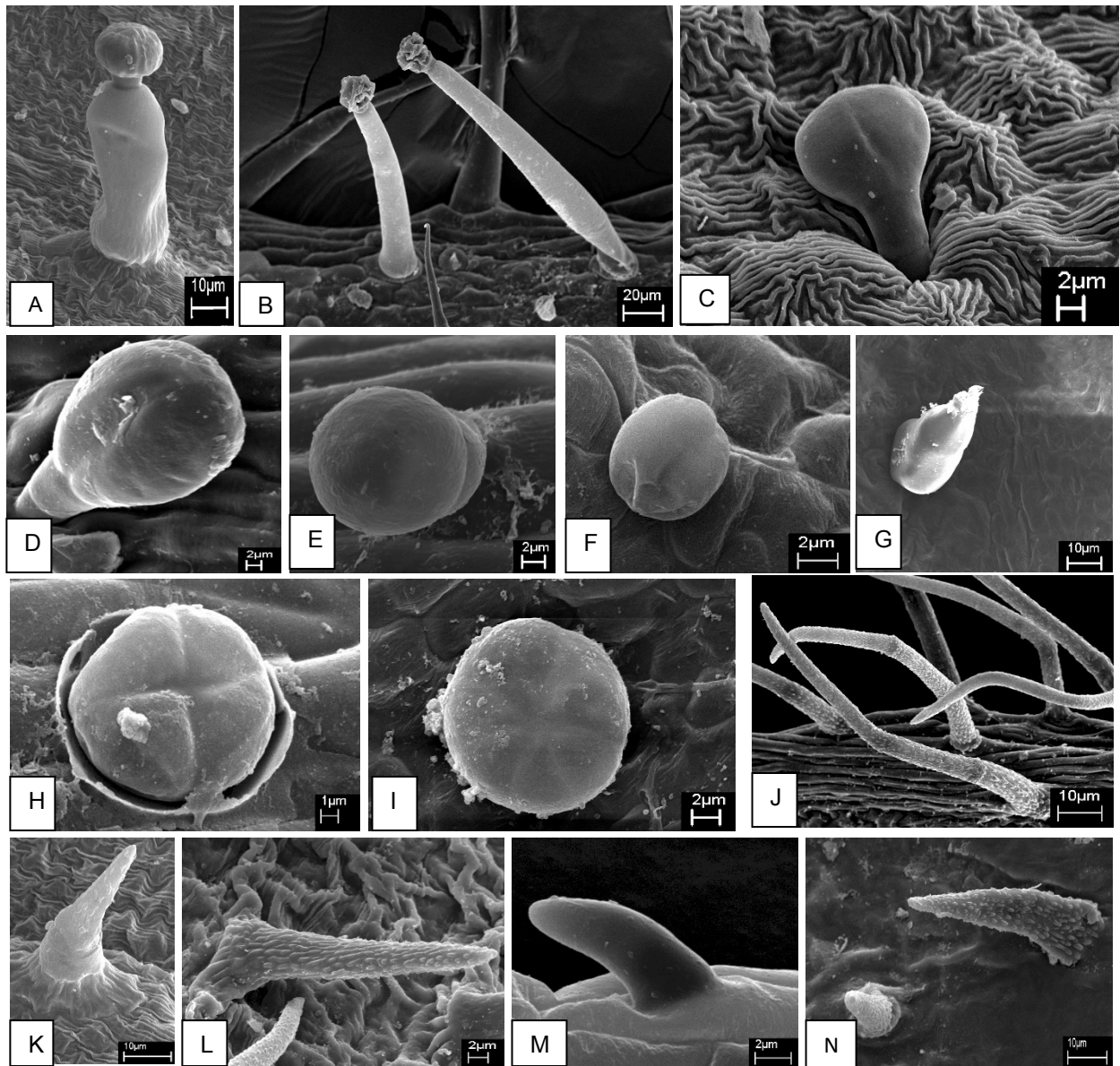


Figure 2. Types of trichomes found in this study. A) Capitulate glandular (long stalk, short middle cell, swollen long base cell – multicellular terminal), B) Capitulate glandular (multicellular long stalk – lobes unicellular terminal), C) Capitulate glandular (multicellular short stalk – multicellular terminal), D) Capitulate glandular (multicellular short stalk – multicellular terminal, 2 cells), E) Capitulate glandular (short stalk – unicellular terminal), F) Peltate glandular (multicellular terminal – 2 cells), G) Simple unicellular (short, conical shape), H) Peltate glandular (multicellular terminal – 4 cells), I) Peltate glandular (multicellular terminal – 8 cells), J) Simple multicellular (short to long, echinate ornamentation), K) Simple multicellular (short, conical shape), L) Simple unicellular (short to long, echinate ornamentation), M) Simple unicellular (short to long, without echinate ornamentation), N) Simple unicellular (short, echinate ornamentation).

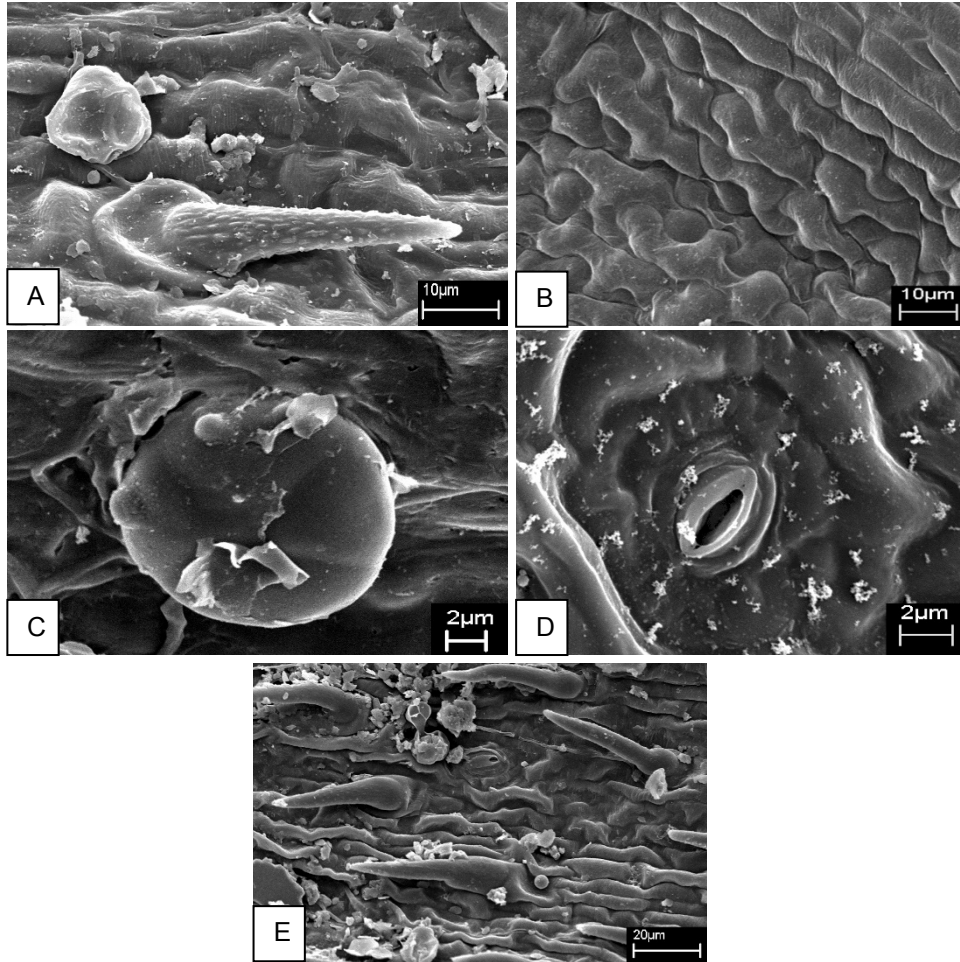


Figure 3. Types of cuticular waxes found in this study. A) Granule, B) Film layer, C) Flakes, D) Crustose, E) Block

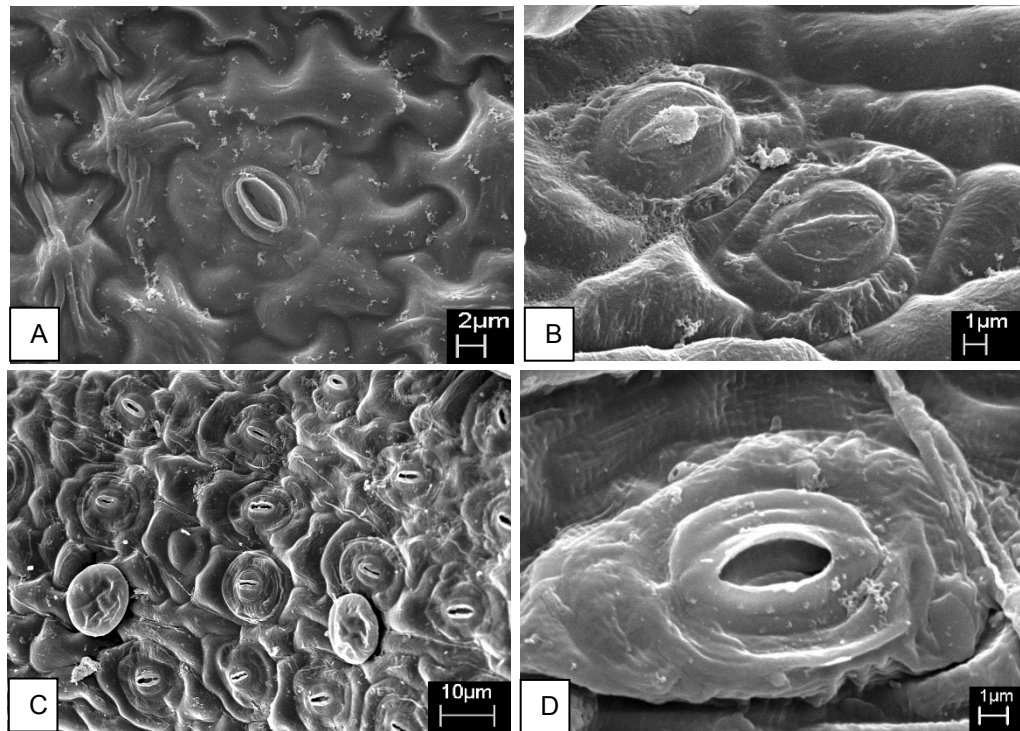


Figure 4. Types of stomata found in this study. A) Anisocytic stomata (paraficial), B) Anisocytic stomata (superficial), C) Diacytic stomata (paraficial), D) Parasitic stomata (superficial).

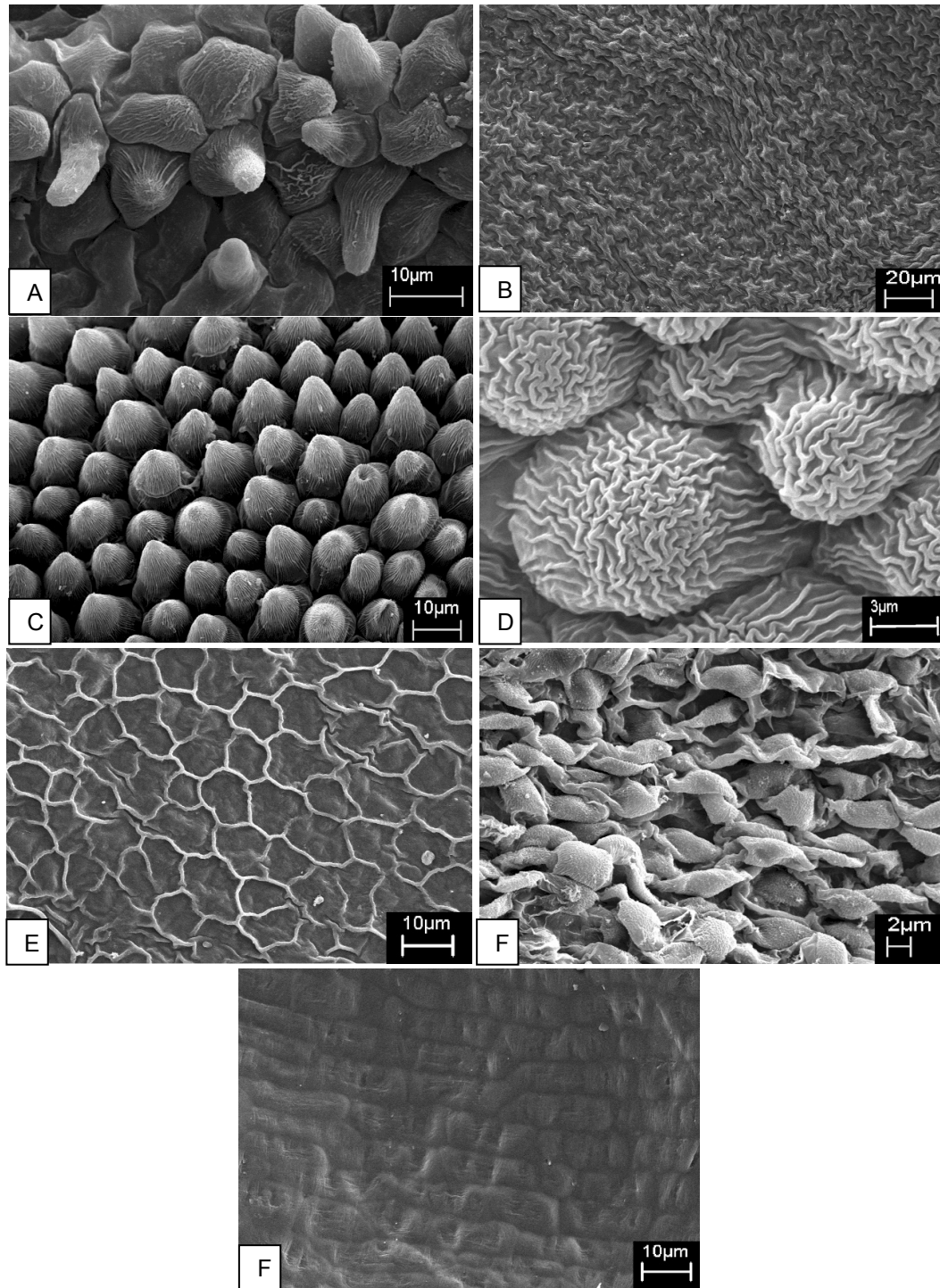


Figure 5. Pattern of anticlinal wall found in this study. A) Tiny striae present at certain part of periclinal epidermal surface and present almost parallel towards the peak of papillae, no striae at the peak of papillae, anticlinal wall sunken, B) Periclinal epidermal emerged and anticlinal epidermal sunken, C) Periclinal wall merged forming conical shape with wide base papillae, striae present on the entire surface of periclinal wall almost parallel towards the peak of papillae, strips on the peak of papillae, anticlinal wall sunken, D) Periclinal wall merged forming lobe-shaped papillae, Striae with rugulate pattern present on the surface of periclinal wall, E) Periclinal wall sunken and anticlinal wall merged forming crest, F) Periclinal wall sunken and anticlinal wall merged forming crest (thin at some part and thick at some part), papillae present, fine unparallel strips present on the papillae, G) Periclinal wall merged and anticlinal wall sunken.

Table 1. Sources of plant material

Taxa	Collector and sources
<i>J. betonica</i>	Che Nurul Aini & Ruzi Abdul Rahman, CNA6, 9.05.2012, Bangi, Selangor
<i>J. carnea</i>	Amirul Aiman & Ruzi Abd Rahman, MAA22, 22.05.2013, Lady Maxwell Road, Fraser's Hill, Pahang
<i>J. comata</i>	Amirul Aiman & Ruzi Abd Rahman, MAA3, 12.10.2012, Bangi, Selangor
<i>J. gendarussa</i>	Amirul Aiman & Ruzi Abd Rahman, MAA66, 8.03.2014, Kuala Woh Forest Reserve, Tapah, Perak
<i>J. procumbens</i>	Amirul Aiman & Ruzi Abd Rahman, MAA63, 7.03.2014, Kuala Woh Forest Reserve, Tapah, Perak
<i>J. ptychostoma</i>	Amirul Aiman & Ruzi Abd Rahman, MAA14, 21.05.2013, Bishop's Trail, Fraser's Hill, Pahang

Table 2: Summary of petal micromorphological characteristics in *Justicia* species studied

Species	Wax		Cuticular striation on epidermis		Stomata		Trichomes	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
<i>J. comata</i>	-Film layer -Granule	-Film layer - Granule	Not clear, periclinal and anticlinal wall cannot be differentiated	Clear, periclinal wall merged at certain area, papillae with conical shape, anticlinal wall sunken, tiny striae present almost parallel towards the peak of papillae, no striae at the peak of papillae.	Absent	Absent	Simple multicellular (short to long, echinate ornamentation)	Peltate glandular trichome (multicellular terminal – 2 cells)
<i>J. betonica</i>	-Film layer -Flakes	-Film layer - Granule	Clear, periclinal epidermal emerged while anticlinal epidermal sunken	Clear, periclinal epidermal emerged and anticlinal epidermal sunken, striae present at certain part of periclinal epidermal forming parallel line heading to the middle part of periclinal epidermal.	Present	Present	Peltate glandular (multicellular terminal – 4 cells), Simple multicellular (short to long, echinate ornamentation)	Simple multicellular (short to long, echinate ornamentation)
<i>J. procumbens</i>	-Film layer -Granule	-Film layer - Granule	Clear, periclinal wall merged and anticlinal wall sunken	Clear, periclinal wall merged forming conical shape with wide base papillae, striae present on the entire surface of periclinal wall almost parallel towards the peak of papillae, strips present on the peak of papillae, anticlinal wall sunken.	Absent	Absent	Simple multicellular (short to long, echinate ornamentation)	Capitate glandular (multicellular short stalk – multicellular terminal)
<i>J. carnea</i>	-Film layer -Granule	-Film layer - Granule	Clear, periclinal wall merged and anticlinal wall sunken, fine parallel cuticle strips present on the abaxial epidermal surface	Clear, periclinal wall merged forming lobe-shaped papillae, anticlinal wall sunken, striae with rugulate pattern present randomly on the surface of periclinal wall	Absent	Absent	Capitate glandular (long stalk, short middle cell, swollen long base cell – multicellular terminal), Simple unicellular (Short, conical shape), Simple	Absent

								multicellular (short to long, echinate ornamentation)	
J. ptychostoma	- Crustose -Granule	-Film layer - Granule	Unclear, periclinal wall merged and anticlinal wall sunken at certain area, anticlinal wall merged and periclinal wall sunken at certain area	Clear, periclinal wall merged and anticlinal wall sunken, striae present and randomly arranged in the epidermal surface	Absent	Absent	Simple multicellular (short to long, echinate ornamentation), Simple unicellular (short, echinate ornamentation)	Absent	
J. gendarussa	-Film layer -Granule	-Film layer - Granule	Clear, periclinal wall merged forming lobe-shaped papillae, anticlinal wall sunken, striate pattern striae present on the surface of periclinal wall	Clear, periclinal wall sunken and anticlinal wall merged forming crest	Absent	Absent	Peltate glandular (multicellular terminal – 2 cells), Peltate glandular (multicellular terminal – 4 cells)	Simple multicellular (short, conical shape)	

Table 3: Summary of sepal micromorphological characteristics in *Justicia* species studied

Species	Wax		Cuticular epidermal		Stomata		Trichomes	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
J. comata	-Film layer -Crustose -Granule	-Film layer -Granule	Clear, periclinal wall merged and anticlinal wall sunken	Clear, periclinal wall merged and anticlinal wall sunken	Present	Absent	Peltate glandular (multicellular terminal – 4 cells), Simple unicellular (short to long, echinate ornamentation), Simple unicellular (short to long, without echinate ornamentation)	Peltate glandular (multicellular terminal – 4 cells), Simple unicellular (short to long, without echinate ornamentation)

<i>J. betonica</i>	-Film layer -Granule	-Film layer -Granule	Not clear, periclinal wall sunken and anticlinal wall merged forming crest, fine strips of cuticle present parallel on the anticlinal and periclinal wall	Not clear, periclinal wall sunken and anticlinal wall merged forming crest (thin at some part and thick at some part), papillae present, fine unparallelled strips present on the papillae	Absent	Absent	Simple unicellular (short to long, echinate ornamentation), Simple multicellular (short to long, echinate ornamentation)	Absent
<i>J. procumbens</i>	-Film layer -Granule -Flakes	-Film layer -Granule -Flakes	Clear, periclinal wall merged and anticlinal wall sunken	Clear, periclinal wall merged and anticlinal wall sunken	Present	Present	Peltate glandular (multicellular terminal – 8 cells), Capitate glandular (short stalk – unicellular terminal), Simple multicellular (short to long, echinate ornamentation), Capitate glandular (multicellular long stalk – lobes unicellular terminal)	Simple multicellular (short to long, echinate ornamentation), Peltate glandular (multicellular terminal – 4 cells)
<i>J. carnea</i>	-Film layer -Granule -Flakes	-Film layer -Granule -Flakes	Clear, with periclinal wall merged and anticlinal wall sunken	Clear, periclinal wall merged and anticlinal wall sunken	Present	Absent	Simple multicellular (short to long, echinate ornamentation), Peltate glandular (multicellular terminal – 8 cells)	Simple multicellular (short to long, echinate ornamentation), Peltate glandular (multicellular terminal – 8 cells)
<i>J. ptychostoma</i>	-Film layer -Block -Flakes	-Film layer -Granule -Flakes	Clear, periclinal wall merged and anticlinal wall sunken	Clear, periclinal wall merged and anticlinal wall sunken	Present	Absent	Simple unicellular (short to long, without echinate ornamentation), Simple unicellular (short to long, echinate ornamentation), Simple unicellular (short to long, echinate ornamentation)	Simple unicellular (short to long, without echinate ornamentation), Simple unicellular (short to long, echinate ornamentation)

J. <i>gendarussa</i>	-Film layer -Granule -Flakes	-Film layer -Granule -Flakes	Clear, periclinal wall merged and anticlinal wall sunken	Clear, periclinal wall merged anticlinal sunken	Present	Present	Simple unicellular (short to long, echinate ornamentation), Peltate glandular (multicellular terminal – 8 cells)	Capitate glandular (multicellular short stalk – multicellular terminal, 2 cells)
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