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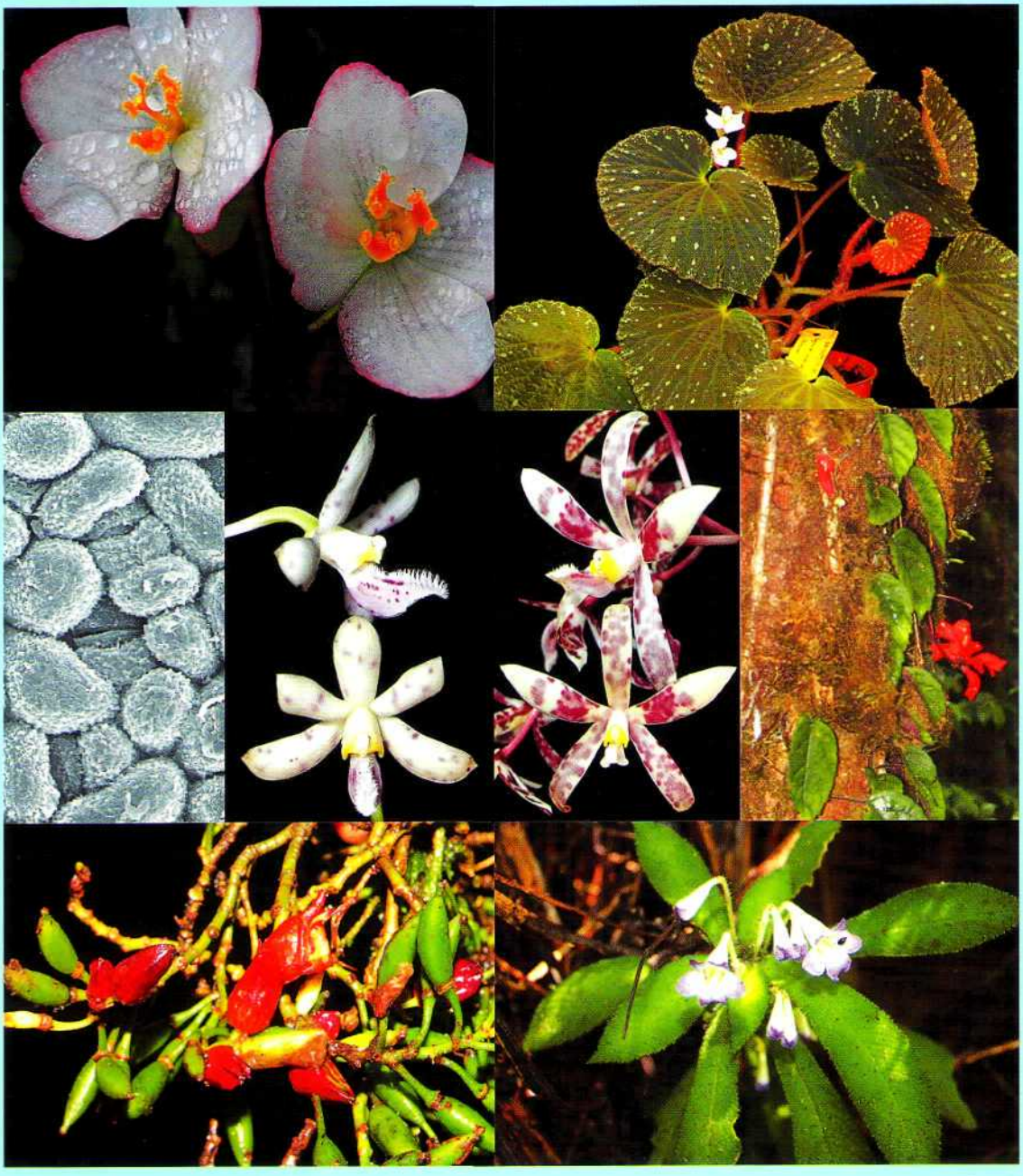
REINWARDTIA

A JOURNAL ON TAXONOMIC BOTANY, PLANT SOCIOLOGY AND ECOLOGY

ISSN 0034 – 365 X



2014 14 (1)

REINWARDTIA

A JOURNAL ON TAXONOMIC BOTANY, PLANT SOCIOLOGY AND ECOLOGY

Vol. 14(1): 1-248, December 23, 2014

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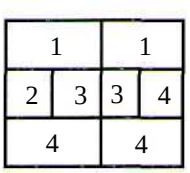

Cover images: 1. *Begonia holosericeoides* (female flower and habit) (Begoniaceae; Ardi *et al.); 2.* Abaxial cuticles of *Alseodaphne rhododendropsis* (Lauraceae; Nishida & van der Werff); *3. Dipodium puspitae, Dipodium purpureum* (Orchidaceae; O'Byrne); 4. *Agalmyla exannulata, Cyrtandra coccinea* var. *celebica, Codonoboea kjellbergii* (Gesneriaceae; Kartonegoro & Potter).

The Editors would like to thanks all reviewers of volume 14(1):

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REINWARDTIA
Vol 14, No 1, pp: 163−170

COMPARATIVE LEAVES ANATOMY OF PANDANUS, FREYCINETIA AND SARARANGA (PANDANACEAE) AND THEIR DIAGNOSTIC VALUE

Received December 11, 2013; accepted August 11, 2014

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ABSTRACT
SANTIKA, Y., TIHURUA, E. F. & TRIONO T. 2014. Comparative leaves anatomy of *Pandanus*, *Freycinetia* and *Sararanga* (Pandanaceae) and their diagnostic value. *Reinwardtia* 14(1): 163 – 170. — Study in leaves anatomy of twenty nine samples of the species classified under *Pandanus*, *Freycinetia* and *Sararanga* of Pandanaceae had been undertaken to unravel generic relationship among of these taxa with a view to provide a set of diagnostic characters for taxonomic identification. The fourth genus of Pandanaceae, *Benstonea* is not included in this analysis since there are no representative samples. Four anatomical diagnostic characters had been identified at the generic level such as present and absent of papillae, stomatal arrangement types, present and absent of bundle sheath extension and hypodermal thickness and its shape. *Pandanus* has papillae, amphistomatous stomata, bundle sheath extension present and hypodermis thin and rectangular; *Freycinetia* lacked of papillae, stomata hypostomatous or amphistomatous, bundle sheath extension absent and hypodermis thick and hexagonal or rounded; meanwhile *Sararanga* has no papillae, stomata amphistomatous, bundle sheath extension absent and hypodermis thin and flatten. An identification key to those genera based on anatomical diagnostic characters is provided.

Key words: *Freycinetia*, leaf anatomy, Pandanaceae, *Pandanus*, *Sararanga*.

ABSTRAK
SANTIKA, Y., TIHURUA, E. F. & TRIONO T. 2014. Perbandingan anatomi daun *Pandanus*, *Freycinetia* dan *Sararanga* (Pandanaceae) serta nilai diagnostiknya. *Reinwardtia* 14(1): 163 – 170. — Studi anatomi daun pada dua puluh sembilan sampel jenis yang diklasifikasikan ke dalam marga *Pandanus*, *Freycinetia* dan *Sararanga* dari suku Pandanaceae telah dilakukan untuk mengungkap hubungan antar marga dengan tujuan menyediakan satu set karakter diagnostik bagi identifikasi taksonomi. Marga keempat dari Pandanaceae, *Benstonea* tidak termasuk dalam kajian kali ini karena ketiadaan sampel. Empat karakter diagnostik telah diidentifikasi pada tingkatan marga yaitu ada tidaknya papila, tipe susunan stomata, ada tidaknya perluasan seludang berkas pengangkut dan ketebalan serta bentuk hipodermis. *Pandanus* memiliki ciri adanya papila, stomata *amphistomatous*, adanya perluasan seludang berkas pengangkut serta hipodermis tipis berbentuk persegi; *Freycinetia* tidak memiliki papila, stomata *hypostomatous* atau *amphistomatous*, seludang berkas pengangkut tidak mengalami perluasan dan hipodermis tebal serta berbentuk heksagonal atau bundar; sementara *Sararanga* tidak memiliki papila, stomata *amphistomatous*, seludang berkas pengangkut tidak meluas, hipodermis tipis dan memipih. Disediakan kunci identifikasi marga berdasarkan karakter anatomi diagnostik.

Kata kunci: Anatomi daun, *Freycinetia*, Pandanaceae, *Pandanus*, *Sararanga*.

INTRODUCTION

Understanding on plant anatomy is a fundamental information to study plant systematic and classification (Evert, 2006). Angiosperm leaves in which display a lot morphological and anatomical diversity have been used in classification and identification of various plant families (Rudall, 2007). Sonibare *et al.* (2006) had used leaf anatomy characters to differentiate *Ficus* species and concluded that leaf anatomy could provide useful character for taxonomic classification.

Leaf anatomical structure of three genera (*Freycinetia*, *Pandanus* and *Sararanga*) of Pandanaceae has been under taken by several authors. Tomlinson in 1965, has proposed five classes of stomata structure using papillae development and distribution on subsidiary or neighbouring cells and epidermis surface. However, no specific character could distinguish those three genera.

Five years later, North & Willis (1970)

discovered stomata and epidermis as useful characters in classifying species under the genus of *Freycinetia* in Solomon island. Similar study also conducted by Lim & Stone (1971), they mentioned that stomata, epidermis and hypodermis cells provide additional characters for the sectional classification of *Freycinetia*. Pasaribu (2010) also concluded that stomata structure could be used for *Freycinetia* infrageneric classification.

Kam (1971) carried out an anatomical comparison among Malayan *Pandanus* species. He found epidermal and stomatal characters as a support data that able to distinguished a considerable value at the generic and infrageneric classification of *Pandanus*. Another *Pandanus* examination was conducted by Rahayu *et al.* (2011) but only stricted to species in Java. Meanwhile, North & Willis (1971) have studied the smallest genus *Sararanga* that comprises two species, namely *Sararanga philippinensis* and *S. sinuosa*. According to their study, they found stellate trichomes as the identity characters of this genus. This was the major separating characters to identify other two genera *Freycinetia* and *Pandanus*.

Those information above indicated that, leaf anatomy only provided partial diagnostic characters for each genus within the family. However, there was no general diagnostic character that is meaningful for genera delimitation within Pandanaceae. Therefore, this study was conducted to review the leaf anatomical characters used by previous authors and provide a set of diagnostic characters for generic identification of *Freycinetia*, *Pandanus* and *Sararanga* in a format of an identification key.

MATERIALS AND METHODS

Twenty nine samples from Indonesia representing 6 species of *Pandanus*, 16 species of *Freycinetia* and 1 species of *Sararanga sensu* Stone (1968) were used. The fourth genus of Pandanaceae, *Benstonea* (Callmander *et al.*, 2013) is not included in this analysis since there are no representative samples. The characters observed were based from previous studies of Tomlinson (1965), North & Willis (1970; 1971), Kam (1971) and Lim & Stone (1971). Those observed characters were (1) stomata, (2) epidermis, (3) the presence of costal and inter-costal zone, (4) hypodermis and (4) vascular bundles.

The specimens used for this study were collected from fieldwork conducted at Java, Sumatra, Kalimantan, Sulawesi and Papua. The collection of fresh leaf samples were preserved in 70% alcohol and only median portion of 7 × 7 mm^2 was used for slide material. Meanwhile voucher specimens were kept as dry specimen and deposited in general BO collection.

Two slide preparation methods were employed in the study. The semi-permanent method using HNO_3 combined with heating was used for observing the leaf surfaces (Cutler, 1978). The permanent slide preparation method using paraffin embedding and safranin-fast green staining was employed to observe leaf structure of transversal section (Sass, 1951). The previous method was applied with free hand section (using new and sharp razor blade), while the latter method sectioning was applied with microtome.

Examination of slides was done in 100× and 400× magnifications using light microscopy Nikon Eclipse 80i equipped with digital camera and LCD viewer. All permanent slides and voucher specimens are deposited in BO. Anatomical description follows Lim and Stone (1971).

RESULTS AND DISCUSSION

Leaf anatomy of *Freycinetia*, *Pandanus* and *Sararanga* described below.

Pandanus

The epidermis cell shapes of the genus, usually square, pentagonal, hexagonal, polygonal and rectangular, sometimes irregular such as in *P. yvanii*; anticlinal cell wall straight or slightly undulate, papillae present or absent with some variation of shape (simple, forked or dendritic) and position (Fig. 1).

Stomata sunken or even to epidermis cells, tetracytic, amphistomatous, scattered on adaxial. In abaxial part, it is found only at inter costal or sometimes scattered thin hypodermis, consist of 1-3 layers of rectangular cells on adaxial and abaxial part as well. There is no sclerenchyma cell found in all species observed, since all samples of this study are different species to Rahayu *et al.* (2011).

Dorsiventral leaf (Fig. 2A), palisade 1-3 layers in adaxial sometimes up to 4 layers, absent or a layer in abaxial, the beneath cells are oval, unordered. The parenchyma tissues with or without intercellular air spaces between vascular bundle.

Raphida $CaCO_3$ crystal present (Fig. 2C), found at mesophyll, prismatic $CaCO_3$ crystal present or absent.

Vascular bundles lay within the mesophyll, enclosed by sclerenchyma and parenchyma tissues. 1-2 xylems tissue located in the middle part, phloem tissue spread out at abaxial part of

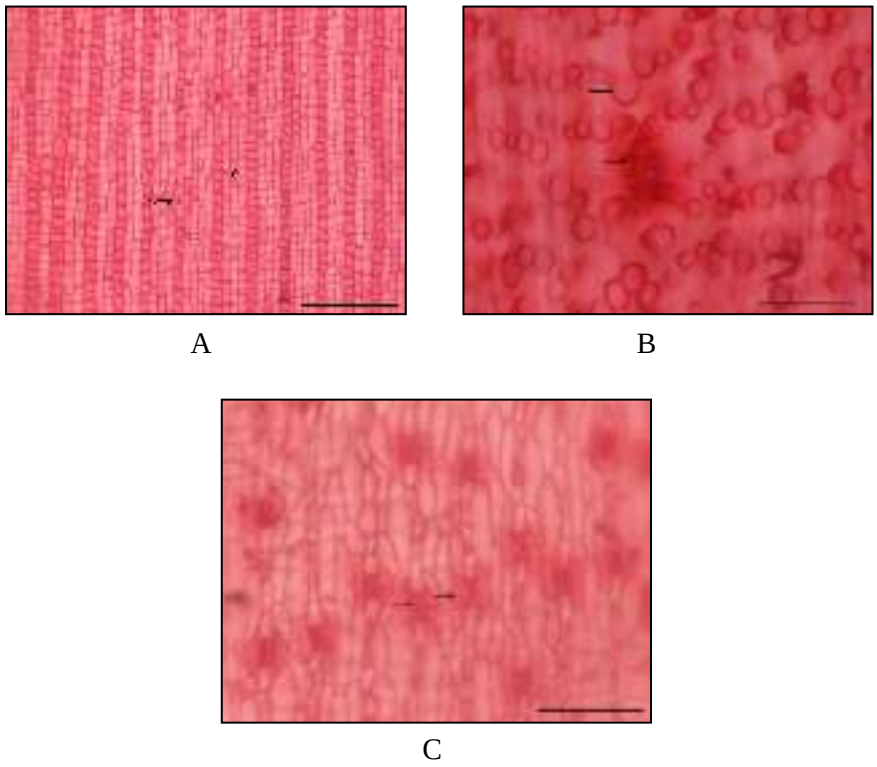


Fig. 1. Upper leaf surface in *Pandanus yvanii* (A), lower leaf surface with papillae (arrow) on *P. helicopus* (B) and *P. yvanii* (C), but some of *P. yvanii* without papillae. e: epidermis, s: sponge. Bar 100 µm (A) and 50 µm (B & C).

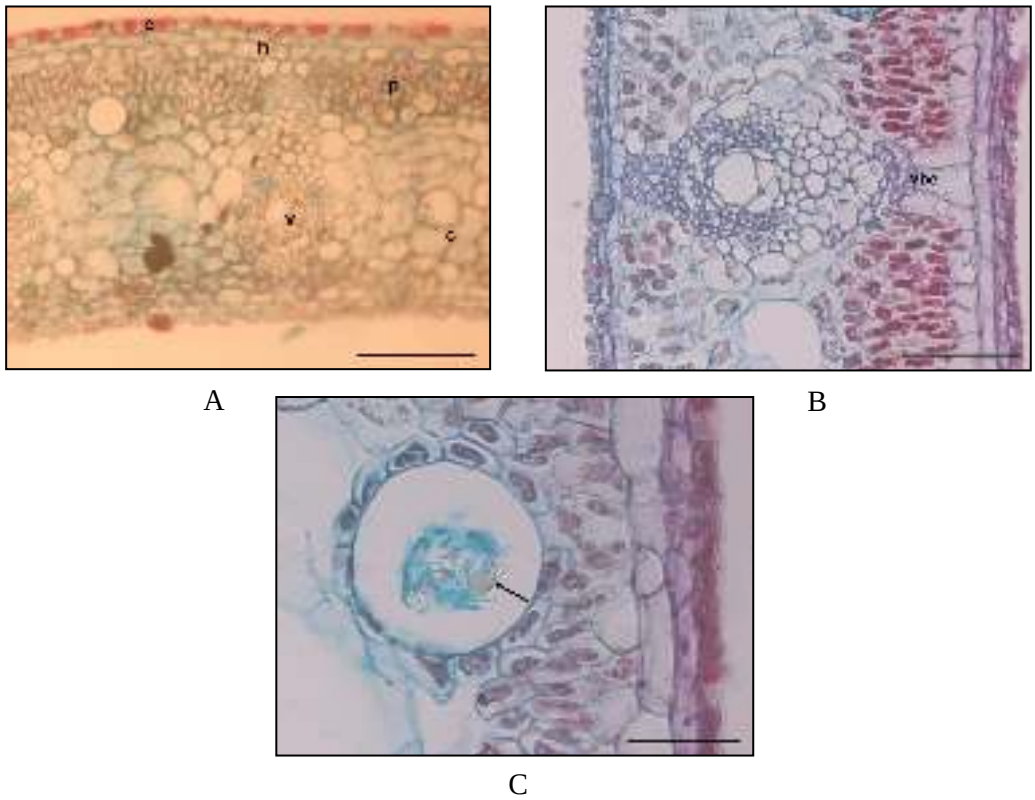


Fig. 2. Transverse leaf section of *Pandanus yvanii* (A) with vascular bundle extension (B) and raphida $CaCO_3$ crystal in the *P. helicopus* (C). c: chlorenchyma, e: epidermis, h: hypodermis, p: palisade, v: vascular bundle, vbe: vascular bundle extention. Bar 100 µm (A & B) and 50 µm (C).

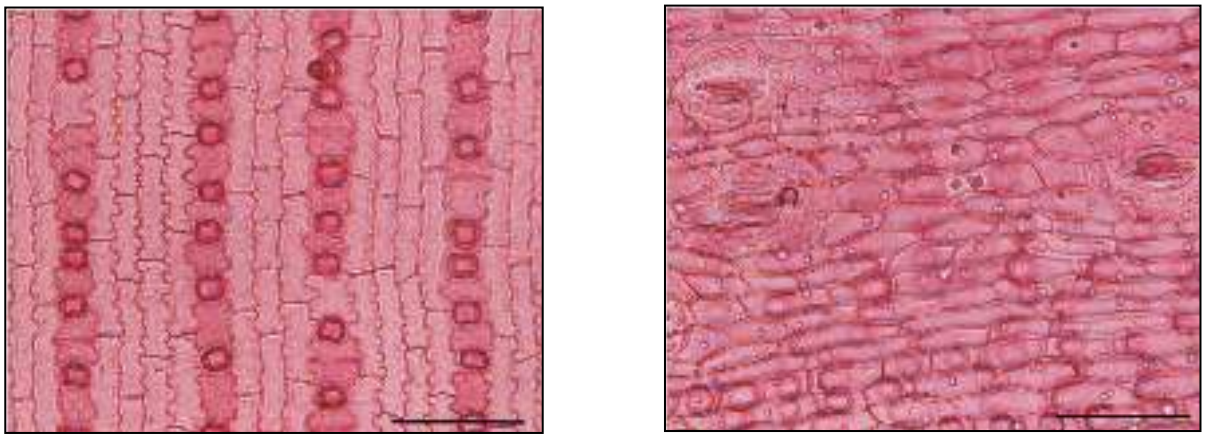
sclerenchyma sheath. The sclerenchyma or parenchyma tissues on *Pandanus* connecting the bundles with hypodermis. This connection makes chlorenchyma concentrate only between 2 vascular bundles in *Pandanus*. Parenchyma tissues found in outer part of the bundles, with bundles sheath extension (Fig. 2B) and can be seen only on mature leaves.

Specimen examined. *Pandanus aristatus (Ary P. Keim* 776); *P. discostigma* (*Ary P. Keim* 765); *P. pachypilus* (*Ary P. Keim* 767); *P. yvanii* (*Ary P. Keim* 777, *Ary P. Keim* 779, *Ina Erlinawati* 58, *Ina Erlinawati* 136, *Ina Erlinawati* 102); *Pandanus* sp. (*Ina Erlinawati* 31); *P. helicopus* (*Ina Erlinawati* 140).

Freycinetia

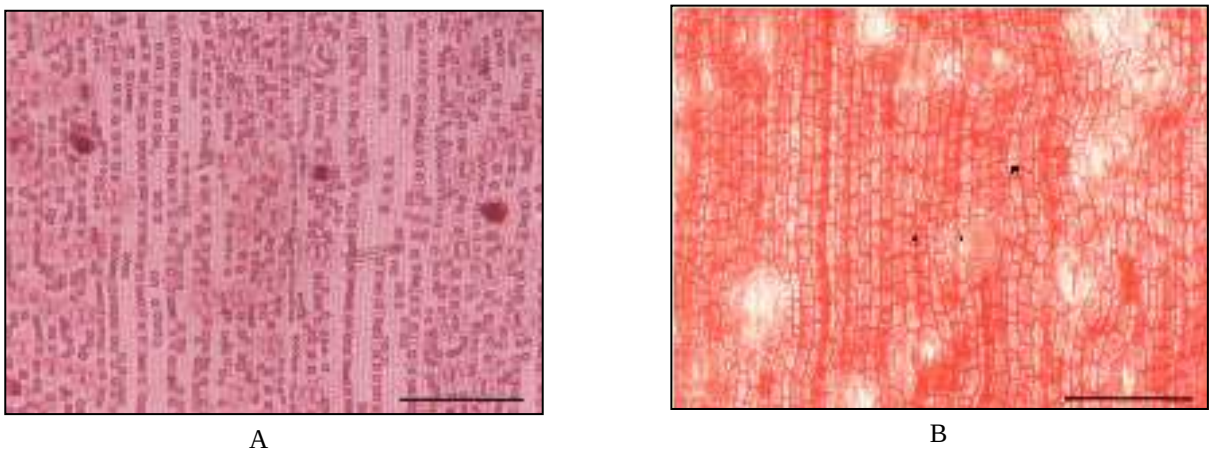
Epidermis cell shape rectangular, square to polygonal, anticlinal cell wall straight or undulate or sinuous; prismatic crystal present or absent; papillae present or absent on epidermis; neighbouring (subsidiary) cell, lateral cell or absent (Fig. 3). Stomata parallel to epidermis or sunken, tetracytic with 4 subsidiary cells in lateral and terminal part, hypostomatous or amphistomatous, concentrate only at intercostal or scattered on abaxial (Fig. 4).

Multilayer hypodermis on adaxial cell is bigger and thicker than abaxial once, hexagonal to rounded, hypodermis wall thickened or not, sclerenchyma near to epidermis (strand) in group or lined. Mesophyll differentiated as palisade, dorsiventral, 1-4 layers above and 1-2 below. Unordered parenchyma cells, ovate, with or without intercellular air spaces, between vascular bundles.



A B

Fig. 3. Leaf surface of *Freycinetia graminifolia* with sinuous anticlinal wall, prismatic crystal but without papillae (A). Leaf of *F. insignis* with straight anticlinal wall and papillae (B). Bar 50 µm.



A B

Fig. 4. Leaf surface with costal and intercostal zone of *Freycinetia graminifolia* (A) and without zone of *F. kartawinatae* (B). e: epidermis, s: sponge. Bar 200 µm (A) and 100 µm (B).

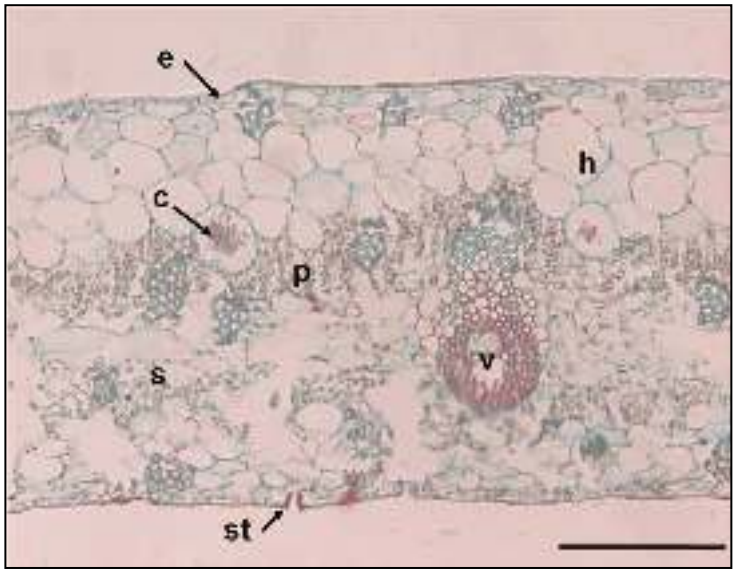


Fig. 5. Transverse leaf section of *F. javanica*. c: raphide $CaCO_3$ crystal inside the idioblast, e: epidermis, h: hypodermis, p: palisade, s: sponge, st: stoma and v: vascular bundle. Bar 200 µm.

Raphide and sclerenchyma often found at chlorenchyma (Fig. 5). Vascular bundle is enclosed by sclerenchyma and parenchyma tissues. 1-2 xylems tissue in the middle, phloem tissue spread out at abaxial part of sclerenchyma sheath, without bundle sheath extension.

Specimen examined. *F. sumatrana* (*Ary P. Keim* 764); *F. insignis* (*Abdulrokhman Kartonegoro* 190); *F. sarawakensis* (*Ary P. Keim* 757); *F. graminifolia* (*Dirman* sn.); *Freycinetia* sp. (*Rugayah* 1543); *Freycinetia* cf. *imbricata* (*Tika Dewi Atikah* 3); *F. scandens* (*Abdulrokhman Kartonegoro* 14); *Freycinetia* cf. *undulata* (*Alex Sumadijaya* 295); *F. minahassae* (*Purwaningsih* 127); *Freycinetia* sp. (*Rugayah* 1538); *Freycinetia* cf. *rigidifolia* (*Ina Erlinawati* 52, *Ary P. Keim* 813); *F. angustifolia* (*Rulyana Susanti sn.*); *F. kartawinatae* (*Ary P. Keim* 770); *F. javanica* (*Ina Erlinawati* 43, *Ary P. Keim* 814); *F. kostermansii* (*Dirman* 22); *F. funicularis* (*Yessi Santika* 263).

Sararanga

Epidermis cell shape square, pentagonal, hexagonal or rectangular; anticlinal cell wall straight; prismatic crystal present; papillae absent (Fig. 6A). Stomata parallel to epidermis; tetracytic, 2 lateral cells and 2 terminal cells; amphistomatous, on abaxial it found only at intercostal zone and scattered on adaxial (Fig. 6B).

Hypodermis thin, multilayer, consist of 2-3 layers of flatten cells, sclerenchyma absent. Mesophyll undifferentiated as palisade and sponge tissue. The parenchyma tissues without intercellular air spaces take place in the middle of two vascular bundle. Raphide crystals are founded at mesophyll (Fig. 7).

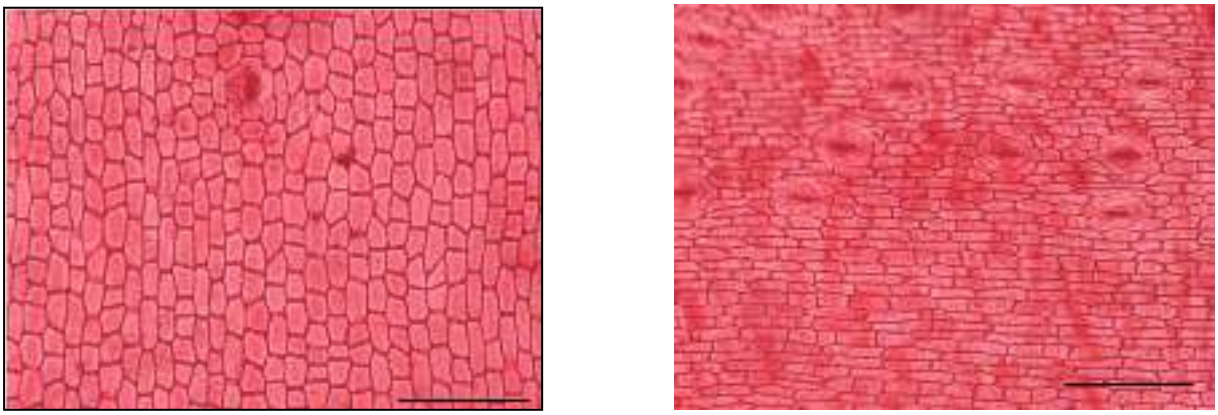
Vascular bundles lay in the mesophyll. It is enclosed by sclerenchyma and parenchyma tissues. 1-2 xylems in the middle, phloem spread at abaxial part of sclerenchyma sheath. Parenchyma tissues on outer part of the bundles, without bundles sheath extension.

Specimen examined. *Sararanga sinuosa* (*Yessi Santika* 321).

Hypodermis on those three genera always present with 2-3 row cells beneath the epidermis. Hypodermis layer in the three genera is always thicker on the adaxial surface than abaxial once. *Pandanus* hypodermis shape is rectangular, sometimes with thickened wall. In contrary, *Freycinetia* hypodermis shape is hexagonal to rounded and sclerenchyma strands distributed within this tissue. An exception occurs in *Sararanga* of which hypodermis cell is flattened and thin.

Compared with two other genera, *Sararanga* has thicker mesophyll while *Pandanus* has dorsiventral mesophyll. Mesophyll in *Pandanus* is only a thin layer of palisade and unordered sponge. Meanwhile, in several *Freycinetia* species, the shape of parenchyma cell in mesophyll is star-like because of its large intercellular air spaces (stellate parenchyma).

The vascular bundles structure for the three genera are almost similar. Vascular bundles in all the genera comprises 1-2 xylems in the middle part, phloem that is spread at abaxial, two sheath encircles of the vascular channel with sclerenchymatous tissue found in inner sheath and parenchymatous tissues in outer part of the bundles. The



A B

Fig. 6. Upper (A) and lower (B) leaf surface of *Sararanga sinuosa* with straight anticlinal wall that showed no papillae on both surfaces. Bar 100 µm.

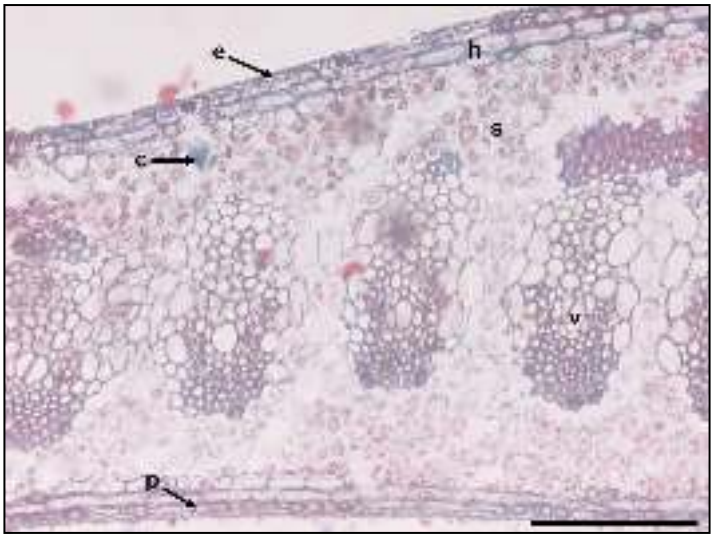


Fig. 7. Transverse leaf sections of *Sararanga sinuosa*. c: raphide $CaCO_3$ crystal, e: epidermis, h: hypodermis with flatten shape, p: prismatic $CaCO_3$ crystal, s: chlorenchyma without differentiation (sponge tissue), v: vascular bundle without sheath extension. Bar 200 µm.

Tabel 1. The comparative leaf anatomical diagnostic characters of *Pandanus*, *Freycinetia* and *Sararanga* (Pandanaceae).

Characters	*Pandanus*	*Freycinetia*	*Sararanga*
Papilla	Generally present	Generally absent	Absent
Stomata	Amphistomatous	Hypostomatous, amphistomatous	Amphistomatous
Zone in abaxial surface	Present or absent	Present or absent	Present
Bundle-sheat extension	Present (sclerenchyma or parenchyma)	Absent	Absent
Shape of hypodermis	Rectangular	Hexagonal to orbicular	Flatten

difference structure is the present or absent of bundle sheath extention. Bundle sheath extension is parenchymatous or sclerenchymatous tissues which connected the bundle sheath with epidermis tissues. It only present on *Pandanus* (Fig. 2B) and absent on the other two genera.

The important character of leaf anatomical structure in the recent study indicated that, epidermal characters especially the presence of papilla and the stomata distribution, can be used to differentiate *Pandanus*, *Freycinetia* and *Sararanga* (see Table 1). This is in according with Tomlinson's work (1968) on *Pandanus* of which and gave satisfactory classification at genus level (Tomlinson, 1968). It is also in line with Kam's work (Kam, 1971) on *Pandanus* that support sectional classification sensu Stone (1968) and also in line to Lim & Stone (1971) on *Freycinetia*.

However, the latter taxonomic treatment only partially agreed to Stone (1968) sectional classification. This partial agreement is due to the nature of *Freycinetia* epidermal tissue in which always homogen.

From this study, papillae usually absent in most of *Freycinetia* epidermal tissue, except in *F. sumatrana* and *F. insignis*. These two species have simple papillae with no variation shape. In contrary, it is very common and vary on *Pandanus*. In *Sararanga* however, no papillae found in this genus. In general, the three genera have stomata on both sides of epidermis, except in some *Freycinetia* species.

Another character from this study is arrangement of abaxial stomata. *Sararanga* and some species of *Pandanus* and *Freycinetia* has stomata only on its intercostal zone. The epidermis with stomata is barried with un-stomata area. Another species has stomata spread on abaxial epidermis (zonation absent).

Based on the diagnostic character in Table 1 and the discussion above, a dichotomous identification key to differentiate *Pandanus*, *Freycinetia* and *Sararanga* of *Pandanaceae* is provided. This key is also useful to identify leaf fragment of *Pandanaceae* using light microscope.

Key to the genera based on leaf anatomical stucture

1.a. Papilla present, complex, dominate on the leaf surface; bundle sheath extension present ……...*Pandanus*
b. Papilla absent, or present with simple shape; bundle sheath extension absent
2.a. Hypodermis hexagonal to round, thick ……..……...……..*Freycinetia*
b. Hypodermis flatten, thin …….*Sararanga*

CONCLUSIONS

Some previous studies only indicated anatomy structure could be used at infrageneric level. Epidermal tissue with some different papillae shape and distribution as the main character (Tomlinson, 1968; Kam, 1971; Lim & Stone, 1971; Pasaribu, 2010; Rahayu *et al.*, 2011). This present study shows that *Pandanus*, *Freycinetia* and *Sararanga* could distinguish by leaf anatomy structure. Four important anatomical diagnostic characters had been identified at the generic level *i.e.* the present and absent of papillae, stomata arrangement type, presence and absence of bundle sheath extension and hypodermal shape.

ACKNOWLEDGEMENTS

We thank colleagues at BO with whom we have discussed this topic, especially Ary P. Keim, leader of *Pandanaceae* research group, Herbarium Bogoriense, Bogor, for his support and willingness to discuss these issues.

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Published by Herbarium Bogoriense, Botany Division, Research Center for Biology, Indonesian Institute of Sciences
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Vol. 14. No. 1.2014
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