

LEAF ANATOMY STUDY OF FREYCINETIA SPP. (PANDANACEAE) WITH REFERENCE TO STONE'S INFRAGENERIC CLASSIFICATION

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Yessi Santika & Eka Fatmawati Tihurua. 2014. Penelaahan Anatomi Daun *Freycinetia* spp. (*Pandanaceae*) Dibandingkan dengan Klasifikasi 'Infrageneric' dari Stone. *Floribunda* 5(1): 21–26. — Telah dilakukan penelaahan anatomi daun *Freycinetia* (*Pandanaceae*) di Kawasan Malesia. Pengamatan dilakukan pada jaringan epidermis, hipodermis dan mesofil daun dari 18 jenis *Freycinetia* spp. Variasi banyak ditemukan pada jaringan epidermis, seperti ada tidaknya papila, susunan stomata, bentuk sel epidermis, dan keberadaan kristal prisma. Berdasarkan beberapa karakter tersebut, *Freycinetia* terbagi kedalam 7 kelompok. Kelompok ini kemudian dibandingkan dengan seksi yang dilakukan oleh Stone berdasarkan karakter morfologinya.

Kata kunci: *Pandanaceae*, *Freycinetia*, anatomi daun.

Yessi Santika & Eka Fatmawati Tihurua. 2014. Leaf Anatomy Study of *Freycinetia* spp. (*Pandanaceae*) with Reference to Stone's Infrageneric Classification. *Floribunda* 5(1): 21–26. — The leaf anatomy of *Freycinetia* (*Pandanaceae*) in the Malesian Region is reviewed. We made observations of epidermal tissue, hypodermic and leaf mesophyll for 18 *Freycinetia* spp. Much variation was seen in the epidermal tissue, e.g., in the existence of papillae, stomata arrangement, shape of epidermis cell and prismatic crystals. We found seven groups within the genus *Freycinetia*, based on these characters and compared these groups to Stone's sections.

Keywords: *Pandanaceae*, *Freycinetia*, leaf anatomy.

Freycinetia is genus of *Pandanaceae* in which all species climbers. Anatomical studies of *Freycinetia* began with Tomlinson (1965), although he examined the whole family *Pandanaceae* and concentrated primarily on *Pandanus*. His study triggered further anatomical studies, especially to find new informative characters.

For *Freycinetia*, North & Willis (1970) examined some species from the Solomon Islands and concluded that anatomy of the genus was relatively uniform, and only varied in degree from *Pandanus*, rather than in fundamental distinction. For *Pandanus*, leaf epidermis tissue can be used to support almost all of Stone's sections (Kam 1971). Lim & Stone (1971) tried to used some characters, especially stomata and epidermal, to support the sections, in six Malayan and eight non Malayan species. Some data supported the section groupings, but in other cases the data seems ambiguous.

Morphologically, fruit is an important character to divide taxa. However, it is difficult to get fertile material in the field, because of the plants' height, and unknown reproductive time. Sterile specimens of some species can be identified by the auricle, but usually they can only can be identified

to genus level. New anatomy characters might hopefully dissolve infrageneric classification problems, and also species complex problems.

MATERIAL AND METHOD

Eighteen leaf samples were studied that was collected during field exploration at Bukit Baka Bukit Raya National Park and Sebangau National Park (Kalimantan), Riau, Java, Halmahera, Sulawesi and Waigeo Island. From 18 samples, 16 samples identified as (*F. sarawakensis* Martelli, *F. cf. imbricata*-Sarawakensis); (*F. sumatrana* Hemsley-Auriculifolia); (*F. scandens*-Oligostigma); (*F. angustifolia*-Racemosiflorae); (*F. cf. graminifolia*-Solmsiella); (*F. cf. kostermansii*; *F. cf. funicularis*-Lateriflorae); (*F. javanica*-Cyrtopoda); (*F. insignis*-Blumeella); (*F. cf. undulata*-Filiformicarpae); (*F. rigidifolia* Hemsley-Hemsleyella), *F. kartawinatae*, *F. minahassae* and two samples unidentified. Mature leaves were chosen and the most width part was used for analysis.

Living material was fixed in 70% alcohol. For paradermal slides, material was boiled in 10% HNO₃ until the epidermal tissue separated, was

washed with distilled water, scraped gently and stained with Safranin O; then again washed and mounted in glycerine for observation.

For transversal section, methods were employed: by paraffin embedding, stained with safranin-fast green (Sass 1951), and by simple hand sections.

Slides were observed with light microscope (Nikon 80 i). All leaf tissue was observed, epidermis, hypodermis and mesophyll but stressed to epidermal tissue, but we concentrated on epidermal tissue.

RESULT

General Description of Anatomical Leaf of *Freycinetia*

Epidermal cell shape is rectangular, square to polygonal with straight anticlinal cell wall sometime undulate or sinuous, dominantly non-papillae, prismatic crystals absent or present. Stomata even or sunken, tetracytic, simple, commonly present on both surface, arrange costal and intercostals regions or not. Hypodermis polygonal at adaxial part and isodiametris below; multiple layers 2–7, mostly 3; fiber present. Mesophyll is differentiated, chlorenchyma cells are arranged by 1–4 rows palisade at adaxial part and 1–2 at lower part, sponge present below the palisade; fiber present; raphide absent or present. Vascular bundle present without bundle sheath extension.

Epidermis

Most species have simple epidermis cells without papillae except in *F. insignis* and *F. sumatrana*. Epidermis cell shape is more variable, rectangular ones (Fig. 1A) were found in *F. minahassae*, *F. angustifolia*, *F. rigidifolia*, *Freycinetia* sp. (R 1538), *F. graminifolia*, *F. kostermansii*, abaxial epidermis of *Freycinetia* cf. *undulata* and adaxial epidermis of *F. insignis*. Square shaped epidermis cells (Fig. 1B) were found in *F. kartawinatae*, *F. javanica*, *F. funicularis*, abaxial epidermis of *F. insignis*, *Freycinetia* sp. (R 1543) and polygonal shapes (Fig. 1C) were found at *Freycinetia* cf. *imbricata*, *Freycinetia* cf. *undulata*. Prismatic crystals are common found, making a line or spread. Simple papillae are only present in abaxial epidermis *F. sumatrana* (Fig. 2A) and *F. insignis* (Fig. 2B).

Stomata are parallel with epidermis cells, tetracytic with two lateral cells and two terminal cells smaller than others. Neighboring cells are not

different with other epidermal cells.

Hypodermal

Multiple layers 2–7 at adaxial and 2–6 at abaxial part, commonly 3–4 layers, isodiametris, thin wall, sometime with thickening. Several species had one flat hypodermis cell, right beneath epidermis tissue.

Mesophyll

Chlorenchyma differentiated into palisade and sponge tissue. Palisade was 1–3 layers in upper and 1–2 in lower part, short rectangular. Sponges were isodiametric, with variation in intercellular space (Fig. 3A). Sometimes, chlorenchyma is found between two vascular bundles as branched tissue or stellate (Fig. 3B). Raphide are present inside the idioblast cell among palisade cells. Sclerenchyma occurs in group among hypodermis or mesophyll.

Vascular tissues were circled by sclerenchyma cells, composed of large xylem as a centre and phloem surrounding the xylem between sclerenchyma cells, without bundle sheath extension.

DISCUSSION

From all samples that were used in this analysis, we can note that *Freycinetia* leaf anatomy is uniform, especially in mesophyll and hypodermis layers. Variation in epidermal shape is significant and sometime could become a species character. Different shapes were found in *F. graminifolia*, *F. kostermansii* and *F. sumatrana*, caused by sinuous and undulate anticlinal cell walls that were not found in other examined species. A similar phenomenon was found by Lim & Stone (1971) in *F. kamiana* from Malaysia. They suggested that this wavy wall was species character. From our study we suspected that wall shape character could be valuable one. Epidermal tissues were simple except *F. insignis* and *F. sumatrana* with papillae in epidermis and neighbouring cells.

Stomata were tetracytic, similar to *Pandanus* (Tomlinson 1965, Kam 1971, Lim & Stone 1971). They could be found in both adaxial and abaxial layers (amphystomatic) or only on abaxial (hypostomatic) as in *F. graminifolia* and *F. kostermansii*. Arrangement of stomata at abaxial divided this genus into two groups, first group with stomata concentrate at intercostal region (costal and intercostal region are seen obviously) (Fig. 4A) and the second group with stomata arranged scattered (without costal and intercostal region) (Fig. 4B).

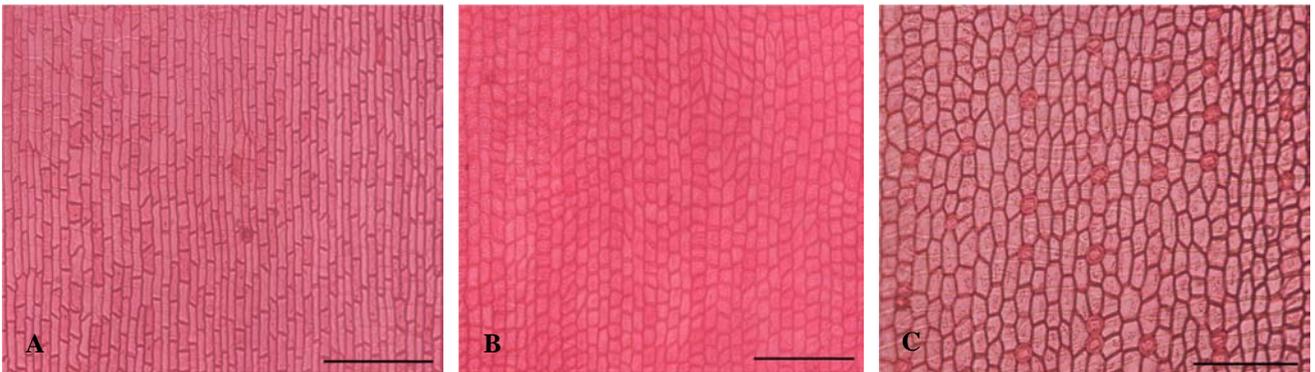


Fig. 1. Shape of epidermis cell. A. Rectangular shape of *Freycinetia* sp. (R1538); B. Square shape of *F. funicularis*; C. Polygonal shape of *F. cf. undulata* (Scale 100 μm).

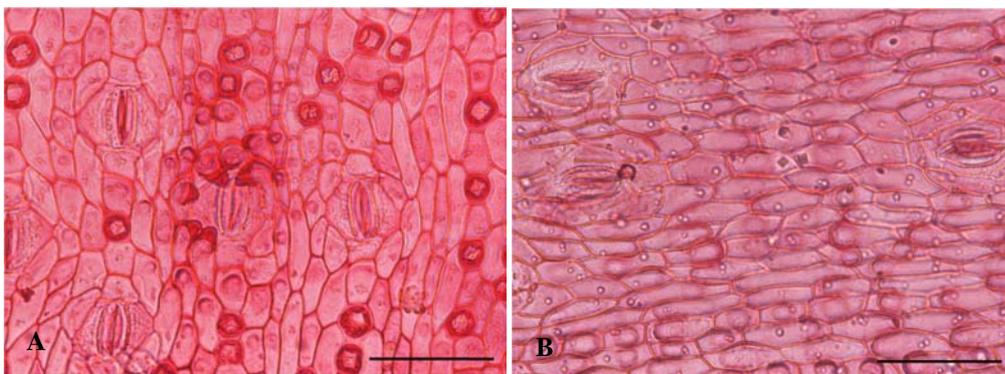


Fig. 2. Simple papillae only present in abaxial epidermis. A. *F. sumatrana*; B. *F. insignis* (Scale 50 μm).

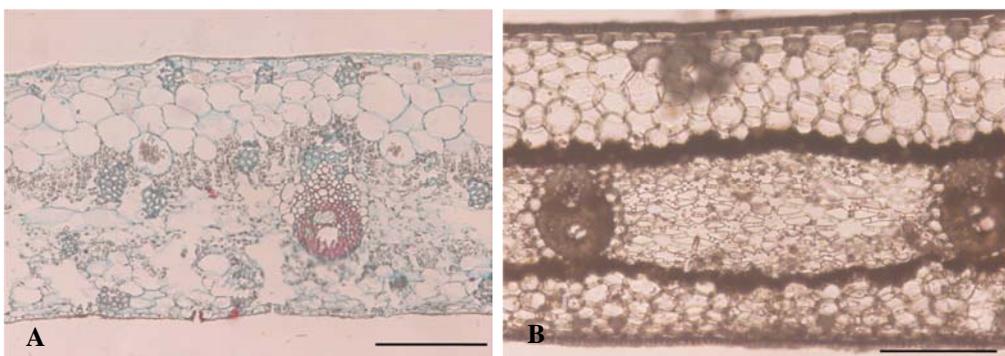


Fig. 3. Transversal cutting of A. *F. javanica* with isodiametric sponge; B. *Freycinetia* sp. (R1543) with stellate like sponge (Scale 200 μm).

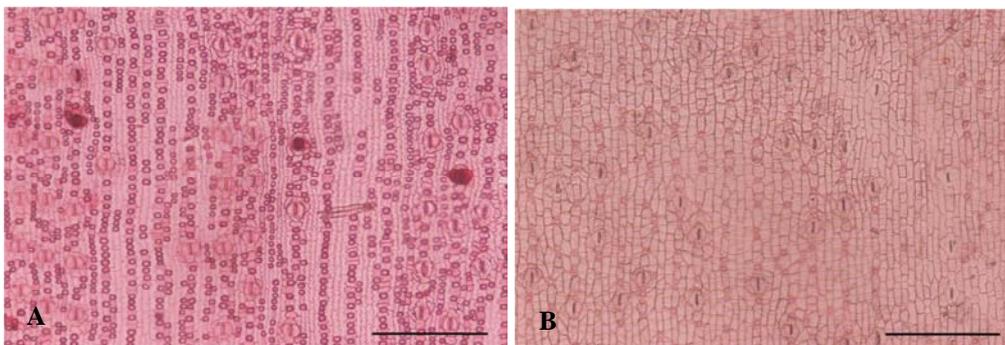


Fig. 4. Stomata arrangement A. Stomata concentrated at intercostals region (*F. graminifolia*); B. Stomata scattered (*Freycinetia* cf. *undulata*) (Scale 200 μm).

According to epidermis, papillae, stomata arrangement, presence of prismatic crystal, presence costal and intercostal region, we can arrange a key to split groups:

- a. Epidermis with papillae 2
- b. Epidermis without papillae 3
- a. Papillae occur in subsidiary cells (Type 1)
- b. Papillae exist in epidermis cells (Type 2)
- a. Stomata present only on lower surface (Type 3)
- b. Stomata distributed on both surfaces 4
- a. Lower epidermis with costal and intercostal regions (Type 4)
- b. Lower epidermis without costal and intercostal regions 5
- a. Granulated epidermis (Type 5)
- b. Non granulated epidermis 6
- a. Prismatic crystals present in both surfaces (Type 6)
- b. Prismatic crystals absent in both surfaces (Type 7)

Type 1

Upper epidermal anticlinal cell wall is straight-undulate, but straight in lower surface; epidermis cell shape is square, pentagonal, hexagonal or rectangular; prismatic CaCO_3 present in the upper and lower surfaces; simple papillae appear in the lower surface especially in the terminal subsidiary cells. Stomata dispersed on both surfaces; placed even to epidermis cells; tetracytic with 2 terminal and 2 lateral subsidiary cells. Costal and intercostal regions are conspicuous. Three layers of hypodermis are arranged in the upper and lower part of leaf. Mesophyll is distinguished to 2–3 layers, palisade in upper and 1–2 layers in lower part; sponge tissue is between both palisade layers; raphide present; sclerenchyma appears in groups and distribute in mesophyll and hypodermis.

Examined specimen: *F. sumatrana* (Ary P. Keim 764).

Notes: Lim & Stone (1971) used *F. sumatrana* and *F. sumatrana* var. *penangiana* which both species have different papillae distribution. Present study, *F. sumatrana*, showed similarity structure with *F. sumatrana* var. *penangiana*.

Type 2

Epidermal anticlinal cell wall is straight; epidermis cell shape is square, pentagonal, hexagonal, polygonal or rectangular; most epidermis cells in the upper surface are long, but shorter in lower surface; prismatic CaCO_3 is absent; simple papillae spread on epidermis cells of the lower surface. Stomata on both surfaces; placed evenly or sunken to epidermis cells; tetracytic type with 2 cells each in terminal and lateral subsidiary cell. Costal and intercostal regions are obvious. Hypodermis 3–4 layers in upper and lower part of leaf. Mesophyll is distinguished to 1–2 layers, palisades in upper part

and sponge tissue below palisade layers; idioblast with raphide crystal spread in mesophyll and hypodermis; sclerenchyma appears in groups and distributed in mesophyll and hypodermis.

Examined specimen: *F. insignis* (Abdurrohman Kartonegoro 190).

Type 3

Freycinetia of this group have epidermal anticlinal cell wall sinuous. Epidermal cell shape is rectangular or irregular (no angles). Prismatic CaCO_3 exists in the upper and lower surface; papillae absent. Stomata restricted to lower surface (hypostomatous); placed even to epidermis cells, tetracytic with 4 subsidiary cells in terminal and lateral. Costal and intercostal zone obviously seen in lower surface. Hypodermis 2–5 layers in upper and 2–6 in lower part of leaf. Mesophyll is distinguished to 1–2 layers palisade in upper part and sponge underneath the palisade; raphide crystal spread in mesophyll and hypodermis; sclerenchyma appear in groups and are distributed in mesophyll and hypodermis.

Examined specimens: *F. graminifolia* (Dirman sn.); *F. kostermansii* (Dirman 22).

Type 4

Epidermal anticlinal cell wall is straight; epidermis cell shape is square, pentagonal, hexagonal, sometimes polygonal and rectangular; prismatic CaCO_3 distributed in both surface, except in *Freycinetia* cf. *imbricata* where present only on lower surface; without papillae. Stomata dispersed on both surfaces, placed evenly on epidermis cells; tetracytic with 2 terminal and 2 lateral subsidiary cells. Costal and intercostal region present on lower surface. Hypodermis 3–7 layers in upper and 2–5 lower part of leaf. Mesophyll is differentiated

into 1–2 palisade layers in upper and single layer/absent in lower part; chlorenchyma of stellate form; with or without raphide; sclerenchyma appears in groups and distributed in mesophyll and hypodermis.

Examined specimens: *Freycinetia* sp. (Rugayah 1543); *Freycinetia* cf. *imbricata* (Tika Dewi Atikah 3); *Freycinetia* cf. *rigidifolia* (Ina Erlinawati 52); *F. sarawakensis* (Ary P. Keim 757).

Notes: *Freycinetia* cf. *rigidifolia* description in this study different to Lim & Stone (1971) description. This specimen and *Freycinetia* sp. (Rugayah 1543) were found sterile.

Type 5

Epidermal anticlinal cell wall is straight; epidermal cell shape is square, pentagonal, hexagonal or rectangular; granular; prismatic CaCO_3 takes place only on the lower surface in *Freycinetia* cf. *rigidifolia* and *F. angustifolia* (AK 813) but absent in *F. angustifolia* (Ruliyana Susanti sn.); papillae absent. Stomata dispersed on both surfaces, placed even to epidermis cells; tetracytic with each 2 subsidiary cells in lateral and terminal. Without costal and intercostal region. Hypodermis 2–4 layers in upper and 2–3 in lower part of leaf. Mesophyll is distinguished to 1–2 layers palisade in upper part and sponge tissue; raphide spread in mesophyll and hypodermis; sclerenchyma appears in groups and distributed in mesophyll and hypodermis.

Examined specimens: *F. angustifolia* (Ary P. Keim 813, Ruliyana Susanti sn.).

Type 6

Epidermal anticlinal cell wall is straight; epidermal cell shape is square, pentagonal, hexagonal, polygonal or rectangular; prismatic CaCO_3 present in both surface; papillae absent. Stomata dispersed on both surfaces; placed even/sunken to epidermis cells; tetracytic with 2 terminal and 2 lateral subsidiary cells. Costal and intercostal region absent. Hypodermis 3–6 layers in upper and 3–5 in lower part of leaf. Mesophyll is distinguished to 1–3 layers palisade in upper and 1 layer in lower part or absent; raphide spread in mesophyll and hypodermis, but absent in *F. minahassae*; sclerenchyma appears in groups and distributed in mesophyll and hypodermis.

Examined specimens: *Freycinetia* cf. *undulata* (Alex Sumadijaya 295); *F. kartawinatae* (Ary P. Keim 770); *F. minahassae* (Purwaningsih 127); *Freycinetia* sp.2 (Rugayah 1538). Notes: *Freycinetia* sp. (Rugayah 1538) is sterile specimen.

Type 7

Epidermal anticlinal cell wall is straight; epidermal cell shape is square, pentagonal, hexagonal, polygonal or rectangular; without prismatic CaCO_3 ; papillae absent. Stomata dispersed on both surfaces; placed even or sunken to epidermis cells; tetracytic with 2 terminal and 2 lateral subsidiary cells. Costal and intercostal region absent. Hypodermis 3–5 layers in upper and 2–4 in lower part of leaf. Mesophyll is distinguished to 1–3 layers palisade in upper and 1 layer in lower part or absent; raphide spread in mesophyll and hypodermis; sclerenchyma occur as groups and distributed in mesophyll and hypodermis.

Examined specimens: *F. javanica* (Ina Erlinawati 43, Ary P. Keim 814); *F. funicularis* (Yessi Santika 263); *F. scandens* (Abdulrohman Kartonegoro 14).

We found that the anatomical characters do not support the Stone's sections in *Freycinetia*, except for *F. sumatrana*-*Auriculifolia* and *F. insignis*-*Blumeella*, which have papillae on their epidermis. *F. kostermansii* and *F. graminifolia* cluster in the same group, Type 3. Even they were placed in different section by Stone, but both of them have similar morphological characters and lateral inflorescence. Anatomically, they have wavy epidermis walls, which are not usually found in most species.

Type 4 including *F. sarawakensis*, *F. cf. imbricata*, *F. cf. rigidifolia* and *Freycinetia* sp.1 have no papilla, but were found to have intercostal regions. The zonation of lower epidermis was inconspicuous found in samples *F. sarawakensis*, *F. cf. rigidifolia* and *Freycinetia* sp.1. We could see the zonation by stomata arrangement, it was spread in intercostal and arrange in order in costal region. Zonation in *F. sarawakensis* is very clear since there are no stomata in its intercostal region. Even Stone placed *F. sarawakensis* and *F. imbricata* in same section, though he was doubtful whether *F. imbricata* really belongs here. Type 5 has only *F. angustifolia* as a group member. It is the only species with racemosa inflorescence.

Four species were placed in type 6, which are *Freycinetia* cf. *undulata*, *F. kartawinatae*, *F. minahassae* and *Freycinetia* sp.2. The only species which was mentioned by Stone (1968) was *F. undulata*. Anatomical characters for these group include: no papilla and granules, intercostal zone absent, but with crystals in epidermis cells.

Three species which belongs to Type 7 were placed in three different sections. *F. javanica* and *F. scandens* were belong to different sections, but in vegetative condition they hardly differ. But ana-

tomically both of them could be differentiated (Santika 2010). *F. funicularis* has lateral flower, opposite with another two species which has terminal flower. Common characters of these group are it has no papilla, intercostal zone absent, it has no granule and crystal in epidermis cell.

CONCLUSION

Most of Stone's section (1968) are not supported by anatomical characters. Low anatomical variation which could be used as characters including lack of papillae in most of species is the reason why it is difficult to support the Stone's section. But anatomical characters still can be used to resolved species complexes in this genus.

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