

Lee Lan LIM* & Benjamin C. STONE*: **Notes on systematic foliar anatomy of the genus *Freycinetia* (Pandanaeae)****

L. L. リム*・B. C. ストーン*: 葉の解剖によるツルアダン属植物の分類学的研究**

Introduction

Until recently the genus *Freycinetia*, with more than 260 binomials and probably more than 160 species, had not been the subject of an elaborate infrageneric classification, but Stone (1968) established a total of 17 infrageneric taxa, all ranked as sections, and later (Stone, 1969, 1970) added three more sections. These taxa were envisioned as closely knit groups of similar and related species, and their formal recognition was considered an important preliminary step in elucidating the interspecific relationships in this comparatively large genus. Since considerable success in testing the validity of such sectional groups had been gained in similar studies of the genus *Pandanus* (Kam, 1969; Kam and Stone 1970) by utilizing anatomical characters, in particular those pertaining to the stomata, epidermis, hypodermis, and other foliar features, it was felt desirable to carry out a pilot study on various species of *Freycinetia*. Fourteen species of *Freycinetia* were chosen on the basis of (a) representation of different generic sections, and (b) availability of living or pickled materials, with emphasis on those occurring in the Malayan peninsula.

Earlier studies by Tomlinson (1965) and North and Willis (1970) suggested that there is a much narrower range of variation in features of the foliar anatomy in *Freycinetia* than in *Pandanus*. Nevertheless, since a very small number of species had been studied, we felt that further investigation was necessary. The results of this study do not disclose any further elaborate types of stomatal structure; as Tomlinson (1965) showed, the

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** Materials for a monograph of *Freycinetia*, XIV. For previous papers in this series, see References below, under Stone, B. C. Most of this work was carried out as an Honours Thesis by Miss Lim Lee Lan in the Division of Botany, School of Biological Sciences, University of Malaya, in 1969.

stomata of various species of *Freycinetia* tend to be either simple and unspecialized (Class I) or the subsidiary and neighboring cells may be more or less papillose (Class II).

In addition to a study of stomatal structure, the following anatomical features were noted: (1) presence or absence of costal and intercostal differentiation in the abaxial epidermis; (2) shape and size of epidermal cells; (3) presence or absence of epidermal papillae; if present, their distribution; (4) number of associated neighboring cells per stomate; (5) presence or absence of silica bodies; if present, their abundance and distribution.

Materials and methods. Six Malayan and eight non-Malayan species were studied. These are listed here by Section, with indication of source and voucher specimens. Species marked * occur in the Malayan peninsula.

- (1) Sect. *Racemosiflorae*: *F. jagorii* Warb. (PNH. 10228).
- (2) Sect. *Sarawakensis*: *F. sarawakensis* Martelli (type species) (Stone 6804, KLU). **F. imbricata* Bl. var. *hispidula* B. C. Stone (Stone, KLU. 9021).
- (3) Sect. *Auriculifoliae*: **F. sumatrana* Hemsl. s. s. (M. Kassim, KLU. 6645). **F. sumatrana* var. *penangiana* B. C. Stone (Stone 5809, KLU).
- (4) Sect. *Filiformicarpae*: *F. mariannensis* Merr. (Stone 5206 b, Guam). *F. minahassae* Koord. (Robinson 6345, US).
- (5) Sect. *Warburgiella*: *F. marantifolia* Hemsl. (Brass 2932. A).
- (6) Sect. *Hemsleyella*: **F. rigidifolia* Hemsl. (Meijer SAN. 59284).
- (7) Sect. *Freycinetia*: *F. rapensis* F. Br. (Chapin 895, NY). *F. arborea* Gaudich. (Craven 104, NY).
- (8) Sect. *Cyrtopoda*: **F. javanica* Bl. (Stone 5623; 6579; 7133, KLU).
- (9) Sect. *Gaudichaudiella*: *F. solomonensis* B. C. Stone (Schodde 4103, LAE).
- (10) Sect. unknown (unassigned): **F. kamiana* B. C. Stone (Dransfield, KLU; Kam, isotype, KLU).—**F. corneri* B. C. Stone (Kam, KLU 8856).

Living materials fixed in F. A. A. of *Freycinetia sumatrana* and *F. javanica* were obtained from suitable habitats in Malaya for study. For the remaining species, either pickled materials or herbarium specimens were utilized. Dry material was restored by boiling gently in a mixture of concentrated lactic acid and glycerol (7: 3), then material for sectioning was fixed in F. A. A. Epidermal peels were obtained by two methods; the simple scraping technique (Metcalf, 1960); or maceration in Jeffrey's solution as

modified by Stace (1965). The prepared materials were then washed in distilled water, in bleach, in 2½% acetic acid, again in water, and then stained with safranin O and Delafield's hematoxylin (after Johansen, 1940) overnight; then again washed, and carried up to 95% ethanol, transferred to xylol, and finally mounted in piccolyte. Macerated peels were washed in 70% ethanol and stained with sudan IV, then mounted in glycerine jelly for observation. (The latter method is simpler but the former gives better

Table 1. Stomatal size and density values in 14 species of *Freycinetia* (Pandanaeae)*

Species (Section)	Density per mm ² .*		Stomatal size in sq. μ	Stomatal class
	Abaxial	Adaxial		
<i>F. javanica</i> (Cyrtopoda)	32	1	1099	I
" "	62	0	—	I
<i>F. marantifolia</i> (Warburgiella)	29	2	901	I
<i>F. corneri</i> (Sect. ?)	48	1	556	I
<i>F. kamiana</i> (Sect. ?)	57	—	475	I
<i>F. sarawakensis</i> (Sarawakenses)	57	1	429	I
<i>F. imbricata</i> (Sarawakenses)	75	1	405	I
<i>F. solomonensis</i> (Gaudichaudiella)	87	1	369	IIa
<i>F. sumatrana</i> var. <i>penan-</i> <i>giana</i> (Auriculifoliae)	131	3	325	IIb
<i>F. mariannensis</i> (Filiformicarpae)	150	3	350	I
<i>F. jagorii</i> (Racemosiflorae)	155	5	204	I
<i>F. sumatrana</i> s. s. (Auriculifoliae)	28	2	270	IIc
<i>F. rapensis</i> (Freycinetia)	66	1	270	IIc
<i>F. rigidifolia</i> (Hemsleyella)	84	2	285	IIc
<i>F. arborea</i> (Freycinetia)	123	3	275	IIc

* All figures rounded off from tenths. In all cases, adult leaves of adult plants were used, from the sixth to the tenth node behind the apex. No juvenile leaves, or juvenile plants or abnormally large or small leaves were used. Measurements are based on the mean of c. 20 readings.

visual differentiation).

Sections and materials fixed in F. A. A. were prepared by means of paraffin embedding, and cut at 12–15 μ on a microtome. The sections were stained in safranin and fast green FCF and mounted in piccolyte.

Measurements of stomata were based on 10–20 readings for both stomatal size and density. The area of the guard cells plus pore was taken as a measure of the stomatal size, and is given by the formula employed by Franco (1939), i. e.: $a \times b \times 0.7854$ (where a = length of stomate, b = width).

Anatomical characters in taxonomy

The species studied are described below in terms of the following five anatomical characters which appear to be of some value, in conjunction, for taxonomic studies: (1) presence or absence of costal and intercostal differentiated regions of the abaxial epidermis; (2) shape of epidermal cells; presence of epidermal papillae, and their distribution when present; (4) number of neighboring cells associated with stomata; (5) presence or absence of silica bodies and their abundance and distribution when present.

Sect. *Racemosiflorae*.

Freycinetia jagorii Warb.—Fig. 10.—Costal vs. intercostal differentiation none. Epidermal cells rectangular, longitudinally elongated in surface view, not papillose. Stomata Class I, not sunken. Neighboring cells 9. Silica bodies in pairs.

This section includes two species, *F. angustifolia* Bl. (the type) and the present species; it is strikingly distinguished by the racemose inflorescence, not present elsewhere in the genus. The foliar anatomy, however, shows no similarly unique features. *F. jagorii* has very small stomata and a high stomatal density value.

Sect. *Sarawakenses*.

Freycinetia sarawakensis Martelli (type species).—Figs. 17–19.—Costal vs. intercostal regions well differentiated. Epidermal cells square to rectangular, not papillose. Stomata Class I, not sunken. Neighboring cells 8 or 9. Silica bodies abundant, usually in pairs, sometimes solitary or in triads, in both costal and intercostal regions.

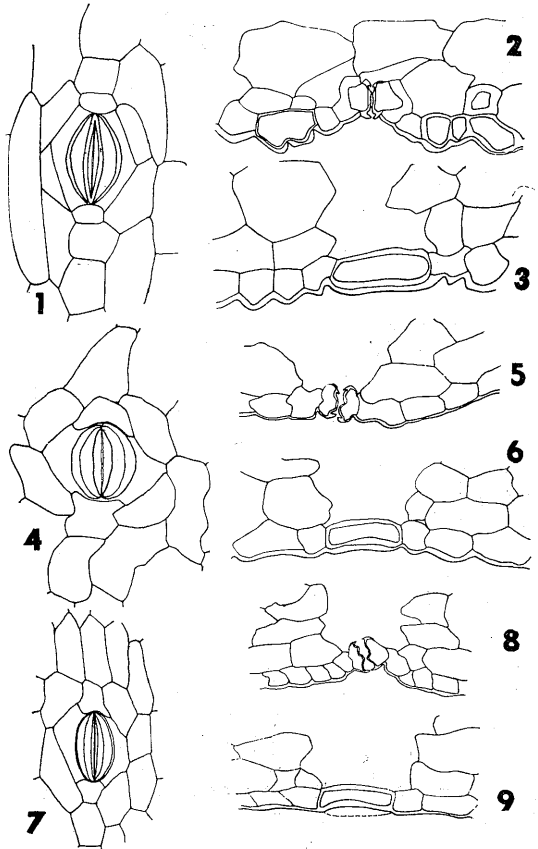
Freycinetia imbricata Bl. var. *hispidula* B. C. Stone—Figs. 7–9.—Costal

vs. intercostal regions well differentiated. Epidermal cells rectangular, not papillose. Stomata Class I, sunken. Neighboring cells 10. Silica bodies usually in pairs, restricted to the intercostal regions.

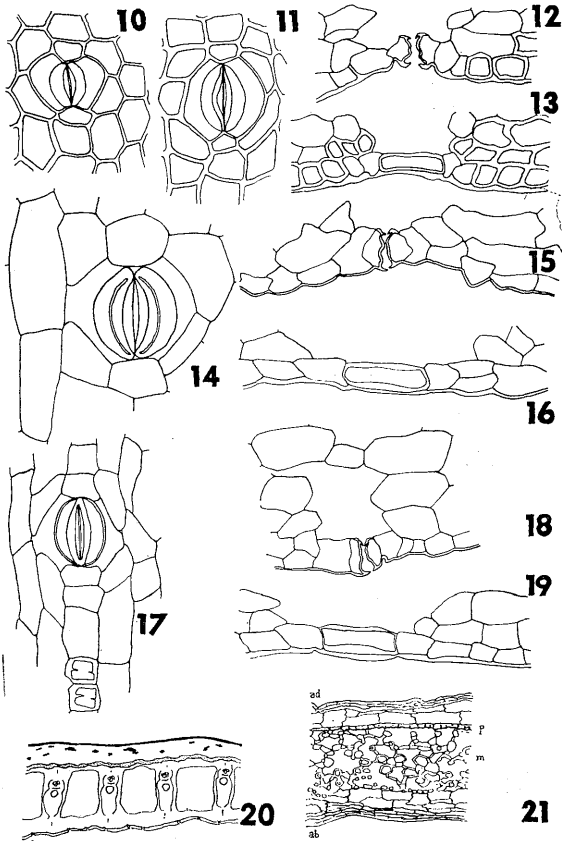
The two species studied share common epidermal characters, and have approximately equal values for stomatal size and density. Although Stone (1968) expressed doubts about including *F. imbricata* in this section, because it may produce cephalia in groups of 2 or 3, the anatomical characters indicate no reason to exclude it.

Sect. *Auriculifoliae*.

Freycinetia sumatrana Hemsl. (type species)—Figs. 33-35.—Costal vs. intercostal regions well differentiated. Epidermal cells rectangular, thick-walled, lignified; costal epidermis low and slightly papillose; intercostal epidermis of tall, collumnar, papillose cells; papillae simple and large adjacent to stomata. Stomata Class IIc, sunken, with outer stomatal chamber. Neighboring cells 10 or 11. Silica bodies not abundant, solitary, or in pairs, in both costal and intercostal regions.



Figs. 1-9.



Figs. 10-21.

F. sumatrana var. *penangiiana* B. C. Stone.—Figs. 20; 30-32.—Costal vs. intercostal regions well differentiated. Epidermal cells rectangular, thick-walled, occasionally lignified, not papillose. Abaxial stomata Class I; adaxial stomata Class IIb. Neighboring cells 10 (abaxial) or 13 (adaxial). Silica bodies abundant, solitary in both costal and intercostal regions, occurring in every third or fourth longitudinal cell file and in every third or fourth cell of the file.

The main anatomical difference be-

tween the two varieties of *F. sumatrana* lies in the stomatal structure and papillosity of the abaxial epidermis. This difference suggests that stomatal structure is not always a reliable taxonomic character at the sectional level, as it may not be uniform within a species. However it must be admitted that var. *penangiiana* may have potentiality to develop stomata of Class IIc.

Sect. *Filiformicarpae*.

Freycinetia mariannensis Merr.—Figs. 11-13.—Costal vs. intercostal regions well differentiated. Epidermal cells rectangular, polygonal, and squarish, not papillose. Stomata Class I. Neighboring cells 7 or 8. Silica bodies rare, solitary.

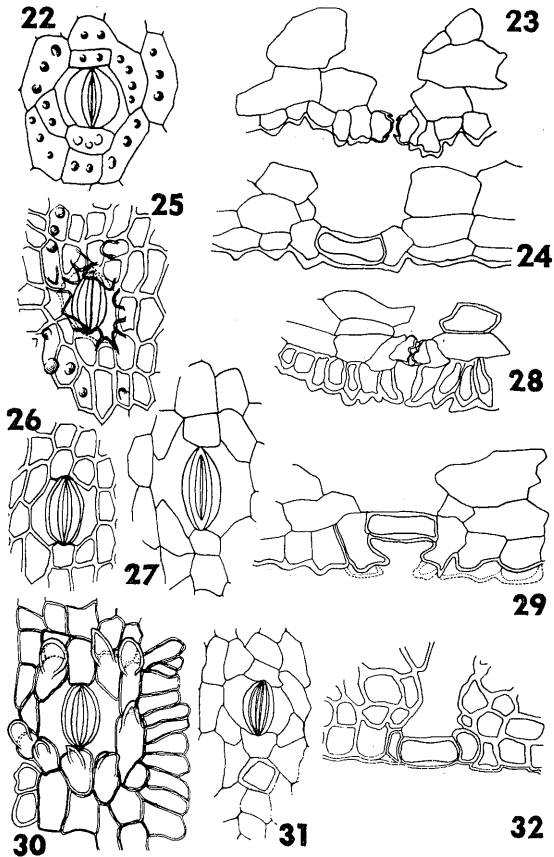
Freycinetia minahassae Koord.—Costal and intercostal regions alike, not differentiated. Epidermal cells nearly polygonal, not papillose. Stomata Class I. Neighboring cells 7-9. Silica bodies abundant, usually in groups of 3, but sometimes paired or solitary.

The two species studied represent two extremes of morphological variation, *F. mariannensis* with small leaf prickles, *F. minahassae* with large coarse ones. They share, nonetheless, unspecialized stomata and non-papillose, which features support their placement in the same section.

Sect. **Warburgiella.**

Freycinetia marantifolia Hemsl.—Figs. 14-16; 21.—Costal vs. intercostal regions alike, not differentiated. Epidermal cells broadly rectangular, not papillose. Stomata Class I, not sunken. Neighboring cells 6-8. Silica bodies rare, occurring in groups of 2 or 3, or solitary.

Notes: North and Willis (1970) report a distinct abaxial layer of palisade tissue for this species. Additional anatomical features characterizing this species are found in the seed, which has a fleshy strophiole (North and Wills 1970, fig. 8 B). Stone (1968) suggested that *F. ja-*



Figs. 22-32.

vanica might find a place in this section, but later rejected this idea, establishing a section *Cyrtopoda* to accommodate it, together with *F. celebica* Koord. and *F. palawanensis* Elmer. The anatomical characters, however, of *F. marantifolia* and *F. javanica* are very similar. (See further notes under Sect. *Cyrtopoda*).

Sect. **Hemsleyella.**

Freycinetia rigidifolia Hemsl.—Figs. 25–29.—Costal vs. intercostal regions distinct, well-differentiated. Epidermal cells rectangular, not papillose, some of them lignified. Stomata Class IIc (marginal stomata Class I). Neighboring cells 10. Silica bodies abundant, occurring in groups of 2 or 3 or solitary. Notes: This small section (2 spp.) is clear-cut morphologically, and the presence of Class IIc stomata with papillae appears to confirm its distinctiveness.

Sect. **Freycinetia.**

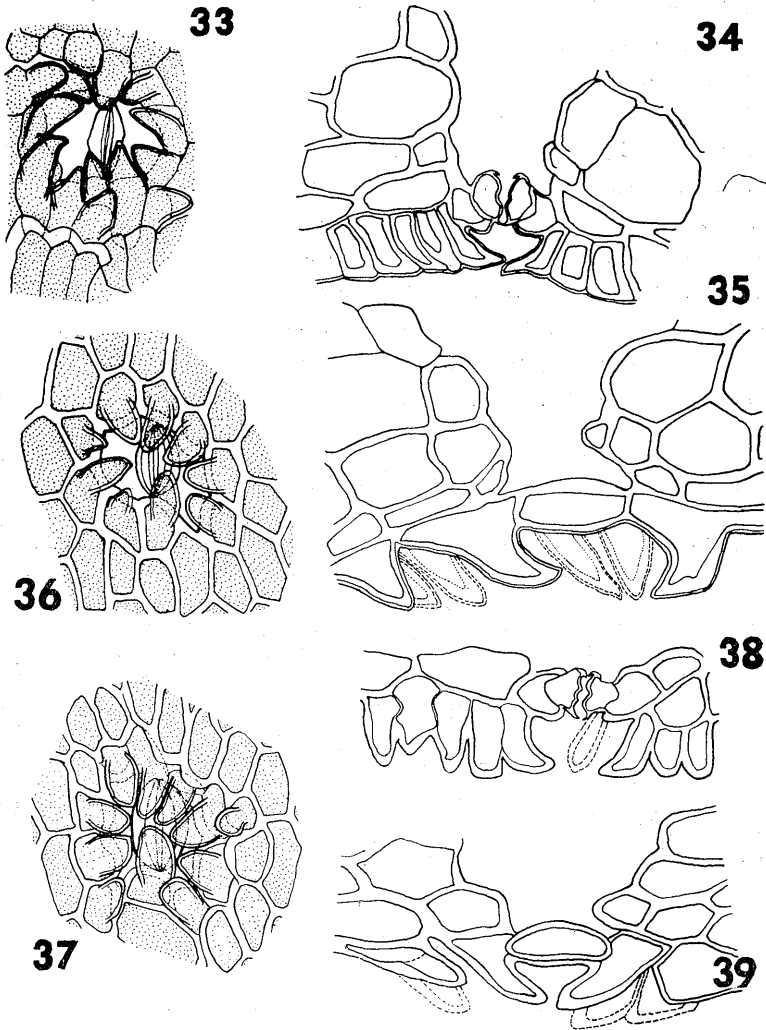
Freycinetia arborea Gaudich. (type species).—Fig. 36.—Costal vs. intercostal regions well differentiated. Epidermal cells rectangular in costal region, from rectangular to polygonal in intercostal region; only neighboring cells and their immediate neighbors are papillose. Stomata Class IIc, sunken, with outer stomatal chamber; papillae almost completely overarching stomata. Neighboring cells 11 or 12. Silica bodies absent.

Freycinetia rapensis F. Br.—Figs. 37–39.—Exactly as for *F. arborea*, except; neighboring cells 11; silica bodies abundant, occurring intercostally in groups of 2 or 3.

Notes: This section includes species which possess rather specialized features. The stomata are the most specialized of any so far noticed in the genus. The epidermal characters support the conclusion that these two species are very closely related. Solla, quoted in Tomlinson (1965), records unspecialized stomata in *Freycinetia banksii* A. Cunn., a species also belonging to Sect. *Freycinetia*. Although we suspect that immature leaves of that species were studied, it is premature to decide whether specialized stomata provide a supporting anatomical character in the definition of this section.

Sect. **Gaudichaudiella.**

Freycinetia solomonensis B. C. Stone.—Figs. 22–24.—Costal and intercostal regions not differentiated. Epidermal cells rectangular, papillae small, 1–4



Figs. 33-39.

per cell. Stomata Class IIa. Neighboring cells 7-9. Silica bodies absent.

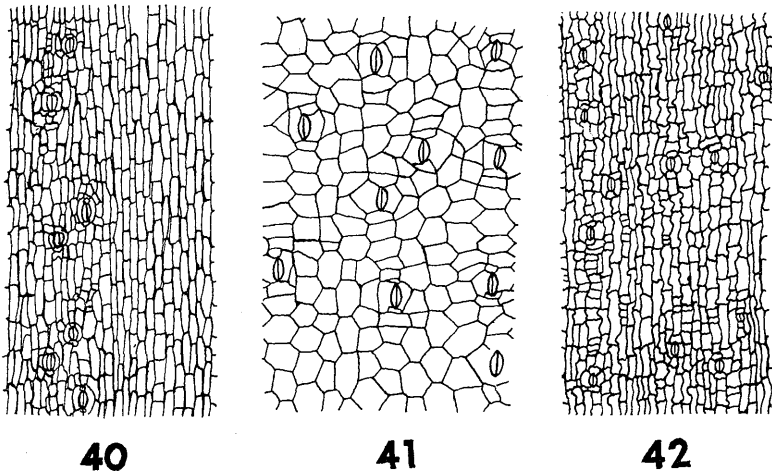
North and Willis (1970) provide some further anatomical information on this species. This section appears close to Sect. Freycinetia both in morphology and in geographical distribution, but the only epidermal feature

indicating affinity is the presence of small papillae in the terminal subsidiary cells and neighboring cells.

Sect. *Cyrtopoda*.

Freycinetia javanica Bl.—Fig. 41.—Costal vs. intercostal regions not differentiated. Epidermal cells polygonal, not papillose. Stomata Class I, not sunken. Neighboring cells 6 or 7. Silica bodies either absent or present, in groups of 2, 3, or 4, in older plants.

Notes: This species shows very large stomata and low stomatal density values (cf. Table I). These features, and the characteristic polygonal epidermal cells, seem useful for purposes of identification. Of the materials studied, one collection (Stone 7133) was of a young plant from comparatively low altitude, and lacking inflorescences; the anatomy confirms its identity.



Figs. 40-42.

As noted above under Sect. *Warburgiella*, *F. marantifolia* and *F. javanica* are very similar in their foliar anatomy, sharing the following characters: costal/intercostal differentiation none; epidermis non-papillose; stomata Class I; silica bodies rare or absent; stomatal size large, density value low. Nonetheless, morphological features appear to substantiate the inclusion of *F. javanica*, along with two related species, in Sect. *Cyrtopoda*, the name alluding to the very short pistillate pedicels. The two sections

are perhaps closely related.

Species not yet assigned to sections.

Freycinetia kamiana B.C. Stone—Fig. 4-6; 42.—Costal vs. intercostal differentiation indistinct. Epidermal cells very long, narrow rectangular, parallel to leaf long axis, the anticlinal walls wavy; not papillose. Stomata Class I. Neighboring cells 6 or 7. Silica bodies abundant, occurring singly or in linear groups of 2-4.

Notes: This species is unique in the presence of wavy anticlinal walls in the epidermal cells. In other respects it conforms fairly well to the anatomical features of Sect. *Cyrtopoda*, but also resembles Sect. *Sarawakensis*. It may perhaps be grouped in one or the other of these sections, but further study is necessary.

Freycinetia corneri B.C. Stone—Fig. 1-3; 40.—Costal vs. intercostal regions well differentiated. Epidermal cells rectangular, not papillose. Stomata Class I. Neighboring cells 6-8. Silica bodies absent.

Notes: This species was placed in Sect. *Freycinetia* in the original publication (Stone 1967), but that was prior to the new infrageneric classification proposed in 1968 by the same author. Morphologically and phytogeographically, as well as anatomically, *F. corneri* cannot be included in Sect. *Freycinetia* as redefined. Its sectional placement is still in doubt.

Anatomical characters in a key to the Malayan species of *Freycinetia*

In the following key, the anatomical features of the leaves, as presented in this paper, are combined with certain other vegetative characters to yield a key to the species of *Freycinetia* which occur in the Malayan peninsula. Since *F. confusa* Ridl. and *F. angustifolia* Bl. have not yet been studied anatomically, other vegetative characters are used to distinguish them. The foliar anatomy of *F. angustifolia* will probably be found to be essentially like that of *F. jagorii*, its partner in Sect. *Racemosiflorae*. (A key to these Malayan species based on standard morphological features is presented in Stone, 1970b).

- A. Leaves narrowly linear, less than 1 cm wide, mostly 20-30 cm long, weakly and sparsely armed. Stems mostly less than 1 cm diam.
- B. Auricles caducous; leaves mostly 5-10 mm wide.—*F. angustifolia*
- B. Auricles subpersistent, fragmenting, finally lost; leaves mostly 4-6 mm wide.—*F. confusa*

- A. Leaves mostly broader than linear, oblong to elliptic, or if linear-ensiform then more than 1 cm wide and up to 1.5 m long; sometimes stoutly armed; stems often 1-2 cm diam.
- C. Stomata simple, tetracytic, with non-papillose neighboring cells.
- D. Abaxial epidermis differentiated into costal and intercostal regions.
- E. Silica bodies absent in abaxial epidermis; neighboring cells 8.—*F. corneri*
- E. Silica bodies solitary or in pairs, restricted to intercostal regions; neighboring cells 10.—*F. imbricata*
- D. Abaxial epidermis not so differentiated.
- F. Epidermal cells polygonal with straight anticlinal walls.—*F. javanica*
- F. Epidermal cells long-rectangular with wavy anticlinal walls.—*F. kamiana*
- C. Abaxial and/or adaxial stomata with papillae from neighboring and terminal subsidiary cells.
- G. Abaxial stomata simple; adaxial stomata with papillose neighboring cells.—*F. sumatrana* var. *penangiana*
- G. Abaxial stomata with papillose neighboring cells.
- H. Papillae of neighboring cells large, almost completely over-arching stomata. Costal and intercostal epidermal cells also papillose.—*F. sumatrana* var. *sumatrana*
- H. Papillae of neighboring cells small, projecting towards but not fully over-arching stomata; marginal stomata simple. Epidermal cells not papillose.—*F. rigidifolia*

Discussion and conclusions

Although only comparatively few species of *Freycinetia* have yet been investigated anatomically with a view to improving the taxonomy of the genus, it appears so far that this approach will prove of some use, especially at the sectional level, and occasionally perhaps for individual species. However there is by no means as wide a range of variation to be exploited for this purpose as has been found in the related genus *Pandanus*, where excellent use of foliar anatomy has been made in support of infrageneric

classification (Kam, 1969; Kam and Stone, 1970). On the other hand, anatomical features of other organs, e. g. seeds, pedicels, fruits and stamens (North and Willis, 1970; Stone, 1970a) suggest a fertile field for investigation in *Freycinetia*. In the present study, the most unusual feature discovered was the long rectangular epidermal cell with wavy anticlinal walls, found only in *Freycinetia kamiana* (Fig. 42).

A somewhat limited use has been made of the presently available information to test the validity of the infrageneric sections. Sometimes the features of foliar anatomy support the grouping of species together (e. g. Sect. *Freycinetia*, Sect. *Sarawakenses*); in other cases the data seems rather ambiguous (Sect. *Filiformicarpae*) or discordant, but in most cases too few species have been examined to justify any conclusions.

On the whole it would seem valuable to continue to accumulate anatomical data in this genus for its own sake and because there is considerable likelihood that, at least in some cases, it can serve as a check on interspecific relationships and thus on the validity of any infrageneric classification. A further use of such data would be more profound comparisons of the genera of the family (*Sararanga*, *Pandanus*, and *Freycinetia*).

Summary

Fourteen species of *Freycinetia* selected to represent a wide range of morphological variation were investigated to discover if the foliar anatomy—especially stomate characters—might throw further light on the problem of infrageneric taxa and relationships. Eight sections of the genus were represented. Stomate structure and epidermal features together proved to be of value systematically. Stomata in *Freycinetia* do not show as wide a range of types as in *Pandanus*. Arbitrarily they are classed in two groups; I: unspecialized tetracytic stomata, and II: specialized tetracytic stomata with papillose terminal subsidiary and neighboring cells. However, within a species, stomatal structure is not always uniform. Nonetheless there is evidence that closely related species have similar foliar anatomy. Stomatal size and density appear not to be useful as taxonomic characters; however there is apparently an inverse relationship between stomatal size and density, and elaborate stomata may be smaller than simple stomata. Further application of anatomical data in the taxonomy of *Freycinetia* will be of

some value.

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タコノキ科のツルアダン属には約 160 種あり、20 節に分けられている。このうち生材料を手に入れ得る各型の代表的な種 14 種 (8 節に分つ) を選び、葉の解剖学的形質を研究した。そのうち気孔の構造と表皮のありさまは分類学上特に重視し得る。気孔はタコノキ属ほどには多くの型がなく、一応 2 群に分けられる。(1) 未分化の 4 細胞性気孔、(2) 柔軟小凸起をもつ周辺の細胞で補助されているもの。ただし同一種のうちでも必ずしも気孔構造は同形ではない。気孔の大きさと密度は分類的特質には使えない。しかしこの二つの形質間には反比例の関係がある。複雑な気孔は簡単なものより小さい。なおマレイ半島産の種の検索表を解剖学的特質を入れて作成した。