

REINWARDTIA

13 (3)

REINWARDTIA

*A JOURNAL ON TAXONOMIC BOTANY,
PLANT SOCIOLOGY AND ECOLOGY*

Vol. 13(3): 221 —315, April 11, 2012

Chief Editor

KARTINI KRAMADIBRATA

Editors

DEDYDARNAEDI (INDONESIA)

TUKTRIN PARTOMIHARDJO (INDONESIA)

JOENI SETIJO RAHAJOE (INDONESIA)

TEGUHTRIONO (INDONESIA)

MARLINAARDIYANI (INDONESIA)

EIZI SUZUKI (JAPAN)

JUN WEN (UNITED STATE OF AMERICA)

Managing editor

HIMMAH RUSTIAMI

Secretary

ENDANG TRI UTAMI

Lay out

DEDEN SUMIRATHIDAYAT

Illustrators

SUBARI

WAHYUDI SANTOSO

ANNE KUSUMAWAIY

Reviewers

BRYAN SIMON (AUSTRALIA), EVE J. LUCAS (UNITED KINGDOM), J.F.VELDKAMP (NETHERLANDS), LAUR-
ENCE SKOG (USA), PIETER BAAS (NETHERLANDS), RUTH KIEW (MALAYSIA), ROBERT J. SORENG (USA), HE-
LENA DUISTERMAAT (NETHERLANDS), LYN A. CRAVEN (AUSTRALIA), RUGAYAH (INDONESIA), MARK
HUGHES (UNITED KINGDOM), MARTIN CALLMANDER (USA), PETER C. VAN WELZEN (NETHERLANDS),
WAYNE TAKEUCHI (USA), NOBUYUKI FUKUOKA (JAPAN).

Correspondence on editorial matters and subscriptions for Reinwardtia should be addressed to:

HERBARIUM BOGORIENSE, BOTANY DIVISION,

RESEARCH CENTER FOR BIOLOGY-LIPI,

CIBINONG 16911, INDONESIA

E-mail: reinwardtia@mail.lipi.go.id

LEAF ANATOMY OF PANDANUS SPECIES (PANDANACEAE) FROM JAVA

Received August 25, 2011; accepted December 27, 2011

SRI ENDARTI RAHAYU

Biology Department, National University, Jl. Sawo Manila 61, Pejaten – Pasar Minggu, Jakarta Selatan, Indonesia.
Email: endarti2004@yahoo.com

KUSWATA KARTAWINATA

Herbarium Bogoriense, Botany Division, Research Center for Biology-LIPI, Cibinong Science Center, Jl. Raya Bogor–Jakarta Km. 46, Cibinong 16911, Bogor, Indonesia.
Botany Department, Field Museum, Chicago, Illinois, USA

TATIEK CHIKMAWATI

Biology Department, Bogor Agricultural Institute, Jl. Raya Dramaga, Bogor, Indonesia.

ALEX HARTANA

Biology Department, Bogor Agricultural Institute, Jl. Raya Dramaga, Bogor, Indonesia.

ABSTRACT

RAHAYU, S. E., KARTAWINATA, K., CHIKMAWATI, T., HARTANA, A. 2012. Leaf anatomy of *Pandanus* species (*Pandanaceae*) from Java. *Reinwardtia* 13(3): 305–313. — The leaf epidermis and mesophyll of fifteen species of *Pandanus* from Java were investigated to assess the value of anatomical features in species identification and classification. Characters of diagnostic importance are epidermal cell shape, differentiation of the abaxial epidermis into costa and intercosta, adaxial anticlinal cell wall outline, occurrence of raphides in the mesophyll, distribution of cubical crystals, palisade cell shape, papillae on epidermal cells, and the stomatal complex. Leaf epidermal anatomy was found to be useful in the identification at species level.

Keywords: anatomy, *Pandanus*, *Pandanaceae*, Java.

ABSTRAK

RAHAYU, S. E., KARTAWINATA, K., CHIKMAWATI, T., HARTANA, A. 2012. Anatomi daun *Pandanus* (*Pandanaceae*) dari Jawa. *Reinwardtia* 13(3): 305–313. — Studi tentang epidermis daun dari 15 jenis *Pandanus* di Jawa dilakukan untuk menentukan kegunaan dari karakter-karakter anatomi ini dan untuk menetapkan nilai manfaatnya di dalam identifikasi jenis dan klasifikasi. Karakter-karakter diagnostik yang penting di dalam klasifikasi *Pandanus* di Jawa adalah bentuk sel epidermis, demarkasi atau diferensiasi epidermis abaksial menjadi kosta dan interkosta, dinding antiklinal sel adaksial, adanya raphid pada potongan melintang, penyebaran kristal berbentuk kubus, bentuk jaringan palisade, adanya papila pada sel epidermis, kompleksitas stomata. Hasil penelitian ini menunjukkan bahwa anatomi dari epidermis daun berguna secara taksonomi dalam identifikasi *Pandanus* pada tingkat jenis.

Kata kunci: anatomi, *Pandanus*, *Pandanaceae*, Jawa.

INTRODUCTION

Pandanus Parkinson is characterized by a number of very obvious features, forming a unique combination of characters. These distinctive characters are generally an erect stem (sometimes sprawling), basally supported by many long stilt and prop roots, branching more or less dichotomously, usually rather prickly because of short, blunt or pointed specialized adventitious roots; leaves in 3 regular, close-set spirals, on the rounded or slightly 3-angled trunks, leaves entire, long, narrow, deeply channelled along the midrib and pleated once on each side, the tip, margin, midrib (below) and the pleats (above, sometimes) set with stout or fine

prickles (Stone, 1965).

Pandanus, with three other genera, *Freycinetia* Gaud., *Sararanga* Hemsl., and *Martellidendron* Callm. & Chassot constitute the family *Pandanaceae*. *Pandanus* contains more than 600 species which are distributed throughout the tropics of the Old World and this large genus is very diverse (Kam, 1971). The last revisions of the *Pandanaceae* from Java were made by Backer & Bakhuizen van den Brink (1968) and Stone (1972). They recognized 16 species and suggested alternative classifications, using morphological characters of the pistillate flowers and fruits as diagnostic features. It is, therefore virtually impossible to identify sterile or staminate plants

(Kam, 1971).

Edeoga and Ikem (2001) showed that leaf epidermal features are as useful in systematic botany as for instance DNA sequences or chemical compounds. The taxonomic value of leaf anatomy features was considered in some detail by Stace (1965). Examining the shape of epidermal, guard and subsidiary cells of stomata may prove useful for the identification of selected plant species (Jakubská, 2007). Stone (1976) constantly reaffirmed that the variation in the epidermal tissue (including stomata) is of great value in systematic botany. Tomlinson (1965) proposed a classification of stomatal types based on progressive complexity, and this system was used by Kam (1971) to demonstrate that there is a correlation between stomatal characters and the sectional delimitation within *Pandanus*. The aims of the present study are to describe the epidermal variation in 15 Javanese species of *Pandanus* and to evaluate the usefulness of the characters for identification and classification purposes.

MATERIALS AND METHOD

The survey was based on fresh material collected from the field, from plants cultivated at the Bogor Botanical Garden and from herbarium material supplied by the Herbarium Bogoriense, Bogor.

Dried leaves were boiled in water for a few minutes to soften the leaf until they became unfolded and were ready for epidermal scrapping. Fresh leaves were fixed in 70% FAA. Leaf samples were prepared according to the modified method of Johansen (1940). The fresh or dried leaves were placed in a tube with 10% nitric acid and kept in boiling water for about 10-15 minutes. Nitric acid softens the leaf tissue and facilitates peeling of the epidermis.

Both epidermal layers were stripped off gently by brushing away unwanted tissue with the help of a pointed needle and forceps, after which the epidermis was stained with safranin. An excess of safranin was washed off and the epidermis was temporarily mounted in an aqueous glycerol solution.

Embedded leaf segments were used for sectioning with a rotary microtome to prepare 15-20 µm thick sections. The ribbons were placed on clean slides smeared with a thin film of Haupt's albumen and allowed to dry, after which a drop of water was added prior to mounting. The slides were placed on a hot plate at 40°C for a few minutes to let the ribbons expand and they were stored overnight. The next day the slides were immersed for 2-5 minutes in a solution of xylene and absolute alcohol (1:1 ratio v/v). The slides were then

transferred to another solution of xylene and alcohol in the ratio 1:3 (v/v) for a few minutes, after which they were washed in a series of 95%, 90%, 70% and 50% alcohol. The slides were stained with a few drops of fast green and counterstained with safranin for two minutes, then dehydrated in a series of 50%, 70%, 80%, 90% xylene/alcohol solution and mounted in Canada balsam. The slides were dried on a hot plate at 30°C (Johansen, 1940), and examined and photographed with a Nikon Eclipse 80i digital microscope.

The voucher specimens are deposited in JHUN Herbarium.

RESULTS

Leaf anatomy

The structure of the leaf as seen in transverse sections of all species are very uniform. All leaves have the same basic construction, *i.e.*, the leaf is isolateral (Fig.1a). In some species, *e.g.* *P. labyrinthicus*, *P. pseudolais* and *P. scabrifolius*, the leaf is truly isolateral: adaxially the palisade tissue is much more developed than abaxially, while in some others, *e.g.* *P. multifurcatus* and *P. tectorius* cv. *sanderi*, the leaf is somewhat dorsiventral which abaxially the palisade tissue is not so developed. This result agrees with earlier conclusion of Tomlinson (1965) and Kam (1971) that the structure of *Pandanus* leaf was dorsiventral.

Epidermis

Pandanus labyrinthicus Kurz has a thick cuticle (5 µm), while the remaining species have a thin cuticle (less than 2 µm). The adaxial epidermis is never differentiated into costal and intercostal regions. The shape of the adaxial and abaxial epidermis cells is similar in *P. amaryllifolius* Roxb., *P. multifurcatus* Fagerl., *P. nitidus* Kurz, *P. tectorius* var. *littoralis* and *P. tectorius* cv. *sanderi*, whereas the adaxial epidermal cells have a very different shape than the abaxial cells in *P. bantamensis* Koord., *P. dubius* Spreng., *P. kurzii* Merr., *P. labyrinthicus* Kurz, *P. odoratissimus* L.f., *P. polycephalus* Lam., *P. pseudolais* Warb., *P. scabrifolius* Martelli, *P. spurius* Miq. cv. *putat* and *P. utilis* Bory.

Six types of epidermal cells could be distinguished adaxially. Each species always shows only a single type. The five types of the epidermal cells of the adaxial surfaces are: (1) rectangular in *P. dubius* and *P. kurzii* (2) squarish in *P. bantamensis* (Fig. 1b), *P. pseudolais*, *P. scabrifolius* and *P. spurius* cv. *putat*, (3) elongated in *P. amaryllifolius*. (5) long cells elongated in *P. nitidus*, *P. multifurcatus*, *P. odoratissimus* and *P. tectorius* cv. *sanderi*. and (6) long cells rectangular were

recorded in *P. labyrinthicus*, *P. polycephalus*, *P. tectorius* var. *littoralis* and *P. utilis*.

The anticlinal walls of the adaxial epidermal cells are straight or undulate. The type of anticlinal walls is constant at the species level. *Pandanus kurzii* shows adaxially undulate anticlinal walls (Fig. 1c), while the remaining species have straight anticlinal walls.

The distribution of cubical crystals in the fifteen species is variable. Cubical crystals are absent in *P. amaryllifolius*, *P. dubius*, *P. kurzii*, *P. labyrinthicus*, *P. tectorius* var. *littoralis*, and *P. bantamensis* (Fig. 1d); present in both epidermides in *P. multifurcatus* and *P. nitidus*; only abaxially present in *P. odoratissimus*, *P. polycephalus*, *P. pseudolais*, *P. scabrifolius*, *P. spurius* cv. *putat*, and *P. tectorius* cv. *sanderi*; and finally, only adaxially present in *P. utilis*.

Stomata occur in the adaxial and abaxial epidermis, but the stomata are always more abundant in the abaxial than in the adaxial epidermis, and the polar subsidiary cells of the latter are slightly larger than those of the abaxial stomata. All species have the tetracytic type of stomata.

There are four subsidiary cells adjacent to the guard cells: two terminal (or polar) subsidiary cells, situated at the ends of the guard cell pairs and smaller in size than the other two, lateral subsidiary cells.

In most species studied the adaxial epidermis has stomata without papillae, but *P. utilis* has stomata with lobed papillae situated on the subsidiary and guard cells (Fig. 1e). Stomatal complex sunk with one ring of papillae over the guard cells and one ring of larger lobes extending from the neighbouring cells above the stomata complex.

For a classification of the stomatal complex in *Pandanus* into 5 classes, see below.

The abaxial epidermis is variable throughout the species studied. It may or may not be differentiated into costal and intercostal regions. The demarcation into zones is very clear-cut in *P. amaryllifolius* (Fig. 1f), *P. dubius*, *P. kurzii*, *P. multifurcatus*, *P. nitidus*, *P. odoratissimus*, *P. polycephalus*, *P. spurius* cv. *putat*, *P. tectorius* var. *littoralis*, *P. tectorius* cv. *sanderi*, and *P. utilis*. However, in *P. labyrinthicus* the demarcation is not so clear, and in *P. bantamensis*, *P. pseudolais* and *P. scabrifolius* the

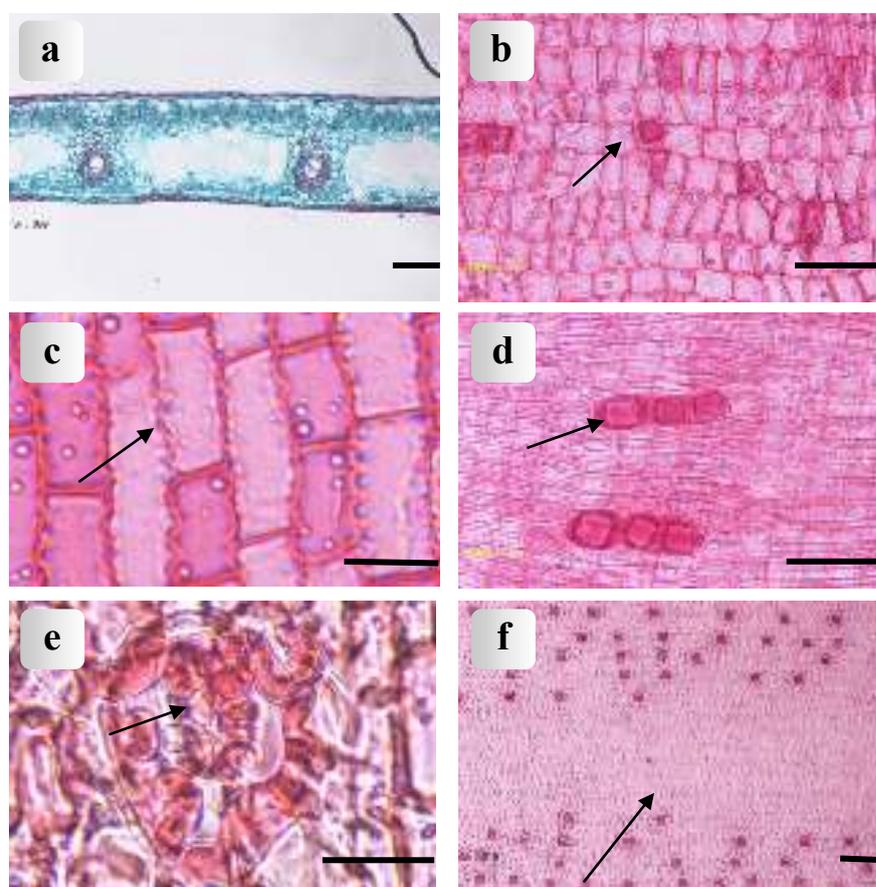


Figure 1. Light micrographs of leaves: (a) Transverse leaf section of *P. multifurcatus* Fagerl.; (b) Squarish abaxial epidermal cells of *P. bantamensis* Koord.; (c) Undulate adaxial anticlinal cell walls of *P. kurzii* Merr.; (d) Adaxial epidermis cells with cubical crystals of *P. bantamensis* Koord.; (e) Adaxial stomata with lobed papillae of *P. utilis* Bory; (f) Costal zone in the abaxial epidermis of *P. amaryllifolius* Roxb. Scale bar for a & f = 100 μ m.; Scale bar for b, c and d = 50 μ m.; scale bar for e = 20 μ m.

abaxial epidermis shows no differentiation into costal and intercostal regions.

The shape of the abaxial epidermal cells can only be divided into three classes, *i.e.* (1) long cells elongated in *P. amaryllifolius*, *P. bantamensis*, *P. dubius* (Fig. 2a), *P. multifurcatus*, *P. nitidus*, *P. pseudolais*, and *P. tectorius* cv. *sanderi*, (2) long cells rectangular in, *P. labyrinthicus*, *P. kurzii*, *P. odoratissimus*, *P. scabrifolius*, *P. spurius* cv. *putat*, *P. tectorius* var. *littoralis* and *P. utilis*; and (3) polygonal in *P. polycephalus*

In most of species, the abaxial epidermis is frequently associated with papillae. The distribution, size and shape of the papillae is highly variable throughout all species studied and can be used with caution for diagnostic purposes.

Papillae, when present may occur on all normal epidermal cells or only on certain cells. Lateral and terminal subsidiary cells of the stomatal apparatus often are papillate. Papillae which occur in lateral subsidiary cells are always arranged in a linear row of three to four as found in *P. tectorius* var. *littoralis* (Fig. 2b), and four to five in *P. amaryllifolius*, *P. scabrifolius* and *P. spurius* cv. *putat*. The papillae vary from simple, globose to elaborately lobed ones, while those present in terminal subsidiary cells vary from simple, or forked to dendritic.

The number of papillae per epidermal cell varies from one to several. One to three globose papillae are found on each epidermal cell of *P. spurius* cv. *putat* (Fig. 2c). In *P. labyrinthicus*, *P. tectorius* var. *littoralis* and *P. utilis* only one papilla is found on each epidermal cell, but the papillae are elaborately lobed at the distal end. *P. kurzii* and *P. polycephalus* have dendritic papillae on the abaxial epidermal cells, while in *P. scabrifolius* and *P. dubius* papillae are absent on the abaxial epidermis.

Hypodermis

One or more cell layers immediately beneath the epidermis are developed as a colourless hypodermis. A hypodermis is most conspicuous in species in which the hypodermis of the lamina is multiseriate, *e.g.* *P. amaryllifolius*, *P. multifurcatus*, *P. pseudolais* and *P. tectorius* cv. *sanderi* consists of at least two layers of colourless cells, three layers of colourless cells are usually present in *P. labyrinthicus* (Fig. 2d).

The adaxial hypodermis is more uniform than the abaxial hypodermis, because it is not interrupted by many stomatal chambers. The adaxial hypodermal layers are usually somewhat thicker than the abaxial ones. The outermost hypodermal layers are sclerotic, while the inner cells are larger and isodiametric and remain thin-walled. Hypodermal cell rows do not coincide with the

epidermal rows. The number of epidermal cells above one of the outermost hypodermal cells in transverse direction can be used as a diagnostic feature for certain species. For example, in *P. labyrinthicus* the cells of the first hypodermal layer are long and correspond to 4 or 5 epidermal cells, 5 or 6 cells in *P. amaryllifolius*, and 7 or 8 epidermal cells in *P. tectorius* cv. *sanderi*.

Crystalliferous cells with rhombic crystals are found in some of the species studied, *viz.* *P. amaryllifolius*, *P. bidur*, *P. labyrinthicus*, *P. multifurcatus*, *P. pseudolais*, *P. tectorius* var. *littoralis* and *P. tectorius* cv. *sanderi*. Crystal cells are generally found in the outermost hypodermis. The crystal cells are distributed uniformly, either solitary or in pairs. In *P. amaryllifolius* and *P. pseudolais* the large rhombic crystals occurs in the outermost layer of the hypodermis (Fig. 2e). while the inner cells are larger and isodiametric and thin-walled. Hypodermal cell rows do not coincide with the epidermal rows. The number of epidermal cells above one of the outermost hypodermal cells in transverse direction can be used as a diagnostic feature for certain species. For example, in *P. labyrinthicus* the cells of the first hypodermal layer are long and correspond to 4 or 5 epidermal cells, 5 or 6 cells in *P. amaryllifolius*, and 7 or 8 epidermal cells in *P. tectorius* cv. *sanderi*.

Mesophyll

The leaves of all species are isolateral (*i.e.* in TS the adaxial side is similar to the abaxial side) to weakly bilateral (adaxial part contains more chlorenchyma and the cells are more palisade like than the abaxial mesophyll). The mesophyll comprises parallel veins separated by large colourless cells, which desintegrate in mature leaves, resulting in the formation of air cavities. In mature leaves there is a wide air cavity between each adjacent pair of veins. The adaxial chlorenchyma may be a two-layered palisade as in *P. pseudolais* or even four-layered palisade in *P. amaryllifolius*; abaxially the palisade is always 1 (or 2) layered. The shape of the palisade cells is variable. Most species show columnar and compactly arranged palisade cells (*P. multifurcatus*, *P. pseudolais* and *P. tectorius* cv. *sanderi*); the only exception is *P. amaryllifolius* with isodiametric palisade cells (Fig. 2f).

Sclerenchyma strands are variable, and occur solitarily or in groups of 2 or 3 cells. The sclerenchyma fibres are hexagonal, rectangular or triangular in transverse section and are present near the adaxial and abaxial epidermis. They are sometimes found within the hypodermis and even within the palisade tissue in *P. amaryllifolius*; in the spongy tissue in *P. tectorius* cv. *sanderi* (Fig.

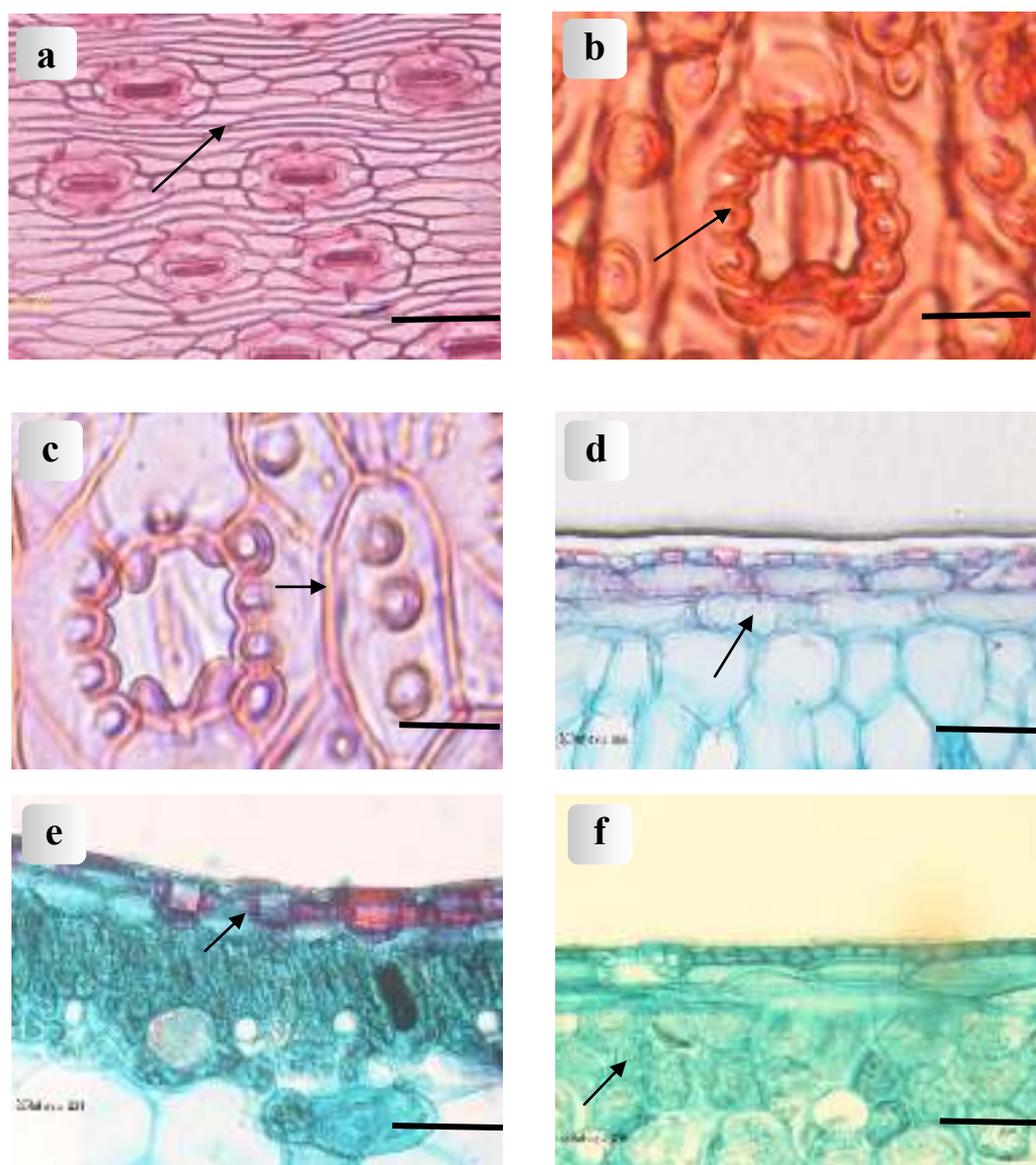


Figure 2. Light micrographs of leaves: (a) Abaxial epidermal cells of *P. dubius* Spreng.; (b) Abaxial papillae in lateral subsidiary cells of *P. tectorius* var. *littoralis*; (c) Abaxial papillae on epidermal cells of *P. spurius* Miq. cv. *putar*; (d) Hypodermis of *P. labyrinthicus* Kurz; (e) Crystal cells in outermost hypodermis of *P. pseudolais* Warb.; (f) Isodiametric palisade cells of *P. amaryllifolius* Roxb. Scale bar for a, d, e. & f = 50 μ m.; scale bar for b & c = 20 μ m

3a), or in the palisade and spongy tissue as in *P. labyrinthicus*. The fibre cells are characterized by concentric wall layering with narrow, circular to oval lumina and in some of the cells also have cone-shaped silica bodies that project into the lumina.

Raphides

Calcium oxalate usually occurs in the form of raphide clusters in distinct raphide sacs. Raphids are bundles of narrow, elongated needle-shaped crystals usually of similar orientation, with pointed ends at maturity. They are usually found in crystals idioblast in parenchymatous tissues (Tomlinson, 1961; Prychid & Rudall, 1999).

Raphids are known to occur in at least 49 monocotyledons and 27 dicotyledon family

worldwide (Dahlgreen & Clifford, 1982). These includes bananas (*Musaceae*), cordyline (*Laxmannia*) and *Pandanus* (*Pandanaceae*) (Osuji & Ndukwu, 2005).

Idioblastic raphide sacs are present in the palisade *e.g.* in *P. amaryllifolius* (Fig. 3b), *P. multifurcatus* and *P. pseudolais*; in the spongy tissue, *e.g.* in *P. tectorius* cv. *sanderi* or in the palisade and spongy tissue, *e.g.* in *P. labyrinthicus*. The individual raphides are as pencil-shaped, *i.e.* flat at one end and pointed at the other.

Stomata

Tomlinson (1965) recorded a considerable range in stomatal structures found in 30 *Pandanus* species, and he provided a general description of the

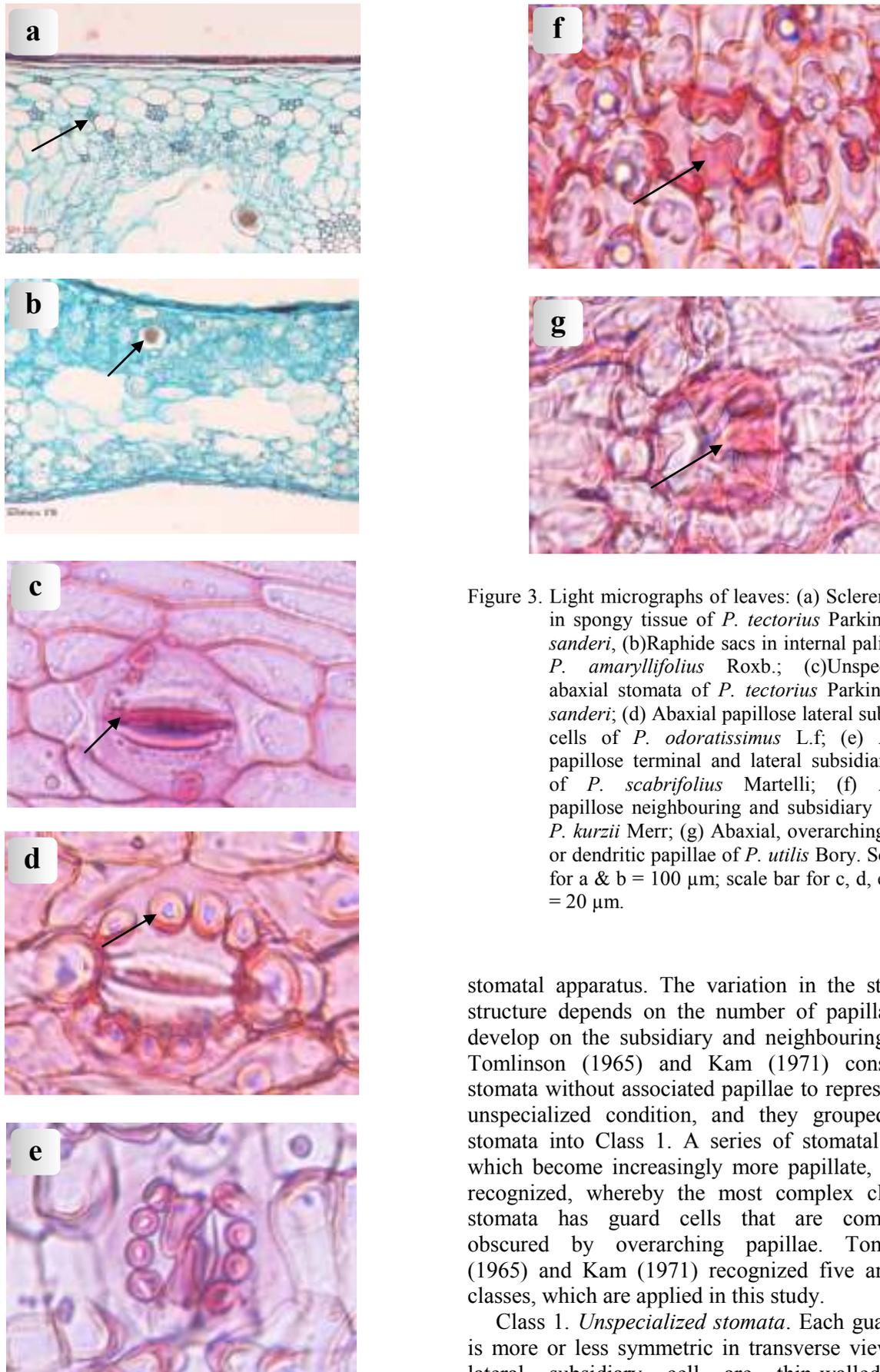


Figure 3. Light micrographs of leaves: (a) Sclerenchyma in spongy tissue of *P. tectorius* Parkinson cv. *sanderi*, (b) Raphide sacs in internal palisade of *P. amaryllifolius* Roxb.; (c) Unspecialized abaxial stomata of *P. tectorius* Parkinson cv. *sanderi*; (d) Abaxial papillose lateral subsidiary cells of *P. odoratissimus* L.f; (e) Abaxial papillose terminal and lateral subsidiary cells of *P. scabrifolius* Martelli; (f) Abaxial papillose neighbouring and subsidiary cells of *P. kurzii* Merr; (g) Abaxial, overarching, lobed or dendritic papillae of *P. utilis* Bory. Scale bar for a & b = 100 μ m; scale bar for c, d, e, f & g = 20 μ m.

stomatal apparatus. The variation in the stomatal structure depends on the number of papillae that develop on the subsidiary and neighbouring cells. Tomlinson (1965) and Kam (1971) considered stomata without associated papillae to represent the unspecialized condition, and they grouped such stomata into Class 1. A series of stomatal types, which become increasingly more papillate, can be recognized, whereby the most complex class of stomata has guard cells that are completely obscured by overarching papillae. Tomlinson (1965) and Kam (1971) recognized five arbitrary classes, which are applied in this study.

Class 1. *Unspecialized stomata*. Each guard cell is more or less symmetric in transverse view. The lateral subsidiary cell are thin-walled, and conspicuously different from normal epidermal cells. Terminal subsidiary cells are short, but otherwise less distinct from normal epidermal cells. The subsidiary and neighbouring cells lack papillae.

This type of stomata is found in *P. dubius*, *P. multifurcatus*, *P. nitidus*, *P. pseudolais*, and *P. tectorius* cv. *sanderi* (Fig. 3c).

Class 2. *Papillose lateral subsidiary cells*. The structure of the guard cells and subsidiary cells is similar to that of class 1 except for the addition of a row of four to six papillae on the outer surface of each lateral subsidiary cell. There is no other stomatal outgrowth, except for a tendency for the terminal subsidiary cells to overarch the lateral subsidiary and guard cells to a greater extent than in class 1. Class 2 stomata are observed together with intermediate stomata, in which papillae occur on one of the two lateral subsidiary cells belonging to a single stoma. At least one full row of papillae is always developed. Class 2 stomata have been observed in *P. bantamensis*, *P. odoratissimus* (Fig. 3d) and *P. spurius* cv. *putat*.

Class 3. *Papillose terminal and lateral subsidiary cells*. The slight tendency in class 2 stomata for the terminal subsidiary cells to protrude over the lateral subsidiary cells is much more pronounced in class 3. Each of the terminal subsidiary cells has prominent papillae, which overarch the stomatal pore. Frequently, the papillae from opposite poles meet and overlap, their ends only mutually overlap or the papillae may even fork to produce short interdigitating branches. Such forking papillae are usually closely adpressed to the stomatal pore, in between the opposite rows of papillae on the lateral subsidiary cells. A species in this category is *P. scabrifolius* (Fig. 3e).

Class 4. *Papillose neighbouring and subsidiary cells*. One step further than class 3 is the development of papillae, which protrude from neighbouring epidermal cells. The external stomatal cavity is larger than in class 3, because the complete stomatal apparatus is sunken into the epidermis. Class 4 stomata are very diverse, because the size and frequency of the papillae varies considerably. In the less papillate types the papillae are not large, so that the outer chamber is shallow, but in more papillate types the papillae are very tall and form a distinct wall surrounding a very deep outer chamber. Tall papillae further show a marked tendency to overarch and hide the outer chamber. The papillae themselves are also diverse. They may be the result of protrusions of the whole outer wall of the epidermal cell, or they may only be formed by a part of the outer wall. Finally, some papillae surrounding the stomata show a tendency to become lobed or shortly branched. Usually this is first noticeable in the terminal subsidiary cells, as in *P. utilis*. This is a transition to class 5. In *P. labyrinthicus* the papillae are very low but often distinctly lobed. The following species included in class 4 are *P. amaryllifolius*, *P. kurzii* (Fig. 3f), *P.*

labyrinthicus, and *P. tectorius* var. *littoralis*.

Class 5. *Overarching papillae lobed or dendritic*. This class includes the most specialized forms. The sinking of the stomata is pronounced, and the deep outer stomatal chamber so formed, is partly or wholly covered by branched papillae of the terminal subsidiary and neighbouring cells. In the least dendritic members the papillae are short and lobed. Increased branching can be seen in a number of species studied, whereby the papillae become taller, distally more elaborately lobed, and the lobes tend to interdigitate and form an incomplete "canopy" above the outer stomatal chamber. The ultimate and the most complex stomatal type in Javanese *Pandanus* was observed in *P. utilis* in which

Stomatal complex sunk with one ring of papillae over the guard cells and one ring of larger lobes extending from the neighbouring cells above the stomata complex. Species in this category include *P. polycephalus* and *P. utilis* (Fig. 3g).

DISCUSSION

The purpose of this leaf anatomical study of fifteen Javanese species of *Pandanus* is to investigate the possibility to identify species using anatomical characters. The information gathered in the present study can only give a rough indication of the anatomical characters, which may prove to be of value taxonomically.

Several anatomical characters that can be used for this purpose (see also key below and results above) are the shape of the epidermal cells, costal-intercostal zones in the abaxial epidermis, the outline of the anticlinal walls of the epidermal cells, the distribution of rhomboidal crystals in the epidermis and hypodermis and the stomatal structure, especially the presence of papillae.

Kam & Stone (1970) and Kam (1971) reported that stomata structure, epidermal zonation, silica-body presence and arrangement, and epidermal papilosity may have value for delimiting sections. By contrast, in this study six species of Javanese *Pandanus*, all belonging to Section *Rykia*, did not have the same features. Zonation is very clear cut in *P. multifurcatus* and *P. nitidus*, but indistinct in *P. labyrinthicus*, and zonation is absent in *P. bantamensis*, *P. pseudolais* and *P. scabrifolius*. Thus, for the Javanese species, zonation can only be used for the identification of species, not sections.

Kam (1971) reported that the abaxial epidermis is very variable in the species studied. However, if we consider the types of epidermal cell shapes that we found, then we cannot confirm the variability reported by Kam. We found that the adaxial epidermal cells are more variable than the abaxial cells, but the variation only consists of five different

shapes for the adaxial epidermal cells, and three shapes for the abaxial cells. Kam (1971) also stated that the transverse length of first layer of hypodermis corresponds to the space occupied by 10 to 12 rows of epidermal cells, but our materials only showed an equivalence of 4-8 cells.

In general, the stomatal structure of the fifteen Javanese *Pandanus* species studied agrees with the earlier conclusions of Tomlinson (1965) and Kam (1971) except for *P. utilis*. *P. utilis* in Tomlinson (1965) sample has elaborate stomata of abaxial epidermis that classified as class 4 (papillose neighbouring and subsidiary cells), but in the present study (*P. utilis* from Java) elaborate stomata in *P. utilis* should be classified as class 5 (papillae overarching and lobed or dendritic). So the shape of elaborate stomata in *P. utilis* could be different if the sample source is different.

In Javanese *Pandanus* anatomical characters appear to provide reliable characters for the identification of species as was already pointed out by Stone (1978). The key underneath can only tentative because their constancy of the features within species was tested on very few specimen only.

KEY TO THE SPECIES OF JAVANESE PANDANUS

1. Costal zonation distinct in abaxial epidermis
 2. Cuticle thick (5µm) *P. labyrinthicus*
 2. Cuticle thin (< 2 µm)
 3. Adaxial epidermal anticlinal cell walls undulate *P. kurzii*
 3. Adaxial epidermal anticlinal cell walls straight
 4. Adaxial stomata elaborate *P. utilis*
 4. Adaxial stomata simple
 5. Cells of adaxial and abaxial epidermis with similar shape
 6. Rhomboidal crystals on both surfaces *P. nitidus*
 6. Rhomboidal crystals on adaxial surface or absent
 7. Raphides in spongy tissue *P. tectorius* cv. *sanderi*
 7. Raphides in internal palisade
 8. Palisade cells circular *P. amaryllifolius*
 8. Palisade cells columnar *P. multifurcatus*
 5. Cells of adaxial and abaxial epidermis different in shape
 9. Adaxial epidermal cell shape are elongated or rectangular
 10. Stomata with papillae on neighbouring epidermal and subsidiary cells *P. tectorius* var. *littoralis*
 10. Stomata with papillae on lateral subsidiary cells *P. odoratissimus*
 9. Adaxial epidermal cell shape are polygonal

11. Stomata without papillae *P. dubius*
11. Stomata with papillae
 12. Papillae on lateral subsidiary cell *P. spurius* cv. *putat*
 12. Papillae lobed or dendritic on neighbouring and subsidiary cell *P. polycephalus*
1. Costal zonation absent in abaxial epidermis
 13. Papillae on stomata absent *P. pseudolais*
 13. Papillae abundant
 14. Long cells of abaxial epidermis is elongated *P. bantamensis*
 14. Long cells of abaxial epidermis is rectangular .. *P. scabrifolius*

ACKNOWLEDGEMENT

The authors deeply thank Prof. Dr. Peter C. van Welzen for improving the manuscript, the directors and curators of the following herbaria: BO, L and Kew. Prof. Dr. Mien A. Rifai for providing valuable suggestion. We wish to thank Dr. Sunaryo, Eka Fatmawati Tihuraa, Widoyanti and Ujang Hapid of Herbarium Bogoriense for helping during laboratory and photographic work. This work was funded by The Directorate General of Higher Education of Indonesia through fundamental research grant number 109/SP2H/PP/DP2M/III/2008.

REFERENCES

- BACKER, C. A. & R. C. BAKHUIZEN VAN DEN BRINK f, 1968. *Flora of Java* 3. Noordhoff, Groningen.
- DAHLGREN, R. M. T. & CLIFFORD, H. T. 1982. *The Monocotyledons. A comparative study*. Academic Press. London.
- EDEOGA, H. O. & IKEM, C. I. 2001. Comparative morphology of leaf epidermis in three species of *Boerhavia* L. *J. Econ. Tax. Bot.* 19: 197-205.
- JAKUBSKA, A. 2007. The analysis of morphological differentiation of the epidermis of selected species of the genus *Epipactis* ZINN, 1757 (*Orchidaceae: Neottieae*). *Wroclaw* 15(2): 41-45.
- JOHANSEN, D. A. 1940. *Plant microtechnique*. McGraw-Hill, Book Company, Inc. New York.
- KAM, Y. K. & STONE, B. C. 1970. Morphological studies in *Pandanaceae* IV. Stomata structure in some Mascarene and Madagascar *Pandanus* and its meaning for infrageneric taxonomy. *Adansonia* 10 (2): 219-246.
- KAM, Y. K. 1971. Comparative systematic foliar anatomy of Malayan *Pandanus*. *Bot. J. Linn. Soc.* 64: 315-353.
- MBAGWU, F. N., NWACHUKWU, C. U. & OKORO, O. O. 2007. Comparative leaf epidermal studies on *Solanum macrocarpon* and *Solanum nigrum*. *Nature and Science* 5(3): 1-4.
- OSUJI, J. O. & NDUKUWU, B. C. 2005. Probable functions and remobilisation of calcium oxalates in *Musa* L. *African Journal of Biotechnology* 4: 1139-1141.

- PRYCHID, C. J. & RUDALL, P. A. 1999. Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. *Annals of Botany* 84: 725-739.
- STACE, C. A. 1965. Cuticular studies as an aid to plant taxonomy. *Bull. Brit. Mus. (Nat. Hist) Bot.* 4: 1 - 78.
- STONE, B. C. 1965. *Pandanus* Stickman in the Malayan Peninsula, Singapore and lower Thailand. *Malay. Nat. J.* 19(4): 203-213.
- STONE, B. C. 1972. A review of Javanese *Pandanaceae* with notes on plants cultivated in Hortus Bogoriensis. *Reinwardtia* 8: 309-318.
- STONE, B. C. 1976. The morphology and systematics of *Pandanus* today (*Pandanaceae*). *Gardens' Bulletin Singapore* 29: 137-142.
- STONE, B. C. 1978. Studies in Malesian *Pandanaceae* XVII on the taxonomy of "Pandan Wangi" A *Pandanus* cultivar with scented leaves. *Economic Botany* 32: 285-293.
- TOMLINSON, P. B. 1961. *Anatomy of the Monocotyledons II Palmae*. Clarendon Press. Oxford.
- TOMLINSON, P. B. 1965. A study of stomatal structure in *Pandanaceae*. *Pacific Science* 19(1): 38-54.

ERRATUM**REINWARDTIA Vol. 13, Part 2, 2010**

1. Please change the existing word in p. 213, LINE 7 on ABSTRAK (written in Bahasa Indonesia version) with the following:

Keberadaan dua jenis terakhir melampaui distribusi yang sebelumnya hanya diketahui di **barat** garis Wallace.

2. Please change the existing epithet name in p, 214, COLUMN 1, LINE 40 on Key to the species of *Marantaceae* in Sulawesi number 5.a. after *Phrynium*:

.....*longispicum*

INSTRUCTION TO AUTHORS

Reinwardtia is a scientific journal on plant taxonomy, plant ecology, and ethnobotany. Manuscript intended for a publication should be written in English represent an article which has not been published in any other journal or proceedings. Every manuscript will be sent to two blind reviewers.

Two printed copies (on A4 paper) of the manuscript of not more than 200 pages together with an electronic copy prepared on Word Processor computer program using Time New Romance letter type and saved in Rich Text File must be submitted.

For the style of presentation, authors should follow the latest issue of *Reinwardtia* very closely. Title of the article should be followed by authors name and mailing address in one-paragraphed English abstract of not more than 250 words. Keywords should be given below each abstract. On a separated paper, author(s) should send the preferred running title of the article submitted.

Taxonomic identification key should be prepared using the aligned couplet type.

Strict adherence to the International Code of Botanical Nomenclature is observed, so that taxonomic and nomenclatural novelties should be clearly shown. Latin description for new taxon proposed should be provided and the herbaria where the type specimens area deposited should be presented in the long form that is name of taxon, authors name, year of publication, abbreviated journal or book title, volume, number and page.

Map, line drawing illustration, or photograph preferably should be prepared in landscape presentation to occupy two columns. Illustration must be submitted as original art accompanying, but separated from the manuscript. On electronic copy, the illustration should be saved in jpg or gif format at least 350 pixels. Legends or illustration must be submitted separately at the end of the manuscript.

Bibliography, list of literature cited or references follow the Harvard system.

W.J.J.O. DE WILDE & BRIGITTA E.E. DUYFJES. <i>Trichosanthes (Cucurbitaceae)</i> in Malesia: additions and corrections, including a new species and a new variety.....	221
DEDEN GIRMANSYAH. Two new species of <i>Begonia (Begoniaceae)</i> from Bukit Tiga-puluh National Park, Riau, Sumatra.....	229
PUDJI WIDODO. New nomenclature in <i>Syzygium (Myrtaceae)</i> from Indonesia and its vicinities.....	235
ALEX SUMADIJAYA & JAN FRITS VELDKAMP. Non-Bambusoid <i>Grasses (Gramineae)</i> from Raja Ampat Archipelago, Papua Barat Province, Indonesia.....	241
ARY PRIHARDYANTO KEIM. New variety, records & discoveries of some species of <i>Pandanus (Pandanaceae)</i> in Sumatra and Kalimantan, Indonesia.....	255
HARRY WIRIADINATA. A new species of <i>Begonia (Begoniaceae)</i> from Sagea Lagoon, Weda Bay, Halmahera Island, North Moluccas, Indonesia.....	263
ARY PRIHARDYANTO KEIM. The Pandan flora of Foja-Mamberamo Game Reserve and Baliem Valley, Papua-Indonesia.....	271
JAN FRITS VELDKAMP. <i>Koordersiochloa</i> Merr. (<i>Gramineae</i>), the correct name for <i>Streblochaete</i> Hochst. ex Pilg.....	299
SRI ENDARTI RAHAYU, KUSWATA KARTAWINATA, TATIEK CHIKMAWATI & ALEX HARTANA. Leaf anatomy of <i>Pandanus</i> species (<i>Pandanaceae</i>) from Java.....	305

Reinwardtia is a LIPI accredited Journal (258/AU 1/P2MBI/05/2010)

Herbarium Bogoriense
Botany Division
Research Center for Biology- LIPI
Cibinong, Indonesia