Notes on leaf anatomy: Additional information for Indonesian Pandanus spp. (Pandanaceae)

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Abstract. In Indonesia, Pandanus spp. were distributed from Sumatra to Papua. These Pandanus species has broader variation of anatomical leaf characters whether epidermis or transversal section traits. This study was conducted to obtain an information of anatomical characters of Pandanus leaf and supporting character on Pandanus identification. Pandanus collections of Herbarium Bogoriense were used for this study, and some of Pandanus species from Papua New Guinea and Malay Peninsula were added as well. Paraffin method and simple peeling of the leaf surface were used to get leaf transversal sections and epidermis characters. In general, anatomical characters of examined species in this study relatively similar to other Pandanus species which were studied by former researchers. Pandanus has broad range of stomata types on the lower leaf surface, from simple stomata to the most specialized stomata. The upper leaf surface of Pandanus spp. has similar stomata type, viz. class 1 (simple). Zonation always presents on all species, except on P. atropurpureus, P. conoideus and P. nitidus. Mostly, crystals CaCO₃ were found in three forms, namely rectangular and needle-like shape in idioblast cells, while prism-shape is scattered in the mesophyll area. These crystal shapes absent in P. odorifer and there is one additional crystal shape, drusse which present in P. conoideus. Silica bodies located in the upper and lower hypodermis of leaf. Bundle sheath extension was seen in all species except P. beguinii, P. conoideus, and P. fusinus.

Keywords: papillae, anatomical character, silica bodies, stomata class, zonation.

1. Introduction
According to [1], there is ca. 450 species of Pandanus which is distributed widespread from Africa to Malesia [2]. Along with wide distribution, this genus has also broad variation on morphological and anatomical characters than other four genera within family [3][4]. [5] stated that anatomical character can be used as identification tool of species within Pandanus and has been studied by [4] and [6] on Pandanaceae leaf surface, [7] Pandanus in Malaysia and [8] on Javan Pandanus. Unfortunately, compared to the number of Pandanus species in Indonesia, the information about leaf anatomy character of Pandanus is not much.

It is commonly known that epidermis traits are good characters for Pandanus diagnosis, especially stomata complex, papillae and the presence of zonation on the lower surface. There are two arguments about the grouping of stomata type on Pandanus [4][6]. [4] classified stomata types into five classes based on the presence of papillae in the subsidiary cells. Unspecialized stomata or stomata present without papillae (class 1), papillae exist on lateral subsidiary cells (class 2), papillae on polar and lateral of subsidiary cells (class 3), papillae on polar and lateral subsidiary cells with additional papillae on
neighboring epidermis cells (class 4) and stomata present with branching or overarching papillae (class 5). While [6] modified the Tomlinson’s stomata class definition and divided stomata class 2 of [4] as two groups and stomata class 5 of [4] became class 6 and 7 based on the papillae morphology.

As the transversal section, there is no significant difference except in the length of hypodermis compared to the length of epidermis in some species [7]. His research used the ratio between the length of hypodermis to the number of epidermis along with silica bodies presence that could distinguish taxa at section level. Furthermore, crystal shapes have never been discussed either for identification tool or its significances. According to those references above, this study is aimed to give some additional informations on the leaf anatomy of Indonesian Pandanus spp. along with some references and specimens from other Malesian regions.

2. Materials and Methods

Nineteen species of Pandanus spp. and one variety of P. tectorius from herbarium specimens were used in this study. All specimens were kept in the Herbarium Bogoriense (BO) (see details in Table 1). Tert-butanol-ethanol series were used as dehydration solution, stained with safranin-fast green and mounted in entellan [9]. Epidermis characters were obtained by simple peeling leaf surface using HNO₃ solution [10] and stained by safranin. Characters that observed were epidermal cell shape, cell wall and stomata classes were described using both [4] and [6] definition. Furthermore, leaf transversal sections were examined on its hypodermis and chlorenchyma layers, the presence of CaCO₃ crystal, silica bodies and bundle sheath extension.

Table 1. Specimen list of Pandanus spp.

| No. | Species                        | Collector               | Origin                        |
|-----|--------------------------------|-------------------------|-------------------------------|
| 1   | *Pandanus amaryllifolius* Roxb. | Hallier s.n.            | Bogor, Java                   |
| 2   | *Pandanus atropurpureus* Merr. & Perry | Ary P. Keim 1276 | Jayawijaya, Wamena, New-Guinea |
| 3   | *Pandanus beccarii* Solms-Laubach | Y. Purwanto, S. Susiarti, R. Polosokan & Suwondo PSU 1 | Gunung Mahawu, Tomohon, north sulawesi, Celebes |
| 4   | *Pandanus begunii* Callm. & A.P. Keim | Oscar Efendi 7 | Central Halmahera, Moluccas |
| 5   | *Pandanus brassii* Martelli     | L. J. Brass 5933        | Dagwa, Oriomo river, western division, New-Guinea |
| 6   | *Pandanus cf limbatus* Merr. & Perry | William Milliken 1235 | Jayawijaya, Wamena, New-Guinea |
| 7   | *Pandanus conoideus* Lamarck    | Ary P. Keim 704         | Jayawijaya, Wamena, New-Guinea, |
| 8   | *Pandanus dubius* Spreng       | Beguin 1651, Indigena s.n., H. J. Lam 3041, H. J. Lutjeharms 5215, W. J. Lutjeharms 5215 | Ternate, Moluccas, Ujung kulon, Banten, Java, Talaud, Celebes |
| 9   | *Pandanus furcatus* Roxb.       | H. A. B. Bunnemeijer 7131 | Lingga, Sumatra |
| 10  | *Pandanus fusinus* Martelli     | W. Meijer & Z. Ariffin AA 1339 | Gunung Sekerat, Borneo |
| 11  | *Pandanus helicopus* S. Kurz Schum. | Nooteboom 4118 | Bukit Raya, Borneo |
| 12  | *Pandanus kraulianus* K. Schum. | Beguin 1812 | Halmahera, Moluccas |
Table 1. Specimen list of *Pandanus* spp. (continued).

| No. | Species              | Collector                        | Origin                      |
|-----|----------------------|----------------------------------|-----------------------------|
| 13  | *Pandanus* leptocarpus Martelli | Versteeg 1101                   | Brasing, New-Guinea         |
| 14  | *Pandanus nitidus* (Miq.) Kurz | Ary P. Keim, Sunardi, Kartam, Himmah R, Rugayah, Atik R, Wardi 416 E. F. de Vogel 4266 | Banyumas, Central Java, Java, Preanger regency |
| 15  | *Pandanus odorifer* (Forssk.) Kuntze | E. F. de Vogel 4266             | Obi, Moluccas               |
| 16  | *Pandanus ornatus* Kurz | Teysmann 116 H.Burkill & Haniff 17541 | Lingga, Sumatra, Pahang, Malay Peninsula |
| 17  | *Pandanus papuanus* Solus-Lautb. | Elizabeth A. Widjaja 2777 | Fakfak, New Guinea          |
| 18  | *Pandanus polycephalus* Lam | Mary Merello, Bilal Sau, Iska G, Idris Haris, Darius Loha 3304 M. van Balgooy & J.Mamesah Arief Hidayat, Yessi Santika, Ujang Hapid Koorders 18466 | Halmahera, Moluccas, Aru Islands, New Guinea, North Kolaka district, Celebes |
| 19  | *Pandanus tectorius* ex Du Roi Parkinson | Koorders 18466 | Manado, Celebes |
| 19  | var. *Pandanus littoralis* tectorius | Susi Susiarti, M. Rahayu, K. Kramadibrata 06-112 | Tasikmalaya, West Java, Java |

3. Results and Discussion

3.1. Sub genus Rykia

All species have upper and lower epidermal cells with straight walls, but wavy cell wall on the upper epidermal cells (Figure 1) also found in *P. furcatus* leaf surface. Epidermal cell shapes are square, rectangular, elongated or not polygonal shape with 4-7 angles. In *P. furcatus*, the epidermal cells have raised-anticinal walls (Figure 1 b). Stomata present on both leaf surfaces (amphistomatic). According to [4] definition, both leaf surfaces showed stomata class 1, except *P. furcatus* which has stomata class 5, *P. nitidus* class 4, whereas *P. helicopus* has both stomata class 4 and 5 (Figures 1).

Whereas, according to [6], stomata class 5 of *P. helicopus* and *P. furcatus* were grouped as class 6 and 7, respectively [Table 2]. Other stomata class that was found in *P. nitidus* is additional information as [8] recorded that this species only has stomata class 1. Meanwhile, [3] recorded that *Rykia’s* subsection has stomata class 1 and sometimes class 2 or 3. In addition, [8] found that besides those three stomata classes, *P. furcatus* also has stomata class 5. The zonation on the lower surface is clearly visible.

Cross section showed that upper and lower hypodermis were arranged by two layers of elongated cells with thick wall (Figure 2 a). In the upper hypodermis, all species also has two layers of oval or isodiametric shape hypodermis cell with thin-walled which located below the upper elongated cell, except *P. nitidus*. Chlorenchyma tissue in the upper side has 1-3 layers of cylindrical shape, palisade, (Figure 2 b) and three layers of flattened chlorenchyma cells, except *P. dubius* from Java which has no flattened cells. There are no palisade cells in the lower side and 1-3 layers of flattened chlorenchyma cells except for *P. nitidus* which has a layer of palisade and even has 1-4 layers of flattened chlorenchyma cells. CaCO₃ crystals are present in three forms, namely prism, rectangular and needle-like, except for *P. nitidus* that does not have rectangular crystals. Rectangular and needle-like shape crystals were included in idioblast cells while prism shape crystal spread in the mesophyll (Figure 2 b). Silica bodies
present at outermost of both upper and lower hypodermis (Figure 2 b). This feature was absent in the lower side of *P. nitidus* and upper side of *P. dubius* from Talaud-Celebes. Bundle sheath extension was shown in all species (Figure 2 b).

**Figure 1.** Upper surface of *P. furcatus* leaf (a and b) and lower leaf surface of *P. furcatus* with stomata class 5 (c) and class 4 on *P. nitidus* (d). ap: arching papillae, h: raised anticlinal cell wall, p: polygonal shape, pp: papillae, r: rectangular, s: square, sw: straight cell wall, ww: wavy cell wall. Scale bar: 60 μm, except A: 30 μm.

**Specimen examined:** Sect. **Hombronia**: *P. dubius* Spreng; Sect. **Rykia**: Subsect. **Rykia**: *P. furcatus* Roxb.; Sect. **Solmsia**: *P. helicopus* S. Kurz; Sect. **Asterodontia**: *P. nitidus*.

**Figure 2.** Hypodermis (h) at leaf lower side and arching papillae (p) of *P. furcatus* (a). Palisade (pa), bundle sheath extension (bse), prism-shape crystal (c) and silica body (s) were showed in *P. helicopus* (b). Scale bar: 60 μm.

### 3.2. Sub genus Lophostigma

The upper epidermis cell shapes are square, rectangular and polygonal with elongated cells or not. The cell walls are straight, slightly wavy, a little bit sinuous and sometime rounded. In *P. beccarii*, some
epidermal cells have raised anticlinal cell walls combined with rounded and irregular wavy wall, therefore the cells epidermal tend to have cylindric-shape. The lower epidermis cells have more uniform cell wall, straight. The epidermis cell shapes are square, rectangular, elongated polygonal cell ((5-6)-7) or not. Stomata type is quite diverse. This subgenus has stomata class 1 (Figure 3 a), class 4 or class 5 [4] and this diversity is happened among or within species. For example, in P. kraulianus, there are two classes of stomata, class 1 (the simplest stomata type) and class 5 (the most specialized stomata type). P. atropurpureus showed two types of stomata in one leaf, class 1 and class 4 (Figure 3 b). This result is additional information for stomata type that has been done by [3]. On [7] study, Pandanus in Malayan, stated that species of this section only have stomata class 5 or 6 or a combination of both. Meanwhile, according to Hyun’s definition, P. beccarii has stomata class 3 i.e. fork-shape with 2-3 heads papillae at polar subsidiary cells. One of P. kraulianus sample has stomata class 7. There was no information of stomata class on Lophostigma before. Zonation in the lower leaf surface was clear-cut (Figure 3 c), except P. atropurpureus which does not have zonation and distinguished this section to Maysops where all examined species have clear-cut zonation.

Hypodermis was composed by 2-3 layers of elongated cells in the upper and 2-3 layers in the lower part. A layer of thin-walled cells located innermost at the upper side, while at the lower side, there is no thin-walled hypodermis cells. Mesophyll on the upper side of the leaf has 1-3 layers of palisade and 1-3 layers of flattened chlorenchyma, except P. beguinii which only has flattened chlorenchyma cells. On the lower side, all species have 1-4 layers of flattened cells and do not have palisade except P. beccarii which has a layer of palisade. CaCO$_3$ crystal present in all species as prism, rectangular and needle-like shapes, except for P. kraulianus which does not have rectangular crystal. Silica bodies was found in both side of hypodermis (Figure d). Bundle sheath extension was shown in all species, except P. beguinii and some part in P. kraulianus leaf from Celebes.

**Species examined:** Sect. Lophostigma: Pandanus atropurpureus Merr. & Perry; Sect. Maysops: Pandanus beccarii Solms-Laubbach, Pandanus beguinii Callm. & A.P. Keim, Pandanus kraulianus K. Schum.

### 3.3. Sub genus Kurzia

The upper epidermis cell wall is straight, but wavy and rounded cell walls are also seen in P. conoideus and even sinuous in Celebes’s P. polypephalus. The shape of upper epidermis is square, rectangular, polygonal which is elongated or not. Unspecialized stomata present on the upper surface. The lower epidermis has more uniform cell wall, straight, and with square shape, rectangular and polygonal ((5-6)-7) elongated or not. Stomata type varies by Tomlinson’s definition i.e. class 1, class 2, class 4 and class 5. The stomata types of P. polypephalus from each region have different stomata types (Table 2; Figure 4 a and b) which indicates differences in the papillae shape. The following description of papillae is based on Hyun’s definition, specimen from Celebes has lobe-shape papillae at lateral dan polar subsidiary cells, whereas specimen from Moluccas has fork-shaped papillae and specimen from Aru Island has two types of papillae viz. fork papillae and branched papillae. According to [8], Javan P. polypephalus has similar stomata type of Aru islands species, that is class 5 in this study. Zonation in lower epidermis is not formed only on P. conoideus.

Species of this sub genus have upper and lower hypodermis which are composed by 1-3 layers of thickened wall-elongated cells. There are 1-2 layers thin wall-isodiametric cells of hypodermis in the upper and a layer in lower part, except in P. conoideus and P. polypephalus from Moluccas. 1-3 layers of cylindrical chlorenchyma in the upper part of P. fusinus and P. polypephalus, but never found in P. conoideus. The latter species only has 4-5 layers of flattened cells, in other hand the other two species have 1-2 layers. In the lower part of the leaf, this sub genus only has flattened chlorenchyma cells (1-3 layers), except P. polypephalus from Celebes which also has a layer of palisade. All three shapes of CaCO$_3$ crystal present in all species as well as silica bodies, but P. polypephalus from Aru Island has no silica bodies neither in the upper or lower part. Additional crystal in drusse-shape is found in P.
conoideus (Figure 5 b). Bundle sheath extension was found only in two specimens, *i.e.* *P. polycephalus* from Moluccas and Celebes.

**Figure 3.** Stomata class 1 on *P. beguinii* (a), whilst there are stomata class 1 (c1) and class 4 (c4) on *P. atropurpureus* (b). Lower surface without zonation on *P. atropurpureus* leaf (c). Transversal section of *P. atropurpureus* leaf with silica bodies, prism-shape and needle-shape crystal (d). Scale bar: 30 μm (a & b), 120 μm (c) and 60 μm (d).

**Species examined:** Sect. *Jeanneretia*: *P. polycephalus* Lam; Sect. *Pulvinistigma*: *P. fusinus* Martelli; Sect. *Microstigma*: *P. conoideus* Lamarck.

### 3.4. Sub genus Pandanus

The upper epidermis cell shapes are square, rectangular, elongated polygonal or not. The epidermis cell wall is straight to sinuous and sometimes there is also found raised-anticlinal cell wall. Stomata distributed on this surface belong to class 1 (unspecialized). The lower epidermis presents with straight and wavy cell walls and has same epidermal cell shape to upper surface. All species have zonation and stomata class 4 or 5. Papillae of epidermis cells was varied, short branched in *P. papuanus* (Figure 4 c), lobed in *P. odorifer* (Figure 4 d) and *P. tectorius* var. *littoralis*. However, *P. odorifer* from Java [8] has stomata type class 2 which only has papillae in the lateral subsidiary cell. *P. brassii*, a species from Papua New Guinea has unusual difference to other species of this subgenus. Stomata on the upper leaf surface of this species has papillae in the lateral and polar subsidiary cell (class 3) and the zonation is also very clear (Figure 4 e and f). Based on [4], stomata class of this sub genus is classified into three groups, although according to [6] it is classified in to two groups.
Table 2. Stomata class and zonation on *Pandanus* spp. leaf surfaces.

| No | Species | Collection Origin | Stomata class | Zonation |
|----|---------|-------------------|---------------|----------|
|    |         |                   | Upper | Lower [4] | Lower [6] |
| 1  | *Pandanus dubius* Spreng | Enggano, Sumatra; Banten, Java; Talaud, Celebes; Halmahera, Moluccas | 1 | 1 | 1 | present |
| 2  | *Pandanus furcatus* Roxb | Lingga, Sumatra | 1 | 5 | 7 | present |
| 3  | *Pandanus helicopus* S. Kurz | Bukit Raya, Borneo | 1 | 5 | 6 | present |
| 4  | *Pandanus nitidus* (Miq.) Kurz | Batu Raden, Java | 1 | 4 | 2 | absent |
| 5  | *Pandanus atropurpureus* Merr. & Perry | Jayawijaya, Wamena, New-Guinea | 1 | 1 | 1 | absent |
| 6  | *Pandanus beccarii* Solms-Laubach | Minahasa, Celebes; Halmahera, Moluccas | 1 | 4 | 3 | present |
| 7  | *Pandanus krualianus* K. Schum. | Banggai, Celebes; Halmahera, Moluccas | 1 | 1 | 1 | present |
| 8  | *Pandanus conoideus* Lamarck | Jayawijaya, Wamena, New-Guinea | 1 | 1 | 1 | absent |
| 9  | *Pandanus fusinus* Martelli | Gunung Sekerat, Borneo; Kolaka, Celebes; Halmahera, Moluccas; Aru Island, Papua | 1 | 5 | 7 | present |
| 10 | *Pandanus polycephalus* Lam | | 1 | 2 | 4 | present |
| 11 | *Pandanus polycephalus* Lam | | 1 | 4 | 3 | present |
| 12 | *Pandanus odorifer* (Forssk.) Kuntze | Obi, Moluccas | 1 | 4 | 2 | present |
| 13 | *Pandanus papuanus* Solus-Lautb. | Papua | 1 | 5 | 7 | present |
| 14 | *Pandanus tectorius* Parkinson ex Du Roi; *Pandanus tectorius* var. littoralis | Manado, Celebes; Tasikmalaya, West Java, Java | 1 | 4 | 2 | present |
Table 2. Stomata class and zonation on Pandanus spp. leaf surfaces (continued).

| No | Species                        | Collection Origin          | Stomata class | Zonation |
|----|--------------------------------|----------------------------|---------------|----------|
|    |                                |                            | Upper | Lower | Lower |       |
| 15 | *Pandanus brassii* Martelli     | Dagwa, Oriomo, New Guinea  | 3     | 3     | 2     |     
|    |                                |                            |       |       |       | present |

Subgenus Acrostigma

16 *P. ornatus* Kurz

|      |                                | Lingga, Sumatra, Malay Peninsula | 1   | 4   | 3 & 5 | present |

Other species

17 *Pandanus amaryllifolius* Roxb.

|      |                                | Bogor, Java                  | 1   | 4   | 2     | present |

18 *Pandanus leptocarpus* Martelli

|      |                                | Brasing, New Guinea          | 1   | 1   | 1     | present |

19 *Pandanus cf limbatus* Merr. & Perry

|      |                                | Jayawijaya, Wamena, New Guinea | 1   | 5   | 7     | present |

Upper hypodermis is arranged by 1-2 layers of elongated cells and a layer thin-walled cells, while the lower hypodermis is composed by 1-2 layers of elongated cells without thin-walled cells. In the upper side of the leaf, mesophyll consists of 2-4 layers of palisade tissue and 1-4 layers of flattened chlorenchyma which exists below the palisade, except *P. odorifer* does not have palisade tissue (Figure 5 a). While at the lower side, the mesophyll consists of flattened chlorenchyma cells except *P. tectorius* that showed a layer of palisade in some parts of the leaf. CaCO₃ crystals were present in three forms, except *P. odorifer* which has none of these three crystal shapes. Silica bodies were found in all species as well as bundle sheath extension.

Species examined: Sect *Intraobtutus*: *P. papuanus* Solus-Lauth.; Sect. *Pandanus*: *P. brassii* Martelli, *P. odorifer*, *P. tectorius*.

3.5. Subgenus Acrostigma

The only species that was observed is *P. ornatus*. The upper epidermis cell wall is straight to fine wavy; it has square and rectangular shape cell, and unspecialized stomata. While the lower epidermis cell has straight wall; rectangular shape and elongated or not polygonal shape; stomata class 4. These epidermal characters are similar to specimen from Malay Peninsula, but slightly differ on the shape of the upper epidermis cell which has additional polygonal on the upper surface and square on the lower surface. Moreover, papillae shape between those two samples also different (Table 2). The Sumatran’s sample has fork-like shape or small lobes at the polar subsidiary cells, whereas Malay Peninsula’s sample has simple lobes or spatula-like shape.

There is layer of each thickened wall-elongated and thin-walled of hypodermis cells at the upper side. While the hypodermis on the lower side is arranged only 1-2 layers of elongated cells. Mesophyll consists of 1-2 layers of palisade tissue on the upper side and a layer of flattened cells, whereas at the lower side, there is 1-2 layers of flattened or isodiametric cells. Only one shape of CaCO₃ crystal was found, prisms, and scattered in the mesophyll (Table 3). Silica bodies were found in outermost of both upper and lower hypodermis. The crystal and silica bodies presence differ to species from Malay Peninsula which does not have silica bodies, but has needle-like shape and rectangular shape of crystal (Table 3).
Specimen examined: Sect. Acrostigma: *P. ornatus*.

3.6. Other species and doubted species

3.6.1. *P. amaryllifolius* Roxb.

This species has straight upper epidermal cell wall with rectangular and polygonal cell shapes. Stomata on this surface present without papillae. The lower epidermis has uniform shape with straight wall, polygonal with 4-6 angles, elongated cell or not. The zonation is clearly visible with stomata type class 4 [4] or class 2 based on [6] with simple lobes papillae.

The hypodermis on the upper side of the leaf consists of a layer of both thick-walled elongated cells and thin-walled cells. Sometimes, elongated cells are not found, therefore the hypodermic tissue layer consists of two layers of thin-walled isodiametric cells. The hypodermis on the lower side consists of a layer of elongated cells. On the upper side, leaf is composed by 1-2 layers of palisade tissue and 3-4 layers of flattened cells, while on the lower side, chlorenchyma cells only show 1-2 layers of isodiametric cells. Crystal CaCO$_3$ present as needle-like, rectangular and prism shapes, silica bodies are found in the hypodermis on both sides. Bundle sheath extensions present toward upper and lower side.

![Figure 4](image_url)

**Figure 4.** Leaf surface characters. Stomata class 2 (a) and class 4 (b) of *P. polycephalus*, short branched papillae (c) of *P. papuanus*, lobes papillae of *P. odorifer* (d). Stomata class 3 present on upper (e) and lower (f) surface of *P. brassii*. Scale bar: 60 μm.
Table 3. The presence of bundle sheath extension, silica bodies and CaCO₃ in *Pandanus* spp leaf.

| No | Species               | Collection origin          | Bundle sheath extension | Silica bodies | CaCO₃ |   |   |   |
|----|-----------------------|----------------------------|-------------------------|---------------|------|---|---|---|
|    |                       |                            |                         |               |      | needle | rectangular | prism | drusse |
|---  |-----------------------|----------------------------|-------------------------|---------------|------|---|---|---|
| 1   | *Pandanus dubius*     | Enggano, Sumatra,          | present                 | present       | present | present | present | present | absent |
|     | Spreng                | Banten, Java, Talaud,     |                         |               |      |   |   |   |
|     |                       | Celebes                   | present                 | Present/absent|       |       |       |       |       |
|     |                       | Halmahera, Moluccas       | present                 | present       | present | present | present | present | absent |
| 2   | *Pandanus furcatus*   | Lingga, Sumatra,          | present                 | present       |       | present | present | present | absent |
|     | Roxb                  |                           |                         |               |      |   |   |   |
| 3   | *Pandanus helicopus*  | Bukit Raya, Borneo        | present                 | present       | present | present | present | present | absent |
|     | S. Kurz               |                           |                         |               |      |   |   |   |
| 4   | *Pandanus nitidus*    | Batu Raden, Java          | present                 | Present/absent| present | present | present | present | absent |
|     | (Miq.) Kurz           |                           |                         |               |      |   |   |   |
| 5   | *Pandanus atropurpureus* | Jayawijaya, Wamena,     | present                 | present       | present | present | present | present | absent |
|     | Merr. & Perry         | New-Guinea                |                         |               |      |   |   |   |
| 6   | *Pandanus beccarii*   | Minahasa, Celebes         | present                 | present       | present |       | present | present | absent |
|     | Solms-Laubach         |                           |                         |               |      |   |   |   |
| 7   | *Pandanus beginii*    | Halmahera, Moluccas       | absent                  | present       | present | present | present | present | absent |
|     | Callm. & A.P. Keim    |                           |                         |               |      |   |   |   |
| 8   | *Pandanus kraulianus* | Banggai, Celebes          | present                 | present       | present | present | present | present | absent |
|     | K. Schum.             |                           |                         |               |      |   |   |   |
|     |                       | Halmahera, Moluccas       | present                 | present       | present | present | present | present | absent |
|---  |-----------------------|----------------------------|-------------------------|---------------|------|---|---|---|
| 9   | *Pandanus conoideus*  | Jayawijaya, Wamena,      | absent                  | present       | present | present | present | present | present |
|     | Lamark                | New-Guinea                |                         |               |      |   |   |   |
| 10  | *Pandanus fusinus*    | Gunung Sekerat, Borneo    | absent                  | present       | present | present | present | present | absent |
|     | Martelli              |                           |                         |               |      |   |   |   |
| 11  | *Pandanus polycephalus* | Halmahera, Moluccas      | present                 | present       | present | present | present | present | absent |
|     | Lam                   | Aru Island, NA            | absent                  | present       | present | present | present | present | absent |
|     |                       | Papua                     |                         |               |      |   |   |   |
Table 3. The presence of bundle sheath extension, silica bodies and CaCO\textsubscript{3} in Pandanus spp leaf (continued).

| No | Species                  | Collection origin          | Bundle sheath extension | Silica bodies     | CaCO\textsubscript{3} |
|----|--------------------------|----------------------------|-------------------------|-------------------|------------------------|
|    |                          |                            |                         | needle            | rectangular | prism | drusse |
| 12 | *Pandanus odorifer*      | Obi, Moluccas               | present                 | present           | absent      | absent | absent |
|    | (Forssk.) Kuntze         |                            |                         |                   |            |        |        |
| 13 | *Pandanus papuanus*      | Papua                      | present                 | present           | present    | present | absent |
|    | Solus-Lautb.             |                            |                         |                   |            |        |        |
| 14 | *Pandanus tectorius*     | Manado, Celebes            | present                 | Present/absent    | present    | present | absent |
|    | Parkinson ex Du Roi      |                            |                         |                   |            |        |        |
|    | *Pandanus tectorius* var.| Tasikmalaya, West Java,    | present                 | present           | present    | present | absent |
|    | *littoralis*              | Java                       |                         |                   |            |        |        |
|    | *Pandanus brassii*       | Dagwa, Oriomo river,       | present                 | present           | present    | present | absent |
|    | Martelli                 | western division, New-Guinea|                         |                   |            |        |        |

Subgenus Acrostigma

| 16 | *P. ornatus* Kurz        | Lingga, Sumatra Malay Peninsula | present | present | absent | absent | present | absent |
|    |                          |                            |          |         |        |        |          |        |

Other species

| 17 | *Pandanus amaryllifolius* | Bogor, Java                | present | present | present | present | present | absent |
|    | Roxb.                     |                            |          |         |        |        |          |        |
| 18 | *Pandanus leptocarpus*    | Brasing, New-Guinea        | present | present | present | present | present | absent |
|    | Martelli                 |                            |          |         |        |        |          |        |
| 19 | *Pandanus cf limbatus*    | Jayawijaya, Wamena, New-Guinea | present | present | present | present | present | absent |
|    | Merr. & Perry             |                            |          |         |        |        |          |        |

3.6.2. *P. leptocarpus* Martelli

Upper epidermis cell wall is straight to narrow wavy; square, rectangular and elongated or not; polygonal shapes and unspecialized stomata. While epidermis cells on lower epidermis present with straight-walled rectangular and elongated or not polygonal shapes. The lower surface of leaf has clear zonation and stomata present as class 1.

The upper hypodermis has layer of thick-wall elongated cells and also single layer of thin-wall oval shape. The lower hypodermis has two layers of elongated cells and 1-2 layers thin-walled cells. Palisade appears as 2-3 layers and single layer at the upper side and lower side respectively. 1-2 layers of flattened
chlorenchyma cells were showed on both side of the leaf. Three shapes of CaCO₃ crystal present in the mesophyll, silica bodies are found in both upper and lower of hypodermis and bundle sheath extension also present in this species.

Figure 5. Chlorenchyma present as isodimetric or oval shape (sp) in *P. odorifer* (a) and drusse-shape of CaCO₃ crystal (c) in *P. conoideus* (b). Scale bar: 120 μm (a) and 30 μm (b).

3.6.3. *Pandanus* cf. *limbatus*

This doubted species has straight epidermis cell wall on both upper and lower surface. The shape of epidermis cell was rectangular, elongated or not polygonal on upper and lower surface. But there is square shape as well on the upper surface. Stomata type was class 1 on the upper surface and class 7 on the lower surface. There was no zonation on lower leaf surface.

2-3 layers of elongated-thickened wall cell compose hypodermis tissue at upper and lower side of leaf. In the innermost of hypodermis, there is a layer of thin-wall cells in upper side, but none in lower side. Palisade at upper side is arranged by 2-3 layers cell and 1-2 layers of flattened chlorenchyma cells, while lower side only has two layers of flattened chlorenchyma cells. All three CaCO₃ crystal shapes and silica bodies present in this species. Vascular bundle has parenchyma bundle sheath extension as well.

4. Conclusion

Some characters such as crystal shape, presence of silica bodies and zonation on the lower leaf surface might be used for describing and classifying above species level or variation within species. The anatomical characters of several species are new to Indonesian *Pandanus* and described here, including *P. papuanus*, *P. beccarii* and *P. beguinii*.

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