doi: 10.11594/jtls.07.03.02
THE JOURNAL OF TROPICAL LIFE SCIENCE
OPEN ACCESS Freely available online
VOL. 7, NO. 3, pp. 197 – 203, September, 2017
Submitted February 2017; Revised May 2017; Accepted June 2017

The Classification of *Bambusa* spp. from Celebes Based on the Micromorphological Characters of Leaf Epidermis

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ABSTRACT

Species of *Bambusa* had widespread in Celebes, especially for *Bambusa striata* and *Bambusa vulgaris*. As an effect of the lacking of flowering, species identification mainly depends on leaf epidermal micromorphology, and vegetative features have proven to be useful in bamboo taxonomy. The objective of this research was to describe the classification of *Bambusa* from Celebes based on the micromorphological characters of leaf epidermis. The specimens were collected from the wild population. The samples of leaf were collected from five members of *Bambusa* i.e.: *Bambusa blumeana*, *Bambusa maculata, B. striata*, *B. vulgaris* and *Bambusa* sp. Micromorphological characters were identified using Scanning Electron Microscope (SEM). Leaf epidermis characters separated *B. blumeana* from other species of *Bambusa*. Furthermore, *B. striata* were closely related to *B. maculata* in a variation of bulliform cells. As an invention, we release peltate hair as a new type of trichome in *Bambusa*. The presence of numerous prickles, trichomes, and bulliform cells may be especially useful in delimiting species.

Keywords: *Micromorphology, scanning electron microscope (SEM), leaf epidermis, Bambusa, Celebes*

INTRODUCTION

Famously, bamboos can take between 7 and 120 years to flower. Because of this rarity of flowering, the identification of bamboo generally using vegetative characters [1]. However, vegetative characters are often influenced by the ecological factors [2]. Therefore, the anatomical technique was undertaken to establish taxonomic studies.

The function of the bamboo epidermis anatomical characters in classification has been known for a long time. Bamboos identification based on leaf anatomical characters were done since 1907 [2]. The nature of the anatomical considered very important in the study of taxonomists at various levels taxa [3, 4, 5].

Furthermore, the researchers offer a variety of leaf epidermal structures as diagnostic characters in the identification of bamboo. The composition of epidermal features along internodal structures can be used to differentiate between species [6]. The leaf epidermis characters of Asian Bamboo was compared with an emphasis on papillae character [7]. The pattern of papillae on the leaf epidermis in Arundinarieae approaches the phylogenetic taxonomy [8]. Micromorphological study of epidermal structures such as silica bodies, bulliform cells and various types of hair can provide valuable information on the anatomical properties of plants and also used in taxonomic studies [9].

This research analyzed the leaf epidermis of *Bambusa blumeana*, *Bambusa maculata, Bambusa striata*, *Bambusa vulgaris* and *Bambusa* sp. to contribute a better understanding of the Bambusoideae and to support taxonomic studies.

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How to cite:
Liana A, Purnomo, Sumardi I, Daryono BS (2017) The classification of *Bambusa* spp. from Celebes Based on the Micromorphological Characters of Leaf Epidermis. J. Trop. Life. Science 7 (3): 197 – 203.

MATERIALS AND METHODS

Plant materials used in this study were collected from a wild population in Celebes. The five species were *Bambusa blumeana, B. maculata, B. striata, B. vulgaris,* and *Bambusa* sp.

For the SEM analysis, leaf pieces were submerged in xylene for at least 4 hours in order to remove the waxy covering from the leaf epidermis. Afterward, materials were dried using hairdryer and then were mounted on stubs coated with gold. After gold sputtering, the specimens were observed in a JEOL (JSM-6510LA). The terminology of epidermis appendages follows Wu (1962), Gomes and Neves (2009) and Zhang et al. (2011) [2, 8, 10].

The data were analysis using Multivariate Statistical Program (MVSP) version 3.1 for identification of cluster analysis and principal component analysis (PCA) of *Bambusa*.

RESULTS AND DISCUSSION

Both the upper and the lower epidermis are constructed of epidermal cells and modified epidermal cells. There are two types of epidermal cells namely long cells and short cells. Some epidermal cells are highly modified and become quite different in appearance from the original cells. They are important in classification and described as hair (macrohair, microhair and prickles), silica bodies, stomata and bulliform cells [2].

Poaceae leaves have traditionally been observed with a light microscope, but some researchers have demonstrated the use of Scanning Electron Microscope (SEM) on the study of the leaf epidermis surface to provide strong support for the taxonomic study of this family [11, 12, 13]. SEM is able to expose some of the structure more clearly, distinguishing aspects of some features. Furthermore, SEM capable to observed more aspects such as papillae, prickles, macrohair, microhair, silica bodies, bulliform cells and stomata apparatus [11]. To confirm the type, the frequency of occurrence, composition, and present or absent of some structures is crucial for taxonomic purposes.

Here is presented characters used to differentiate species in the genus *Bambusa* (Table 1). The characters include trichomes, silica cells, bulliform cell, and stomata structure. Analysis of leaf epidermis on *Bambusa* was done based on the structure of the epidermis on both surfaces, a variety of shapes and sizes.

The analysis of leaf epidermis in *Bambusa* species revealed that epidermal elements on both surfaces vary in structures. Leaf epidermis cells on the upper epidermis showing silica bodies, bulliform cells and trichomes. Silica bodies present in *B. maculata*, *B. striata*, *B. vulgaris* and *Bambusa* sp., but absent in *B. blumeana*. Bulliform cells are clearly present in the intercostal zones of all spesies *Bambusa* were tested. Stomatal apparatuses, papillae, and various trichomes observed on the lower epidermis. The leaf surface characteristics of all the examined species are shown in Figure 1 and 2.

There are three types of trichomes observed in *Bambusa* leaf epidermis namely prickles, microhair and peltate hair. Prickles have two types, the first type is nearly elliptical at the base and cuspidate at the apex, are found on both surface of leaves over the costal zone; the second type is rounded at the base and acute at the apex, are

Table 1. Comparison of leaf epidermis characters among species of *Bambusa*

Characters	*Species*				
	B. blumeana	*B. maculata*	*B. striata*	*B. vulgaris*	*Bambusa* sp.
Adaxial surface					
1. trichomes					
Prickles in costal zone	present	absent	absent	present	Absent
Prickles in leaf blade	absent	absent	absent	present	absent
Peltate hair	present	absent	absent	absent	present
2. Silica bodies	absent	present	present	present	present
3. Bulliform cell					
Rows	3	4	4	3	3
Middle cell of 3-rowed bulliform cell	polygonal	polygonal	elongate	elongate	elongate
Abaxial surface					
1. trichomes					
Prickles	present	present	present	present	Present (different type)
2. stomata					
Rows of stomata	2	5	3	4	3

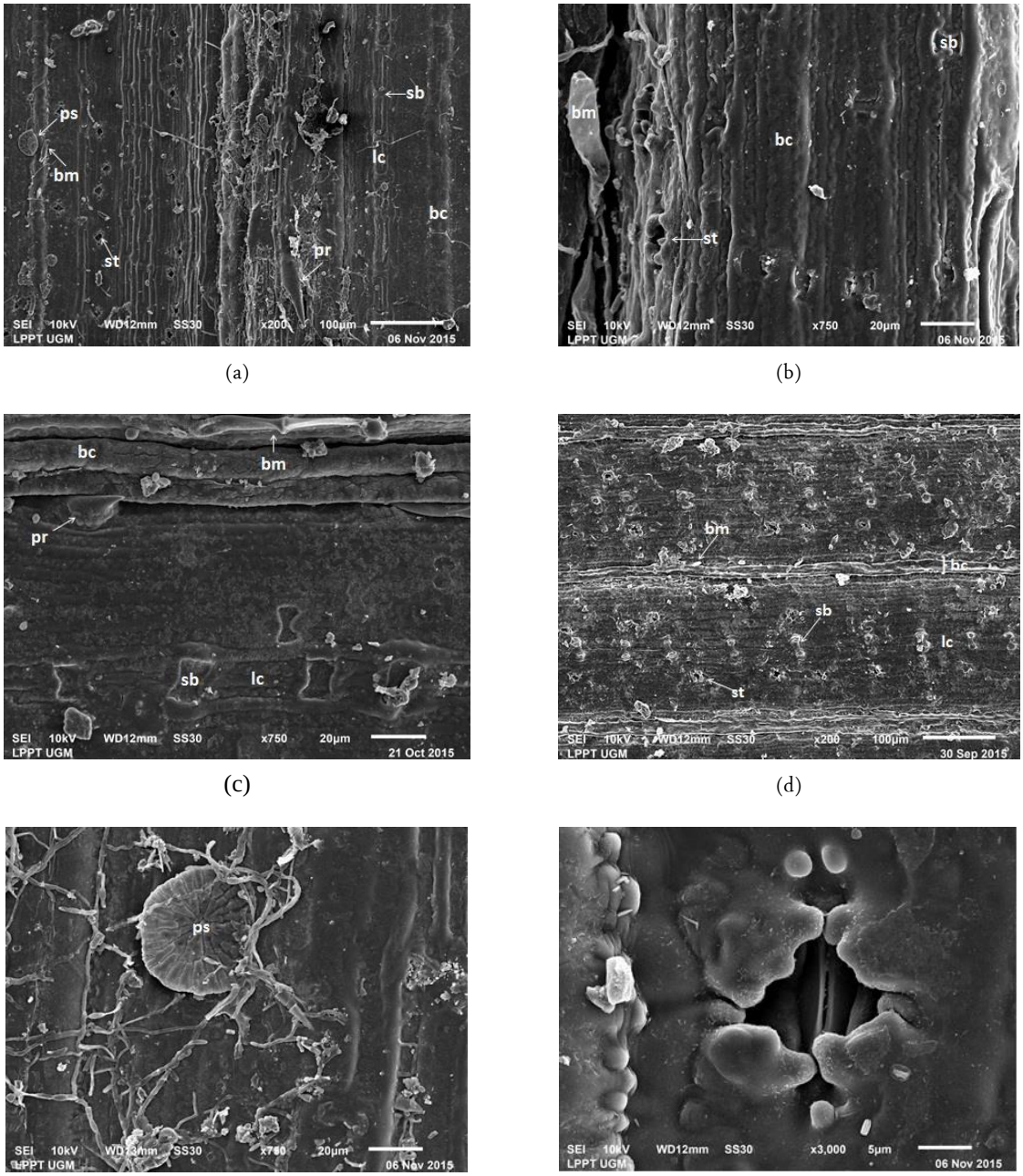

Figure 1. Micromorphology of the upper epidermis of *Bambusa* using scanning electron microscope. *B. blumeana* (a), *B. maculata* (b), *B. vulgaris* (c), *B. striata* (d), *Bambusa* sp. (e), and stomata of *B. blumeana* (f). Abbreviations: bm: bicellular microhair; bc: bulliform cells; sb: silica bodies; st: stomata; ps: peltate hair; lc: long cell; pr: prickles

found in the lower epidermis over the intercostal zone. Microhairs are bicellular. The apical cell has a very thin wall, which may be separated during plant material preparation [10]. Microhair is distributed both of upper and lower epidermis of all species *Bambusa* observed. Peltate hairs are multicellular. Peltate hairs consist of a short stalk and a flat disk. Each partition on the disk is a single cell. This type generally saved secretory fluids to protect the plant from external factors [15]. Peltate hairs are new trichome observed in this study. This trichome type has not been reported found on *Bambusa*. Peltate hairs found on the upper epidermis of *B. blumeana* and *Bambusa* sp.

Silica bodies are derivatives of epidermal cells. There is a short cell consists of two types of cells, the silica cells, and cork cells. Silica cells and cork cells are often

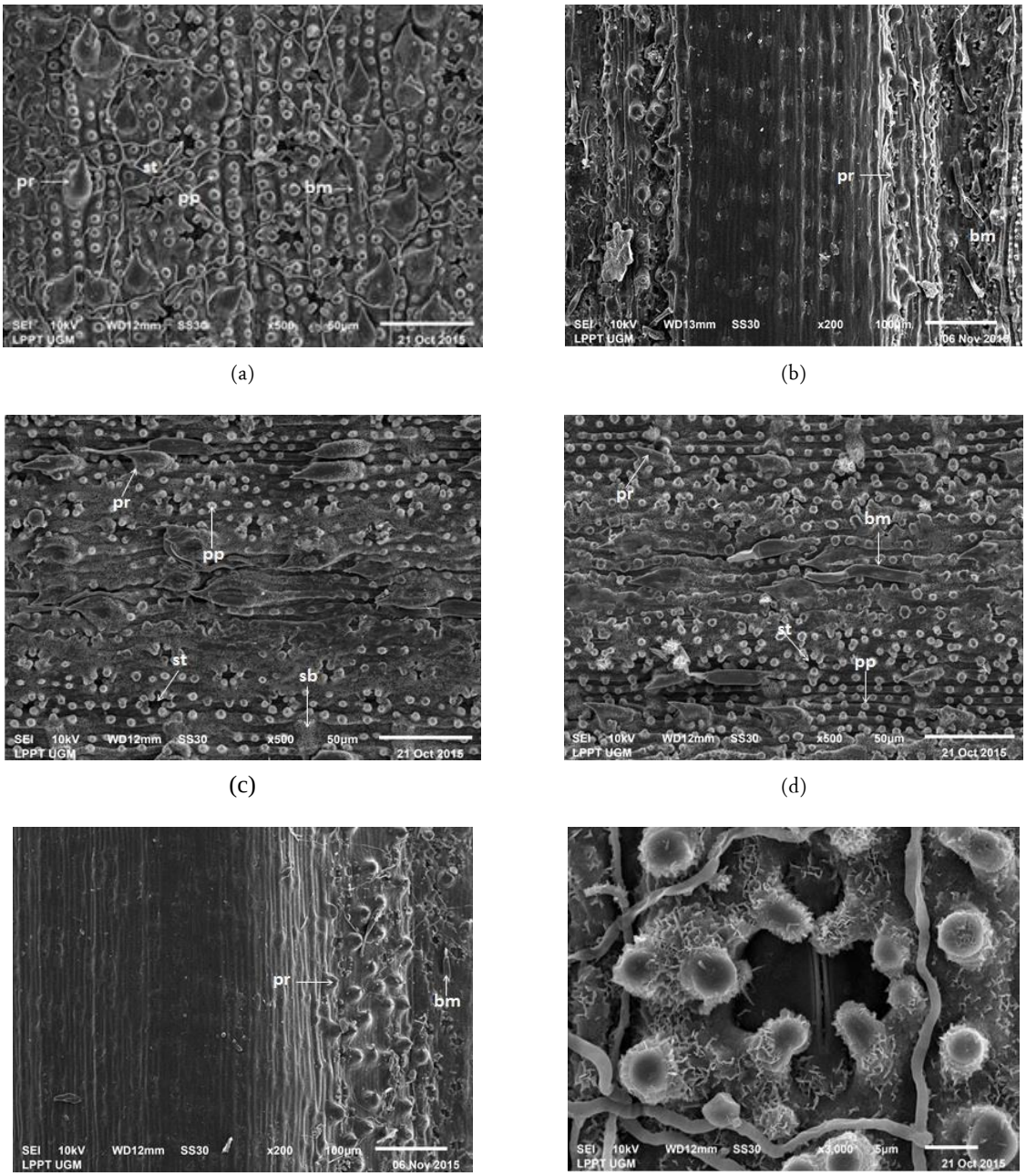

Figure 2. Micromorphology of the lower epidermis of *Bambusa* using scanning electron microscopy. *B. blumeana* (a), *B. maculata* (b), *B. vulgaris* (c), *B. striata* (d), *Bambusa* sp. (e), and stomata of *B. blumeana* (f). Abbreviations: bm: bicellular microhair; bc: bulliform cells; sb: silica bodies; st: stomata; ps: peltate hair; lc: long cell; pr: prickles

successively formed in pairs along the leaf. The cells are developed from isotropic silica mass and usually containing microscopic granules in the middle of structures. Silica cells are thought to have protective mechanisms of plants from pests and environmental stresses [15]. The silica cells on the upper epidermis are dumbbell of all species *Bambusa* were studied. These results are consistent to Wu (1962) that *Bambusa* has a dumbbell shape silica cells in the costal zone of upper epidermis [2]. Thus the silica cells on the upper epidermis cannot be used to differentiate species of *Bambusa*. Silica cells in the costal zone of lower epidermis are rectangular found in all species tested, while silica cells on intercostal zone of lower epidermis found in all *Bambusa* species, except in *Bambusa* sp.

Some types of leaves may curl in dry air or others

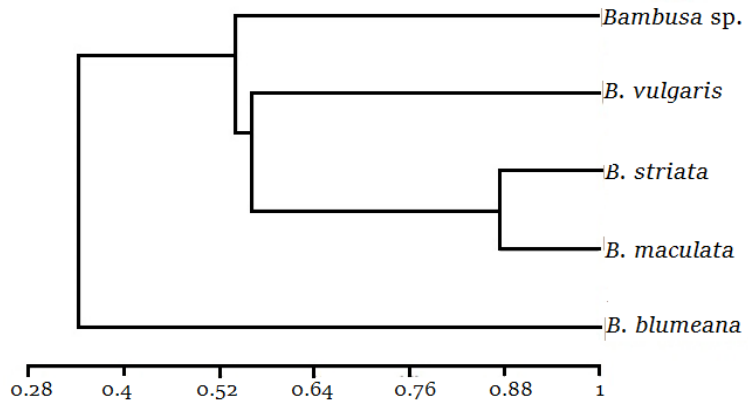

Figure 3. Dendrogram of *Bambusa* Celebes based on micromorphological characters

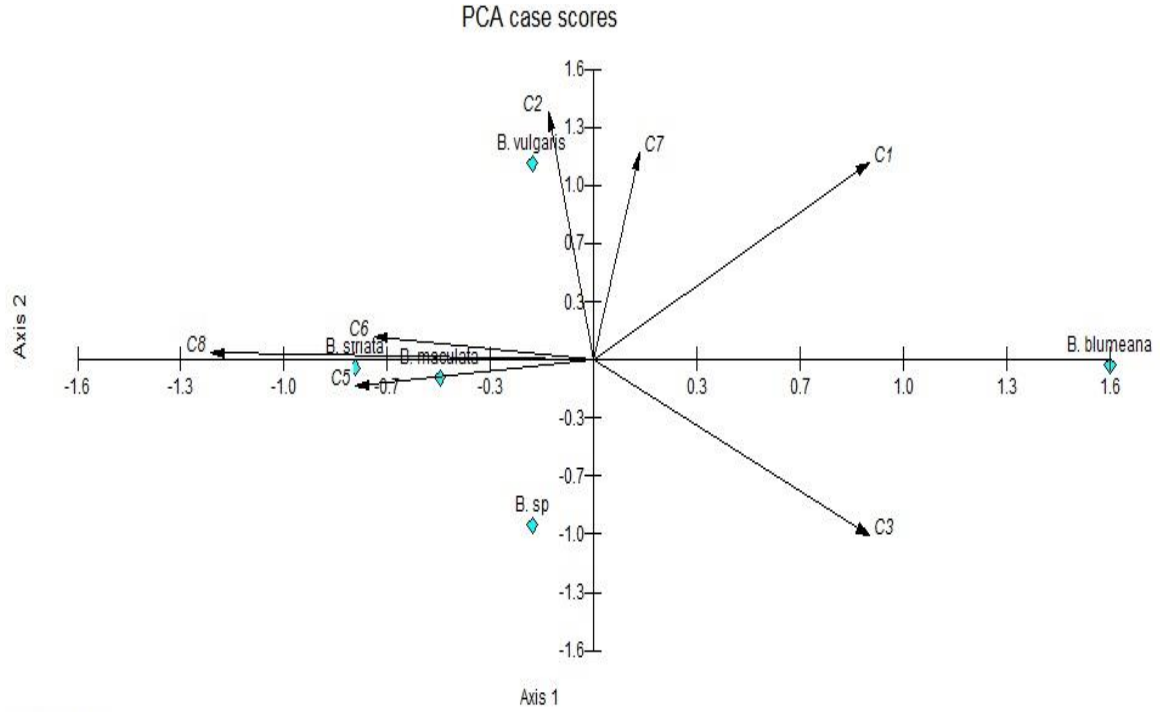

Figure 4. PCA based on micromorphological characters

environmental conditions. This mechanism supported by bulliform cells. The shape, size, and disposition of these cells can be used for classification and identification purpose [16]. *Bambusa* species has a wide variety of bulliform cells types, the number of rows constituting the band varies three or four, with a polygonal or an elongated of middle cell. Hence the bulliform cells structure are useful for classification.

Stomata in Poaceae lined up on the leaf blade and Bamboo generally on lower epidermis [15]. Stomata are very difficult to observe because of the growth of papillae that cover the surface [10]. In many studies, papillae character is considered appropriate to be a differentiator, taxonomic representative. However, the character of papillae not appropriate to distinguish members of Bamboo tropical Asia [7]. In this study showed that *Bambusa* only has four finger-like protuberances with the same structure.

B. blumeana is a member of *Bambusa* with the lowest coefficient of similarity to another species *Bambusa*, namely 0.587 (Figure 3). Accoding to morphological classification, *B. striata* is a variety of *B. vulgaris*. However on micromorphological data *B. striata* to be closely related to *B. maculata*. *B.*maculata has been considered as part of *B. vulgaris* for a long time, but because of differences in the vegetative and generative characters, it is

quite distinct [17].

Defined role of each micromorphological characters in the grouping of accession can clearly see in the scatter of Principal Component Analysis (PCA) diagram (Figure 4). These diagram is showing direction and role of each character, which showing as vectors with different length. Based on eigenvalues is known to form a group of species consisting of *Bambusa* species, except *B. blumeana* that split its own. *B. blumeana* were split due to prickles character and present of peltate hair. This result is supported by field observation that among members of *Bambusa*, *B. blumeana* have different morphological characters since main branches elongated and vinelike, spreading horizontally and coiled around culm.

Key to *Bambusa* species based on leaf epidermis characters:

1. No peltate hairs on upper epidermis 2
1. A few peltate hairs on upper epidermis 3
2. Silica cells absent on the upper epidermis, middle cells polygonal in a 3-rowed bulliform band . . . . . *B. blumeana*
2. Silica cells present on the upper epidermis, middle cells elongate in a 3-rowed bulliform band . . . . . *Bambusa* sp.
3. Prickles present on upper epidermis over costal zone . . . . . *B. vulgaris*
3. Prickles absent on upper epidermis over costal zone 4
4. Three rows of stomata on each side of costal on lower epidermis . . . . . *B. striata*
4. Five rows of stomata on each side of costal on lower epidermis . . . . *B. maculata*

CONCLUSION

From above explanation, we conclude that leaf epidermis characters (i.e., prickles, trichomes, and bulliform cells) were useful for distinguishing between *Bambusa* species. Therefore, the use of anatomical characters to complete the morphological data on species identification may be suggested.

ACKNOWLEDGMENT

We would like to thank Dr. Harini, Integrated Research and Testing Laboratory, Universitas Gadjah Mada Yogyakarta for her skillful technical assistance in scanning electron microscope operation.

REFERENCES

1. Kelchner SA, Bamboo Phylogeny Group (2013) Higher level phylogenetic relationship within the bamboos (Poaceae: Bambusoideae) based on five plastid markers. Molecular Phylogenetics and Evolution. 67 (2): 404 – 413. doi: 10.1016/j.ympev.2013.02.005.
2. Wu MC (1962) The classification of bambuseae based on leaf anatomy. Botanical Bulletin of Academia Sinica 3: 83 – 114.
3. de Villiers BJ, Tilney PM, van Wyk EB (2010) The taxonomic significance of leaf anatomical characters in Cussonia and related genera (Araliaceae). Botanical Journal of the Linnean Society 164 (3): 246 – 263. doi: 10.1111/j.1095-8339.2010.01085.x.
4. Zarrei M, Wilkim P, Ingrouille MJ et al. (2010) The systematic importance of anatomical data in Gagea (Liliaceae) from the Flora Iranica area. Botanical Journal of the Linnean Society 164 (2): 155 – 177. doi: 10.1111/j.1095-8339.2010.01081.x.
5. Liu W, Zhu XY (2011) Leaf epidermis characters and taxonomic revision of *Schizophragma* and *Pileostegia* (Hydrangeaceae). Botanical Journal of the Linnean Society 165 (3): 285 – 314. doi: 10.1111/j.1095-8339.2010.01101.x.
6. Pattanath PG, Rao KR (1969) Epidermal and internodal structure of the culm as aid to identification and classification of bamboo. In: recent advices in the Anatomy of Tropical seed plants eds. New Delhi, Hindustan Publishing Corporation, pp 79 – 196.
7. Yang H, Wang H, Li D (2008) Comparative morphology of the foliage leaf epidermis, with emphasis on papillae characters, in key taxa of woody bamboos of the Asian tropics (Poaceae: Bambusoideae). Botanical Journal of the Linnean Society 156 (3): 411 – 423. doi: 10.1111/j.1095-8339.2007.00736.x.
8. Zhang HY, Yang YM, Liu XZ (2011) Bamboo species relations revealed by random amplified polymorphism chloroplast DNA. African Journal of Agricultural Research 6 (5): 1241 – 1245. doi: 10.5897/AJAR10.794.
9. Pole M (2010) Cuticle morphology of Australasian Sapindaceae. Botanical Journal of the Linnean Society. 164 (3): 264 – 292. doi: 10.1111/j.1095-8339.2010.01086.x.
10. Gomes DMS, Neves LJ (2009) Scanning electron microscopy of the leaf epidermis of *Merostachys* Spreng. (Poaceae: Bambusoideae). Acta Botanica Brasilica 23 (2): 516 – 525. doi: 10.1590/S0102-33062009000200023.
11. Palmer PG, Tucker AE (1981) A scanning electron microscope survey of the epidermis of East African grasses I. Washington, Smithsonian Institution Press.
12. Palmer PG, Tucker AE (1983) A scanning electron microscope survey of the epidermis of East African grasses II. Washington, Smithsonian Contributions to Botany. doi: 10.5479/si.0081024X.53.
13. Palmer PG, Gerbeth-Jones S, Hutchison S (1985) A scanning

electron microscope survey of the epidermis of East African grasses III. Washington, Smithsonian Contributions to Botany. doi: 10.5479/si.0081024X.55.

14. Ascensão L, Marques N, Pais MS (1995) Glandular trichomes on vegetative and reproductive organs of *Leonotis leonulus* (Lamiaceae). Annals of Botany 75 (6): 619 – 626. doi: 10.1006/anbo.1995.1067.
15. Judziewicz EJ, Clark LG, Londono X, Stern MJ (1999) American bamboos. Washington, DC, Smithsonian Institution Press.
16. Cutler DF, Botha T, Stevenson DW (1939) Plant anatomy: An applied approach. New Jersey, Blackwell Publishing.
17. Widjaja EA (1997) New taxa in Indonesian bamboos. Reinwardtia 11 (2): 57-152.