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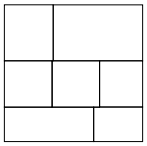

Cover images: *Nepenthes calcicola* Gary W.Wilson, S.Venter & Damas. Top left: Habit with lower pitcher. Top right: Habit with mid-level pitcher. Middle left: Habit with male inflorescence. Middle mid: A rosette of pitchers. Middle right: Field assistant Siwi with mid level rosette. Below: Underside of the lid of mid level pitcher. Photos by S. Venter.

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# COMPARATIVE MICROMORPHOLOGY LEAF SURFACE OF SELECTED *HOYA* SPP. (APOCYNACEAE) FROM SARAWAK

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**ABSTRACT**
KHALEEDA, R. & MEEKIONG, K. 2023. Comparative micromorphology leaf surface of selected *Hoya* spp. (Apocynaceae) from Sarawak. *Reinwardtia* 22(2): 69–77. — Traditionally, morphological parameters have been used for several taxonomic and ecological identifications. The morphological characteristics alone would be difficult to distinguish the evidence for species identification. Hence, a study of leaf anatomy was conducted to compare the structural characteristics which focused on the epidermal cells, stomata, and trichomes by using a Compound Microscope and Scanning Electron Microscope (SEM). Four selected species of *Hoya* from various localities within western of Sarawak were studied: *H. caudata, H. coronaria*, *H. omlorii,* and *H. verticillata*. The results showed stomatal types of *Hoya* species have stephanocytic stomata, except for two species which have slightly significant differences: *H. coronaria* has anomocytic stomata and *H. omlorii* has paracytic stomata. Meanwhile, in terms of the stomatal presence, all species possessed hypostomatic stomata, only *H. verticillata* has amphistomatic stomata which stomata presence on both sides of the adaxial and abaxial surfaces. Highest stomatal density was recorded in *H. omlorii* whereas the lowest were recorded in *H. coronaria.* Stomatal index were calculated and *H. caudata* has the highest with 12.80% and *H. coronaria* has the lowest value which is 6.28%. All four species were completely absence of trichomes except for *H. coronaria* which has simple unicellular non-glandular trichomes. The result indicates that the anatomical characteristics provide additional information and could be a great assist in the distinction within *Hoya* species.

**Key words**: Amphistomatic, Borneo, epiphyte, systematic, wax plant.

**ABSTRAK**
KHALEEDA, R. & MEEKIONG, K. 2023. Perbandingan morfologi mikro permukaan daun *Hoya* spp. (Apocynaceae) terpilih dari Sarawak. *Reinwardtia* 22(2): 69–77. — Secara konvensional, parameter morfologi telah digunakan untuk mengidentifikasi secara taksonomi dan ekologi. Karakter morfologi saja sulit dipakai untuk membedakan bukti identifikasi jenis. Oleh karena itu, kajian anatomi daun telah digunakan untuk membandingkan karakter struktur sel epidermis, stomata, dan trikoma dengan menggunakan Mikroskop Majemuk dan Mikroskop Pemindai Elektron (SEM). Empat jenis *Hoya* terpilih dari berbagai lokasi di bagian barat Sarawak telah dipelajari yaitu *H. caudata*, *H. coronaria, H. omlorii*, dan *H. verticillata.* Hasil penelitian menunjukkan kebanyakan tipe stomata keempat jenis *Hoya* mempunyai stomata stefanositik, kecuali dua jenis yang mempunyai perbedaan yang sedikit, yaitu *H. coronaria* yang mempunyai stomata anomositik dan *H. omlorii* mempunyai stomata parasitik. Hasil pengamatan stomata menunjukkan semua jenis mempunyai stomata hipostomatik dan hanya *H. verticillata* yang mempunyai stomata amfistomatik yaitu adanya stomata pada kedua belah permukaan adaksial dan abaksial. Kerapatan stomata tertinggi terdapat pada *H. omlori*, sedangkan yang terendah pada *H. coronaria.* Indeks stomata tertinggi pada *H. caudata* ialah 12,80% dan terendah pada *H. coronaria* yaitu 6,28%. Trikoma tidak terdapat pada keempat jenis *Hoya* yang diperiksa, kecuali pada *H. coronaria* yang mempunyai trikoma tanpa kelenjar uniselular yang sederhana. Hasil penelitian menunjukkan karakter anatomi dapat memberi informasi tambahan dan dapat menjadi alat bantu untuk membedakan jenis-jenis *Hoya*.

**Kata kunci**: Amfistomatik, Borneo, epifit, sistematik, tumbuhan lilin.

## INTRODUCTION

The genus *Hoya* belongs to the family Apocynaceae (subfamily Asclepiadoiceae) which is known as the wax-plant, wax-vine, wax-flower, or simply *Hoya*. *Hoya* R.Br. is a large and complex genus with approximately 350–450 species (Rodda, 2015). The Indomalesian-Australian-Western Pacific Region is home to this plant (Rodda & Simonsson, 2011), followed by Philippines, Borneo, and New Guinea which serving as diversity hotspots (Cabactulan *et al.,* 2017). Recent study by Rahayu (2021) revealed that Indonesia leads the most diverse *Hoya* which recorded with more than 110 species. Therefore, multiple papers describing new *Hoya* species have been published previously (Lamb *et al.,* 2014; Rodda, 2015) resulting increasing the number of *Hoya* taxa occurring in Borneo

which updated up to 85 species and four subspecies (Rodda & Rahayu, 2022). Most of the descriptions of these species are mainly focused on the morphological characteristics where Lamb & Rodda (2016) has listed a preliminary checklist consisting of 32 species occurring in Sarawak alone. *Hoya* is highly appreciated for its horticultural values and also believed to have a potential in medicinal uses (Rahayu, 2011; Silalahi *et al*., 2015). As ornamental plants, conservation and sustainable commercialization of these native species are important in the country of origin because it facilitates reintroductions if wild populations decline in numbers for any reason.

Stomata are important structures in plant biology and have been the subject of extensive research to understand their regulation, response to environmental and their role in plant physiology and adaptation. According to Cowan & Farquhar (1977) differences in stomatal characteristics may indicate adaptive relates to the environmental conditions in which plants grow, given the critical function that stomata play in managing the conflicting demands of carbon uptake and regulating the amount of water loss from the leaf. Stomata and epidermal cell characteristics have also been extensively employed as taxonomic data to support plant grouping, for instance in *Hoya* species (Hakim *et al.,* 2013), orchid species (Rompas *et al.,* 2011) and *Begonia* species (Efendi, 2019). Therefore, various studies have proven that stomatal density with light environment (James & Bell, 2000) and carbon dioxide concentration (Hetherington & Woodward, 2003) as well as stomatal size and shape (Jordan *et al.,* 2015), have been identified as adaptive or beneficial under specific conditions. As such, this study was carried out to differentiate the micromorphology features of leaves surfaces within four *Hoya* species that may contribute to a beneficial enhancement for species delimitation and useful parameter for taxonomic difficult in systematic investigations and species management between *Hoya* genus.

## MATERIALS AND METHODS

**Samples collection.** This study were involved a field surveys and laboratory works. The field survey comprises a convenience sampling where subjected to a small scale of several areas in Western Sarawak: Kuching, Kota Samarahan, and Serian Divisions respectively (Fig. 1). Global Positioning System (GPS) was used to record coordinates localities for every species collected which adapted to different types of environments and habitats. A total of four species were successfully collected: *H. caudata, H. coronaria, H. omlorii,* and *H. verticillata* as shown in Table 1. The identification was done and compared based on specimen in Herbarium of Sarawak (SAR).

**Anatomical preparation.** A modification of Cutler's method (1978) in preparing a cuticular epidermal cell was applied. A free-hand sectioning was employed to gather the structural organization of the leaf of *Hoya* species. A fresh and matured leaves were taken randomly from all parts of the leaves of each species. For comparison and consistency, at least 3 replicates of approximately 1 $cm^2$ of fresh samples were used. Each leaf part was subjected to a cuticle epidermal clearing using 15% nitric acid ($NHO_3$). The leaf was slowly heated under hotplate until the adaxial and abaxial surfaces of the leaf were split completely. The samples were removed by using a brush, placed into a petri dish and washed with distilled water. All the mesophyll were gradually washed and became a clean piece of tissue. The clean tissues were placed in a petri dish containing sodium hypochloride (NaOCl) until decolourized. The tissues were then washed again with distilled water and transferred into another petri dish containing safranin and staining for 3–5 minutes. The tissues were then dehydrated using a series of ethanol (50%, 60%, 70%, 80%, 90%, and 100%). The tissues were then placed on a clean slide and glued with Canada balsam. Labelled the slides accordingly and dried using the slide drier (at 60°C) overnight until the slides were completely dried and ready for observation. The specimens then were observed under compound microscope. The cell arrangement was observed and images taken. Each of the *Hoya* species was used to compare their differences in the cell structure.

**Determination stomatal density and index.** The determinations were done according to Salisbury (1927) methods. The stomatal index (SI %) was determined using the formula (S/S+E) × 100, where the S and E stand for the number of stomata and epidermal cells, respectively. Whereas, the stomatal density was obtained by using formula as described by Wilmer (1983 as cited in Hakim *et al*., 2013).

$$\text{Stomatal density} = \frac{\Sigma\ \text{Stomata}}{\textit{Field of view area } (mm^2)}$$

$$\text{Stomatal index} = \frac{\Sigma\ \textit{Stomata}}{\Sigma\ \textit{stomata} + \Sigma\ \text{epidermis } \textit{cell}} \times 100\%$$

**Scanning Electron Microscope (SEM).** The middle part of the samples was cut into 5 $mm^2$ and cleaned thoroughly with distilled water for the Scanning Electron Microscopes (SEM). The samples were then fixed with 4% gluteraldehyde overnight before being dehydrated with a series of ethanol 50%, 60%, 70%, 80%, 90%, and 100%. The


Fig. 1. The study sites of *Hoya* species within Western of Sarawak.

Table 1. Localities and ecology of *Hoya* species in Western of Sarawak.

| No | Species | Localities | Altitude | Habitat |
|---|---|---|---|---|
| 1 | *H. caudata* | Padawan, Kudung | 8 m | MDF |
| 2 | *H. coronaria* | Siburan, Serian | 19 m | MDF |
| 3 | *H. omlorii* | Kota Samarahan | 17 m | Mangrove Forest |
| 4 | *H. verticillata* | Bako NP, Kuching | 38 m | Kerangas Forest |

Notes. MDF: Mixed Dipterocarp Forest, NP: National Park.

samples underwent Critical Point Drying (CPD) to make sure the tissues were totally dried. Both surfaces of dried samples were fixed with double-sided adhesive tape on stub and labelled accordingly. A Sputter Coater was used to coat the leaf specimen with platinum. The samples were analysed under JSM – 6390 LA Analytical Scanning Electron Microscope (SEM) and the images were captured at various magnifications to examine the characteristics and to obtain the measurement of parameters.

## RESULTS

### Epidermal cells

The examination of the epidermal cells of four species studied revealed a similarity in the shape and the type of anticlinal cell wall. Three species including *H. caudata, H. coronaria*, and *H. verticillata* shared the same characteristic of the epidermal cell which appeared as a thin and straight cell wall with a polygonal shape of five to eight sides on both abaxial and adaxial surfaces. Nonetheless, only *H. omlorii* exhibited a characteristic and showed a different shape on the abaxial surfaces where the anticlinal cell wall arrangement was slightly undulate compared to the actual polygonal shapes (Fig. 2F).

### Stomata

From the observation, leaves of *H. caudata*, *H. omlorii,* and *H. coronaria* are generally found hypostomatic, with the distribution distance between the stomata varies in some species where much closer to each other and some species vice versa. With an exception of all four species, *H. verticillata* observed has an amphistomatic leaves with the presence of stomata on both abaxial and adaxial surfaces. However, the stomata distribution on the adaxial surfaces is not as dense as on the abaxial surfaces (Figs. 2G & 2H). This may be considered a distinct character for species identifi-

Table 2. Comparison of the leaf anatomical characters of *Hoya* species.

| Species / Characteristics | *H. caudata* | *H. coronaria* | *H. omlorii* | *H. verticillata* |
|---|---|---|---|---|
| Type of anticlinal cell wall | Straight | Straight | Slightly undulate | Straight |
| Presence of stomata | Hypostomatic | Hypostomatic | Hypostomatic | Amphistomatic |
| Position of stomata | Raised | Same level | Same level | Raised |
| Type of stomata | Stephanocyctic | Anomocytic | Paracytic | Stephanocyctic |
| Size of stomata (µm) | 30.05–34.48 × 27.67–33.67 | 32.76–38.18 × 27.95–31.51 | 27.29–29.01 × 15.45–20.88 | 26.60–36.66 × 30.67–34.96 |
| Stomatal density ($mm^2$) | 148.15 | 83.95 | 187.65 | 9.88 (Adaxial) & 167.90 (Abaxial) |
| Stomatal index (%) | 12.80 | 6.28 | 11.56 | 10.34 |
| Presence of trichomes | Absent | Present | Absent | Absent |
| Presence of ornamentation | Papillae | Absent | Absent | Papillae |

cation within the genus. Thus, it appears that plants with hypostomatic leaves have to face habitats with water stress which need to withstand and thrive in harsh environment. The stomatal types in this study showed a substantial variation among the four *Hoya* species. *H. coronaria* was noticed as anomocytic stomata while *H. omlorii* has paracytic stomata with two subsidiary cells, one with a short cell on each side of the guard cells and one with a wide cell at each pole. The stomata are randomly and evenly distributed on the abaxial leaves surfaces. The biggest stomata were noticed in *H. coronaria*, which is 32.76–38.18 µm length and 27.95–31.51 µm width. Contrasted with *H. omlorii* which recorded to be the smallest stomata cells compared to the others species measuring 27.29–29.01 µm length and 15.45–20.88 µm width respectively. Figures 2E & 2F illustrated the stomata on abaxial surfaces of the species examined.

**Stomatal density & stomatal index**

Stomatal density for the abaxial part of four *Hoya* leaves, the highest record was observed in *H. omlorii*, which is 187.65 $mm^2$ and the lowest stomatal density was 83.95 $mm^2$ which shown in *H. coronaria*. Whereas, *H. verticillata* which presence stomata on both of the leaves surfaces represent 9.88 $mm^2$ and 167.90 $mm^2$ on adaxial and abaxial respectively (Table 1). Although *H. verticillata* was considered as amphistomatic stomata, but the stomatal distribution on the adaxial surfaces are scarce compared to the abaxial surfaces. Stomatal index shows the ratio between the number of stomata with the number of the stomata and the epidermal cells. From the observation, the highest stomatal index was shown in *H. caudata* with value of 12.80% and the lowest stomatal index was found in *H. coronaria* with 6.28% as shown in Table 1.

**Trichome**

The presence of trichomes only observed in *H. coronaria*, where the trichomes occur singly, unicellular with non-glandular, long and acute apical cells on both sides of the leaves epidermis.

**Papillae**

Papillae ornamentation was observed in *H. caudata* and *H. verticillata*. However, only on the abaxial surfaces of both species showed abundance density of papillae that raised directly over the cells (Figs. 2B & H).

## DISCUSSION

**Epidermal cells**

According to Salasiah & Meekiong (2018), a polygonal are the two-dimensional shapes with straight lines which are consist of rectangular, pentagonal, hexagonal, heptagonal and octagonal on the cells. The majority of anticlinal cell walls of dicotyledon epidermal cells are straight, curved, or sinuous in shape (Cutler *et al*., 2008). However, the distinct characters of anticlinal cell wall shown in *H. omlorii* are believed to be taxonomically valuable in separating species within this genus which also found was reported by Fontenelle *et al.* (1994) to identify species of *Eugenia* (Myrtaceae) and by Moraes *et al.* (2009) to distinguish *Simira sampaioana* (Rubiaceae) one species to another by using the outline of the anticlinal cell wall characteristics.

**Stomata**

Stomata occurred either on both sides of surfaces amphistomatic or only on the upper surfaces hyperstomatic or more commonly on the lower surfaces hypostomatic of leaves (Serna *et al*., 2002; Parveen *et al.,* 2007). Mbagwu *et al.* (2008) report-

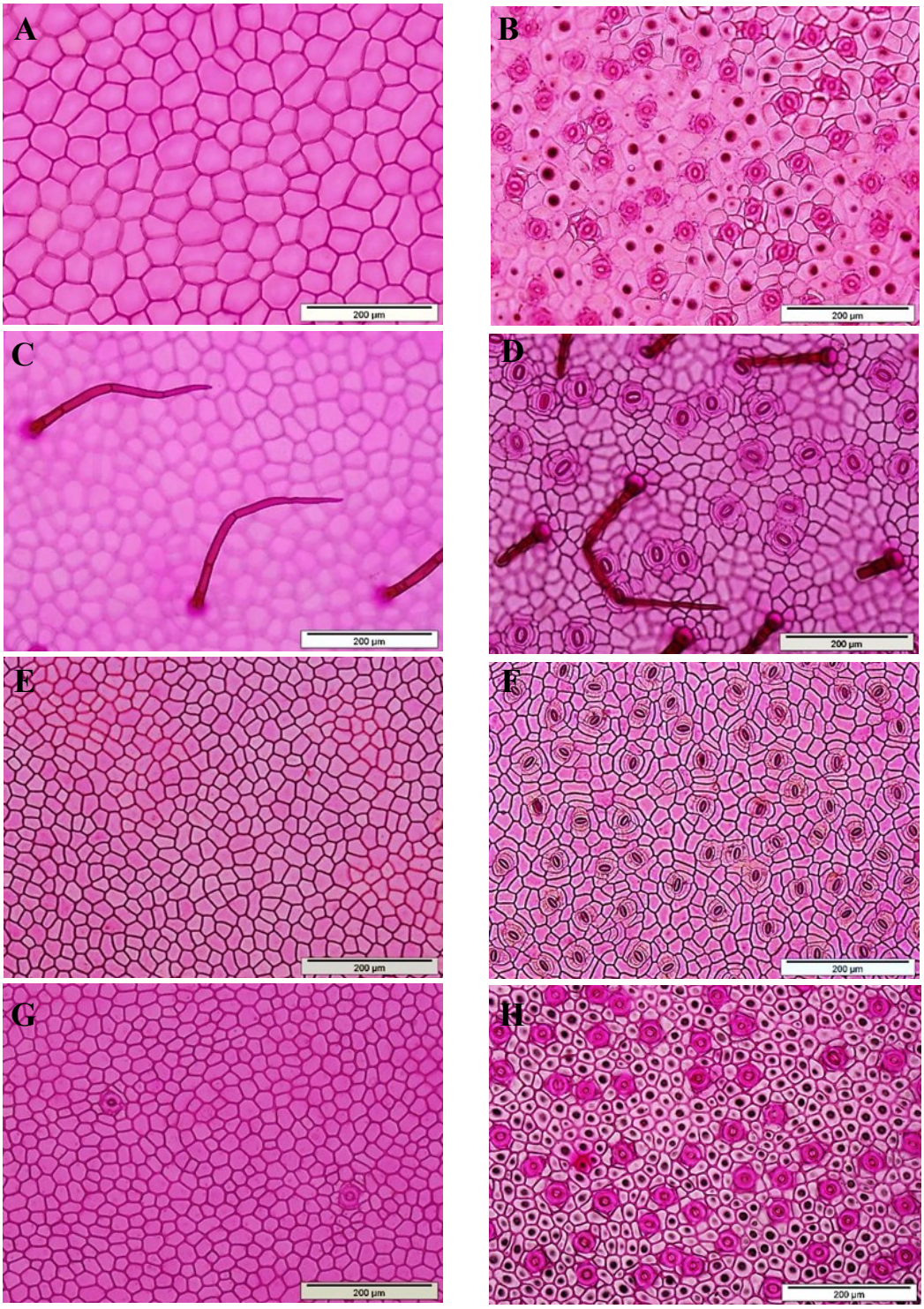


Fig. 2. Comparison leaf epidermis of four *Hoya* species by using compound microscope. A-B. The adaxial and abaxial of *H. caudata*; C-D. The adaxial with unicellular trichome and abaxial of *H. coronaria*; E-F. Adaxial and abaxial surfaces of *H. omlorii*. G-H. The adaxial with rarely presence of stomata and abaxial surfaces of *H. verticillata.*

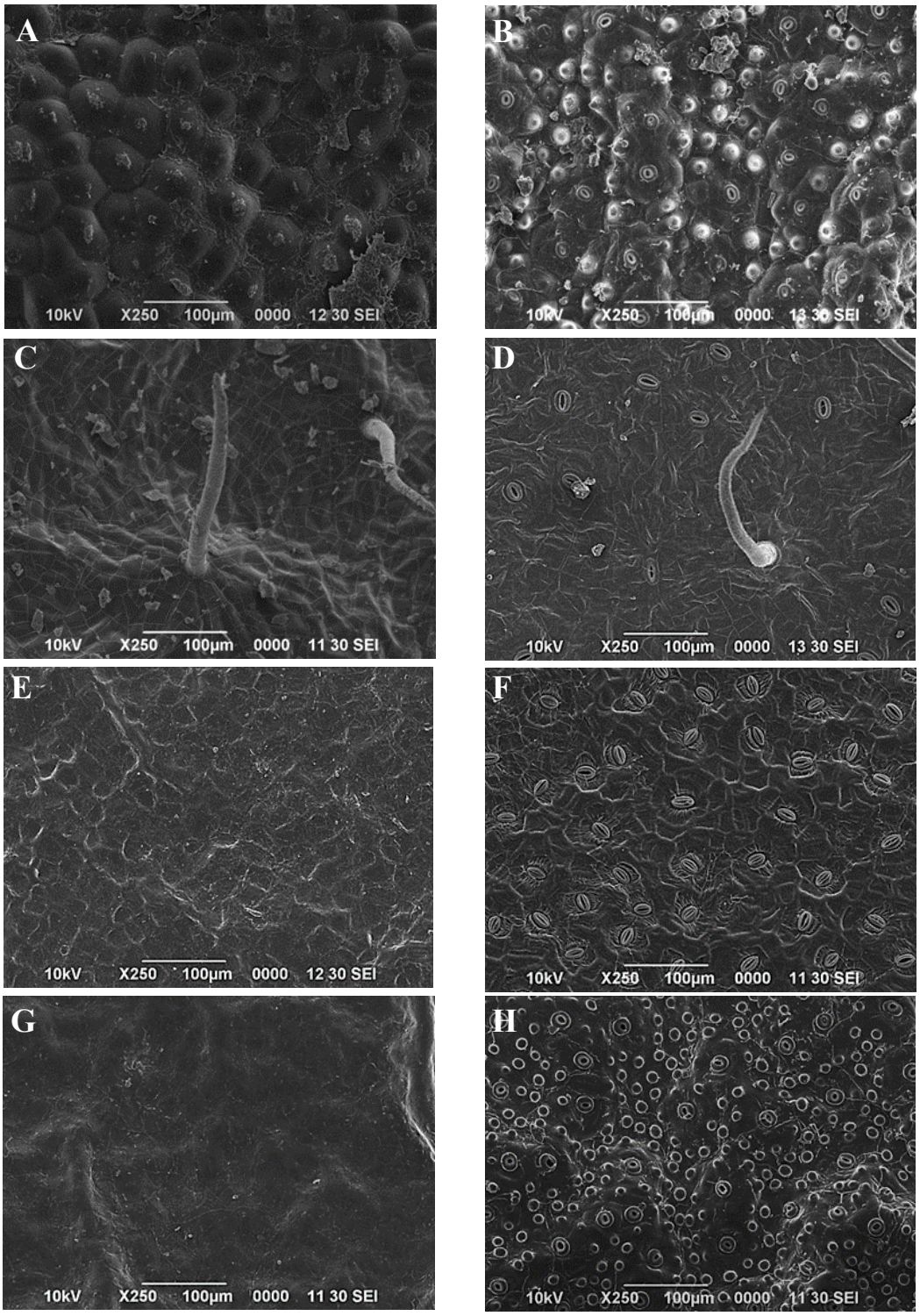


Fig. 3. Comparison leaf epidermis of four *Hoya* species by using Scanning Electron Microscope (SEM). A-B. The adaxial and abaxial surface with raised of stomata of *H. caudate*. C. The adaxial surface with unicellular trichome of *H. coronaria.* D. Stomata and trichomes of *H. coronaria.* E-F. Adaxial surface and same level of stomata of *H. omlorii*. G-H. The adaxial surface and raised stomata and abundance papillae of *H. verticillata.*

ed that species with hypostomatic leaves adapt this strategy to allow efficient gases exchange while minimize the water loss since the limited stomatal occur at the lower part of leaves which can caused by the convection movements or current of air that could remove water vapour from the leaves surfaces. According to Mott *et al. (*1982), the greater number of stomata in amphistomatic leaves, resulting a greater capacity to absorb carbonic acid in order to achieve a high rate of photosynthesis. Generally, plants with amphistomatic leaves are correlated with habitats without water stress and considered as sun shade plants (Rao & Rammaya, 1981). A stephanocytic type were clearly observed in *H. caudata* and *H. verticillata* where the stomata were surrounded by four or more weakly differentiated subsidiary cells forming a broader, less clear ring and less elongated (Baranova, 1987). Variations of stomata within the Apocynaceae family have also been reported by Salas *et al.* (2018) recently who stated that actinocytic stomata in *H. incrassata* and cyclocytic stomata in *H. soligamiana*. Nisa *et al.* (2019) advocated this statement with an investigation of the stomatal multiplicity in the family Apocynaceae, revealing a different stomatal types such as paracytic, anisocytic, tetracytic, actinocytic, anomocytic, laterocytic, stephanocytic, pseudoholoparacytic, and brachyparahexacytic.

**Stomatal density & stomatal index**

The density of stomata on plant surfaces can vary depending on factors such as species, environmental conditions, and plant adaptation to specific habitats. As such, plants in arid environments may have fewer stomata or specialized adaptations to minimize water loss, while plants in humid environments may have more stomata to maximize gas exchange. The higher the size of the stomata, the lower stomatal density, vice versa, the lower the size of stomata, was resulting the higher stomatal density on the species. However, stomatal density can influence a plant's ability to regulate gas exchange and water loss (Chaves *et al.,* 2016). According to Miskin *et al*. (1972), plants with a high stomatal density will have a higher transpiration rate than plants with low stomatal density which closely associated with photosynthetic and growth characteristics in plants. Wilmer (1983) reported that, stomatal density to be said has correlation and also effected by the size of the stomata. As reported by Mulyani (2006) the lower stomatal density, with high number of epidermal cells, will result in a low value of stomata index. Contradictly, higher stomatal density, with low number of epidermal cells, will affect a higher value of stomatal index. The differences in the stomatal index were doubt could be related to physiological reactions to various environmental conditions (Adegbite, 2008; Aworinde *et al*., 2012). The distribution and frequency of stomata were considerable as taxonomic significance, though sometimes may be connected with the ecology of the species (Stace, 1965).

**Trichomes**

Ghahreman *et al.* (1999) mentioned that this characteristic is also believed to be a useful indicator to differentiate between the genus. Furthermore, Gabr *et al.* (2015) investigated species within Apocynaceae family showed 15 different types of trichomes variations with glandular hairs and non-glandular hairs. Densely trichomes characters are always associated with protection and adaptation to high light intensity (Ichie *et al.*, 2016).

**Papillae**

Papillae are small projections found on the external walls of epidermal cells (Moraes *et al.*, 2011). According to Judd *et al.* (2008), the occurrence of papillae is an important systematic character. Although many theories have been proposed, Patterson (1964) and Proctor (1981) conclude that the function of papillae remains unclear, ranging from light and temperature control for adaptation to xerophytic conditions.

## CONCLUSION

The study highlighted that *Hoya* can be differentiated despite of several shared characteristics. The micro-morphology studies of *Hoya* species leaf surfaces proved useful for the Apocynaceae family's systematics, providing important distinguishing features such as the different types of epidermal cells, differences in stomatal type, the stomatal density and index, the presence of trichrome and the presence of papillae. The different features of leaves anatomy studies of *Hoya* species act as complement for existing morphological data, supplementary information that should be utilised and implemented for species delimitation in the future.

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# COMPARATIVE LEAF ANATOMY AND MICROMORPHOLOGY OF *ASYSTASIA GANGETICA* T.ANDERSON SUBSP. *MICRANTHA* (NEES) ENSERMU AND *RHINACANTHUS NASUTUS* (L.) KURZ (JUSTICIINAE, ACANTHACEAE) FROM PENINSULAR MALAYSIA

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**ABSTRACT**
AMRI, C. N. A. C., ZAKARIA, S. M., SHAHARI, R., TAJUDIN, A. A. B. M., TALIP, N., RAHMAN, M. R. A. & SIAM, N. A. 2023. Comparative leaf anatomy and micromorphology of *Asystasia gangetica* T.Anderson subsp. *micrantha* (Nees) Ensermu and *Rhinacanthus nasutus* (L.) Kurz (Acanthaceae) from Peninsular Malaysia. *Reinwardtia* 22(2): 79–89. — Acanthaceae family has been used traditionally for medicinal purposes, especially amongst the native communities in Peninsular Malaysia. Nowadays, many taxonomists have difficulties in the identification of the Acanthaceae species due to its morphological similarities and when there is an incomplete part of plants obtained from the field sampling. But until now, there is no comprehensive study that has been documented especially on the Acanthaceae family, specifically for *A. gangetica* subsp. *micrantha* and *R. nasutus*. To avoid incorrect species identification, a systematic study that involved the leaf anatomy and micromorphology parts is being used for the identification and classification of plants in the Acanthaceae. Therefore, the main objective of this present study is to identify the leaf anatomical and micromorphological characteristics that can be used in plant identification and for supportive data in plant classification. The leaf anatomical and micromorphological studies that are conducted on species studied involve several procedures such as cross-section using a sliding microtome, and observation under a light microscope and scanning electron microscope. The anatomical and micromorphological characteristics observed that have been used to identify each species studied include patterns of petiole and midrib vascular bundles, leaf margin, leaf lamina, presence of cuticular striae, and the presence of trichomes. The results of this study showed that the cystolith cells can be found only in midrib of *A. gangetica* subsp. *micrantha* while it also recorded in petiole, midrib, and the leaf lamina of *R. nasutus*. Observation under the light microscope revealed nine types of trichomes in *R. nasutus* meanwhile seven trichomes were recorded in *A. gangetica* subsp. *micrantha.* Other than that, the present of cuticular striae only recorded at the abaxial epidermis of *A. gangetica* subsp. *micrantha.* In conclusion, results showed that anatomical and micromorphological characteristics have taxonomic significance that can be used in the identification and classification, especially at the species level.

**Key words**: Acanthaceae, *Asystasia gangetica* subsp. *micrantha*, cystolith, *Rhinacanthus nasutus*.

**ABSTRAK**

AMRI, C. N. A. C., ZAKARIA, S. M., SHAHARI, R., TAJUDIN, A. A. B. M., TALIP, N., RAHMAN, M. R. A. & SIAM, N. A. 2023. Perbandingan anatomi dan mikromorfologi daun *Asystasia gangetica* T.Anderson subsp. *micrantha* (Nees) Ensermu dan *Rhinacanthus nasutus* (L.) Kurz (Acanthaceae) dari Semenanjung Malaysia. *Reinwardtia* 22 (2): 79–89. — Suku Acanthaceae telah digunakan dalam pengobatan tradisional, terutama bagi masyarakat asli di Semenanjung Malaysia. Sampai dengan saat ini, ahli taksonomi mengalami kesulitan terutama dalam determinasi jenis suku Acanthaceae karena ciri morfologi yang hampir sama terutama untuk spesimen yang tidak lengkap saat pengambilan sampel di lapangan. Namun hingga saat ini, kajian anatomi daun yang komprehensif khususnya *A. gangetica* subsp. *micrantha* dan *R. nasutus* masih sangat terbatas. Dalam rangka menghindari kesalahan dalam determinasi jenis, kajian sistematik khususnya anatomi dan mikromorfologi daun telah digunakan sebagai data tambahan bagi determinasi dan pengelompokkan jenis-jenis Acanthaceae. Kajian ciri anatomi menggunakan metode dengan irisan mikrotom geser (ciri anatomi tangkai, helaian, tulang dan tepi daun), sedangkan kajian ciri mikromorfologi menggunakan metode dengan melihat secara langsung permukaan epidermis abaksial dan adaksial daun di bawah mikroskop pemindaian elektron. Ciri anatomi dan mikromorfologi yang telah direkam adalah ciri berkas pengangkut pada tangkai dan tulang daun, tepi dan helaian daun, keberadaan kutikula serta keberadaan trikom pada setiap jenis yang diteliti. Hasil penelitian menunjukkan keberadaan sel sistolit hanya pada tulang tengah *A. gangetica* subsp. *micrantha*, tangkai, tulang, dan helaian daun *R. nasutus*. Hasil pengamatan di bawah mikroskop menunjukkan sembilan jenis trikom pada *R. nasutus* dan tujuh jenis trikom pada *A. gangetica* subsp. *micrantha*. Selain itu juga, kehadiran kutikel hanya dijumpai pada permukaan epidermis abaksial *A. gangetica* subsp. *micrantha.* Hasil penelitian menunjukkan bahwa ciri anatomi dan mikromorfologi daun dapat digunakan sebagai data tambahan khususnya untuk determinasi jenis yang diteliti.

**Kata kunci**: Acanthaceae, *Asystasia gangetica* subsp. *micrantha, Rhinacanthus nasutus,* sistolit.

## INTRODUCTION

The Acanthaceae family, which is known as a large pan-tropical family is mainly herbaceous and shrubs. However, some of the members are climbers or liana, whilst a few species are woody plants (Metcalfe & Chalk, 1965; Scotland *et al.,* 1995). Acanthaceae family belongs to the order Lamiales consists of at least 3000 species in some 250 genera with the centre of distribution in Indo-Malaysia, Africa (including Madagascar), Northern South America, Central America, and Mexico; with thirty-five genera are native or naturalized in Peninsular Malaysia (Keng, 2003).

Mc Dade *et al.* (2008) divided Acanthaceae into four families: Acanthoideae, Andrographideae, Nelsonioideae, and Thunbergioideae. Whislt Vollesen (2008) later elevated the tribe Ruellieae to the subfamily Ruellioideae. New findings by Schwarzbach & Mc Dade (2008) and Borg (2008) have suggested that the genus *Avicennia* has a sister relationship with Acanthaceae through the floral characteristics that have been shared between *Avicennia* and Thunbergioideae. Stevens (2017) and Manzitto-Tripp *et al.* (2021) recognized the updated classification and the placement of *Avicennia* into Acanthaceae as in Angiosperm Phylogeny Website (APweb) by which the classification of Acanthaceae is divided into four subfamilies (Nelsonioideae, Acanthoideae, Thunbergioidae, and Avicennioideae).

*Asystasia gangetica* subsp. *micrantha* is recognized as weed and locally known as Chinese violet or rumput Israel in Malaysia. This plant species is native in Africa, India, and Sri Lanka (Hsu *et al.,* 2005). *Asystasia*, define as inconsistency that refers to the characteristics of corolla which is remarkable trait of uncommon characters for Acanthaceae family. Meanwhile, the origin of the word "gangetica" is from the Ganges River. This species also has been used as cover plant in orchards because it can prevent the growth of noxious weed and soil erosion (Gopal *et al*., 2013). This weed plant also are very useful in ethnobotanical study which can treat rheumatism, stomach pain, heart problems, and asthma (Hamid *et al*., 2011).

*Rhinacanthus nasutus*, commonly known as snake jasmine, *pokok kepala sari* or *ubat kurap*. This plant is native to India and widely distributed in Malaysia, Laos, Indonesia, Thailand, Myanmar, and Vietnam. It is subshrubs or perennial herbs that grow up to 1.5 m tall. The capsule is pubescent with gland-tipped trichomes, and the seeds are papillose (Chia-chi *et al.,* 2011). Munavvar *et al.* (2004) mentioned that the leaf of *R. nasutus* is commonly used in traditional medicine preparations to treat various skin problems. For instance, the leaves of *R. nasutus* are pounded with benzoin and sulphur and applied externally to the area infected by ringworm. In Thailand, *R. nasutus* is traditionally used to treat various cancers such as colon (Kupradinum *et al.,* 2009), and cervical and liver cancers (Rojanapo *et al*., 1990).

Besides, the Acanthaceae is also recognized with the occurrence of cystoliths that are visible with the magnifying lenses as rod-shaped, especially in the epidermis surfaces of the leaves. Hence, it is very significant to have an additional

tool to provide information about the inner part of the plants. Previous study by Maisarah *et al.* (2020) reported four main types of cystoliths cells based upon 41 taxa studied in Acanthaceae. Four types of cystoliths identified as Type 1 (solitary cystolith with rounded-shaped), Type 2 (solitary cystolith with blunt-end), Type 3 (solitary cystolith elongated with one end pointed), and Type 4 (solitary cystolith elongated with end pointed both). It was also observed that the absence of cystoliths occurred in 13 taxa out of 41 taxa in Acanthaceae from genera of *Acanthus*, *Avicennia*, S*taurogyne*, and *Thunbergia*, thus recognized the significance of cystoliths in the identification and classification of Acanthaceae species.

This present study thereby involved the investigation of the leaf anatomy and micromorphological characteristics of *A. gangetica* subsp. *micrantha* and *R. nasutus* in Peninsular Malaysia that possessed taxonomic significance and can be used as additional data to avoid misidentification of the species.

## MATERIALS AND METHODS

Fresh leaf samples of *A. gangetica* subsp. *micrantha* and *R. nasutus* were collected at several open areas such as in Nilai, Negeri Sembilan, Selangor, and Perak. Five replicates were used throughout this research. Voucher specimens were deposited at Universiti Kebangsaan Malaysia Herbarium (UKMB). Fresh specimens were fixed in 3:1 AA Solutions (70% Alcohol: 30% Acetic Acid). In this study, four parts were observed including petiole, midrib, lamina, and marginal parts. These parts were sliced by using a sliding microtome composed of a fixed sample holder known as polystyrene. The slicing was made by using a disposable knife (Leica 818) at a range of thickness of 10–40 µm. The cut parts were immersed into a petri dish filled with bleach 'Clorox' solution for 10 minutes until the original pigment at the specimen vanished. Next, the sliced specimens were washed three times with distilled water to remove any residue left. The staining process involved two stages of staining solution which are alcian blue and safranin. Then, the specimens obtained from the sliding microtome were undergone a dehydration process in a series of alcohol and mounted in Euparal. All slides were covered with coverslips and kept in the oven for two weeks at about 60°C for drying purposes. Anatomical images were captured using a video (3CCD) camera attached to a Leitz Diaplan microscope using Cell^B software. Suitable modifications in terms of fixation and embedding followed the method by Johansen (1940) and Saas (1958). For the micromorphology study, the scanning electron microscopy (SEM) method was applied. The specimens were taken from a dried sample of the herbarium in which 1 $cm^2$ lamina portions of leaf sample were cut and mounted on a mounting holder. The specimens were then coated with gold by using a sputter-coated machine. The observation of micromorphological characteristics was done under Scanning Electron Microscope Zeiss Model Evo 50.

## RESULTS

Figure 1 shows the characteristics of leaf anatomy and micromorphology of *A. gangetica* subsp. *micrantha.* The descriptions of the leaf anatomical and micromorphological characteristics for *A. gangetica* subsp. *micrantha* are summarized as below:

**Petiole.** Adaxial outline: concave with V-wide shape and two ear-like at the left and right side of the petiole outline. Abaxial outline: ¾ round shape. Vascular tissue: main vascular tissue (opened system with non-continuous ring of vascular bundle) with four additional vascular bundles are situated at the above left and right side of the main vascular bundle. Parenchyma cells: *ca.* 7 –9 layers of parenchyma cells. Sclerenchyma cells: clusters of sclerenchyma cells present at the vascular bundles. Collenchyma cells: 4–9 layers present under the epidermis of abaxial and adaxial surfaces. Mucilage cell: Present at the parenchyma cortex. Trichomes: peltate glandular trichomes (terminal unicellular), simple unicellular trichomes (short, pointed end), multicellular trichomes (long, pointed end) and multicellular trichomes (long, tapered end) present at the epidermis of abaxial and adaxial surfaces (Fig. 1A).

**Midrib**. Adaxial outline: slightly concave hump with ½ inverted rectangle shape. Abaxial outline: ½ round shape. Vascular tissue: main vascular tissue (opened system with non-continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle. Sclerenchyma cells: clusters of sclerenchyma cells present at the vascular bundles. Collenchyma cells: 2–4 layers present under the epidermis of abaxial and adaxial surfaces. Mucilage cell: Present at the parenchyma cortex. Cystolith cells: rounded, solitary cystolith cells present at the epidermis of abaxial and adaxial surface. Trichomes: peltate glandular trichome (terminal multicellular), simple unicellular trichomes (short, pointed end), multicellular trichome (short, pointed end) and multicellular trichome (short, tapered end), present at the epidermis of abaxial and adaxial surfaces (Fig. 1B).

**Leaf Margin.** Outline: slightly tapered, 40º recurved downwards to the abaxial side (Fig. 1C).

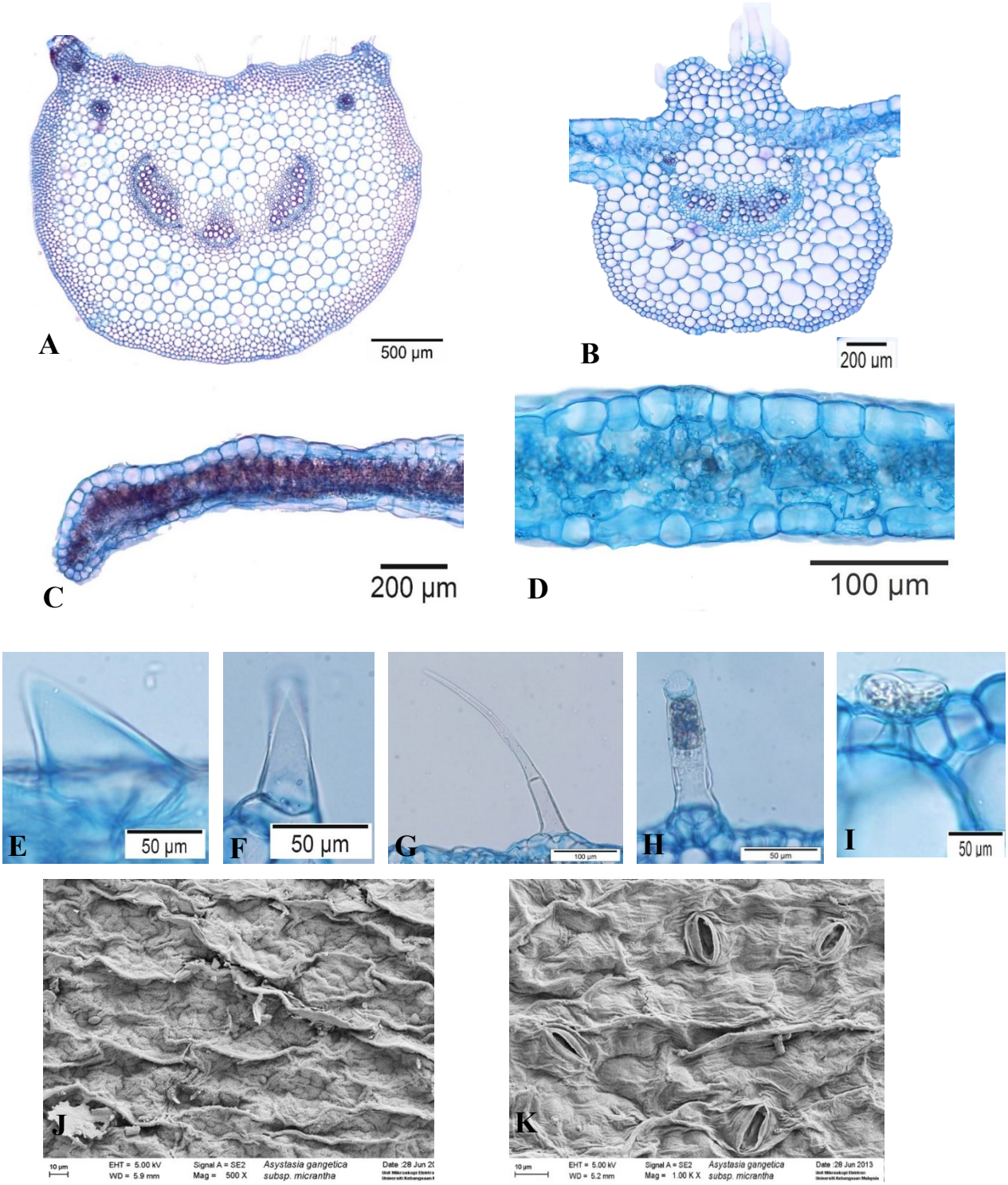


Fig. 1. *Asystasia gangetica* subsp. *micrantha*: A. Petiole cross-section. B. Midrib cross-section. C-D. Lamina and margin cross-section. E. Simple unicellular trichome (short, pointed end). F. Simple multicellular trichome (short, tapered end). G. Simple multicellular trichome (long, pointed end). H. Simple multicellular trichome (short, tapered end). I. Peltate glandular trichome. J. Adaxial surface. K. Abaxial surface. Scale: J & K 10 µm.

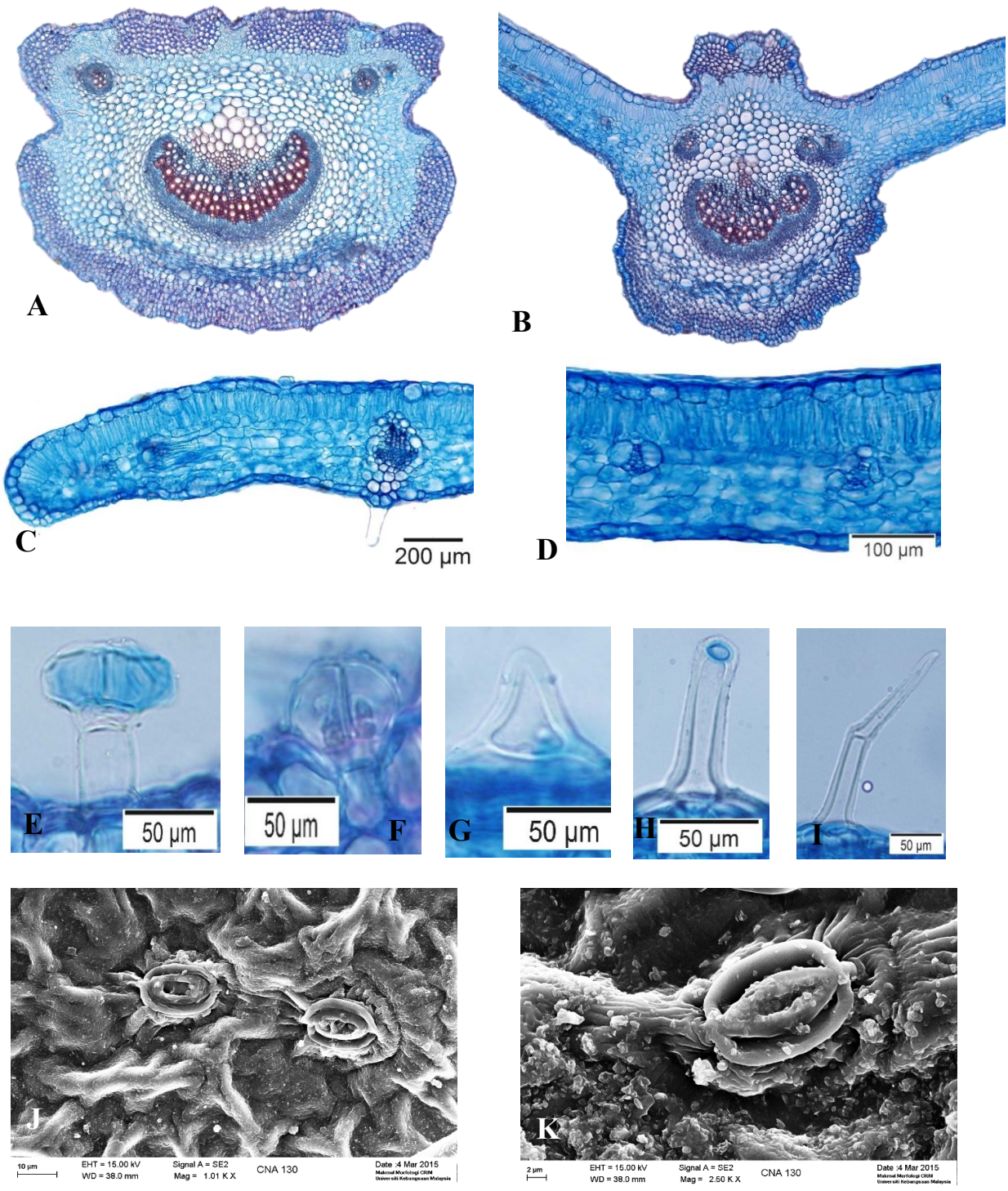


Fig. 2. *Rhinacanthus nasutus*. A. Petiole cross-section. B. Midrib cross-section. C-D. Lamina and margin cross-section. E. Capitate glandular trichome. F. Peltate glandular trichome. G. Simple multicellular trichome (short, tapered end). H. Simple multicellular trichome (long, tapered end). I. Simple multicellular trichome (long, pointed end). J. Adaxial surface. K. Abaxial surface. Scale: J. 10 µm. K. 2 µm.

**Leaf Lamina.** Cuticular layer: relatively thin. Adaxial and abaxial epidermis: single layer with height: width ratio – 1:1 and 1:2. Chlorenchyma cells: mesophyll palisade: two layers filling ½ part of the height of leaf lamina. Spongy mesophyll: 2–3 layers of spongy mesophyll. Vascular bundles: simple vascular bundles. Parenchyma cells: single layer encircling each vascular bundle. Trichome: peltate glandular trichome (terminal multicellular), simple unicellular trichomes (short, pointed end), multicellular trichome (short, pointed end) and multicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces (Fig. 1D).

**Leaf Epidermis.** Epicuticular waxes: films, granules, flakes, and crustose present at the epidermis of adaxial surface, verrucate and buttress-like wax present at the epidermis of abaxial surface. Cuticular striation: anticlinal and periclinal wall can not be differentiated on both sides, cuticular striae present at the epidermis of abaxial. Stomata: diacytic, superficial, elliptic shape. Stomata size range: H × W (15.79–18.95 μm) × (10.98–13.07 μm). Trichome: Peltate glandular trichome (terminal multicellular) present at the adaxial surface only while peltate glandular trichome (terminal unicellular) and multicellular trichomes (long, pointed end, echinate ornamentation) present at both sides (Figs. 1J & 1K) .

Figure 2 shows the characteristics of leaf anatomy and micromorphology of *R. nasutus*. The descriptions of the leaf anatomical and micromorphological characteristics for *R. nasutus* are summarized as below:

**Petiole.** Adaxial outline: straight with two ear-like at the left and right side of the petiole outline. Abaxial outline: ¾ oblong shape. Vascular tissue: main vascular tissue (opened system with continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle. Parenchyma cells: *ca.* 9–12 layers of parenchyma cells. Sclerenchyma cells: clusters of sclerenchyma cells present at the vascular bundles. Collenchyma cells: 3–10 layers present under the epidermis of abaxial and adaxial surfaces. Mucilage cell: Present at the parenchyma cortex. Cystolith cells: rounded, solitary cystolith cells present at the epidermis of abaxial and adaxial surface. Trichomes: capitate glandular trichomes (terminal multicellular, rounded), peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end), and unicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces (Fig. 2A).

**Midrib.** Adaxial outline: convex with slightly hump. Abaxial outline: U shaped. Vascular tissue: main vascular tissue (opened system with continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle. Sclerenchyma cells: clusters of sclerenchyma cells present at the vascular bundles. Collenchyma cells: 3–10 layers present under the epidermis of abaxial and adaxial surfaces. Mucilage cell: Present at the parenchyma cortex. Cystolith cells: rounded, solitary cystolith cells present at the epidermis of abaxial and adaxial surface. Trichomes: capitate glandular trichomes with multicellular stalk, peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end), unicellular trichomes (long, tapered end), and unicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces (Fig. 2B).

**Leaf Margin.** Outline: slightly tapered, 30–45º recurved downwards to the abaxial side (Fig. 2C).

**Leaf Lamina.** Cuticular layer: relatively thin. Adaxial and abaxial epidermis: single layer with height: width ratio – 1:1 and 1:2. Chlorenchyma cells: mesophyll palisade: two layers filling ½ part of the height of leaf lamina. Spongy mesophyll: 5–6 layers of spongy mesophyll. Vascular bundles: simple vascular bundles. Parenchyma cells: single layer encircling each vascular bundle. Cystolith cells: rounded, solitary cystolith cells present at the epidermis of adaxial surface. Trichome: peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (long, tapered end), unicellular trichomes (long, pointed end), and multicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces (Fig. 2D).

**Leaf Epidermis.** Epicuticular waxes: granules, crustose present at the epidermis of adaxial surface, verrucate and granules present at the epidermis of abaxial surface. Cuticular striation: anticlinal and periclinal wall can be differentiated directly on both sides. Stomata: diacytic, superficial, elliptic shape. Stomata size range: H × W (16.55–20.51 μm) × (12.12–15.75 μm). Trichome: Peltate glandular trichome present at both sides while multicellular trichomes (long, pointed end, echinate ornamentation) present at the abaxial surface only (Figs. 2J & 2K).

## DISCUSSION

The result of this study showed the significance of leaf anatomical and micromorphological characteristics that can be useful in identification of species studied. The results of this study showed that the cystolith cells can be found only in midrib of *A. gangetica* subsp. *micrantha* while it also recorded in petiole, midrib, and the leaf lamina of *R. na-*

Table 1. Summary of leaf anatomical and micromorphological variation of species studied

| Anatomical characteristics | *Asystasia gangetica* subsp. *micrantha* | *Rhinacanthus nasutus* |
|---|---|---|
| Petiole | Adaxial outline: concave with V-wide shape and two ear-like at the left and right side of the petiole outline; abaxial outline: ¾ round shaped. | Straight with two ear-like at the left and right side of the petiole outline; abaxial outline: ¾ oblong shaped. |
| | Main vascular tissue (opened system with non-continuous ring of vascular bundle) with four additional vascular bundles are situated at the above left and right side of the main vascular bundle. | Main vascular tissue (opened system with continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle. |
| | Peltate glandular trichomes (terminal unicellular), simple unicellular trichomes (short, pointed end), multicellular trichomes (long, pointed end) and multicellular trichomes (long, tapered end) present at the epidermis of abaxial and adaxial surfaces. | Capitate glandular trichomes (terminal multicellular, rounded), peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end) and unicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces |
| Midrib | Adaxial outline: slightly concave hump with ½ inverted rectangle shape; abaxial outline: ½ round shape. | Adaxial outline: convex with slightly hump; abaxial outline: u shaped. |
| | Main vascular tissue (opened system with non-continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle. | Main vascular tissue (opened system with continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle. |
| | Peltate glandular trichome, simple unicellular trichomes (short, pointed end), multicellular trichome (short, pointed end) and multicellular trichome (short, tapered end) | Capitate glandular trichomes with multicellular stalk, peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end), unicellular trichomes (long, tapered end), and unicellular trichomes (long, pointed end) |
| Leaf margin | Slightly tapered, 40º recurved downwards to the abaxial side | Slightly tapered, 30–45º recurved downwards to the abaxial side |
| Leaf lamina | Spongy mesophyll: 2–3 layers of spongy mesophyll.<br>Peltate glandular trichome, simple unicellular trichomes (short, pointed end), multicellular trichome (short, pointed end) and multicellular trichomes (long, pointed end) | Spongy mesophyll: 5–6 layers of spongy mesophyll.<br>Peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (long, tapered end), unicellular trichomes (long, pointed end), and multicellular trichomes (long, pointed end) |
| Leaf epidermis | Epicuticular waxes: films, granules, flakes, and crustose present at the epidermis of adaxial surface, verrucate and buttress-like wax present at the epidermis of abaxial surface. | Epicuticular waxes: granules, crustose present at the epidermis of adaxial surface, verrucate and granules present at the epidermis of abaxial surface. |
| | Anticlinal and periclinal wall cannot be differentiated on both sides, cuticular striae present at the epidermis of abaxial. | Anticlinal and periclinal wall can be differentiated directly on both sides. |
| | Peltate glandular trichome present at the adaxial surface only while peltate glandular trichome (terminal unicellular) and multicellular trichomes (long, pointed end, echinate ornamentation) present at both sides | Peltate glandular trichome present at both sides while multicellular trichomes (long, pointed end, echinate ornamentation) present at the abaxial surface only |

*sutus*. The occurance of cystolith cells in all species studied supported previous research by Metcalfe & Chalk (1965), Nurul-Aini *et al.* (2018), and Maisarah *et al.* (2020) which recorded the presence of cystoliths cells in Acanthaceae species. The type of cystoliths also varied even within the same species either in petiole, midrib, or lamina. The previous research that has been done by Metcalfe & Chalk (1965) stated that the presence of cystolith cells is known as one of the important characteristics that can be used to identify and classify certain plant families such as Acanthaceae, Moraceae, Urticaceae, and Boraginaceae.

Hare (1942) reported that the vascular tissue system has significant value in plant taxonomy. Previous studies on three species of the genus *Shorea* by Rojo (1987) support the research findings by Hare (1942) by which the pattern of vascular tissue on the petiole part can be used to distinguish the three species of *Shorea.* Furthermore, a study by Ruzi *et al.* (2009) on the genus *Dipterocarpus* (family Dipterocarpaceae) showed the arrangement of vascular tissue has taxonomic value, especially in the classification of species at the genus level. Additionally, Nurul-Aini *et al.* (2013) also reported on the presence of high variation of vascular tissue patterns in the genus *Microcos* that can be used in species classification. Apart, the results of this study also showed that the type of vascular bundles in the midrib and petiole can be used to identify species studied.

O'Neill (2010) stated that the thickness of collenchyma cells makes the stems to be strong for protection against the wind. Nurul-Aini *et al.* (2018) recognized the presence of collenchyma cells in the petiole and midrib of several Acanthaceae species such as *Acanthus ebracteatus*, *Andrographis paniculata*, and *Chroesthes longifolia* which is very useful, especially in the identification of Acanthaceae family. Three to ten layers of collenchyma cells have been recorded in *R. nasutus*, meanwhile four to nine layers of collenchyma cells occurred in *A. gangetica* subsp. *micrantha.* Thus, supporting the previous research that has been done by Verdam *et al.* (2012) and Nurul-Aini *et al.* (2018). According to Leroux (2012), collenchyma cells are highly dynamic compared with sclerenchyma cells, whereby the collenchyma cells become more rigid due to changes in cell wall deposition or may undergo sclerification through lignification of newly deposited cell wall materials.

Furthermore, the results also showed that the mucilage cells were present either in the petiole, lamina or even in the midrib of all species studied. A study on the Shoreae tribe by Noraini (2006) reported the presence of mucilage cells or canals as a common feature of the tribe. Previously, Bass & Gregory (1985) explained that the presence of mucilage cells is a diagnostic characteristic of some plant species. Metcalfe & Chalk (1950) also recognized the presence of mucilage and oil cells in Lauraceae, thereby this feature giving additional data to characterize *R. nasutus*. Noraini *et al.* (2005) mentioned that even though the leaf margin is rarely used in the systematic study of plants, the results on leaf anatomy of the genus *Alpinia* from the family Zingiberaceae showed that the leaf margin has taxonomic value in the identification and classification of species. Results of the leaf lamina showed that *R. nasutus* have slightly tapered, 30–45º recurved downwards to the abaxial side. However, *A. gangetica* subsp. *micrantha* have slightly tapered with 40º recurved downwards to the abaxial side.

Inamdar (1967) stated that the characteristics of trichomes can be used for species delimitation. Trichomes are the physical structure that is present in plants (Levin, 1929) and can be used as one of the characteristics to identify certain plant species (Metcalfe & Chalk, 1979). Observation under the light microscope revealed nine types of trichomes in *R. nasutus* (Figs. 2E–2I) which are capitate glandular trichomes with multicellular stalk, capitate glandular trichomes (terminal multicellular, rounded), peltate glandular trichome (terminal multicellular), simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end), unicellular trichomes (long, tapered end), unicellular trichomes (long, pointed end), multicellular trichomes (long, pointed end), and multicellular trichomes (long, pointed end, echinate ornamentation). Meanwhile seven trichomes were recorded in *A. gangetica* subsp. *micrantha* which are peltate glandular trichomes (terminal unicellular), peltate glandular trichomes (terminal multicellular), simple unicellular trichomes (short, pointed end), multicellular trichomes (long, pointed end), multicellular trichomes (long, tapered end), multicellular trichomes (short, pointed end), and multicellular trichomes (long, pointed end, echinate ornamentation) (Figs. 1E–1I). Zainab Sholehah *et al.* (2022) also mentioned the systematic significance based on the type of trichomes, especially to resolve the taxonomic conflicts of the species. Even previous research by Amirul-Aiman *et al.* (2014) agreed that the presence and types of trichomes have systematic value as in some petals of Acanthaceae species.

Besides, Barthlott (1990) recorded the presence of epicuticular wax in the cuticle layer which has significant variation as well as high taxonomic value in the angiosperm, gymnosperm, pteridophyte, and bryophyte groups. The results of a study by Nurhanim *et al.* (2014) showed that variation in wax types is significant and useful in the species identification and classification for *Schoutenia*. Results showed three types of wax present in *R. nasutus* which are granules, verrucate and crustose layers, meanwhile, in *A. gangetica* subsp. *micrantha*, types of wax such as films, granules, flakes, and crustose present at the epidermis of adaxial surface, verrucate, and buttress-like wax

present at the epidermis of abaxial surface. Thus, giving good criteria to recognize each species studied. A previous study conducted by Patil & Patil (2011) on 22 species of the family Acanthaceae reported the presence of hypostoma-tic and diacytic stomata. Besides, preliminary studies by Metcalfe & Chalk (1965) also recorded the presence of diacytic type stomata either on both surfaces of the epidermis (amphistomatic) or on the apical epidermal layer only (hypostomatic), thereby giving evidence of the presence of amphistomatic and diacytic type of stomata in all species studied. Barthlott (1981) mentioned that morphological characteristics of the leaf surface, such as the presence or absence of cuticular ornamentations, can be used to classify genus and species level. Result of this study showed the present of cuticular striae only at the abaxial epidermis of *A. gangetica* subsp. *micrantha*. Therefore, the results of this study support previous studies where the characteristics of stomata have significant value in the classification of species studied.

The dichotomous key that has been constructed from the present study thus gives evidence on the significance of leaf anatomy and micromorphology characteristics as supportive data in the identification and classification of species studied.

1 Peltate glandular trichome (terminal unicellular) and (terminal multicellular); absence of capitate glandular trichomes; presence of cuticular striae at the abaxial epidermis; adaxial outline: midrib slightly concave hump with ½ inverted rectangle shape; abaxial outline: ½ round shape; verrucate and buttress-like wax present at the epidermis of abaxial surface……. *A. gangetica* subsp. *micrantha*

1 Peltate glandular trichome (terminal multicellular); presence of capitate glandular trichomes with multicellular stalk, capitate glandular trichomes (terminal multicellular, rounded); absence of cuticular striae at the abaxial epidermis; midrib adaxial outline: convex with slightly hump; abaxial outline: U shaped; verrucate and granules present at the epidermis of abaxial surface……..… *R. nasutus*

## CONCLUSION

Given that *A. gangetica* subsp. *micrantha* and *R. nasutus* is well known as a medicinal plant, systematic evidence obtained from the study of leaf anatomy and micromorphology characteristics is useful in providing significant distinctive characters for plant identification and classification. The results of this present study reported potential important leaf anatomy and micromorphological characteristics of species studied. Thus, the findings of this study suggest that the characteristics such as patterns of petiole and midrib vascular bundles, the presence of cuticular striae, type of stomata, types of trichomes and types of epicuticular waxes can be used to identify each species studied. As such, the anatomical and micromorphological tools are proven to be important tools in the systematic study.

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# PRELIMINARY STUDY OF THE POLLEN MORPHOLOGY OF MALAYSIAN ZINGIBERACEAE (TRIBE ALPINIEAE) AND THE TAXONOMIC RELATIONSHIP

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**ABSTRACT**
MOHAMAD, S., MEEKIONG, K & SEDEK, A. S. 2023. Preliminary study of the pollen morphology of Malaysian Zingiberaceae (tribe Alpinieae) and the taxonomic relationship. *Reinwardtia* 22(2): 91–102. — The family Zingiberaceae forms an important herbaceous layer in the tropical forests of Malaysia. From a phylogenetic point of view, certain genera of the problematic tribe Alpinieae are non-monophyletic and in need of more taxonomic evidence to support the classification. This study demonstrates how the palynological data could correlate with the proposed phylogenetic data, using representatives from the Malaysian species. The pollen morphology of 21 species from the tribe Alpinieae was investigated. Parameters including polarity, symmetry, shape, size, apertures, exine ornamentation, size of spine, type of spine apex, spine density, and distance between spine were analysed. The results demonstrated that the studied species were conveniently divided into two major groups based on the exine sculpturing of the spheroidal pollens, either psilate as in *Etlingera* and *Hornstedtia*, or echinate as in *Alpinia, Conamomum, Meistera, Plagiostachys, Sundamomum*, and *Sulettaria.* Hence, as far as the study is concerned, the main sculpturing is considered useful to generally distinguish the genera in the tribe.

**Key words**: Diversity, Johor, systematics, Sarawak, taxonomy, wild gingers.

**ABSTRAK**
MOHAMAD, S., MEEKIONG, K & SEDEK, A. S. 2023. Penelitian awal morfologi serbuk sari Zingiberaceae Malaysia (puak Alpinieae) dan hubungan taksonominya. *Reinwardtia* 22(2): 91–102. — Suku Zingiberaceae membentuk lapisan herba penting di hutan tropis Malaysia. Dari sudut pandang filogenetik, marga tertentu dari puak Alpinieae yang bermasalah adalah non-monofiletik dan membutuhkan lebih banyak bukti taksonomi untuk mendukung klasifikasi. Studi ini menunjukkan data palinologi dapat berkorelasi dengan data filogenetik yang diusulkan, menggunakan perwakilan dari jenis Malaysia. Morfologi polen dari 21 jenis dari puak Alpinieae telah diamati. Parameter yang dianalisis termasuk polaritas, simetri, bentuk, ukuran, bukaan, perhiasan eksin, ukuran duri, tipe ujung duri, kerapatan duri, dan jarak antar duri. Hasilnya menunjukkan bahwa jenis yang diteliti dengan mudah dibagi menjadi dua kelompok besar berdasarkan perhiasan eksin serbuk sari yang membulat, baik *psilate* seperti pada *Etlingera* dan *Hornstedtia*, atau *echinate* seperti pada marga *Alpinia, Canamomum, Meistera, Plagiostachys, Sundamomum*, dan *Sulettaria*. Oleh karena itu, perhiasan utama dianggap berguna untuk membedakan marga dalam puak secara umum.

**Kata kunci**: Jahe liar, Johor, keragaman, Sarawak, sistematika, taksonomi.

## INTRODUCTION

Zingiberaceae is the largest family among the eight families in the monophyletic order, Zingiberales and has at least 60 genera and 1,900 species (POWO, 2022; Banaticla-Hilario & Altamirano, 2023). The species occur in the tropics and subtropics, of which the Asian tropics hold the highest diversity and number of taxa (Lamb *et al.,* 2013; POWO, 2022). Most Zingiberaceae are evergreen plants that form the important herbaceous layer of the tropical rainforest, especially in forest gaps where light is not scarce. Within Zingiberaceae, diverse genera such as *Alpinia* and *Amomum* are often dominant in humid tropical lowlands, while *Etlingera, Hornstedtia,* and *Plagiostachys* can pre-

dominate in habitats that provide more sunlight, such as disturbed areas or secondary forests (Lamb *et al.*, 2013). In Malaysian Borneo, as botanical exploration progresses, more than 250 named taxa in Zingiberaceae have been reported and subfamily Alpinioideae encompassed the highest number of species (De Boer *et al.*, 2018; Mohamad & Meekiong, 2019). On top of that, several Alpinieae genera are highly distributed or endemic to Sarawak, in particular *Epiamomum* (all six species, 100%), *Sundamomum* (10 species out of 16.63%), and *Plagiostachys* (13 taxa out of 33.38%) (POWO, 2020; WCVP, 2020; Mohamad *et al.*, 2020; Mohamad & Meekiong, 2020). Of *Etlingera* in Borneo, 32 taxa out of the known 42 (80%) occur in Sarawak, 30 taxa in Sabah (70%), 25 taxa (65%) in Kalimantan, and 13 taxa (33%) in Brunei (Poulsen, 2006). It is difficult to compare the species richness of Borneo with that of other islands, as only a relatively small number of detailed studies have been carried out (Poulsen, 2006). By 1999, Peninsular Malaysia had documented a lower count of Zingiberaceae species, with a total of 160 species across 18 genera, as reported by Larsen *et al.* (1999). In 2001, Khaw conducted a revision of *Etlingera* in Peninsular Malaysia, which initially comprised 12 species (Khaw, 2001). Subsequently, in the same year, Lim's report added two more species to the list, totaling 14 species (Lim, 2001).

A new classification of Zingiberaceae was proposed by Kress *et al.* (2002) based on the DNA sequence data who recognised four subfamilies and six tribes, namely Siphonochiloideae (tribe Siphonochileae), Tamijioideae (tribe Tamijieae), Alpinioideae which comprises most of the former tribes Alpinieae (tribe Alpinieae and tribe Riedelieae), and Zingiberoideae which includes the former tribes Hedychieae and Globbeae (tribe Zingibereae and tribe Globbeae). The subfamily Alpinioideae is diagnosed by the plane of distichous leaves perpendicular to the rhizome and the absence or reduction of the two lateral staminodes. The taxonomic status of the tribe Alpinieae still needs further clarification, especially for the non-monophyletic genera, including *Alpinia* which consists of different clades (Kress *et al.*, 2005), *Plagiostachys* (nested deep within *Zerumbet* clade IV in *Alpinia*) (Kress *et al.*, 2005; Julius *et al.*, 2008), and *Hornstedtia* (two species are nested within the *Amomum* clade and *H. leonurus* is nested within the *Etlingera* clade) (Pedersen, 2004; De Boer *et al.*, 2018). In the current development, species that were formerly placed under *Elettaria* have been moved to a new genus, *Sulettaria* based on works by Poulsen *et al.* (2019). In the long-term, the species in these genera may have to be transferred to another genus, but for the time being, they are ideally classified based on morphological characters.

The pollen morphology in Zingiberaceae have been investigated in certain genera and several characteristics, including shape, size, symmetry, and polar aperture of the pollen grains, were discovered as beneficial in identifying the species of the particular genus (Larsen *et al.*, 1998; Saensouk *et al.*, 2009; Chen & Chia, 2011; Saensouk *et al.*, 2015) and for classification at the generic and sectional levels (Theilade *et al.*, 1993). In 1990, Mangaly & Nayar, who studied the South Indian Zingiberaceae, reported that the shape of pollen grains ranged from spheroidal, subspheroidal, ovoid, and ellipsoid, whilst the sculpturing of the exine can be echinate, regulate, scabrate, striate, psilate, or verrucate. In addition, they proposed that the thin layer of exine does not provide mechanical strength for the pollen grain, thus most of the taxa have low resistance to acetolysis treatment (Mangaly & Nayar, 1990). Previously, the pollen of Zingiberaceae was regarded as exineless and inapertu-rate (Dahlgren *et al.*, 1985), but this was proved untrue by Mangaly & Nayar (1990) who disco-vered that among the 21 taxa studied, an exine was only absent in *Kaempferia galanga.* All other taxa portrayed a distinct exine layer covering the pollen grain, as found in other genera in the Zingiberales order*: Heliconia* (Heliconiaceae), *Strelitzia* (Strelit-ziaceae) and *Tapeinochilos* (Costaceae). In contrast, the intine consisted of different layers that are well developed and lamellated (Mangaly & Nayar, 1990).

Of the tribe Alpinieae, Mangaly & Nayar (1990) recorded that the pollen shapes of the seven studied species were either spheroidal or subspheroidal, while the exine sculpture was spinulosed in most of the tribe, *viz*. *Alpinia calcarata, A. galanga, A. zerumbet, Amomum hypoleucum, A. pterocarpum*, and verrucate as in *Elettaria cardamomum*. In contrast, the pollen shapes of *Zingiber* (tribe Zingibereae) were either spheroidal with rugulate ornamentation or ellipsoidal and striate, which closely followed the subgeneric boundaries. Among the different taxa studied, the well developed exine only occurred in *Zingiber.* The presence or absence of ornamentation, however, clarified that there is no correlation to the thickness of the exine layer (Mangaly & Nayar, 1990). Variation in pollen morphology was shown as following the taxonomic borders, even though the tribes encompassed more than one pollen type.

Theilade *et al.* (1993), who explored the pollen structure and morphology of 18 *Zingiber* species from Peninsular Malaysia, Thailand, and Myanmar reported two groups of pollen structure, particularly spherical with cerebroid or reticulate sculpturing in section *Zingiber* and section *Dymezewiczia,* and ellipsoidal with a spiro-striate surface for section *Cryptanthium.* The pollen morphology of *Zingiber* offered a more useful criterion than the ambiguous inflorescence habit for the classification into sec-

tions. The present subdivision of the genus is based on the inflorescence habit, whereby the inflorescences of sections *Zingiber* and *Cryptanthium* are radical, and apical for section *Dymezewiczia.* Therefore, based on the pollen morphology, she had proposed that the section *Dymezewiczia* should be included in the section *Zingiber.*

In comparison, Kaewsri & Paisooksantivatana (2007), who investigated 14 taxa of *Amomum* from Thailand, have revealed that the pollen grains are spherical to subspherical, inaperturate, and exine sculptures of psilate and echinate. The exine sculpturing was proven to be significant in dividing the genus into two groups. Additionally, the palynology study of *Curcuma* (tribe Zingibereae) by Saensouk *et al.* (2015) on 14 taxa has described that the pollen shapes ranged from subspheroidal, prolate spheroidal, spheroidal, subprolate, and prolate. The various shapes of pollen grains were proven to be beneficial as a supplementary character to distinguish the species. However, the taxonomic groups based on palynological characters were found to be incoherent with those based on morphological characters. Later, Kajornjit *et al.* (2018) revealed that the size, shape, exine sculpturing, and type of spine apex of 22 taxa of Thai *Globba* could be used to distinguish groups in the genus. However, pollen morphology has low taxonomic value since all taxa are quite similar in all aspects. Pollen characters alone were quite insignificant in resolving the classification of *Globba* as they can not be used to identify all species, but they can provide some information to differentiate some taxa.

Palynology could provide supportive and descriptive information in systematics study. However, most species in Malaysia, especially in the eastern part, have not had their pollen morphology thoroughly studied, and it is a fact that, the complex tribe Alpinieae in the subfamily Alpinioideae encompassed the highest number of species in Sabah and Sarawak. Thus, a study that describes the comparative pollen morphology among the complex genera to provide useful characteristics concerning taxonomy is highly needed.

## MATERIALS AND METHODS

**Specimens Collection and Identification.** Plant specimens were collected and documented from various localities in the states of Sarawak (East Malaysia) and Johor (West Malaysia) as presented in Table 1. The study encompassed a total of three primary vegetation sampling sites, consisting of five totally protected areas, namely Similajau National Park in Bintulu, Niah National Park, and Lambir Hills National Park in Miri, Kubah National Park in Kuching, and Endau Rompin Johor National Park in Johor. Additionally, two forest reserves were included: Fairy Cave Nature Reserve in Bau and Ayer Hitam Utara Forest Reserve in Johor. Furthermore, the research covered five secondary disturbed areas. Twenty-one species of the tribe Alpinieae were retrieved and documented with preference to fertile material. Morphological characters of each collected plant were measured and assessed, especially on floral and reproductive parts, to provide a primary basis for species identification. Specimens were described and verified with type materials through examination of specimens from several herbaria, digital images of types (K, E), protologues, and taxonomic data from online databases (POWO, 2020; BHL, 2020; IPNI, 2020; Newman, 2022; WVCP, 2022) and published materials on related species. Herbarium specimens were deposited at the Herbarium of Forest Department Sarawak (SAR) while the duplicates were kept at the herbaria in Universiti Malaysia Sarawak and Universiti Tun Hussein Onn Malaysia. Figure 1 provides photographs of the studied species in their original habitat.

**Pollen Micromorphology Assessment.** Pollen grains from the anther of blooming flowers of selected taxa were studied. For each species, about 15–20 pollen grains were measured to ensure consistent and reliable data. Pollens were stored in 70% ethanol. Samples were then dehydrated in an alcohol series of 70%, 80%, 95%, and 100%. Later, the pollen grains in absolute alcohol were dried on aluminium stubs with double-sided carbon tape. Samples were sputter-coated with gold-palladium and examined using an analytical scanning electron microscope (SEM) (JEOL: JSM-6390LA). Parameters of pollen grains that were analysed were polarity, symmetry, shape, size, apertures, exine ornamentation, size of spine, type of spine apex, spine density, and distance between spine. The terminology adopted to describe the pollen morphology was made based on Punt *et al.* (2007), Chen & Xia (2011), and Saensouk *et al.* (2015), while the pollen shape and size classification was made following Erdtman (1969).

**Hierarchical Clustering.** The key pollen morphology were scored to evaluate the phenetic similarities and interrelationships among the species. Table 1 provides details of the scored key characters. Phenetic similarities of variable characters were enumerated through clustering using PAST3 (PAlentological STatistics) Software Version 3.22. A dendrogram was constructed using the algorithm of the unweighted pair-group method using arithmetic average (UPGMA) based on Bray-Curtis similarity index (Sokal, 1986). The clustering process is estimated by the cophenetic correlation coefficient. The Bray-Curtis index is based on shared similarities divided by total similarities, which can be calculated using the formula below:

Table 1. Scored pollen characters for clustering analysis.

| | Pollen characters | Scores |
|---|---|---|
| 1 | Shape | 0. Oblate-sphereoidal; 1. Spheroidal; 2. Prolate-spheroidal; 3. Prolate |
| 2 | Size | 0. Medium; 1. Large |
| 3 | Exine sculpturing | 0. Echinate-rugulose; 1. Echinate-psilate; 2. Psilate-rugulose; 3. Psilate-reticulate |
| 4 | Spine length | 0. ≤ 3 μm; 1. ≥ 3 μm; 2. Absent |
| 5 | Spine width | 0. ≤ 3 μm; 1. ≥ 3 μm; 2. Absent |
| 6 | Distance between spine | 0. ≤ 3 μm; 1. ≥ 3 μm; 2. Absent |
| 7 | Spine apex | 0. ≤ Blunt; 1. Sharp; 2. Absent |

$$BC_{ij} = \Sigma |(n_i - n_j)| / \Sigma (n_i + n_j)$$

In which,
$BC_{ij}$ = Bray-Curtis Dissimilarity of two species i and j
ni = number of characters present in i
nj = number of characters present in j
$BC_{ij}$ Similarity Index = $(1 - BC_{ij}) \times 100$

## RESULTS

### Pollen Micromorphology

Pollen grains from 21 selected species were classified as monad (single dispersal unit), radial symmetry, apolar, and inaperturate. The pollen shape of Alpinieae species was mainly spheroidal, which was essentially divided into three subgroups: spheroidal, oblate-spheroidal, or prolate-spheroidal. Two main types of exine sculpturing or ornamentation were revealed, in particular, echinate (as shown in *Alpinia* (Figs. 2A–2D and 3A–3D), *Conamomum* (Figs. 2E–2F and 3E–3F), *Meistera* (Figs. 2L and 3L), *Plagiostachys* (Figs. 2M–2N and 3M–3N), *Sundamomum* (Figs. 2O–2P and 3N– 3O), and *Sulettaria* (Figs. 2Q–2R and 3P–3Q) and psilate as in *Etlingera* (Figs. 2G–2J and 3G–3J) and *Hornstedtia* (Figs. 2K and 3K). In detail, the ornamentation can be further defined as echinate with a psilate surface between spine, echinate with a rugulose surface between spine, or psilatereticulate surface.

The pollen sizes were mainly large (50–100 μm) based on the longest axis, except for *A. aquatica* (Figs. 2A and 3A), *A. ligulata* (Figs. 2D and 3D), *S. longipilosa* (Figs. 2Q and 3P), and all species of *Plagiostachys* that displayed mediumsized pollen grains. For echinate pollens, the spine properties, *i.e.*, spine apex (sharp or blunt), size, and distance, were distinguishable between species. The length of the spine varied in each species, from the shortest at approximately 1.96 μm as in *P. glandulosa* (Figs. 2N and 3M), to the longest as in *S. polycarpa* (Figs. 2R and 3Q), at approximately 5 μm. The width of the spine bases was also diverse, with the smallest at 2.02 μm as in *A. aquatica* to a maximum width of 5.12 μm as in *S. polycarpa*. The shortest distance between the spine was displayed by *S. laxesquamosum* (Figs. 2P and 3O), measuring 1.33 μm compared to *C. xanthophlebium* (Figs. 2F and 3F), having the longest distance of 4.20 μm. Table 1 provides details on the pollen morphological characteristics of the studied species. Table 2 displays the studied pollen morphological characteristics in detail. While the SEM micrographs of selected pollen grains were illustrated in Figs. 2 and 3.

### Hierarchical Clustering

The dendrogram in Fig. 4 illustrates the phenetic relationship among studied Alpinieae species, utilising scored key pollen characteristics and four main clusters were produced. Exine sculpturing, type of spine apex, as well as spine morphology (distance, width, and length), were essential in determining the final placement of the species, though they were not exactly grouped in accordance with their generic boundaries. Species of *Etlingera* and *Hornstedtia* were closely clustered together (> 95% similarity) based on psilate pollens .

## DISCUSSION

### Pollen Micromorphology

*Alpinia* displayed two types of echinulate ornamentation: echinate-psilate for *A. latilabris* (Figs. 2C and 3C), *and A. ligulata* (both from *Zerumbet* clade) and echinate-rugulose for *A. aquatica* (*Zerumbet* clade) and *A. galanga* (Figs. 2B and 3B) (*Galanga* clade). Although *A. aquatica* is classified under the *Zerumbet* clade, the exine sculp-


Fig. 1. Inflorescences and infructescences of the studied taxa in their respective habitats. A. *Alpinia aquatica.* B. *A. galanga.* C. *A. latilabris*. D. *A. ligulata*. E. *Conamomum cylindrostachys*. F. *C. xanthophlebium*. G. *Etlingera coccinea*. H. *E. elatior*. I. *E. inundata*. J. *E. nasuta*. K. *Hornstedtia leonurus.* L. *H. reticulata*. M. *Meistera gyrolophos*. N. *Plagiostachys crocydocalyx.* O. *P. glandulosa*. P. *P. strobilifera* var. *conica.* Q. *Sundamomum laxesquamosum.* R. *S. corrugatum*. S. *Sulettaria longipilosa*. T. *S. polycarpa.* Photos by Salasiah Mohamad.

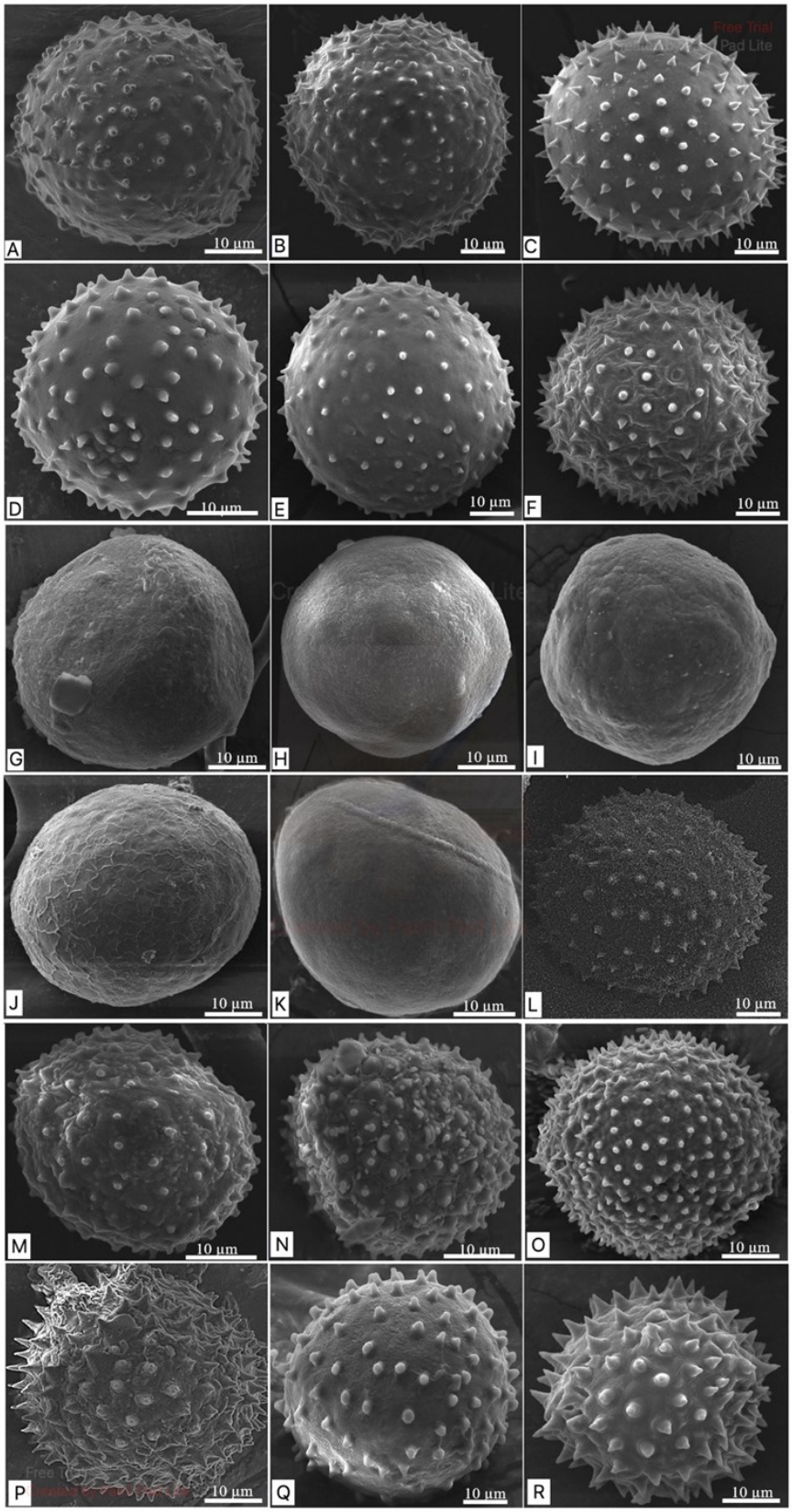


Fig. 2. SEM micrographs of pollen grains of the studied Alpinieae. A. *A. aquatica.* B. *A. galanga.* C. *A. latilabris*. D. *A. ligulata*. E. *C. cylindrostachys*. F. *C. xanthophlebium*. G. *E. coccinea*. H. *E. elatior*. I. *E. inundata*. J. *E. nasuta*. K. *H. reticulata*. L. *M. gyrolophos*. M. *P. strobilifera* var. *conica*. N. *P. glandulosa*. O. *S. corrugatum*. P. *S. laxesquamosum*. Q. *S. longipilosa.* R. *S. polycarpa.* Magnification: B, C, F, H, I, J, L= 1000×. O= 1400×. A, P, R= 1500×. E= 1600×; G= 1700×. D, K, M, N, Q= 2000×.

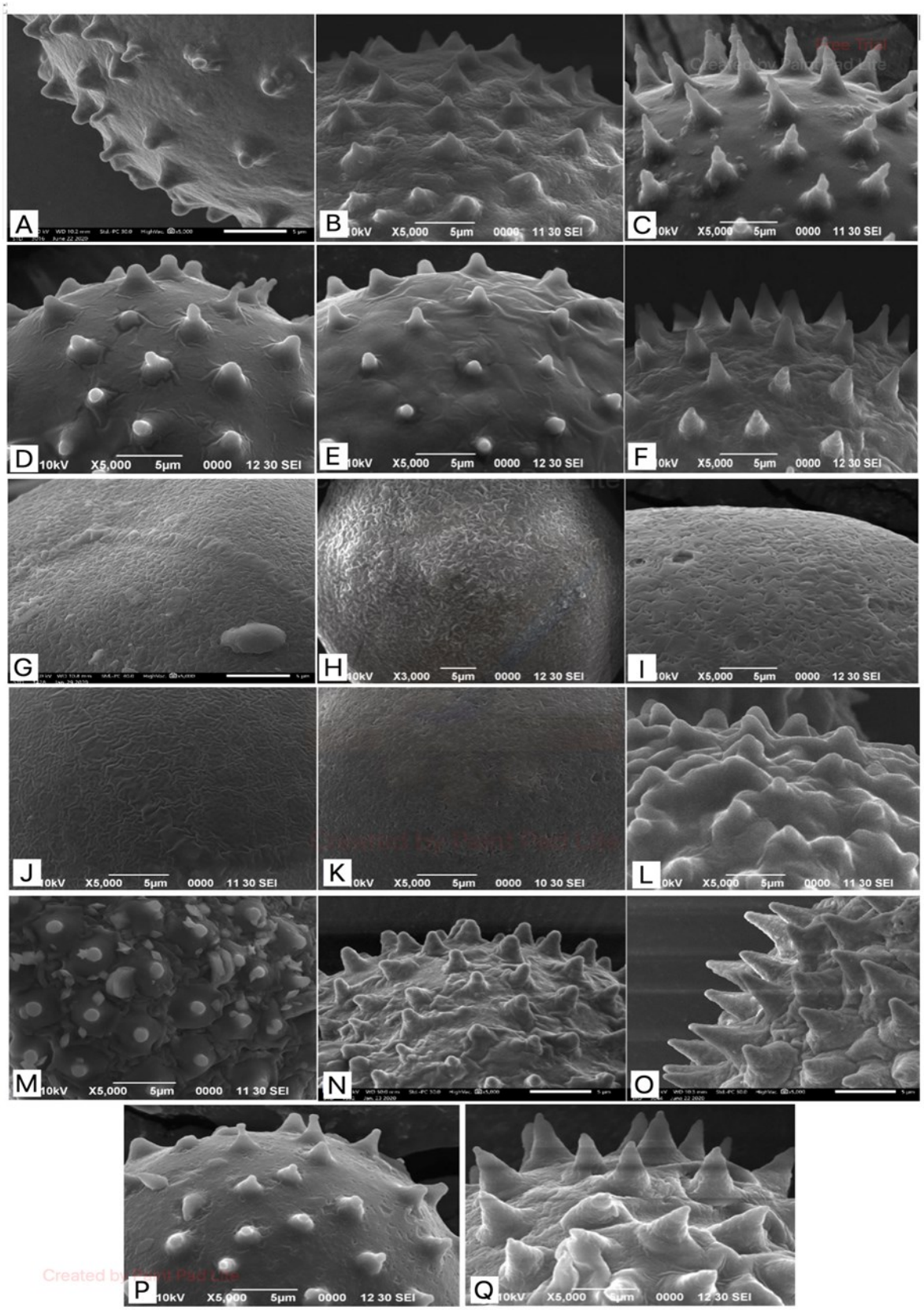


Fig. 3. SEM micrographs close-up of pollen sculpture of the studied Alpinieae. A. *A. aquatica.* B. *A. galanga.* C. *A. latilabris.* D. *A. ligulata.* E. *C. cylindrostachys.* F. *C. xanthophlebium.* G. *E. coccinea.* H. *E. elatior.* I. *E. inundata.* J. *E. nasuta.* K. *H. reticulata.* L. *M. gyrolophos.* M. *P. glandulosa*. N. *S. corrugatum.* O. *S. laxesquamosum*. P. *S. longipilosa.* Q. *S. polycarpa* .

ture and spine distance slightly resemble *A. galanga.* Perhaps their floral morphology, such as smaller flowers, requires similar mechanisms of pollination that decide the characteristics of the pollen. Likewise, two species of *Conamomum* with different labellum sizes unveiled slightly different echinulate sculpturing of either echinate-rugulose or echinate-psilate. However, this presumption relies solely on a limited taxonomic sample, necessitating further testing across various taxa in the respective generic groupings.

Species of *Etlingera, Hornstedtia, Plagiostachys, Sulettaria* and *Sundamomum*, however, recorded uniformly stable and reasonably similar ornamentation. The pollen shape of the studied *Etlingera* seemed to be correlative with their genus groupings based on morphology, whereby the oblatespheroidal pollen was observed in *E. elatior* (Figs. 2H and 3H) (Group A in Sakai & Nagamasu, 2003), while the remaining species (Figs. 2G, 2I, 2J and 3G, 3I, 3J) from Group C'(Sakai & Nagamasu, 2003) exhibited prolate-spheroidal pollen shape. Additionally, three species, including *A. galanga*, *E. elatior*, and *H. leonurus*, that were obtained from two distinct geographical regions (East and West Malaysia), exhibited similar pollen micromorphological characteristics.

**Hierarchical Clustering**

Although the clustering of pollen morphology was less useful for the entire generic classification, this study still provides information that will be useful in future related research in species delineation since pollen characteristics are helpful in characterising and identifying a specific taxon. Nonetheless, the pollen size, the pollen shape, as well as the spine properties (for echinate pollens), *i.e.*, apex form, distance, width, and length, are considerably functional in species recognition and groupings of some genera, including *Alpinia, Amomum, Conamomum, Meistera, Plagiostachys, Sundamomum,* and *Sulettaria.* Additionally, relying on pollen characters alone is unwise as they cannot be used to identify all species, but they do provide supplementary traits relevant to a particular taxon.

Pollen morphology of the polyphyletic *Alpinia,* unveiled spheroidal, medium- to large-sized, and echinate ornamentation with varying spine characteristics. The current pollen grain results were rather limited in distinguishing the species from two *Alpinia* clades. Cluster analysis of the pollen morphology in Fig. 4 revealed the close relationship between *A. aquatica* and *A. galanga* which probably correlated to their affinity in floral morphology (size of flower and labellum).

Likewise, species of *Hornstedtia* displayed similar pollen morphology, of having spheroidal psilate-reticulate with those of *Etlingera*; in fact, *H. leonurus* was clustered together with some species of *Etlingera* on the basis of molecular phylogenetic analysis. Other comparable data by Lam *et al.* (2010) on Bornean species (*H. havilandii* and *H. tomentosa*) and Acma & Mendez (2018) (on *H. conoidea*) have also recorded similar pollen morphology, thus it may also suggest a stable pollen morphology in members of *Hornstedtia* which are nearly allied to *Etlingera.*

The findings of Furness & Rudall (1999) regarding the widespread occurrence of inaperturate pollen among monocotyledonae were also confirmed through this study. The main pollen ornamentation and shape of Alpinieae species were consistent with previously related reports by Mangaly & Nayar (1990) and Kaewsri & Paisooksantivatana (2007) on *Alpinia* and *Amomum s.l.* species, as well as Lam *et al.* (2010) and Acma & Mendez (2007) on selected *Etlingera* and *Hornstedtia* in Borneo and the Philippines, respectively. Adding to the current data of the family Zingiberaceae, there are about five types of pollen sculpturing reported thus far, *i.e.*, echinate as observed in *Boesenbergia* (Chen & Xia, 2011; Mangaly & Nayar, 1990) and *Globba*, although very rarely, psilate in one species (Kajornjit *et al.*, 2018), verrucose in *Elettaria cardamomum* (Mangaly & Nayar, 1990), reticulate or rugulose in *Curcuma* (Saensouk *et al.*, 2015), and cerebroid and spirostriate in *Zingiber* (Theilade *et al.*, 1993). Other than the main spheroidal shape, *Curcum*a and *Zingiber* appeared to be of ovoid to ellipsoidal shape (Saensouk *et al.*, 2015; Theilade *et al.*, 1993; Mangaly & Nayar, 1990), which was not observed in any Alpinieae species so far. Pollen study on more species, including examination of the exine thickness, would provide more information that may help in defining subgroups or sections of a particular genus, as reported by Theilade *et al*. (1993) on *Zingiber*, Kaewsri & Paisooksantivatana (2007) on *Amomum*, and Kajornjit *et al.* (2018) on *Globba*.

The pollen sculpturing in plants might also be influenced by pollination systems. The pollen sculpture acts as an adhesion enhancer to the pollinators' bodies in the presence of pollen kitt (Grayum, 1986; Pacini & Hesse, 2005). According to the data on gingers pollination guilds by Sakai *et al.* (1999), it was shown that species with echinate pollen sculpturing (*C. cylindrostachys, M. gyrolophos, P. crocydocalyx, P. glandulosa*, and *S. polycarpa*) were associated to Halictic- or Amegilla-guilds, whereas species with psilate ornamentation (*H. leonurus* and *H.reticulata*) were allied to Spiderhunter-guild. Besides the floral characters, *i.e.*, sizes of floral tube, labellum, pistil, and stamen, which played vital roles in the ginger pollination mechanism, the pollen sculpture was also consistent with the pollination guild system. Although the interaction between pollen ornamentation and type of pollinator has been commonly reported in angiosperms, Sannier *et al.* (2009), based on their comparative analysis of other monocots of the Ara-

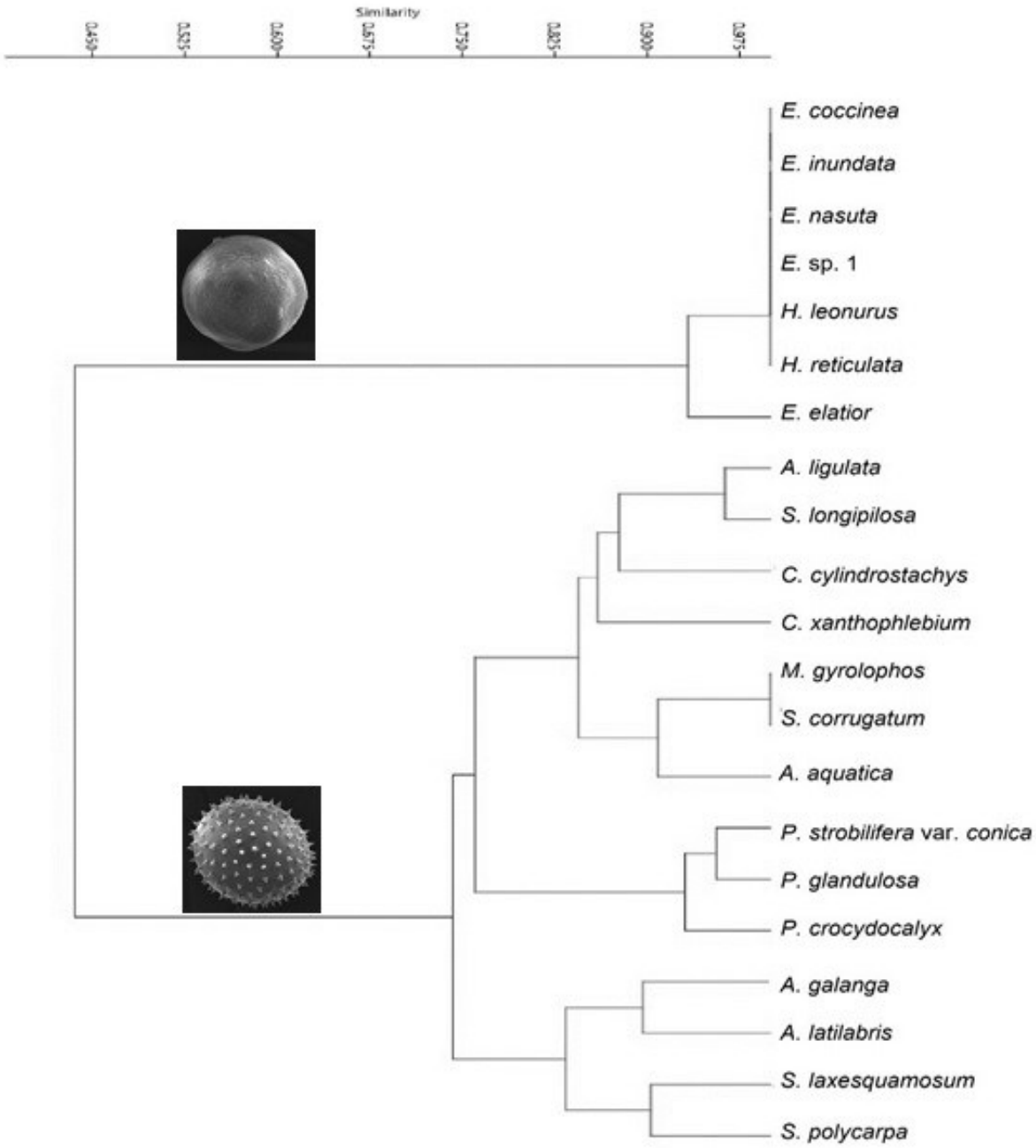


Fig. 4. Dendrogram indicates phenetic relationship of selected Alpinieae species based on pollen characteristics using Bray-Curtis and UPGMA algorithm.

Table 2. Comparison of pollen grain characteristics in the studied Alpinieae.

| | Taxa | Localities | PA (µm) | EA (µm) | P/E ratio | Shape | Size | ES | SL (µm) | SW (µm) | SA | DS (µm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | ***Alpinia*** | | | | | | | |
| 1 | *A. aquatica* | SNP | 48.4 | 48.1 | 1.00 | SP | M | E-RU | 2.12 | 2.02 | B | 2.75 |
| 2 | *A. galanga* | NNP, AHUFR | 68.8 | 68.8 | 1.00 | SP | L | E-RU | 2.59 | 3.43 | SH | 2.71 |
| 3 | *A. latilabris* | US | 68.9 | 69.1 | 1.00 | SP | L | E-P | 3.60 | 3.01 | SH | 3.70 |
| 4 | *A. ligulata* | FNCR | 43.3 | 41.7 | 1.04 | PS | M | E-P | 2.57 | 2.58 | B | 3.10 |
| | | | | | ***Conamomum*** | | | | | | | |
| 5 | *C. cylindrostachys* | LHNP | 51.0 | 53.0 | 0.96 | OS | L | E-P | 2.20 | 2.58 | B | 4.03 |
| 6 | *C. xanthophlebium* | SNP | 58.9 | 57.5 | 1.02 | PS | L | E-RU | 3.14 | 2.37 | SH | 4.20 |
| | | | | | ***Etlingera*** | | | | | | | |
| 7 | *E. coccinea* | LHNP | 50.9 | 49.5 | 1.03 | PS | L | P-RE | - | - | - | - |
| 8 | *E. elatior* | US,AHUFR | 51.1 | 52.1 | 0.98 | OS | L | P-RE | - | - | - | - |
| 9 | *E. inundata* | LHNP | 57.7 | 57.0 | 1.01 | PS | L | P-RE | - | - | - | - |
| 10 | *E. nasuta* | KNP | 52.2 | 49.7 | 1.05 | PS | L | P-RE | - | - | - | - |
| 11 | *Etlingera* sp. 1 | SNP | 63.7 | 58.9 | 1.08 | PS | L | P-RE | - | - | - | - |
| | | | | | ***Hornstedtia*** | | | | | | | |
| 12 | *H. leonurus* | LHNP, ERJNP | 52.3 | 51.5 | 1.02 | PS | L | P-RE | - | - | - | - |
| 13 | *H. reticulata* | KD | 59.4 | 54.9 | 1.08 | PS | L | P-RE | - | - | - | - |
| | | | | | ***Meistera*** | | | | | | | |
| 14 | *M. gyrolophos* | LHNP | 57.7 | 60.4 | 0.96 | OS | L | E-RU | 2.34 | 2.42 | B | 2.10 |
| | | | | | ***Plagiostachys*** | | | | | | | |
| 15 | *P. strobilifera* var. *conica* | SNP | 42.8 | 44.0 | 0.97 | OS | M | E-RU | 2.03 | 2.32 | B | 3.01 |
| 16 | *P. crocydocalyx* | LHNP | 41.0 | 42.3 | 0.97 | OS | M | E-RU | 2.01 | 2.15 | B | 2.69 |
| 17 | *P. glandulosa* | NNP | 40.1 | 41.1 | 0.99 | OS | M | E-RU | 1.96 | 4.75 | B | 1.69 |
| | | | | | ***Sundamomum*** | | | | | | | |
| 18 | *S. corrugatum* | TB | 65.6 | 64.3 | 1.02 | PS | L | E-RU | 2.66 | 2.23 | B | 2.07 |
| 19 | *S. laxesquamosum* | GP | 59.5 | 59.1 | 1.00 | SP | L | E-RU | 4.55 | 5.10 | SH | 1.33 |
| | | | | | ***Sulettaria*** | | | | | | | |
| 20 | *S. longipilosa* | SNP | 40.0 | 39.2 | 1.02 | PS | M | E-RU | 2.55 | 2.78 | B | 3.11 |
| 21 | *S. polycarpa* | TT | 54.9 | 54.9 | 1.00 | SP | L | E-RU | 5.00 | 5.12 | SH | 1.52 |

SNP - Similajau National Park Bintulu, NNP - Niah National Park Miri,AHUFR - Ayer Hitam Utara Forest Reserve Johor, ERJNP – Endau Rompin Johor National Park Johor, Fairy Cave Nature Reserve, Bau, LHNP - Lambir Hills National Park Miri, KNP - Kubah National Park Kuching, US - Ulu Sebauh Bintulu, KD – Kidurong Bintulu, TB - Tubau Bintulu, GP - Gunung Podam Bau, TT - Tatau Bintulu, PA - polar axis, EA - equatorial axis, ES - exine sculpture, SL - spine length, SW - spine width, SA - spine apex, DS - distance between the spine, OS - oblate-spheroidal, PS - prolate-spheroidal, SP - spheroidal, L - large, M - medium, E - echinate, P - psilate, RE - reticulate, RU - rugulose, B - blunt, SH – sharp.

ceae and Arecaceae, suggested that pollen ornamentation alone was not the most significant element that determines the pollination system. Other pollen properties, such as pollen kitt or aroma, could also play a role. The relationship between plant and pollinator literally differs between plant taxa that may be affected by geographical area or derived from ancestral characters or as results of adaptative mechanism.

## CONCLUSION

Pollen grains of selected Alpinieae species were examined under SEM to facilitate in defining the infrageneric and intergeneric variation. The shape was predominantly spheroidal, either oblate-spheroidal, prolate-spheroidal, to prolate, and the size ranged from medium (< 50 μm) to large (50–100 μm). Hierarchical clustering of the studied species was rather inconsistent with the group boundaries, although it has preliminarily divided the tribe into two major clusters based on pollen ornamentation, *i.e.* psilate and echinate. The main sculpturing is useful to generally distinguish the genera; in particular, the psilate-reticulate pollen is a characteristic of *Etlingera* and *Hornstedtia,* whilst the echinate pollen is common for members of *Alpinia, Conamomum, Meistera, Plagiostachys, Sulettaria,* and *Sundamomum*.

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# *NEPENTHES CALCICOLA* (NEPENTHACEAE), A NEW PITCHER PLANT FROM GULF PROVINCE, PAPUA NEW GUINEA

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**ABSTRACT**
WILSON, G. W., VENTER, S. & DAMAS, Q. K. 2023. *Nepenthes calcicola* (Nepenthaceae), a new pitcher plant from Gulf Province, Papua New Guinea. *Reinwardtia* 22(2): 103–109. — A new species of pitcher plant from rain forest on limestone karst in the Purari River catchment in the Gulf Province of Papua New Guinea is described and illustrated as *Nepenthes calcicola* Gary W.Wilson, S.Venter & Damas. It is distinguished from *N. neoguineensis* on the basis of its distribution, ecology, habitat, and pitcher and inflorescence morphology. The new species is illustrated, and description is here given. The species is assessed as Vulnerable (VU) according to IUCN criteria.

**Key words**: Karst, Nepenthaceae, Papua New Guinea, pitcher, taxonomy.

**ABSTRAK**
WILSON, G. W., VENTER, S. & DAMAS, Q. K. 2023. *Nepenthes calcicola* (Nepenthaceae), tanaman kantong semar baru dari Provinsi Teluk, Papua Nugini. *Reinwardtia* 22(2): 103–109. — Sebuah jenis baru kantong semar dari hutan hujan di batugamping di DAS Sungai Purari di Provinsi Teluk Papua Nugini dipertelakan dan diilustrasikan sebagai *Nepenthes calcicola* Gary W.Wilson, S.Venter & Damas. Jenis ini dibedakan dari *N. neoguineensis* berdasarkan distribusi, ekologi, habitat, dan morfologi kantong dan perbungaannya. Pertelaan dan ilustrasi dari jenis baru ini disajikan dalam naskah ini. Status konservasi jenis ini berdasarkan kriteria IUCN adalah jenis rentan (VU).

**Kata kunci**: Batugamping, kantong, Nepenthaceae, Papua Nugini, taksonomi.

## INTRODUCTION

The genus *Nepenthes* L. (1753) comprises approximately 181 species, with the greatest diversity and endemic species in the Philippines, Sumatra and Borneo (Gronemeyer *et al.,* 2014; Cheek, 2015; Murphy *et al*., 2020).

At a time when so much biodiversity is being lost or is under threat, exploration of the island of New Guinea is providing a wealth of new and often spectacular species of flora and fauna. In particular, targeted Rapid Assessment trips by teams of expert observers to remote and difficult to access locations have revealed many new species. This has included *Nepenthes* pitcher plants, with several taxa recently being found or recognised *e.g.*, *N. monticola* (Robinson *et al*., 2011; McPherson, 2011), from mountains in West Papua, Indonesia. During fieldwork in Papua New Guinea in November 2011 the second author made two collections of a *Nepenthes* taxon that did not key to any known species. Here we describe *Nepenthes calcicola* a new species of pitcher plant from the Purari River catchment in the Gulf Province.

## MATERIALS AND METHODS

Georeferencing was made using a Garmin GPSMAP 64sx handheld unit with dual GPS and GLONASS telemetry enabled. Taxonomic descriptions are based on morphometric and qualitative data from dried specimens and from living plants in the field. A total of 15 plants were examined in the wild across two different sites. Plants of the new species were systematically compared with morphologically allied *Nepenthes* species. Specimens of *Nepenthes* were examined in the collections of BRI, CANB, CBG, CNS, K, LAE, and NSW. The type material was deposited in LAE and CNS (herbarium codes follow Thiers, 2022).

Material for prey investigation was sampled from both forest floor pitchers and from intermediate pitchers.

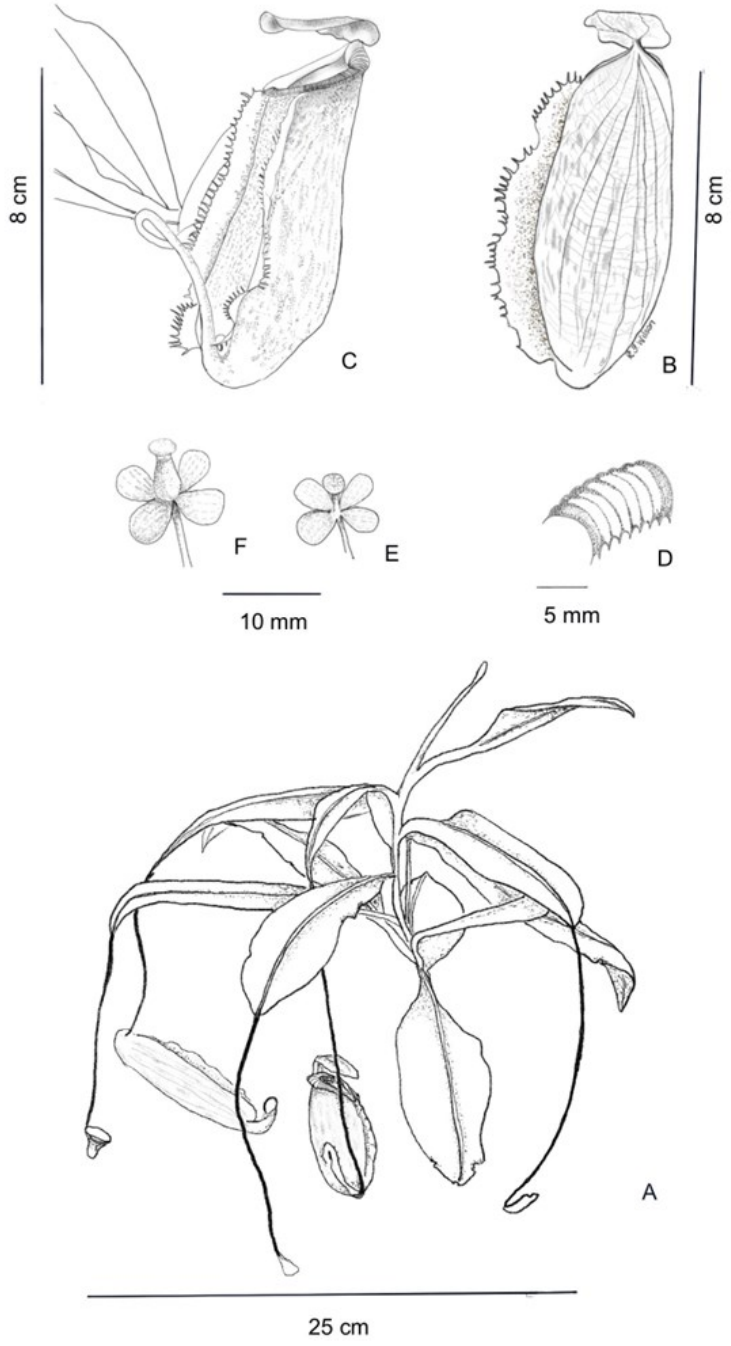


Fig. 1. *Nepenthes calcicola* Gary W.Wilson, S.Venter & Damas. A. Habit with mid-level pitchers. B. Mid-level pitcher showing alae with fimbriae. C. Lower pitcher showing alae with fimbriae. D. Section of peristome showing teeth on inner margin. E. Male flower. F. Female flower. Drawings by R. F. Wilson.

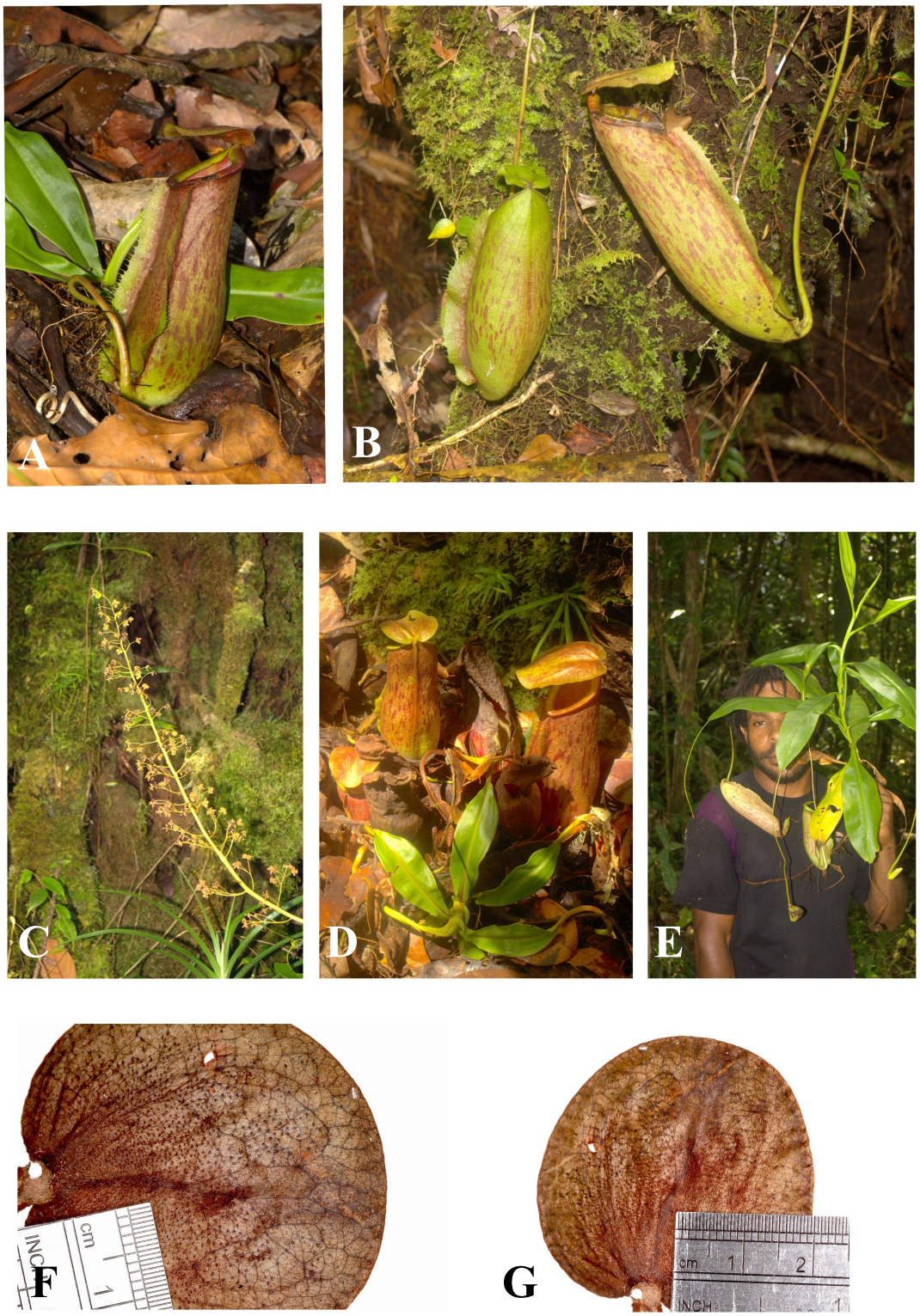


Fig. 2. *Nepenthes calcicola* Gary W.Wilson, S.Venter & Damas. A. Habit with lower pitcher. B. Habit with mid-level pitchers. C. Habit with male inflorescence. D. A rosette of pitchers E. Field Assistant Siwi with mid-level rosette. F-G. Images of the underside of the lid of mid-level pitcher showing the distribution of the nectar gland. (from the type, Collection S. Venter 14170 lodged at CNS). Photos by S. Venter.

Table 1. Diagnostic morphological characteristics of *N. calcicola* and *N. neoguineensis*

| Character | *N. calcicola* | *N. neoguineensis* |
|---|---|---|
| Habitat | Low light undisturbed Closed Forest | Higher light regime, often disturbed habitats, not known from Closed Forest |
| Stem | To 20 m long in the canopy | To 10 m long or more |
| Stems below leaf litter | Present | Absent |
| Pitcher-bearing leaves | No curl in the tendril | Always a curl in the tendril |
| Longitudinal nerves on each side of midrib | 1–2 | 3–4 |
| Vestiture of young pitcher | Glabrous | Covered in stellate hairs |
| Spur shape | Terete and recurved | Dorsiventrally flattened, not recurved |
| Intermediate pitcher wing width (mm) | 7.9–10.0 | 5.0–8.0 |
| Short stems and rosettes of pitchers | Present | Absent |
| Male inflorescence peduncle length (mm) | 8.0–14.0 × 3.0–5.0 | 4.0–12.0 × 2.5–4.0 |
| Female inflorescence peduncle length (mm) | 160–190 × 3.0–4.0 | 120–150 × 2.0–2.5 |
| Tepal shape | Obovate to broadly-obovate | Orbicular-elliptic |

## RESULTS AND DISCUSSION

**Nepenthes calcicola** Gary W.Wilson, S.Venter & Damas, *spec. nov*. Figs. 1 & 2 — TYPE: PAPUA NEW GUINEA: Gulf Province: limestone karst along Mua River, Purari River Catchment, 16 October 2011. *S. Venter 14170*. (Holotype: LAE!, isotype: CNS!).

*Nepenthes calcicola* differs from *N. neoguineensis* in having stems that grow beneath the layer of leaf litter (*vs.* stems above leaf litter); short stems and rosettes of pitchers present (*vs.* absent); spur terete and recurved (*vs.* dorsiventrally flattened and not recurved; female inflorescence peduncle 160–190 × 3.0–4.0 mm (*vs.* 120–150 × 2.0–2.5 mm); tepals obovate to broadly-obovate (*vs.* orbicular-elliptic).

*Terrestrial climber*, reaching to *ca.* 20 m, stems running horizontally underneath leaf litter forming rosettes of leaves with erect pitchers which are sometimes half buried in the litter, before climbing to the canopy. *Stems* 6–8 mm diameter, internodes 20–40 cm on climbing stems, glabrous. *Leaves* sessile, coriaceous, broadly lanceolate-oblong, 180–320 × 30–75 mm, midrib 1–5 mm wide, cross-section flat, apex acute, margin straight, entire, longitudinal nerves 2–3 on each side of midrib, in outer ⅓ of lamina, obscure or moderately obvious, pennate nerves not obvious, upper surface glossy, glabrous, lower surface matt, glabrous, tendril insertion simple, straight, 150–220 × 1.0–1.5 mm, sometimes greater than the length of the lamina blade, not coiled. *Terrestrial pitchers* growing on the forest floor, 70–100 × 40–50 mm, base flat, ovoid in lower part, constriction above mid-point, wings 3–9 mm wide along the length of the pitcher, simple in cross section, filaments to 1.5 mm long, mouth oblique and ovate, peristome to 3 mm wide, rounded, ribs to 0.15 mm high and 0.2 mm apart, inner edge with teeth to 0.3 mm long; lid ovate-orbicular, 16–30 × 16–30 mm, base cordate, attachment 2.8–3 mm wide; spur simple, straight or curved, vestigial or to 3.0 mm long, nectar glands orbicular occasionally ovate, 0.15–0.2 mm in size, most dense about centre line and base of lid; pitcher exterior and lid most often red in colour and lighter interior but some uniform light green. *Intermediate pitchers* most often in rosettes on elevated portions of the stem, 0.5–2 m above ground and on normally developed leaves

(Fig. 2D), pitchers 75–150 × 35–100 mm, wings 8–10 mm wide along the length of the pitcher, filaments to 3.0 mm long, mouth oblique and ovate, peristome to 6.5 mm wide, rounded, ribs to 0.15 mm high and 0.2 mm apart, inner edge with teeth to 0.35 mm long; lid suborbicular-orbicular, 21–50 × 31–50 mm, base cordate; spur simple, straight or curved, vestigial or to 3.0 mm long, nectar glands sunken, circular or oval, 0.15–0.2 mm in size, surrounded by a light-coloured annulus, most dense, to 150/cm$^2$, about centre line and base of lid; pitchers red in colour, darkening with age, mid-age pitchers have lighter and contrasting peristomes, and mature-age pitchers a dark-red peristome. Upper pitchers not produced. *Male inflorescence* with peduncle 8–14 cm long, 3–5 mm diameter, bearing 30–55 partial peduncles, 10–30 mm long, evenly scattered along its length, 3–6 flowered in the basal third, bracts absent, pedicels divergent 10–17 mm long. Tepals 4, obovate to broadly obovate, 3.5–4.0 × 3.0–4.0 mm, lower surface with reddish-brown sub-appressed branched and stellate dense hairs, upper surface with elliptic nectar glands, glabrous, live colour green. Androphore 4.0 × 2.5 mm, glabrous. Anther-head white, globose, 1.3–1.8 mm. *Female inflorescence* peduncle 160–190 × 3.0–4.0 mm, partial-peduncles 10–35 × 0.8–1.0 mm, 2–3 flowered. Bracts absent. Pedicels 8.0–18.0 mm long. Tepals 4, ovoid, 4.4–5.5 × 1.8–2.5 mm, upper surface with elliptic nectar glands, ovary sessile. Fruit valves 4, narrowly elliptic, 18.5–35.0 × 3.5–4.2 mm, outer surface covered in fine reddish-brown hairs.

The *Nepenthes* species most similar *to N. calcicola* is *N. neoguineensis* Macfarl. (1910) (Table 1).

**Distribution**. *Nepenthes calcicola* is known only from the type locality in limestone karsts along the Mua River, a tributary of the Purari River, Gulf Province, Papua New Guinea. Specific collection locations not revealed to reduce the likelihood of poaching.

**Habitat and Ecology**. This species grows in Closed Low Lowland Hill Rainforest (Hammermaster & Saunders, 1995; Paijmans, 1976) with *Pometia pinnata* J.R.Forst. & G.Forst. and *Syzygium* P.Br. ex Gaert. spp. as emergents, sensu Shearman *et al*. (2008), on the margins of limestone karsts at elevations of 250–270 m. Epiphytes from many genera, and moss and liverwort species are common. Dominant tree genera are *Aglaia* Lour., *Syzygium* P.Br. ex Gaertn., *Ficus* Tourn., *Myristica* Gron. and *Terminalia* L. No other *Nepenthes* taxon is present in the collection area. The soil is a humus-rich clay loam and varies considerably in depth. The leaf litter layer is well developed and to 20 cm deep. Most surface rock (limestone) is covered in vegetation. Only a small number of *Nepenthes* species occur on limestone, mostly from Borneo and one Thai species (Cheek, 2015). Both populations of *Nepenthes calcicola* are restricted to limestone. Examination of the contents of terrestrial and intermediate pitchers shows the species traps invertebrate fauna, including ants, cockroaches, snails and slugs, and katydids.

**Etymology**. The specific epithet describes the calcareous substrate the plants grow in.

**Conservation status**. Vulnerable (VU) with Criteria D1,2 (IUCN 2012) as *Nepenthes calcicola* is known from only two populations, one kilometre apart, on karst limestone in the Purari River catchment in the Gulf District in Papua New Guinea. The area of occupancy (AOO) is <100 km$^2$ and the number of individuals is <1,000.

**Notes**. This is the first record of a *Nepenthes* species from closed forest habitat on karst limestone in Papua New Guinea (see Jebb, 1991), a habitat type recognised for its distinct biodiversity (Clements *et al*., 2006). *Nepenthes treubiana* Warb. and *Nepenthes* sp. Misool, described by McPherson (2009) as 'similar to the extremely variable *N. neoguineensis'* also grow on limestone sea-stacks and cliffs in West Papua, Indonesia (McPherson, 2009) but in soil-depauperate exposed sites. *Nepenthes* typically occur in high light regime habitats, the notable exception being *N. ampullaria*, which also occurs in forest habitats in New Guinea but derives most of its nitrogen from leaf detritus (Moran *et al*., 2003), and has not been found at or near the collection site of *N. calcicola*.

The species that *N. calcicola* may be confused with on basis of gross morphology is *N. neoguineensis* (Table 1) which occurs throughout New Guinea and the d'Entrecasteaux Archipelago (Cheek & Jebb, 2001). *Nepenthes neoguineensis* grows in habitats from open grassland at sea level to heath forest on ridge tops at *ca.* 1400 m (Jebb, 1991; Cheek & Jebb, 2001; McPherson, 2009) but has not been recorded from primary closed forest. In addition, *N. neoguineensis* produces functional aerial pitchers with wings (McPherson, 2009), a morphology typical of terrestrial pitchers of other taxa and of intermediate pitchers in *N. calcicola.* In contrast, *N. calcicola* has a restricted distribution in rain forest on limestone hills below 300 m altitude and despite careful searching aerial pitchers have not been found; suggesting they are restricted to the canopy. Surveys of the *N. calcicola* collection location has not revealed the presence of *N. ampullaria*, the most likely other

A portion of the key of *Nepenthes* in New Guinea & neighbouring islands in Cheek and Jebb (2001) modified to include *N. calcicola* is presented here.

4a Leaves decurrent to at least ½ way down the internode ……... 5

4b Leaves distinctly petiolate, never decurrent ……. 7

5a Stem triangular, peristome >0.8 cm in width …….. 35. *N. insignis*

5b Stem rounded, peristome <0.8 cm in width …….. 6

6a Inflorescence a raceme; grows on ultramafics at >1000 m altitude ……... 38. *N. lamiii*

6b Inflorescence a panicle; grows on limestone at <300 m altitude …… *N. calcicola*

7 Margins of lower leaf blades fimbriate; upper pitchers not winged …….. 49. *N. mirabilis*

species in closed forest habitat, or of *N. neoguineensis.*

**Additional specimens examined**. PAPUA NEW GUINEA: Gulf Province: limestone karsts along Mua River, Purari River Catchment, 17 October 2011. *S. Venter 14169* (CNS, LAE) and Hill east of Mua Creek, 20 October 2011, *S. Venter 14210* (CNS, LAE).

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# MOLECULAR AND MORPHOLOGICAL ANALYSIS SUPPORTS THE TRANSFER OF THE MONOTYPIC INDONESIAN GENUS *SEPTOGARCINIA* KOSTERM. TO *GARCINIA* (CLUSIACEAE)

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**ABSTRACT**
SARI, R., CRAYN, D., DILLON, N., GADEK, P. & ABELL, S. 2023. Molecular and morphological analysis supports the transfer of the monotypic Indonesian genus *Septogarcinia* Kosterm. to *Garcinia* (Clusiaceae). *Reinwardtia* 22(2): 111–129. — Based on molecular phylogenetic analysis and an assessment of fruit and pollen characters, the transfer of *Septogarcinia sumbawaensis* Kosterm., endemic to Sumbawa Island, Indonesia and the sole member of the genus *Septogarcinia*, to *Garcinia* is strongly supported. The formal transfer of *S. sumbawaensis* to *Garcinia* (as *G. sumbawaensis*; the current name is *G. septogarcinia*) was based on morphological studies only. Phylogenetic analysis of nuclear internal transcribed spacer (ITS) sequences supports a placement of *G. septogarcinia* in *Garcinia* Section *Brindonia.* The distinctive dehiscent fruit, cited by Kostermans as justification for erecting *Septogarcinia*, is interpreted as an autapomorphy for this species in *Garcinia.* Pollen exine ornamentation is similar to *G. griffithii, G. gummi-gutta* var. *gummi-gutta, G. mestonii, Garcinia* sp. (Maluku) and *Garcinia* sp. (Batulanteh, Sumbawa).

**Key words**: Batulanteh, *Septogarcinia sumbawaensis,* Sumbawa.

**ABSTRAK**
SARI, R., CRAYN, D., DILLON, N., GADEK, P. & ABELL, S. A. 2023. Analisis molekuler dan morfologi mendukung perpindahan marga monotipik Indonesia *Septogarcinia* Kosterm. to *Garcinia* (Clusiaceae). *Reinwardtia* 22(2): 111–129. — Berdasarkan analisis molekuler dan penilaian terhadap karakter buah dan serbuk sari, perpindahan *Septogarcinia sumbawaensis* Kosterm., endemik di Pulau Sumbawa, Indonesia dan anggota tunggal dari marga *Septogarcinia*, ke *Garcinia* sangat didukung kuat. Perpindahan secara formal *S. sumbawaensis* ke *Garcinia* (sebagai *G. sumbawaensis*; nama saat ini adalah *G. septogarcinia*) hanya didasarkan atas studi morfologi. Analisis filogenetik dari sekuens *internal transcribed spacer* (ITS) inti sel mendukung hubungan kekerabatan yang dekat antara *Garcinia septogarcinia* dengan *Garcinia* Seksi Brindonia. Perbedaan karakter buah yang terbelah, dikutip oleh Kostermans sebagai dasar untuk memisahkan *Septogarcinia*, diinterpretasikan sebagai otomorfi untuk jenis ini dalam *Garcinia.* Ornamentasi eksin serbuk sari mirip dengan *G. griffithii*, *G. gummi-gutta* var. *gummi-gutta*, *G. mestonii*, *Garcinia* sp. (Maluku) dan *Garcinia* sp. (Batulanteh, Sumbawa).

**Kata kunci:** Batulanteh, *Septogarcinia sumbawaensis*, Sumbawa.

## INTRODUCTION

*Septogarcinia* Kosterm. (Clusiaceae) was erected by Kostermans (1962) for a single species *S. sumbawaensis* Kosterm. based on material from Sumbawa Besar Island, Nusa Tenggara Barat, Indonesia (Figs. 1 & 2). Kostermans noted that *Septogarcinia* exhibited an unusual character state for Clusiaceae –fruits dehiscent (Fig. 3) at maturity– but in other respects was very similar to *Garcinia* L. He considered the dehiscent fruit of *S. sumbawaensis* to be morphologically similar to those found in the genera *Tovomita* Aubl. and *Rheedia* L. *Rheedia* fruit, however, do not dehisce. Another genus in Clusiaceae that has dehiscent fruit is *Clusia* L. but Kostermans did not compare *Septogarcinia* with *Clusia*.

Kostermans (1962) considered *Septogarcinia* to be related to *G. septata* from Celebes (Sulawesi). However, the name *G. septata* has not been published, nor are there any known specimens annotated with this name. It is possible that Kostermans was referring to *G. segmentata* Kosterm. (Kostermans, 1956) which was collected from South Sulawesi. This species has fleshy fruits that shrink when dried causing the fruits to split into many segments.

*Septogarcinia sumbawaensis* was informally included in *Garcinia* by Corner (1976), Jones (1980), and Stevens (2007) based on a suite of morphological characters. Corner (1976) noted that the flesh covering the seeds is typical of *Garcinia*, Jones (1980) found that the pollen is similar to that of *Garcinia* species in Section *Brindonia*, and Stevens (2007) assessed inflorescence characters and concluded that *Septogarcinia* could not be separated from *Garcinia.* Despite the morphological evidence none of these authors formally transferred *Septogarcinia* to *Garcinia.*

The morphological analysis of *Garcinia* by Ruhfel *et al*. (2013) placed *Septogarcinia* sister to *G. morella* (Gaertn.) Desr. but with weak support. Medellin-Zabala & Marinho (2015) followed previous studies of *Garcinia,* mainly by Sweeney (2008) and Ruhfel *et al*. (2013) and undertook a morphological study using the isotype of *S. sumbawaensis*. On the basis of these studies, Medellín-Zabala and Marinho transferred *S. sumbawaensis* to *Garcinia* as *G. sumbawaensis* (Kosterm.) Medellín-Zab. & L.Marinho (Medellín-Zabala & Marinho, 2015). However, Nazre (2018) considered that *G. sumbawaensis* and *G. sumbawensis* Lauterb. were synonyms. Turner & Jennings (2021) disagreed and provided clear morphological evidence that *G. sumbawaensis* (Kosterm.) Medellín-Zab. & L.Marinho and *G. sumbawensis* Lauterb. are distinct taxa. Further they considered *G. sumbawaensis* (Kosterm.) Medellín-Zab. & L. Marinho to be a later homonym of *G. sumbawensis* Lauterb. and erected the replacement name *G. septogarcinia* I.M. Turner & L.V.S. Jenn. for *G. sumbawaensis*. Although POWO does not accept this, we do and use the name *G. septogarcinia* henceforth.

Despite *S. sumbawaensis* having been transferred to *Garcinia*, its taxonomic status has not been tested against a molecular dataset and its relationships and position within *Garcinia* remains unresolved. In this study we use a phylogenetic analysis of molecular and morphological data to evaluate the taxonomic status of *Septogarcinia*, in particular its position in relation to *Garcinia.*

## MATERIALS AND METHODS

### Molecular Analysis

#### DNA extraction, ITS amplification and sequencing

Total genomic DNA was extracted from silica gel-dried leaf material or herbarium specimens of 75 taxa using the DNeasy Kit (Qiagen, Germany). Extractions were performed according to the manufacturer's instructions, with a minor modification: at the second wash using AW2 buffer samples were centrifuged at 13,000 rpm instead of 14,000 rpm. The ITS regions were amplified by polymerase chain reaction (PCR) using forward primer ITS-I (Urbatsch *et al*., 2000) and reverse primer ITS4 (White *et al*., 1990) on a Bio-Rad TM-100 Thermal Cycler (BIO-RAD, Hercules, California, USA). Removal of unincorporated primers and degradation of unincorporated nucleotides from PCR products was done using the FastAP kit (Thermo Fisher, California, USA) following the manufacturer's instructions. The dried templates were sequenced at the Australian Genome Research Facility (AGRF, Brisbane) by capillary electrophoresis on AB3730xl instruments (Life Technologies, California, USA).

Herbarium vouchers were deposited in the Australian Tropical Herbarium (CNS) except for two samples from India (*G. indica* and *G. talbottii*) which were deposited in Krishna Mahavidyalaya Herbarium, Shivaji University, Maharastra, India (SUK), and one sample of *Garcinia* sp. (Batam Island) that was deposited in Herbarium Bogoriense (BO). GenBank accession numbers for all sequences are provided in Table 1.

#### Alignment and phylogenetic analysis

The sequences were aligned using Multiple Alignment using Fast Fourier Transform (MAFFT, Katoh *et al*., 2002; Katoh & Standley, 2013) in Geneious® version 9.1.6 software (Kearse *et al.*, 2012). Phylogenetic analyses were undertaken using Bayesian Inference (BI) in Geneious® version 9.1.6. (Kearse *et al*., 2012) with the following settings: General-Time-Reversible (GTR) substitution model, rate variation = gamma, gamma categories = 4, chain length = 1,000,000, heated chains = 4, heated chain temperature = 0.2, subsampling fre-


Fig. 1. Map of Indonesia and the position of Sumbawa Island as indicated by the arrow and the box (Google Earth Data SIO, NOAA, U.S. Navy, NGA, GEBCO Landsat/Copernicus IBCAO U.S.).

Fig. 2. Map of Nusa Tenggara Barat Province showing the location of Sumbawa Island (Google Earth Data SIO, NOAA, U.S. Navy, NGA, GEBCO Landsat/Copernicus IBCAO U.S. 8°08’15” S, 116°46’27” E).

Fig. 3. The dehiscent fruit of *G. septogarcinia* at maturity, split from the base as indicated by the arrow. Photo by A. Aris.

Table 1. Details of samples used in the molecular study.

| Species | Origin | Collector No. | Garden/Herbarium Accession No. | Sample Source |
|---|---|---|---|---|
| *Clusia major* L. | C. America | R. Sari RI1403 | - | FBG |
| *Cratoxylum sumatranum* (Jack) Blume | Jambi, Sumatra, Indonesia | R. Sari RI1437 | XIX.F.116 | BBG |
| *Mammea siamensis* T.Anderson | Maluku, Indonesia | R. Sari RI1443 | VII.D.85 | BBG |
| *Pentadesma butyracea* Sabine | Africa | R. Sari RI1444 | VI.C.246a | BBG |
| *Garcinia balica* Miq. | Lesser Sunda Island, Indonesia | R. Sari RI1438 | XIX.N.23 | BBG |
| *G. bancana* Miq. | Bangka Belitung, Sumatra, Indonesia | R. Sari RI1366 | VI.C.392b | BBG |
| *G. binucao* (Blanco) Choisy | Philippines | R. Sari RI1378 | VI.C.161 | BBG |
| *G. brassii* C.T.White | N. Queensland, Australia | S.J. Worboys 825 | CNS-130985 | ATH |
| *G. celebica* L. | E. Java, Indonesia | R. Sari RI1410 | XII.G.2 | PBG |
| *G. cymosa* (K.Schum.) I.M.Turner & P.F.Stevens | New Guinea | R. Sari RI1394 | XXIV.A.92 | BBG |
| *G. daedalanthera* Pierre | N. Sulawesi, Indonesia | R. Sari RI1369 | VI.C.429 | BBG |
| *G. dulcis* (Roxb.) Kurz. | Kai Island, Maluku, Indonesia | R. Sari RI1404 | XVII.K.II.40 | PBG |
| *G. echinocarpa* Thwaites | Sri Lanka | R. Sari RI1380 | VI.A.36 | BBG |
| *G. fruticosa* Lauterb. | S. Papua, Indonesia | R. Sari RI1395 | VI.C.217 | BBG |
| *G. graminea* Kosterm. | Papua New Guinea | S.A. James | SAJ1369 | ATH |
| *G. gummi-gutta* (L.) N. Robson var. *gummi-gutta* (yellow fruit) | India | R. Sari RI1425 | 20050100 | SW |
| *G. gummi-gutta* (L.) N.Robson var. *gummi-gutta* (red fruit) | India | R. Sari RI1424 | 20050099 | SW |
| *G. hombroniana* L. | Belitung Island, Sumatra, Indonesia | R. Sari RI1370 | IX.D.286 | BBG |
| *G. humilis* (Vahl.) C.D.Adams | S. America | R. Sari RI1438 | - | PU |
| *G. intermedia* (Pittier) Hammel | S. America | R. Sari RI1433 | - | SW |
| *G. jensenii* W.E.Cooper | N. Queensland, Australia | W.E. Cooper | CNS-G01401 | ATH |
| *G. kola* Heckel | C. Africa | R. Sari RI1428 | 20100018 | SW |
| *G. kydia* Roxb. | Kalimantan, Indonesia | R. Sari RI1393 | VII.D.84 | BBG |
| *G. lateriflora* Blume | Java, Indonesia | R. Sari RI1442 | IX.D.278B | BBG |
| *G. latissima* Miq. | S. Papua, Indonesia | R. Sari RI1381 | VI.C.338 | BBG |
| *G. leggeae* W.E.Cooper | N. Queensland, Australia | W.E. Cooper | CNS-G01399 | ATH |
| *G. linii* C.E.Chang | Taiwan | W.H. Hu | 4503 | TBG |
| *G. livingstonei* Anderson | Tropical Africa | R. Sari RI1382 | VI.A.30 | BBG |
| *G. loureiri* Pierre | Vietnam | R. Sari RI1397 | VI.C.60 | BBG |

| | | | | |
|---|---|---|---|---|
| *G. malaccensis* Hook.f. ex T.Anderson | Jambi, Sumatra, Indonesia | R. Sari RI1371 | 2012000038 | BBG |
| *G. mangostana* L. | Java, Indonesia | R. Sari RI1372 | XIII.E.10 | BBG |
| *G. megaphylla* Verdc. | Brazil, S. America | R. Sari RI1386 | VI.A.45 | BBG |
| *G. mestonii* F.M.Bailey | N. Queensland, Australia | W.E. Cooper | CNS-G01403 | ATH |
| *G. multiflora* Champ. ex Benth. | Taiwan | S.H. Wu | - | TBG |
| *G. nervosa* Miq. | Jambi, Sumatra, Indonesia | R. Sari RI1374 | IX.D.269 | BBG |
| *G. nigrolineata* Planch. | Bangka Island, S. Sumatra, Indonesia | R. Sari RI1398 | VI.C.37 | BBG |
| *G. picrorhiza* Miq. | Ambon Island, Maluku, Indonesia | R. Sari RI1405 | VI.A.27 | BBG |
| *G. porrecta* Wall. | W. Java, Indonesia | R. Sari RI1388 | VI.A.79 | BBG |
| *G. porrecta* Wall. var. *schizogyna* Boerl. | Ambon Island, Maluku, Indonesia | R. Sari RI1389 | VI.A.50 | BBG |
| *G. prainiana* King | Malaysia | R. Sari RI1432 | - | SW |
| *G. rigida* Miq. | N. Sulawesi, Indonesia | R. Sari RI1375 | XXIII.A.221 | BBG |
| *G. russellii* W.E.Cooper | N. Queensland, Australia | W.E. Cooper | CNS-G196 | ATH |
| *G. schomburgkiana* Pierre | Thailand | R. Sari RI1431 | 20050097 | SW |
| *G. septogarcinia* I.M. Turner & L.V.S. Jenn. | Batudulang, Sumbawa Besar, NTB, Indonesia | R. Sari RI1461 | - | BSB |
| *G. sizygiifolia* Pierre | Sarawak, Malaysia | R. Sari RI1399 | VI.C.325 | BBG |
| *Garcinia* sp. (Batam Is.) | Batam Island, Indonesia | I.P. Astuti | - | BI |
| *Garcinia* sp. (Kai Is.) | Kai Island, Maluku, Indonesia | R. Sari RI1455 | XVII.K.II.10 | PBG |
| *Garcinia* sp. (E. Java) | E. Java, Indonesia | R. Sari RI1421 | XVII.J.II.27-a | PBG |
| *Garcinia* sp. (Halmahera Is.) | Halmahera Island, Maluku, Indonesia | R. Sari RI1415 | XVII.K.II.39 | PBG |
| *Garcinia* sp. (Buru Is.) | Buru Island, Maluku, Indonesia | R. Sari RI1414 | XVII.J.II.16 | PBG |
| *Garcinia* sp. (N. Sumatra) | N. Sumatra, Indonesia | R. Sari RI1413 | IX.D.299 | BBG |
| *Garcinia* sp. (C. Kalimantan) | C. Kalimantan, Indonesia | R. Sari RI1422 | XVII.J.II.33-ab | PBG |
| *Garcinia* sp. (E. Kalimantan) | E. Kalimantan, Indonesia | R. Sari RI1446 | XVII.J.II.26 | PBG |
| *Garcinia* sp. (S. Morotai) | S. Morotai, N. Maluku, Indonesia | R. Sari RI1478 | XVII.J.II.14-a | PBG |
| *Garcinia* sp. (Seram Is.) | Seram Island, Maluku, Indonesia | R. Sari RI1450 | VI.C.379 | BBG |
| *Garcinia* sp. (Bukit Lawang) | Bukit Lawang, N. Sumatra, Indonesia | R. Sari RI1477 | - | MRT |
| *Garcinia* sp. (Bengkulu) | Bengkulu, Sumatra, Indonesia | R. Sari RI1447 | XIX.F.102 | BBG |
| *Garcinia* sp. (Batudulang) | Batudulang, Sumbawa Besar, NTB, Indonesia | R. Sari RI1462 | - | BSB |
| *Garcinia* sp. 1 (Maluku) | Maluku, Indonesia | R. Sari RI1368 | XVII.K.II.31 | PBG |
| *Garcinia* sp. 2 (Maluku) | Maluku, Indonesia | R. Sari RI1423 | XVII.K.II.35-ab | PBG |
| *Garcinia* sp. 1 (Papua) | Papua, Indonesia | R. Sari RI1376 | IX.D.283 | BBG |
| *Garcinia* sp. 2 (Papua) | Papua, Indonesia | R. Sari RI1377 | IX.D.295 | BBG |

| | | | | |
|---|---|---|---|---|
| *Garcini*a sp. 3 (Papua) | Papua, Indonesia | R. Sari RI1406 | IX.D.295a | BBG |
| *Garcinia* sp. 4 (Papua) | Papua, Indonesia | R. Sari RI1419 | IX.D.302 | BBG |
| *Garcinia* sp. 5 (Papua) | Papua, Indonesia | R. Sari RI1449 | IX.D.303 | BBG |
| *Garcinia* sp. 6 (Papua) | Papua, Indonesia | R. Sari RI1445 | XVII.J.II.24 | PBG |
| *Garcinia* sp. 1 (S. Sulawesi) | S. Sulawesi, Indonesia | R. Sari RI1408 | IX.D.287a | BBG |
| *Garcinia* sp. 2 (S. Sulawesi) | S. Sulawesi, Indonesia | R. Sari RI1420 | IX.D.274 | BBG |
| *Garcinia* sp. 1 (W. Sumatra) | W. Sumatra, Indonesia | R. Sari RI1448 | IX.D.294 | BBG |
| *Garcinia* sp. 2 (W. Sumatra) | W. Sumatra, Indonesia | R. Sari RI1451 | VI.C.463 | BBG |
| *G. subelliptica* Merr. | Taiwan | W.H. Hu | 4502 | TBG |
| *G. tetrandra* Pierre | Philippines | R. Sari RI1391 | VI.C.108 | BBG |
| *G. warrenii* F.Muell. | N. Queensland, Australia | D. Warmington | - | CNS |
| *G. xanthochymus* Hook.f. ex T.Anderson | India | R. Sari RI1392 | VI.A.52 | BBG |
| *G. zichii* W.E.Cooper | N. Queensland, Australia | W.E. Cooper | CNS 138512.1 | ATH |

quency = 1,000, burn-in = 200,000 and random seed = 4,654. *Clusia rosea* was used as the outgroup.

**Morphological Analysis**

**Pollen**

Sampling of taxa for the pollen study was based on the availability of male flowers. Male flowers of 72 species were obtained fresh, or from herbarium or spirit material. Fresh samples were air dried and spirit samples oven dried at 40ºC in tea bags prior to preparation for microscopy. The presence of mature, fully formed pollen grains was confirmed in 32 of the 72 samples using light microscopy (Wild M7 S, Wild Heerbrugg, Pty. Limited, Australia; Nikon Eclipse E100, Nikon Corporation, Tokyo, Japan) (Table 1). Pollen from these 32 samples was mounted on IA023 carbon tabs (PSA, Thuringowa, Australia) on aluminium stubs, coated with gold using a SPI Module Sputter Coater (SPI, West Chester, Pennsylvania, USA) then observed and imaged using a JEOL JSM 6300 (CAE, Austin, Texas, USA) scanning electron microscope operating at 5 kV with Semaphore imaging software.

**Fruit**

The scoring of fruit morphological characters was carried out using the naked eye on fresh material in the field. Measurements were made using a ruler and calipers.

**Morphological character scoring**

Variation in pollen and fruit morphology was scored as four characters as follows. The morphological character matrix is provided as Table 2.

1. Pollen ornamentation. Six states were observed and scored: psilate (0), surface smooth; scabrate (1), sculptural elements of varying shapes, <1µm in diameter; verrucate (2), sculptural elements wart-like (usually broader than high and never constricted at the base), >1 µm in diameter; echinate (3), sculptural elements pointed, >1 µm high; gemmate (4) sculptural elements the same width as height and constricted at their bases, >1 µm high; pilate (5) sculptural elements rod-like with swollen or knob-like heads (capita), >1 µm high (Moore *et al*., 1991). All character states are illustrated in Fig. 4 (A–F).
2. Pollen aperture. Seven states were observed and scored: monocolpate (0), having a single elongated aperture (colpus); tricolpate (1); tetracolpate (2); tetraporate (3), having four pore-like apertures; tetra-pentacolpate (4); penta-hexacolpate (5); hexacolpate (6) (Moore *et al*., 1991). All character states are illustrated in Fig. 5 (A–G).
3. Fruit segmentation: non-segmented (0), exocarp smooth, without grooves; segmented (1), fruit segmented or grooved.
4. Fruit dehiscence: indehiscent (0); dehiscent (1).

Of the 32 taxa included in the pollen study, 14 were included in the molecular analysis. Ancestral state reconstruction of the pollen and fruit characters was undertaken using maximum likelihood in Mesquite ver. 3.40 (Maddison & Maddison, 2018) using the Bayesian tree from the molecular analysis pruned to include only those taxa for which the morphological data were scored (*i.e.* taxa in Table 1) and the following settings: current probability models, max. number of mappings per character per tree = 50. The proportional likelihood of each character state is indicated for each node using pie graphs.

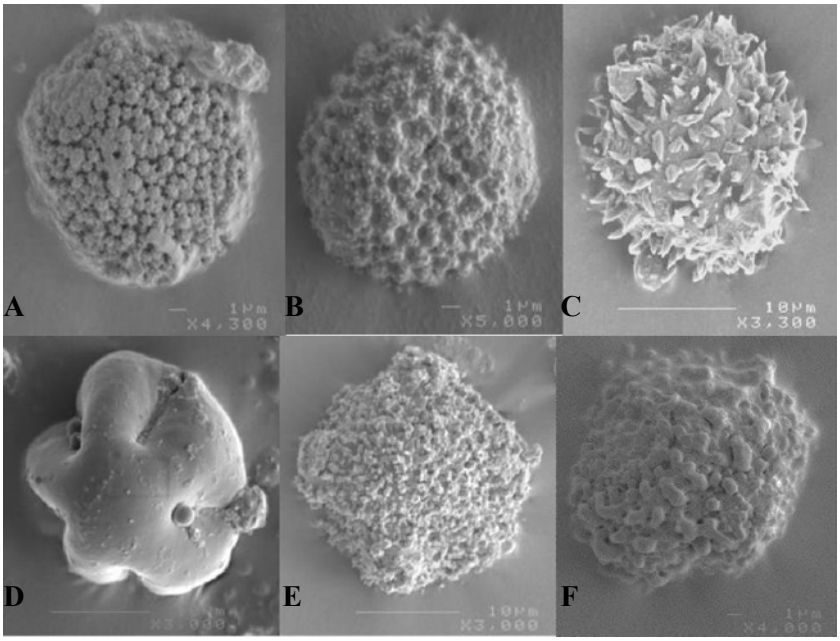


Fig. 4. Pollen ornamentation in *Garcinia* observed in this study. A. Gemmate (*G. binucao*). B. Pilate (*G. griffithii*). C. Echinate (*G. latissima*). D. Psilate (*G. nervosa*). E. Scabrate (*G. porrecta*) and F. Verrucate (*G. hombroniana*). Micrographs by R. Sari.

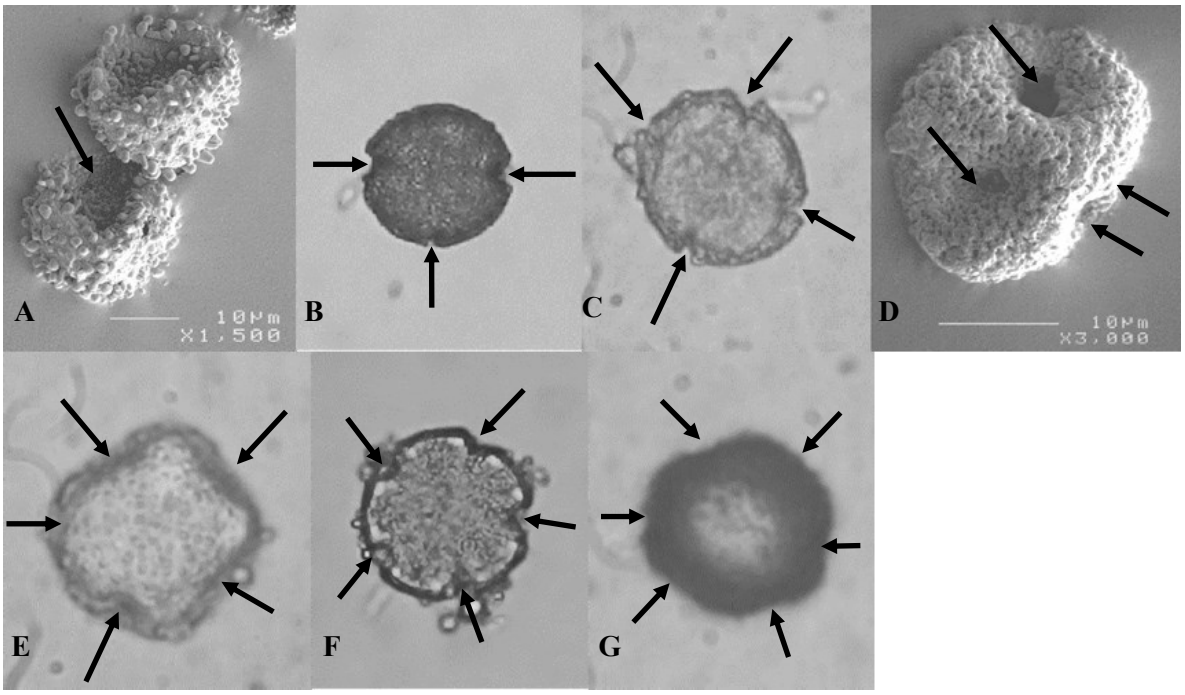


Fig. 5. Pollen apertures observed in this study as indicated by arrows. A. Monocolpate (*G. zichii*, SEM). B. Tricolpate (*G. griffithii*, light microscope 100×). C. Tetracolpate (*G. binucao*, light microscope 100×). D. Tetraporate (*Garcinia* sp. (Batudulang, Sumbawa, SEM). E. Tetra-pentacolpate (*G. malaccensis*, light microscope 100×). F. Penta-hexacolpate (*G. nervosa*, light microscope 40×). G. Hexacolpate (*G. warrenii*, light microscope 100×). Micrographs by R. Sari.

## RESULTS

### Molecular Analysis

The aligned ITS sequences generated in this study ranged in length from 761–1,808 bp, with 87.2% pairwise identity. Bayesian analysis of these data resolved two major lineages with maximum support among the ingroup: (1) *Cratoxylum* + *Mammea* (posterior probability, PP, 1.0); and (2) *Garcinia* + *Pentadesma* (PP 1.0) (Fig. 6). The position of *Pentadesma* is unresolved at the base of *Garcinia*, potentially rendering *Garcinia* non-monophyletic. Within the *Garcinia* clade 11 subclades were resolved, although some lineages were unresolved on polytomies such as *Garcinia* sp. (Halmahera Island, Indonesia), *Garcinia* sp. 6 (Papua, Indonesia), *G. echinocarpa* (Sri Lanka), *G. mestonii* (N. Queensland, Australia), *G. bancana* (Bangka Belitung, Sumatra, Indonesia) and *Garcinia* sp. (Seram, Maluku, Indonesia).

*Garcinia septogarcinia* clustered within a maximally supported clade comprising species assigned to Section *Brindonia* according to Jones (1980). This clade includes 10 samples representing described species, and four samples of unknown species identity. Together, these 14 samples represent a wide geographical range, including Australia (*G. leggae*, *G. mestonii*), India (2 samples of *G. gummi-gutta* var. *gummi-gutta*, representing red-fruited and yellow-fruited forms), Indonesia (*G. bancana, G. nigrolineata*, *Garcinia* sp. (Bengkulu), *Garcinia* sp. (Bukit Lawang), *Garcinia* sp. 1 (W. Sumatra), *Garcinia* sp. (Seram Is.), Malaysia (*G. sizygiifolia*, from Sarawak, Borneo), Philippines (*G. binucao*, *G. tetrandra*, the latter also widely distributed in Sulawesi), and Vietnam (*G. loureiri*). Within this clade *G. septogarcinia* is sister to *G. sizygiifolia.*

### Morphological Analysis

The pollen of *G. septogarcinia* is tetracolpate with pilate ornamentation. Among the 21 taxa examined in this study this combination of character states is found in two other taxa: *G. mestoni* and *G. gummi-gutta*. Tetracolpate pollen is also found in *G. binucao*, which has scabrate ornamentation. Pilate pollen ornamentation is found in three other taxa: *G. echinocarpa*, *Garcinia* sp. (Maluku), and *Garcinia* sp. (Batudulang, Sumbawa Besar) (Table 3).

The ancestral state reconstruction of pollen ornamentation is shown in Fig. 7. Three character states are autopomorphic, namely: state 1, psilate (*G. nervosa*); 2, verrucate (*G. hombroniana*); and 4, gemmate (*G. zichii*). The other character states are spread among the clades and are not monophyletic. In this analysis, the pollen ornamentation of *S. sumbawaensis* (5, pilate) is resolved as ancestral for the Section *Brindonia* clade which includes *G. septogarcinia* (marked with an asterisk in Fig. 7) with a high proportional likelihood of 0.97525734 (other proportional likelihoods are state 0: 0.00123573; 1: 0.01972518; 2: 0.00124 669; 3: 0.0013019; 4: 0.00123317). Within the *Brindonia* clade, scabrate pollen is resolved as ancestral for a subclade comprising *G. leggae, G. binucao* and *G. tetrandra*.

Of the pollen aperture character states only state 6, hexacolpate, occurred in a single clade (Fig. 8). The proportional likelihood is 0:0.00081357; 1: 0.01300851; 2: 0.9829738*; 3: 0.0007924; 4: 0.00 078478; 5: 0.00079117; 6: 0.00083577. Cha-racter states 3, tetraporate and 4, tetra-pentacolpate are each autapomorphic, for *Garcinia* sp. (Sumbawa, NTB, Indonesia) and *G. malaccensis* respectively, which are sisters in the tree. All other character states have evolved more than once and are shared among the taxa. The pollen aperture of *G. septogarcinia* is categorized as state 2, tetracolpate.

"Fruit segmented" is found only in sister taxa *G. septogarcinia* and *G. gummi-gutta* var. *gummi-gutta* (Fig. 9). "Fruit dehiscent" was only found in *G. septogarcinia* (Table 2, Fig. 10) in this study but it might occur in some other species that have not been included here such as *G. segmentata* Kosterm. which Kostermans considered a close relative of *S. sumbawaensis*.

### Variation within *G. septogarcinia*

During field work undertaken during this study the first author discovered two variants of *G. septogarcinia* distinguished by characters of the fruit (colour, shape, and texture of the stigma) and leaves (pedicel length and colour, and lamina dimensions) (Table 4, Fig. 11). Flowers of variant 2 were not available so floral morphology could not be compared. Further field, herbarium and laboratory studies are required to determine the appropriate taxonomic status of these variants.

## DISCUSSION

The morphological and molecular analyses confirmed previous morphological studies that showed *S. sumbawaensis* belongs in the genus *Garcinia*. In our molecular analysis, ITS was used as previous studies showed it is an appropriate marker to analyse phylogenetic relationships in *Garcinia* (Sari, 2000; Nazre *et al*., 2007; Sweeney, 2008). Most specimens were successfully amplified in the PCR, but several failed such as *G. atroviridis* and *G. gibbsieae*. This may be due to poor primer match or the use of herbarium specimens for DNA extraction in a few species. For the next study it is recommended to design additional primers for *Garcinia* and to obtain fresh or silica gel-dried samples for the remaining taxa where possible. We also attempted to locate material of *G. sumbawensis* Lauterb. in a few herbaria but it appears that the only material of *G. sumbawensis* is in the Wroclaw Herbarium in Poland (see Turner & Jen-


Fig. 6. A strict consensus tree of 71 samples of *Garcinia* based on Bayesian analysis of ITS sequence data showing *G. septogarcinia* (in red) nested within *Garcinia* Section *Brindonia*. Sections *Brindonia* and *Garcinia* are marked by the two lines. Numbers associated with nodes are posterior probability values.

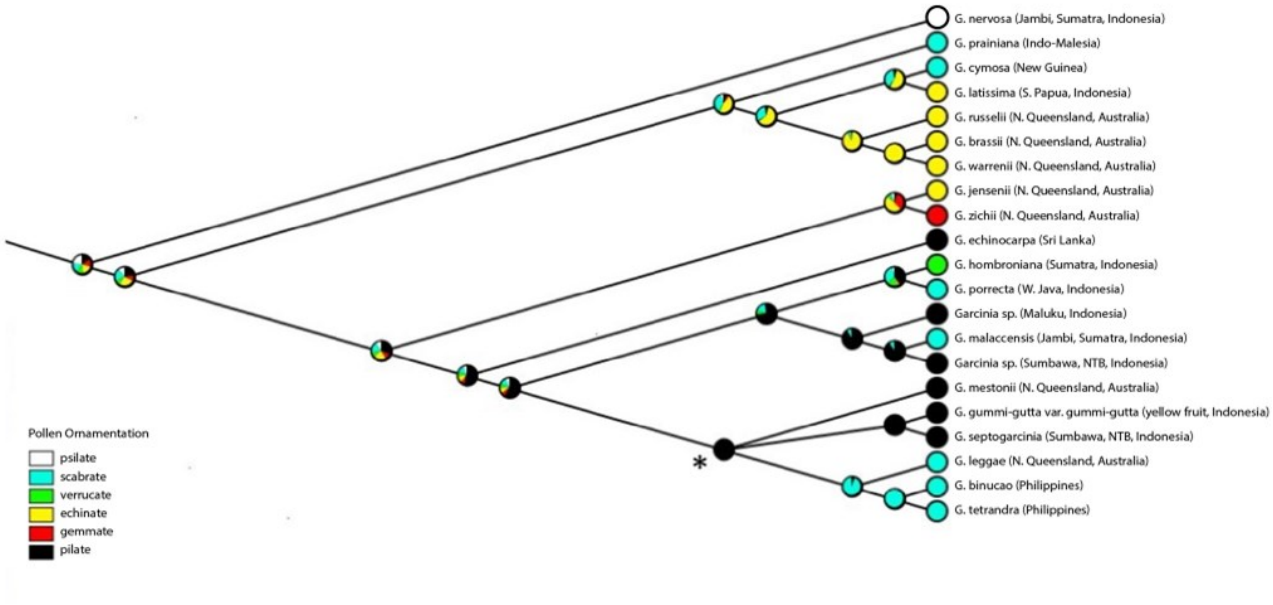


Fig. 7. Ancestral state reconstruction analysis of pollen ornamentation in 21 species of G*arcinia.*

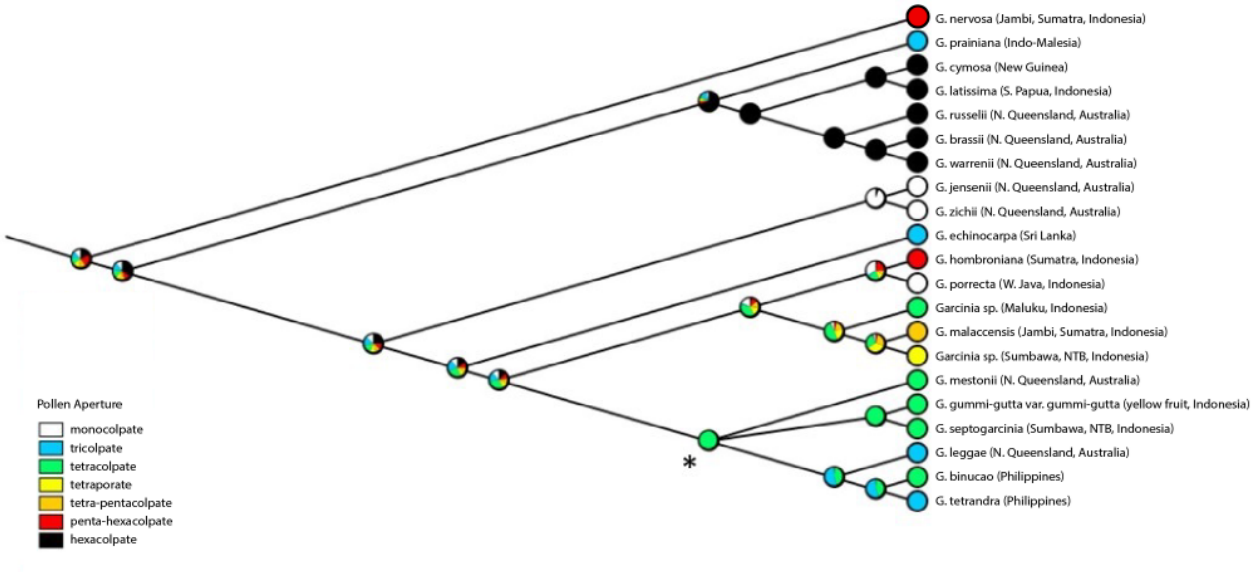


Fig. 8. Ancestral state reconstruction analysis of pollen aperture in 21 species of *Garcinia.*

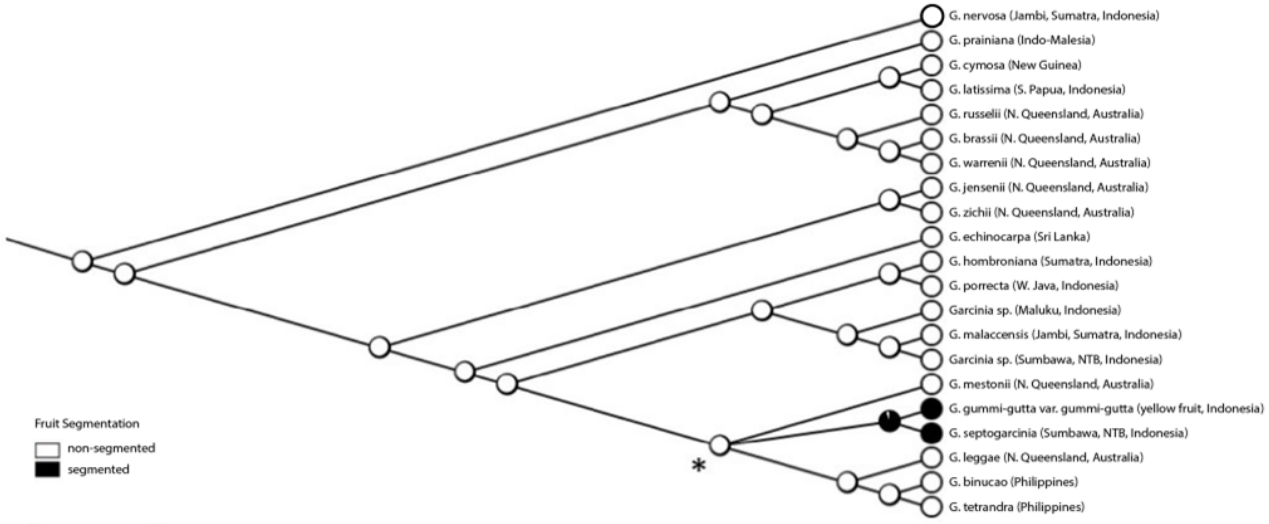


Fig. 9. Ancestral reconstruction of segmented fruit of 21 species of *Garcinia.*

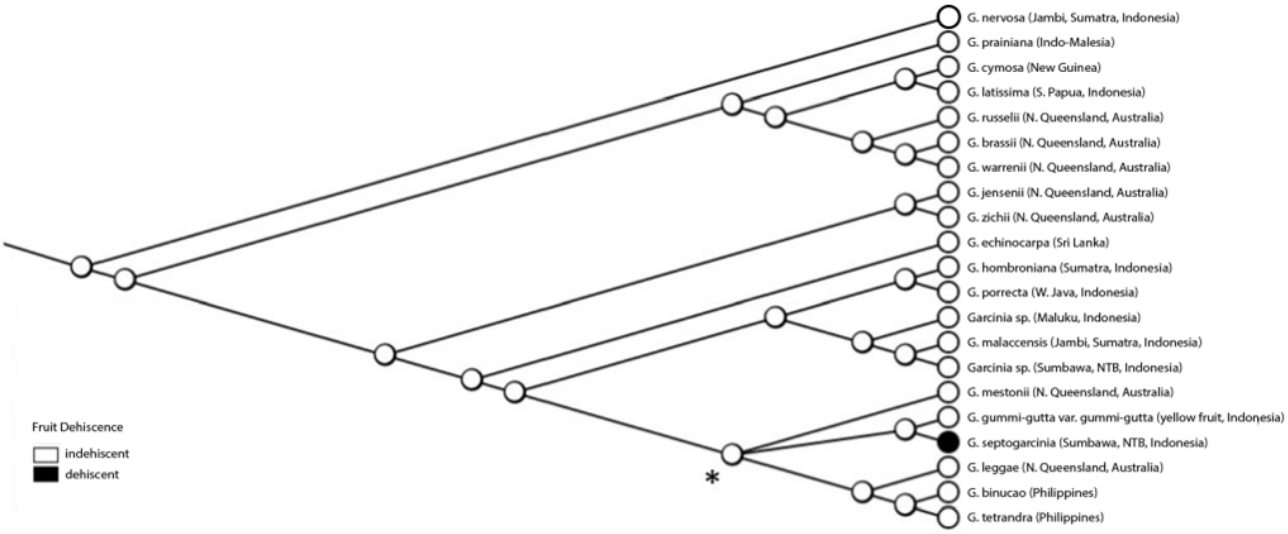


Fig. 10. Ancestral state reconstruction analysis of fruit dehiscence in 21 species of *Garcinia.*

Table 2. Morphological character matrix. The source of material for pollen were from herbarium specimen (H), spirit collections (S) or fresh (F). Fruit characters were observed on fresh fruit or taken from the literature.

| Species | Pollen ornamentation | Pollen apertures | Fruit segmentation | Fruit dehiscence | Pollen source material |
|---|---|---|---|---|---|
| *Garcinia binucao* Choisy | 1 | 2 | 0 | 0 | H |
| *G. brassii* C.T.White | 3 | 6 | 0 | 0 | S |
| *G. cymosa* (K.Schum.) I.M.Turner & P.F.Stevens | 1 | 6 | 0 | 0 | F |
| *G. echinocarpa* Thwaites | 5 | 1 | 0 | 0 | F |
| *G. gummi-gutta* (L.) N.Robson var. *gummi-gutta* (yellow fruit) | 5 | 2 | 1 | 0 | F |
| *G. hombroniana* Pierre | 2 | 5 | 0 | 0 | H |
| *G. jensenii* W.E.Cooper | 3 | 0 | 0 | 0 | H |
| *G. latissima* Miq. | 3 | 6 | 0 | 0 | H |
| *G. leggeae* W.E.Cooper | 1 | 1 | 0 | 0 | S |
| *G. malaccensis* Hook.f. | 1 | 4 | 0 | 0 | F |
| *G. mestoni* F.M.Bailey | 5 | 2 | 0 | 0 | S |
| *G. nervosa* (Miq.) Miq. | 0 | 5 | 0 | 0 | H |
| *G. porrecta* Laness. | 1 | 0 | 0 | 0 | F |
| *G. prainiana* King | 1 | 1 | 0 | 0 | F |
| *G. russellii* W.E.Cooper | 3 | 6 | 0 | 0 | S |
| *Garcinia* sp. (Batudulang, Sumbawa Besar) | 5 | 3 | 0 | 0 | F |
| *Garcinia* sp. (Maluku) | 5 | 2 | 0 | 0 | F |
| *G. septogarcinia* I.M.Turner & L.V.S.Jenn. | 5 | 2 | 1 | 1 | F |
| *G. tetrandra* Pierre | 1 | 1 | 0 | 0 | F |
| *G. warrenii* F.Muell. | 3 | 6 | 0 | 0 | H |
| *G. zichii* W.E.Cooper | 4 | 0 | 0 | 0 | H |

nings, 2021). The condition of the specimen seems inadequate to obtain DNA suitable for Sanger sequencing, however future studies using phylogenomic approaches may have a greater chance of successfully obtaining sequence data.

The pollen characters in this study support Jones' (1980) findings of similarity between *G. septogarcinia* (as *Septogarcinia sumbawaensis*) and some *Garcinia* species. Despite the limited availability of male flowers used in this study, the pollen of *G. septogarcinia* is most similar to species in Section *Brindonia* (Jones, 1980). However, reticulate ornamentation does not occur only in Section *Brindonia* but also in species of Section *Garcinia*. For instance, *Garcinia* sp. from Batudulang, Sumbawa, which obviously belongs to Section *Garcinia*, has pollen similar to that of *G. septogarcinia*. Pollen ornamentation in *G. septogarcinia* is similar to six other species included in this study, three of them in Section *Brindonia*

Table 3. The pollen ornamentation and aperture of 21 *Garcinia* species.

| Species | Ornamentation | Apertures |
|---|---|---|
| *G. binucao* | Scabrate | Tetracolpate |
| *G. brassii* | Echinate | Hexacolpate |
| *G. cymosa* | Scabrate | Hexacolpate |
| *G. echinocarpa* | Pilate | Tricolpate |
| *G. gummi-gutta* var. *gummi-gutta* var. fruit yellow | Pilate | Tetracolpate |
| *G. hombroniana* | Verrucate | Penta-hexacolpate |
| *G. jensenii* | Echinate | Monocolpate |
| *G. latissimi* | Echinate | Hexacolpate |
| *G. leggeae* | Scabrate | Tricolpate |
| *G. malaccensis* | Scabrate | Tetra-pentacolpate |
| *G. mestonii* | Pilate | Tetracolpate |
| *G. nervosa* | Psilate | Penta-hexacolpate |
| *G. porrecta* | Scabrate | Monocolpate |
| *G. prainiana* | Gemmate | Tricolpate |
| *G. russelii* | Echinate | Hexacolpate |
| *G. tetrandra* | Scabrate | Tricolpate |
| *G. warrenii* | Echinate | Hexacolpate |
| *G. zichii* | Gemmate | Monocolpate |
| *Garcinia* sp. (Batudulang, Sumbawa Besar) | Pilate | Tetraporate |
| *Garcinia* sp. 1 (Maluku) | Pilate | Tetracolporate |
| *G. septogarcinia* | Pilate | Tetracolpate |

(see also Jones, 1980). However, the similarity between pollen characters of *G. septogarcinia* and *Garcinia* sp. (Batudulang, Sumbawa) revealed that pollen characteristics in *Garcinia* might have some exceptions as infrageneric grouping delimitation characters. One of those, *Garcinia* sp.1 (Maluku), was provisionally named *G. cylindrocarpa* by Kostermans in the Type Specimen Collection in the Herbarium Bogoriense but this name has not yet been published. Further studies of pollen in *Garcinia* will likely yield additional morphological characters that support the infrageneric groupings.

SEM is a suitable technique to analyse the pollen that provides detailed images of the outer morphological characters. Despite some anthers having been contaminated with fungi, there was plentiful pollen. Another challenge was that the pollen from the herbarium specimens, particularly the immature ones, tended to be brittle and easily damaged in the coating process rendering some of the features difficult to observe as has been reported previously (Sari, 2000). In this study, the flowers of *G. septogarcinia* were dried at room temperature from fresh flowers and the pollen was well-coated, and using mature pollen resulted in a positive outcome for pollen analysis using SEM.

Examination of the morphological characters of *G. septogarcinia*, and the ancestral state reconstruction analyses, are consistent with a placement of *Septogarcinia* within *Garcinia. Garcinia septogarcinia* is most readily distinguished from other species in having dehiscent, segmented fruit.

Table 4. Characteristics of the green- and red-fruited variants of *G. septogarcinia.*

| Character | Variant 1 | Variant 2 |
|---|---|---|
| *Leaves* | | |
| Leaf blade length (cm) | 8.6–9.4 | 8.5–8.7 |
| Leaf blade width (cm) | 6.0–6.4 | 5.0–5.6 |
| Pedicel length (cm) | 2.0–2.3 | 3.0–3.1 |
| Pedicel colour | green | green with a red flush |
| *Fruit* | | |
| Young fruit colour | green | red |
| Mature fruit colour | yellowish green | red |
| Fruit shape | subglobular to subconical | subconical |
| Stigma surface texture | smooth | corrugated |

However, segmented indehiscent fruit occurs in some other species of *Garcinia* including in at least three OTU's in the same cluster as *G. septogarcinia*: *G. gummi-gutta* var. *gummi-gutta* (yellow fruited and red fruited forms) and *Garcinia* sp. (Bukit Lawang) (Fig. 12). Other species that have segmented fruit are *G. atroviridis* Griff. ex T.Anderson*, G. cowa* Roxb. ex Choisy and *G. griffithii* T.Anderson which are mainly distributed in SE Asia (Corner, 1952). Among these three species, dehiscent fruits occur only in *G. atroviridis*. Compared to *G. septogarcinia* the fruit of *G. atroviridis* is much bigger (*ca.* 7.9–10.5 cm diameter; Sari & Sutrisno, 2005), dehisces differently, and is green when young, ripening yellow. In *G. septogarcinia* the fruit dehisces septicidally from the base toward the top of the fruit (acropetally) and the seeds remain attached to the placenta when the segments fall (Kostermans, 1962), while in *G. atroviridis* the whole fruit drops to the ground when fully ripe causing the fruit to split septicidally into segments containing the seeds (David Warmington, Cairns pers. comm. 2018). Kostermans remarked that the splitting in *Septogarcinia* was a unique character that differentiated it from other *Garcinia.* Unfortunately, the DNA sample of *G. atroviridis* could not be amplified in this study therefore its relationships remain unknown.

Other morphological characters not analysed in the present study provide further insights into the relationships of *G. septogarcinia*. The yellow, sticky exudate of *G. septogarcinia* appears to be plesiomorphic in *Garcinia.* Sticky latex is a distinct character of the family Clusiaceae, and the latex glands and canals are found in all parts of the fruit (Stevens, 2007). The decussate arrangement of leaves and branches, the presence of two black dots (glands) at the base of the petiole, the petiole base that clasps the twig, and dioecy are all common characters of this genus (Sari, 2000; Cooper, 2013).

Both male and female flowers of *G. septogarcinia* are sessile, have four small bracts, and four sepals and petals. The sepals are green and in pairs, connected via a short tube. Four petals is the most common flower character in *Garcinia* (Stevens, 2007). Certain sections in *Garcinia* have three or five petals, but flowers of other taxa in the same cluster with *G. septogarcinia* in the phylogenetic tree, *G. bancana, G. gummi-gutta* var. *gummi-gutta* (yellow fruit), *G. leggeae*, *G. mestonii* and *G. nigrolineata,* have four sepals and petals (Fig. 13).

The results of the morphological and the molecular analyses indicate that *G. septogarcinia* strongly clusters within *Garcinia* Section *Brindonia*, and that the dehiscent fruit character is autapomorphic for this species. Therefore, the results strongly support the recent placement of *Septogarcinia* in the synonymy of *Garcinia* (Medellín-Zabala & Marinho, 2015).

**Comments of the status of *G. sumbawensis* Lauterb. versus *G. sumbawaensis* (Kosterm.) Medellín-Zab. & L.Marinho.**

In 1923 Lauterbach described *Garcinia sumbawensis* Lauterb. from the same island as *Septogarcinia sumbawaensis* Kosterm. and tentatively placed it in section *Discostigma* (Lauterbach, 1923, p. 26). Nazre (2018) considered *G. sumbawensis* Lauterb. to be conspecific with *G. sumbawaensis* (Kosterm.) Medellín-Zab. & L.Marinho (=*Septogarcinia sumbawaensis*). A comparison of the morphology of the two species based on the


Fig. 11. Comparison of fruits and leaves of *G. septogarcinia* variant 1 and variant 2. A. Fruits of variant 1. B. Fruits of variant 2. C. Polar view of fruits of variant 1 (left) and variant 2 (right). D. Lateral view of fruits of variant 1 (left) and variant 2 (right). E. Leaves of variant 1. F. leaves of variant 2. Photos by R. Sari.


Fig. 12. Fruits of *Garcinia.*. A. Young fruit of *G. septogarcinia.* B. Young fruit of *G. gummi-gutta* var. *gummi-gutta* (fruit yellow). C. Mature fruit *G. gummi-gutta* var. *gummi-gutta* (fruit red). D. Young fruit of *Garcinia* sp. (Bukit Lawang). Photos by R. Sari.

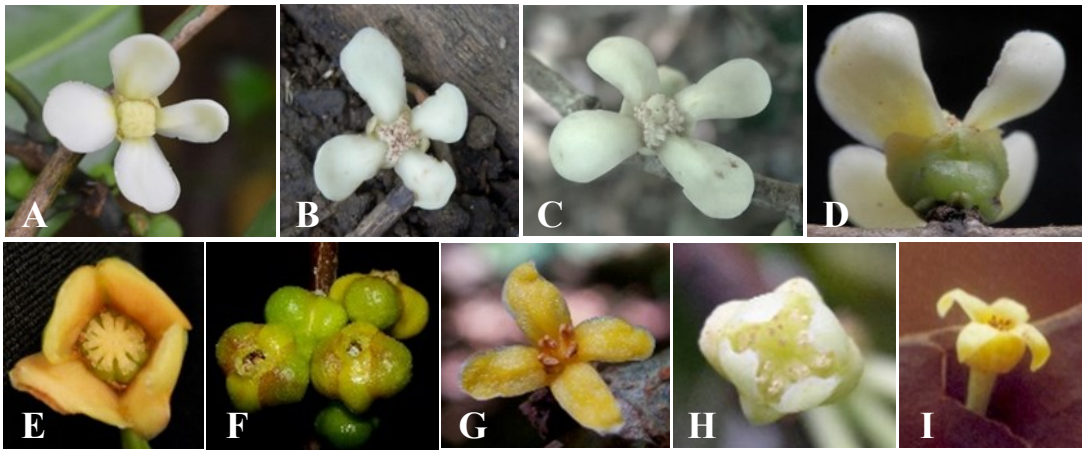


Fig. 13. Flowers of *Garcinia.* A. Female flower of *G. septogarcinia.* B & C. Two types of male flowers of *G. septogarcinia*. D. Bract, sepals, and petal of female flower of *G. septogarcinia*. E. Female flower of *G. gummi-gutta* var. *gummi-gutta* (yellow fruit). F. Male flower of *G. mestonii*. G. Male flower of *G. leggeae*. H. Male flower of *G. bancana* and I. Male flower of *G. nigrolineata.* Photos by G. Sankowsky, R. Jensen, R. Sari.

protologues and observations by the first author is presented in Table 5.

*Garcinia sumbawensis* was described as having leaves 8–10 × 3–4 cm, petiole 8–12 mm long, leaf tip 5 mm long (Lauterbach, 1923) whereas *G. sumbawaensis* is described by Kostermans (1962; as *Septogarcinia sumbawaensis*) as having leaves 7–13 × 5–6.5 cm, petiole 1.5–2 cm (15–20 mm), and apex obscurely acuminate. Based on these data the leaves of *G. sumbawensis* are smaller and have significantly shorter petioles with a distinct leaf tip compared with *G. sumbawaensis*.

According to Lauterbach (1923) the male flowers of *G. sumbawensis* are *ca.* 15 mm, the outer sepals 2 mm, the inner sepals 4 mm, the petals 8 mm long and 5 mm wide in the upper third, the androecium 3.5 mm and the anthers 0.7 mm. Kostermans (1962) stated that the male flower of *G. sumbawaensis* (as *Septogarcinia sumbawaensis*) has a light green calyx, sepals 4, pairwise opposite, persistent, obovate to rotundate, the lower ones 3–4 mm long, attached much lower than the 8 mm long upper ones. To generate comparable data, we examined male flowers on an herbarium specimen (BO.0120127, Herbarium Bogoriense) collected by Kostermans. The male flowers were observed to be 15 mm in diameter, the outer sepals 2.5 mm long, the inner sepals 4 mm long, the petals 8 mm long and 4.5 mm wide, the androecium 4 mm long and the anthers 1 mm long. Based on field observations the male flower diameter was 18–22 mm, the sepals 3.5–5 mm long, petals 6–7.2 mm long and 2.5–5 mm wide, stamen bundles 6.5 mm in diameter, and flowers fragrant. Female flowers of *G. sumbawensis* were not available for comparison.

The male flowers of *G. sumbawensis* seemed to be close to *G. terpnophylla* Thwaites of Sri Lanka which is considered as a transition to the sections *Mangostana* or *Peltostigma* (Lauterbach, 1923). Section *Mangostana* was recognised by Choisy (1824), Vesque (1894), Pierre (1883) and Engler (1925) and Section *Peltostigma* by the last three (not Choisy). The two sections were merged as Section I *Garcinia* by Jones (1980). This section is marked by having four stamen bundles in the male flower which do not occur in *G. sumbawaensis*. Section *Brindonia* is characterised by numerous

Table 5. Comparison of morphological characters in *Garcinia sumbawaensis* (Kosterm.) Medellín-Zab. & L.Marinho (= *G. septogarcinia*) and *G. sumbawensis* Lauterb. based on the species protologues, examination of specimen BO.0120127 (Herbarium Bogoriense) and field observations.

| Character | *Garcinia sumbawaensis* (Kosterm.) Medellín-Zab. & L.Marinho (= *G. septogarcinia*) | | | *Garcinia sumbawensis* Lauterb. |
|---|---|---|---|---|
| | Kostemans, 1962 | This study - BO.0120127 | This study – field observations | Lauterbach, 1923 |
| *Leaves* | | | | |
| Leaf length (cm) | 7–13 | 3.35-14 | 4.75-12.15 | 8–10 |
| Leaf width (cm) | 5–6.5 | 3.45-7.85 | 2.55-6.6 | 3–4 |
| Petiole length (mm) | 15–20 | 14-28 | 0.55-0.95 | 8–12 |
| Leaf tip | obscurely acuminate | rotundate, acute, acuminate | rotundate, acute, acuminate | 5 mm long |
| *Male flower* | | | | |
| Diameter (mm) | NA | 15 | 18–22 | 15 |
| Outer sepal length (mm) | 3–4 | 2.5 | 3.5 | 2 |
| Inner sepal length (mm) | 8 | 4 | 5 | 3 |
| Petal length (mm) | NA | 8 | 6–7.2 | 8 |
| Petal width (upper third) (mm) | NA | 4.5 | 2.5–5 | 5 |
| Androecium length (mm) | NA | 4 | NA | 3.5 |
| Anther length (mm) | NA | 1 | 0.5–0.6 | 0.7 |

free stamens (Sweeney, 2008). These two anther characters clearly can be differentiated.

Whereas Nazre (2018) considered *G. sumbawensis* and *G. sumbawaensis* to be conspecific, the comparison above indicates that they are distinct, as Turner & Jennings (2021) argued. Indeed, they are treated as such in the Plants of the World Online (POWO, 2023).

From the protologue, it seems that *G. sumbawensis* was found in Sambor, 1,300 m above sea level (Lauterbach, 1923). The name Sambor does not exist today on the island nor do authorised people recognise the name. It may possibly be Tambora, a mountain on Sumbawa Island which reaches a height of up to 4,300 m (https://en.wikipedia.org/wiki/Mount_Tambora). The highest peak in Batulanteh forest is 1,200 m above sea level which is lower than the altitude where *G. sumbawensis* was recorded. The precise location of Sambor remains unresolved, and it would be interesting to find out whether it was collected from the same location where *S. sumbawaensis* grows.

## CONCLUSION

The results of analyses of morphological and molecular data support the transfer of *Septogarcinia* to the synonymy of *Garcinia* (Medellín-Zabala & Marinho, 2015). Further, comparisons of published morphological data and new observations in the herbarium and field indicate that *G. sumbawensis* Lauterb. should be maintained as a species distinct from *G. sumbawaensis* Kosterm. (= *G. septogarcinia*), and revealed the existence of two variants of *G. septogarcinia* distinguished on leaf and fruit characters. Further research is required to determine the appropriate taxonomic status of these two variants.

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# ETHNOBOTANICAL ANALYSIS OF PHYTONYMS AND PLANT-RELATED GLOSSES MENTIONED IN BUJANGGA MANIK, A PRE-ISLAMIC SUNDANESE TEXT (15TH CENTURY JAVA, INDONESIA)

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**ABSTRACT**
MULYANTO, D., ISKANDAR, B. S., ISKANDAR, J., INDRAWARDANA, I. & AUFA, A. A. 2023. Ethnobotanical analysis of phytonyms and plant-related glosses mentioned in Bujangga Manik, a pre-Islamic Sundanese text (15th century Java, Indonesia). *Reinwardtia* 22(2): 131–143. — This study aimed to identify and analyze ethnobotanical data on phytonym, utilization, and cultural value of plants mentioned in an Old Sundanese text. Since plants are mentioned with their vernacular names, identification was based on an exhaustive bibliographical search of the respective scientific name. A comprehensive investigation led to the identification of a total of 85 Old Sundanese phytonyms, which represented vernacular names for 79 distinct plant species. Furthermore, by considering the number of identified species from the plant-related glosses, 93 species belonging to 57 genera and 44 plant families were registered. Among these plant families, Arecaceae (12 species), Poaceae (nine species), and Fabaceae (six species) exhibited the highest number of identified species. In addition to the phytonyms, 36 phytotoponyms, which utilize vernacular phytonyms to designate settlements, hermitages, ports, mountains, and rivers were identified. It was worth noting that the majority of the plants associated with these phytotoponyms were indigenous. Meanwhile, only 13 species were traced back to the pre-Columbian exchange period during the Austronesian migration and the Indianization-Sinicization era in Indo-Malayan history. The result showed that the predominantly mentioned utilization included beverage production, textile manufacturing, vessel craftsmanship, betel quid preparation, perfume creation, and incense production. Among the identified species, *Areca catechu* emerges as the most frequently mentioned in the text, along with *Graptophyllum pictum* and *Cordyline fruticosa*, which were also considered as possessing profound spiritual value due to association with heavenly realms. Furthermore, the text highlighted that the production of plant-based fragrance products, also referenced in the celestial domain, held significant prominence in global trade during the 15th century.

**Key words**: Anthropobotanic relations, historical ethnobotany, phytonymy, Old Sundanese.

**ABSTRAK**
MULYANTO, D., ISKANDAR, B. S., ISKANDAR, J., INDRAWARDANA, I. & AUFA, A. A. 2023. Analisis etnobotani fitonim dan nilai budaya tumbuhan yang disebutkan dalam Bujangga Manik, teks Sunda pra-Islam (abad ke 15 Jawa, Indonesia). *Reinwardtia* 22(2): 131–143. — Tujuan artikel ini adalah mengidentifikasi data etnobotani tentang fitonim, pemanfaatan, dan nilai budaya tumbuhan yang disebutkan dalam teks Sunda pra-Islam. Mengingat tumbuhan disebutkan dengan nama lokal mereka, identifikasi didasarkan pada pencarian kepustakaan lengkap dari nama ilmiah masing-masing. Sebanyak 85 fitonim ditemukan yang teridentifikasi sebagai nama lokal untuk 79 jenis berbeda. Digabungkan dengan jumlah jenis yang teridentifikasi dari peristilahan terkait tumbuhan, total terdaftar 93 jenis dan 57 marga dari 44 suku. Suku dengan jenis terbanyak adalah Arecaceae (12), Poaceae (sembilan), dan Fabaceae (enam).

Kami juga menemukan 36 toponim yang memakai nama lokal tumbuhan dan mengacu pada 33 jenis berbeda untuk menamai permukiman, pertapaan, pelabuhan, gunung, dan sungai. Sebagian besar (20) tumbuhan yang nama lokalnya dipakai dalam fitotoponimi adalah tumbuhan asli, dan hanya 13 eksotik atau diperkenalkan pada periode sebelum Pertukaran Kolumbian, mungkin selama migrasi penutur bahasa-bahasa Austronesia dan periode India-nisasi-Sinisisasi dalam sejarah Indo-Malaya. Pemanfaatan tumbuhan yang paling banyak disebutkan terkait dengan minuman, produksi tekstil, pembuatan kapal, sajian sirih-pinang, parfum, dan dupa. Melalui fitonim dan istilah terkait tumbuhannya, *Areca catechu* adalah jenis yang paling sering disebutkan dalam teks. Jenis ini, bersama *Graptophyllum pictum* dan *Cordyline fruticosa*, juga dianggap memiliki nilai spiritual tinggi karena penyebutan keberadaannya di surga. Produk wewangian nabati, juga disebutkan terdapat di surga, adalah produk-produk terpenting dalam perdagangan dunia abad ke-15.

**Kata kunci**: Etnobotani historis, fitonimi, hubungan antropobotanik, Sunda kuno.

## INTRODUCTION

Ethnobotany is the multidisciplinary exploration of the plants within a given region and their practical applications through the traditional knowledge embedded in the local culture and its people, both historical and contemporary. An ethnobotanist diligently records the indigenous customs associated with the practical utilization of local flora across different aspects of life (Martinez *et al.*, 2019). A significant approach within the field of ethnobotany is historical ethnobotany, which focuses on examining the intricate connection between human populations in a particular geographical location and plants. This approach relies on utilizing historical documents such as manuscripts, books, iconographies, and inscriptions as valuable sources of evidence to construct comprehensive narratives and scholarly discussions (Silva *et al.*, 2014; Medeiros, 2014; 2016; 2020). Furthermore, it focuses on the repertoires of the anthropobotanic relationships which are stayed in time and space to understand the ways of society at the time under study.

Historical ethnobotany analyzes the understanding that interrelationships between humans and plants evolve and adapt over time, influenced by dynamic ecological and cultural contexts. The recognition of the ever-changing nature of these interrelationships has sparked a renewed interest among ethnobotanists in exploring the historical anthropobotanical connections. This interest has been fueled by the prospect of using historical documents as valuable resources for conducting ethnobotanical studies (Castro *et al.*, 2013; Silva *et al.*, 2014; Alves & Ming, 2015; Fatur, 2019; Svanberg *et al.*, 2019; Petran *et al.*, 2020; Coimbra & Welch, 2020; Ford, 2020; Dafni *et al.,* 2021; Liu *et al.*, 2021; Saraci & Damo, 2021). The renewal of interest is generated because exploring information within historical documents promotes a chronological perspective on the development of plant usage. This contributes significantly to the clarification of the present cultural conception of plants within specific societies (Jákl, 2015a; Dafni & Bock, 2019; Hoogervorst & Jákl, 2020; Leonti *et al.*, 2020; Wagner *et al.*, 2020).

The past anthropobotanic relationship is often recorded in literary works (Pardo-de-Santayana *et al.*, 2006; Ryan, 2018; 2020), encompassing folksongs and poems (Cardano & Herrero, 2014; Herrero & Cardano, 2015; Fernandez-Llamazares & Lepofsky, 2019; Ivanova *et al.*, 2021; Fiser, 2022). Even though literary texts from the past may not possess the same level of authority as historical sources, they can still be regarded as ethnobotanical documents. This is because such texts often serve as valuable reflections of customs, capturing the thoughts, beliefs, and traditions prevalent during that period (Sorokin, 2019).

There are studies on ethnobotanical aspect of ancient literary works from pre-Islamic Java (Jákl, 2015a; 2015b; 2016; 2017; Hoogervorst & Jákl, 2020; Mulyanto *et al.*, 2023). However, these works, similar to the majority of studies conducted on ancient Java, relied heavily on texts originating from Middle and East Java or Bali Island. For instance, Mulyanto *et al.* (2023) discovered a wealth of ethnobotanical knowledge regarding the diversity of fruits in ancient Java by examining a single text, namely Kakawin Ramayana, written in Middle Java around the 10$^{th}$ century.

One of the valuable remains of Old Sundanese text from the western part of Java Island is the story of Bujangga Manik. However, the academic literature on Bujangga Manik is not extensive. As a historical document, the poem has been the subject of surprisingly limited academic reviews. Noorduyn (1982) and Noorduyn & Teeuw (1999) discussed its topographical information. West (2017) worked on toponymic aspects and concluded that its deployment of listed toponyms was part of a widespread pattern or trope in Austronesian or more specifically Malayo-Polynesian literature. Several of the passages from Bujangga Manik, specifically those related to textile production in Old Sundanese texts, were also analyzed by Gunawan (2019). From a historical ethnobotanical perspective, these works provided only limited information. The encyclopedic character and presentation of daily life and material culture made them helpful sources for accounts of pre-Islamic Sunda-

nese ethnobotany. West (2021) also collected and identified some phytonyms mentioned in Bujangga Manik. However, not all phytonyms and plant-related glosses were identified and analyzed ethnobotanically. This study, then, aimed to identify all phytonyms that were directly and indirectly extracted from phytotoponyms, as well as those obtained from phytonym based glosses mentioned in Bujangga Manik. The utilization of plants and the cultural context of plant-related material cultures and activities were analyzed using historical ethnobotanical methods.

## MATERIALS AND METHODS

### Source

Data were obtained from Bujangga Manik, an Old Sundanese narrative poem about a Hindu ascetic's travels composed in West Java during the late fifteenth century. Bujangga Manik, as a *codex unicus*, was one of several surviving Old Sundanese poems written in octosyllabic meter. Its sole surviving manuscript, MS Jav. B.3. (R) was preserved in the Bodleian Library at the University of Oxford since 1627 or 1629. The unfinished text consisted of 1630 extant lines, which were inscribed scripto continua on both sides of thirty thin leaves (Noorduyn, 1982; Noorduyn & Teeuw, 1999; West, 2017; 2021). This study utilized the newest edition of Bujangga Manik which was romanized and translated into English by West (2021), with an earlier edition by Noorduyn & Teeuw (2006) as a comparison.

### Data collection

All lines in Bujangga Manik were successively reviewed, and the fragment of the poem was recorded. A database was created and organized into the fields of directly and indirectly extracted phytonyms from phytotoponymy, glosses related to plant-based objects or products, plant-processing activities, the number of citations, and the uses and symbolic values attributed to the plant.

### Identification of OS phytonyms and plant-related glosses

Several vernacular phytonyms were found to correspond to botanical taxa in the modern scientific sense. However, when working with Old Sundanese, the absence of any unified system of phytonyms in the era of Bujangga Manik text creation should be considered. One of the most crucial methods for disclosing the meaning of ancient phytonyms is the analysis of the context of the quotations. The frequency of encountering a specific phytonym and the diversity of contexts directly correlates to the amount of information obtained regarding the plants described. Consequently, the precise identification of botanical taxa associated with ancient phytonyms that appear only once in the corpus becomes a challenging endeavor (Sorokin, 2019). The majority of phytonyms referenced in the text are mentioned only once or twice, with virtually no botanical description accompanying them, aside from their utilization or cultural representation.

To identify the botanical identity of Old Sundanese phytonyms, sources of information outside the text were utilized. Old Sundanese was a member of Malayo-Polynesian languages, a subgroup of the Austronesian language family, spoken by people in the western part of Java Island before colonial era. Vernacular phytonyms in numerous Malayo-Polynesian languages spoken by other native Indonesians, specifically in Java Island and its vicinity, were the most important source of information. The Old Sundanese phytonym parallels in another Malayo-Polynesian plant vocabulary, such as Malay and other Sumatran, Javanese, Madurese, and Balinese, allowed this study to put forward several hypotheses. Relevant sources of information were early botanical works on Malesian plants such as Blume (1825), Hasskarl (1844), Miquel (1856), Teijsmann (1866), Van den Burg (1885), de Clercq (1909), and Heyne (1916-1927). These works not only described, and classified botanical taxa in a Linnaean way but also recorded vernacular phytonyms collected from local inhabitants, as well as information about their utilization by the native population. In addition to early Sundanese lexicographic works such as Rigg (1862), Geerdink (1875), and Coolsma (1913), Zoetmulder (1982), Old Javanese-English Dictionary was also used to find botanical vocabulary parallel to Old Sundanese phytonyms found in the text.

To verify a vernacular name into a botanical scientific nomenclature system, identification was not only carried out in consultation with these works but also cross-checking the field by taking samples of plant specimens in the form of vouchers for each specimen according to its vernacular name. Based on this collection of specimens, then, authentication be requested from plant taxonomist at Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran.

Finally, to determine the origin of a species and the phytogeographic zones used to determine a species is native or exotic to Java, authors consulted Plants of the World Online (https://powo.science.kew.org/).

### Data analysis

After the botanical identity of all phytonyms and plant-related glosses was identified, a list of plant species was compiled alphabetically based on their current scientific names. This list was followed by information about respective plant

families, Old Sundanese phytonym, and the number of mentions.

The direct identification of plant species was limited, as a significant portion of the glosses related to plant-based products and plant-processing activities did not explicitly mention any phytonyms, thereby hindering their straightforward classification. Plant species identified from the examination of these two categories of glosses, which did not directly contain any specific phytonym, were presented in a separate table. This presentation was accompanied by a discussion associated with the sociocultural context of plant-based products and plant-processing activities.

## RESULTS

### Directly identified phytonyms

A total of 85 Old Sundanese phytonyms were identified, referring to 79 different plant species. These phytonyms were categorized into four groups, namely (A) phytonyms directly mentioned as a plant name, (B) phytonyms directly mentioned as part of a toponym, (C) phytonyms directly mentioned as adjectives for plant-based products, and (D) phytonyms extracted from phytonym-based verbs. Furthermore, 78 phytonyms were classified exclusively under one category, including 26 (33.33%), 29 (37.18%), 18 (23.07%), and 5 (6.41%) under category A, B, C, and D, respectively. There were 9 phytonyms mentioned in more than one category, namely 5 in A and C, 3 in B and C, 1 in A and D, and 1 in A and B, as shown in Table 1.

An additional explanation is not required for phytonyms and glosses belonging to categories A, B, and C, as they are directly referred to without any transformation. Several phytonyms under category D are positioned as the object for a verb in certain phrases. For example, from the phrase ‘ngela sepang’ (line 164), the process of boiling (a certain part) of a plant known as ‘sepang’ for dyeing purposes, the phytonym: *sepang* can be identified immediately. The name was identified as sappanwood, *Biancaea sappan* (L.)Tod. Similarly, with the phrase ‘ngangeun hayam’ (line 164), the process of stewing (certain parts) of a plant called ‘hayam’, the phytonym, namely *hayam* can be identified. The phytonym refers to *Gynochthodes umbellata* (L.) Razafim. & B. Bremer, named by Malay people as ‘akar perut ayam’ which is its root bark used to dyeing yarn with yellow colour.

Further discussion is warranted for certain phytonyms that fall under category D, as they have undergone morphological transformations. In the Sundanese language, it is common for verbs to be formed by adding a prefix to a noun. Therefore, in the context of phytonyms, several words may have been derived from plant names through this morphological process. By separating the prefix and the noun, the identity of the noun can be derived. Several verbs in Bujangga Manik are formed by adding a prefix to a noun that is the name of a particular plant. The active verb ‘nyangkuduan’, for example, is formed by adding prefix ‘nya-‘ and the noun ‘cangkudu’. The context of the verb ‘nyangkuduan’ is the activity related to dyeing, hence, it can be interpreted as “process of dyeing cotton yarn with ‘cangkudu’. The term 'cangkudu', identified as *Morinda coreia* Buch.-Ham. (syn. *Morinda tinctoria* Roxb.), refers to a specific plant species. In particular, 'cangkudu' is a mononomial used to describe this plant. The plant is known for its root bark, which, when decocted, is used to dye yarns or fabrics yellow-red. In some cases, pepper and ash may be added during the dyeing process to enhance the colour's durability (de Clercq 1909: 283).

Similarly, Old Sundanese phytonyms ‘hingul’ and ‘jerinang’ are derived from the active verbs ‘ngahingul’ and ‘ngajerinang’. The terms in question pertain to the colouration of the wood in a specific section of the vessel. This colouration is the result of a meticulous process involving particular plants. The first term, 'hurung kena ngahingul' (line 112, 150, and 910), can be understood as "[this part] is imbued with a fiery-red hue through the application of *hingul*-wood". The second term, 'siyang kena ngajerinang' (line 113, 153, and 911), can be translated as ‘[this part] is endowed with a bright-red shade through the utilization of *jerinang*-resin’. The phytonym ‘hingul’ is a synonym of ‘suren’ that refers to *Toona sureni* (syn. *Cedrela febrifuga* Blume, de Clercq 1909: 197), while ‘jerinang’ refers to *Calamus draco* (syn. *Daemonorops draco* Blume, de Clercq 1909: 217), a rattan species producing red resin commercially known as ‘dragon’s blood’.

### Identified plant species from composite plant-based products

The inclusion of plant species in Table 1 is not comprehensive since it does not cover all the likely plant species found in the Bujangga Manik depiction. This discrepancy arises due to the absence of phytonyms in certain Old Sundanese glosses identified as plant-related glosses. The subsequent section examines and analyzes some of these glosses under scrutiny.

In Bujangga Manik, several glosses refer to something made from or composed of several plants. For example, the noun ‘seupaheun’ can be translated as betel quid. This particular noun, along with the related verbs 'nyeupah' and 'mucang', typically involves three vital components, namely the seed of the *Areca catechu*, the leaf of *Piper betel*, and a mixture of lime powder and water (calcium hydroxide) blended to form a paste-like consistency, making it suitable for chewing. Other ingredients may be added depending on availability and preference (Rooney, 1993; Singh *et al.*,

Table 1. List of Old Sundanese phytonyms and the botanical identity.

| Scientific name, and family | Old Sundanese phytonym | Number of mentioned under category | | | | |
|---|---|---|---|---|---|---|
| | | A | B | C | D | Σ |
| *Abelmoschus moschatus* Medik., Malvaceae | *Kasturi* | 0 | 1 | 1 | 0 | 2 |
| *Acalypha caturus* Blume, Euphorbiaceae | *Lawu* | 0 | 2 | 0 | 0 | 2 |
| *Aegle marmelos* (L.) Corrêa, Rutaceae | *Maja* | 0 | 1 | 0 | 0 | 1 |
| *Alyxia stellata* (J.R.Forst. & G.Forst.) Roem. & Schult, Apocynaceae | *Palasari* | 0 | 1 | 0 | 0 | 1 |
| *Alpinia malaccensis* (Burm.f.) Roscoe, Zingiberaceae | *Kamisadi* | 2 | 0 | 0 | 0 | 2 |
| *Amomum maximum* Roxb., Zingiberaceae | *Bungarésa* | 3 | 0 | 0 | 0 | 3 |
| *Areca catechu* L., Arecaceae | *Pinang, pucang* | 11 | 0 | 0 | 3 | 14 |
| *Arenga pinnata* (Wurmb.) Merr., Arecaceae | *Kawung* | 1 | 0 | 1 | 0 | 2 |
| *Artocarpus elasticus* Reinw. ex Blume, Moraceae | *Teureup, koja* | 0 | 1 | 1 | 0 | 2 |
| *Artocarpus heterophyllus* Lam., Moraceae | *Nangka* | 0 | 1 | 0 | 0 | 1 |
| *Averrhoa bilimbi* L., Oxalidaceae | *Balingbing* | 0 | 1 | 0 | 0 | 1 |
| *Averrhoa carambola* L., Oxalidaceae | *Calingcing* | 0 | 0 | 1 | 0 | 1 |
| *Bambusa vulgaris* Schrad. ex J.C.Wendl., Poaceae | *Haur kuning* | 1 | 0 | 0 | 0 | 1 |
| *Bambusa vulgaris* Schrad. ex J.C.Wendl., Poaceae | *Haur séyah* | 1 | 0 | 0 | 0 | 1 |
| *Biancaea sappan* (L.) Tod., Fabaceae | *Sepang* | 0 | 0 | 0 | 1 | 1 |
| *Blumea balsamifera* (L.) DC., Asteraceae | *Sembung* | 0 | 2 | 0 | 0 | 2 |
| *Borassus flabelifer* L., Arecaceae | *Taal* | 0 | 1 | 0 | 0 | 1 |
| *Calamus* L., Arecaceae | *Hoé* | 0 | 1 | 0 | 0 | 1 |
| *Calamus caesius* Blume, Arecaceae | *Hoé walatung* | 4 | 0 | 0 | 0 | 4 |
| *Calamus ciliaris* Blume, Arecaceae | *Hoé muka* | 2 | 0 | 0 | 0 | 2 |
| *Calamus draco* Willd., Arecaceae | *Jerinang* | 0 | 0 | 0 | 3 | 3 |
| *Calamus javensis* Blume, Arecaceae | *Hoé omas* | 2 | 0 | 0 | 0 | 2 |
| *Cananga odorata* (Lam.) Hook.f. & Thomson, Annonaceae | *Wangsana* | 0 | 0 | 1 | 0 | 1 |
| *Chenopodium album* L., Amaranthaceae | *Diheng* | 0 | 1 | 0 | 0 | 1 |
| *Chrysopogon zizanioides* (L.) Roberty, Poaceae | *Narawastu* | 2 | 0 | 0 | 0 | 2 |
| *Cinnamomum camphora* (L.) J.Presl., Lauraceae | *Kapur* | 0 | 0 | 1 | 0 | 1 |
| *Citrus x aurantium* L., Rutaceae | *Jerukmanis* | 0 | 2 | 0 | 0 | 2 |
| *Colocasia esculenta* (L.) Schott, Araceae | *Tales, bayabon* | 1 | 0 | 1 | 0 | 2 |
| *Cocos nucifera* L., Arecaceae | *Kalapa* | 0 | 2 | 0 | 0 | 2 |
| *Cordyline fruticosa* A.Chev., Asparagaceae | *Hanjuang, handong* | 2 | 0 | 0 | 0 | 2 |
| *Corypha utan* Lam., Arecaceae | *Pucuk* | 1 | 0 | 0 | 0 | 1 |
| *Cryptocarya massoy* (Oken) Kosterm., Lauraceae | *Masui* | 0 | 0 | 1 | 0 | 1 |
| *Cucumis sativus* L., Cucurbitaceae | *Bonténg* | 0 | 0 | 2 | 0 | 2 |
| *Curculigo latifolia* Dryand, Amaryllidaceae | *Parasi* | 0 | 2 | 0 | 0 | 2 |
| *Dendrocalamus asper* (Schult. & Schult.f.) Backer, Poaceae | *Beutung* | 1 | 1 | 0 | 0 | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Dioscorea hispida* Dennst., Dioscoreaceae | *Gadung* | 0 | 1 | 0 | 0 | 1 |
| *Dolomiaea costus* (Falc.) Kasana & A.K.Pandey, Asteraceae | *Pucuk* | 0 | 0 | 1 | 0 | 1 |
| *Dryobalanops aromatica* C.F.Gaertn., Dipterocarpaceae | *Kapur Barus* | 0 | 0 | 3 | 0 | 3 |
| *Durio zibethinus* L., Malvaceae | *Kadu* | 0 | 1 | 0 | 0 | 1 |
| *Embelia ribes* Burm.f., Primulaceae | *Kacambang* | 0 | 0 | 1 | 0 | 1 |
| *Ficus benjamina* L., Moraceae | *Caringin* | 0 | 2 | 0 | 0 | 2 |
| *Gigantochloa apus* (Schult.f.) Kurz ex Munro, Poaceae | *Apus* | 5 | 0 | 3 | 0 | 8 |
| *Gigantochloa verticillata* (Willd.) Munro, Poaceae | *Awi gombong* | 2 | 0 | 0 | 0 | 2 |
| *Gnetum gnemon* L., Gnetaceae | *Tangkil* | 0 | 1 | 0 | 0 | 1 |
| *Graptophyllum pictum* (L.) Griff., Acanthaceae | *Handeuleum* | 1 | 0 | 0 | 0 | 1 |
| *Gynochthodes umbellata* (L.) Razafim. & B.Bremer, Rubiaceae | *Hayam* | 0 | 0 | 0 | 1 | 1 |
| *Marsdenia tinctoria* R.Br., Apocynaceae | *Tarum* | 0 | 4 | 0 | 0 | 4 |
| *Magnolia champaca* (L.) Baill. ex Pierre, Magnoliaceae | *Kembang* | 0 | 2 | 0 | 0 | 2 |
| *Mimusops elengi* L., Sapotaceae | *Tanjung* | 0 | 1 | 0 | 0 | 1 |
| *Momordica charantia* (L.) Descourt., Cucurbitaceae | *Payanggu* | 0 | 1 | 0 | 0 | 1 |
| *Morinda coreia* Buch.-Ham., Rubiaceae | *Cangkudu* | 0 | 0 | 0 | 2 | 2 |
| *Murraya paniculata* (L.) Jack, Rutaceae | *Kamuning* | 2 | 0 | 0 | 0 | 2 |
| *Musa acuminata* Colla, Musaceae | *Jantung* | 0 | 0 | 1 | 0 | 1 |
| *Myristica fragrans* Houtt., Myristicaceae | *Pala* | 0 | 1 | 0 | 0 | 1 |
| *Myristica iners* Blume, Myristicaceae | *Kayu laka* | 2 | 0 | 0 | 0 | 2 |
| *Nypa fruticans* Wurmb., Arecaceae | *Nipah* | 1 | 0 | 0 | 0 | 1 |
| *Oncosperma tigillarium* (Jack) Ridl., Arecaceae | *Haliwung* | 0 | 4 | 0 | 0 | 4 |
| *Oroecnida integrifolia* (Gaudich.) Miq., Urticaceae | *Nangsi* | 0 | 1 | 0 | 0 | 3 |
| *Pandanus amaryllifolius* Robx. ex Lindl., Pandanaceae | *Jaksi* | 3 | 0 | 0 | 0 | 1 |
| *Pandanus tectorius* Parkinson, Pandanaceae | *Pandan* | 1 | 0 | 0 | 0 | 1 |
| *Papaver somniferum* L., Papaveraceae | *Candu* | 0 | 0 | 1 | 0 | 1 |
| *Parkia speciosa* Hassk., Fabaceae | *Peuteuy* | 0 | 1 | 0 | 0 | 1 |
| *Phyllanthus acidus* (L.) Skeels, Phyllanthaceae | *Ceremay* | 0 | 3 | 0 | 0 | 3 |
| *Phyllanthus emblica* L., Phyllanthaceae | *Malaka* | 0 | 1 | 0 | 0 | 1 |
| *Piper betel* L., Piperaceae | *Seureuh* | 2 | 0 | 2 | 0 | 4 |
| *Pogostemon cablin* (Blanco) Benth., Lamiaceae | *Pupur* | 0 | 0 | 2 | 0 | 2 |
| *Quercus infectoria* G.Olivier, Fagaceae | *Majakané* | 0 | 0 | 2 | 0 | 2 |
| *Rosa* sp., Rosaceae | *Mawar* | 0 | 0 | 2 | 0 | 2 |
| *Santalum album* L., Santalaceae | *Candana* | 3 | 0 | 2 | 0 | 5 |
| *Saraca indica* L., Fabaceae | *Dédés* | 3 | 0 | 0 | 0 | 3 |
| *Schizostachyum iraten* Steud., Poaceae | *Tamiang* | 0 | 1 | 0 | 0 | 1 |
| *Schleichera oleosa* (Lour.) Oken, Sapindaceae | *Laka* | 0 | 0 | 2 | 0 | 2 |
| *Sesamum indicum* L., Pendaliaceae | *Lenga* | 0 | 0 | 2 | 0 | 2 |
| *Sesbania sesban* (L.) Merr., Fabaceae | *Janten* | 0 | 0 | 2 | 0 | 2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Sundacarpus amarus* (Blume) C.N.Page, Podocarpaceae | *Taji* | | 0 | 1 | 0 | 0 | 1 |
| *Styrax benzoin* Dryand, Styracaceae | *Kamenyan* | | 0 | 0 | 1 | 0 | 1 |
| *Syzygium polycephalum* (Miq.) Merr. & L.M.Perry, Myrtaceae | *Kupa* | | 1 | 0 | 0 | 0 | 1 |
| *Tectona grandis* L.f., Lamiaceae | *Jati* | | 0 | 3 | 2 | 0 | 4 |
| *Toona sureni* (Blume) Merr., Meliaceae | *Hingul* | | 0 | 0 | 0 | 3 | 3 |
| *Ziziphus mauritiana* Lam., Rhamnaceae | *Darah* | | 0 | 1 | 0 | 0 | 1 |
| Unidentified, Poaceae | *Awi nyowana* | | 2 | 0 | 0 | 0 | 2 |
| | | Σ | 62 | 50 | 39 | 13 | 163 |
| | | % | 38 | 30 | 24 | 8 | 100 |

2020). In Java, gambier, an extract derived from the leaves of *Uncaria gambir*, a climbing shrub native to tropical Southeast Asia, is usually used as an addition. Among the lower classes, it is often made from the leaves and bark of *Ficus ribes* Reinw. ex Blume (Uphof, 1959).

There are many terms related to fabric mentioned in Bujangga Manik, such as *boéh*, *bédong*, *hasiwung*, *heuyeuk*, *kaén*, *kantéh*, *kasang*, *simbut sulam*, *sinjang*, and *tapih*. However, there is no mention of their basic material. The basic material of native Indo-Malayan weaving tradition is cotton. According to Pleyte (1912: 46), there are two main species bred in Western Java to make fabric, namely ‘kapas honje’ (*Gossypium herbaceum* L.) and ‘kapas mori’ (*G. micranthum* Cav.). In recent taxonomic work, the latter is considered a subspecies of the former, *G. herbaceum* subsp. *herbaceum* L.

**Identified plant species from plant-processing verbs**

Some verbs related to plant-processing activities are derived from noun prefixes that represent specific plant names to directly identify the phytonym. However, there are also verbs such as 'neuleum' (line 162 and 282) and its corresponding noun 'teuleum' (line 542) that do not follow this pattern. These terms describe the process of dyeing cotton yarn with indigo dye, which involves soaking in a container filled with a mixture of warm water, certain plant parts, and other inorganic materials. According to Pleyte (1912: 74), in West Java, the indigo dye used in this process does not come from *Indigo tinctoria.* Instead, it is derived from *Marsdenia tinctoria* R.Br, which the Sundanese people of the early 20$^{th}$ century referred to as 'tarum areuy'.

Other verbs related to textile production, such as ‘nuar’, involve plant elements from several different species. The original noun of the verb ‘nuar’ is ‘tuar’ or ‘tuwar’ which means “kneading and oiling of white goods” (De Kat Angelino, 1930: 216). According to Pleyte (1912: 73), after being cooked, the yarn remains greasy and takes on paint poorly. The degreasing agent is found in a weak soap bath (cituar) in which the yarn is dipped and kneaded before drying in the sun. The active ingredients are oil and lye with a binder. Additionally, lye is made by charring the straw of withered rice stalks and then leaching with water. The oil used in the degreasing process is derived from various plant species. It forms a mixture that incorporates products from multiple plants when combined with lye. Pleyte (1912: 73) provides a list of plants involved in this process, along with their botanical identities (Table 2).

In the *nyangkuduan* activity (line 162 and 282), once the cloth has dried and hardened properly, it is dyed using a filtered decoction of cangkudu. However, instead of immersing the cloth in the dye, it is applied by smearing onto the cloth using a brush made of flapping peel or *pandan laut*. After the cloth is dry, the process is repeated until the desired level of redness is achieved. A post-coating can be applied using an extract of *geugeunteulan* and *gambir* (Loeber, 1914: 22) when a darker colour is preferred. *Pandan laut*, *geugeunteulan*, and *gambir* are Sundanese phytonyms that respectively refer to *Pandanus odorifer* (Forssk.) Hasskarl, *Diospyros frutescens* Blume, and *Uncaria gambir* (W.Hunter) Roxb. So, in the process of *nyangkuduan*, apart from *Morinda coreia* or *M. citrifolia*, there are three other plants whose products are involved.

Concerning glosses that pertain to plant-based products, it is common to be made using various types of plants. For instance, the noun 'tuak' (line 632) denotes a lightly alcoholic beverage produced through the fermentation of sap obtained from certain palm trees. In Java, this beverage can be prepared using the sap from either the palmyra palm (*Borassus flabelifer*) or the sugar palm (*Arenga pinnata*). Based on personal observations, in western Java, particularly among the Sundanese people, 'tuak' is typically made using sugar palm.

Table 2. List of plant in plant-processing activity 'nuar'.

| Sundanese name | Scientific name | Family | Part used |
|---|---|---|---|
| *Cabe-areuy* | *Piper betel* L. | Piperaceae | Leaf |
| *Dadap* | *Erythrina subumbrans* (Hassk.) Merr. | Fabaceae | Leaf |
| *Jahe* | *Zingiber officinale* Rosc. | Zingiberaceae | Rhizome |
| *Jeruk nipis* | *Citrus x aurantiifolia* (Christm.) Swingle | Rutaceae | Fruit |
| *Kaliki* | *Ricinus communis* L. | Euphorbiaceae | Seed |
| *Kapas* | *Gossypium herbaceum* L. | Malvaceae | Seed |
| *Pare* | *Oryza sativa* L. | Poaceae | Stalk |
| *Pedes* | *Piper nigrum* L. | Piperaceae | Fruit |
| *Picung* | *Pangium edule* Reinw. | Achariaceae | Fruit |
| *Suuk* | *Arachis hypogaea* L. | Fabaceae | Seed |
| *Wijen* | *Sesamum indicum* L. | Pedaliaceae | Seed |

**On phytotoponyms**

A total of 36 phytotoponyms were documented (Table 1), with 26 referring to places, six to mountains, and four to rivers, respectively. Furthermore, 33 different plant species were identified from the phytonyms of the phytotoponyms. Among them, 20 plant species were native to the region, while 13 were exotic or introduced during pre-Columbian exchange, possibly during Austronesian migration. Examples of these introduced species included coconut (*kalapa*, *Cocos nucifera*), wild rhea (*nangsi*, *Oroecnida integrifolia*), and durian (*kadu*, *Durio zibethinus*). Some plants were introduced during the Indianization-Sinicization period of western Java's history, such as jujube (*darah*, *Ziziphus mauritiana*), teak (*jati*, *Tectona grandis*), pummelo (*jeruk manis*, *Citrus x aurantium*), and bael (*maja*, *Aegle marmelos*). Several of phytotoponyms mentioned in Bujangga Manik were still used in some parts of western Java without any morphological transformation such as Citarum, Citeureup, Gunung Ceremai, Palasari, Gunung Sembung, or with transformation such as Cihaliwung (now Ciliwung, a river). However, almost all phytonyms used to form phytotoponym mentioned in Bujangga Manik were utilized in many parts of western Java.

**Utilization and cultural value of plant**

From the description above, several forms of plant utilization were identified related to beverage, textile production, vessel craft, and betel quid. Betel chewing was firmly embedded in the traditions of Southeast Asia. In ancient times it was seen as a sign of lavish luxury and enjoyed amongst royalty (Clarence-Smith, 2018). In Bujangga Manik, glosses related to betel quid such as 'seupaheun', 'nyeupah', 'seupah', 'mucang', and 'pasileman', were the terms that appear most often. This was related to the social background of individuals from the nobility circle. The phytonym 'pinang' (refer to *Areca catechu*) was also the most often referred to in Bujangga Manik, as shown in Table 1. The areca palm tree held high cultural value as an essential ingredient of the betel quid, which served multiple social functions in Old Sundanese society. It was not only given as an offering to special guests but also presented during proposal ceremonies. The cultural significance was vividly depicted in the presence of this tree in the portrayals of the places that the main character, Bujangga Manik, traversed on his journey to heaven (lines 1457–1469). This highlighted the deep historical and symbolic importance attributed to the areca palm tree in Old Sundanese culture. In addition to the areca palm, there are other plants mentioned in this study. These include *handeuleum* (*Graptophyllum pictum*), *hanjuang* or *handong* (*Cordyline fruticosa*), and *parasi* (*Curculigo latifolia*). These plants are referenced alongside the areca palm, highlighting their presence and significance within the narrative.

Plant-based fragrance products, such as *candana* (sandalwood, *Santalum album*) and *kulit masui* (massoy bark, *Cryptocarya massoy*), held significant cultural value as they were depicted as a parable for the fragrant soul of a saint ascending to heaven (lines 1640–1643). These fragrances, along with other scents such as *kapur Barus* (Barus camphor, *Dryobalanops aromatica*), *dédés* (flower-based musk, *Saraca indica*), *lenga* (sesame oil, *Sesamum indicum*), *wangsana* (cananga oil, *Cana-*

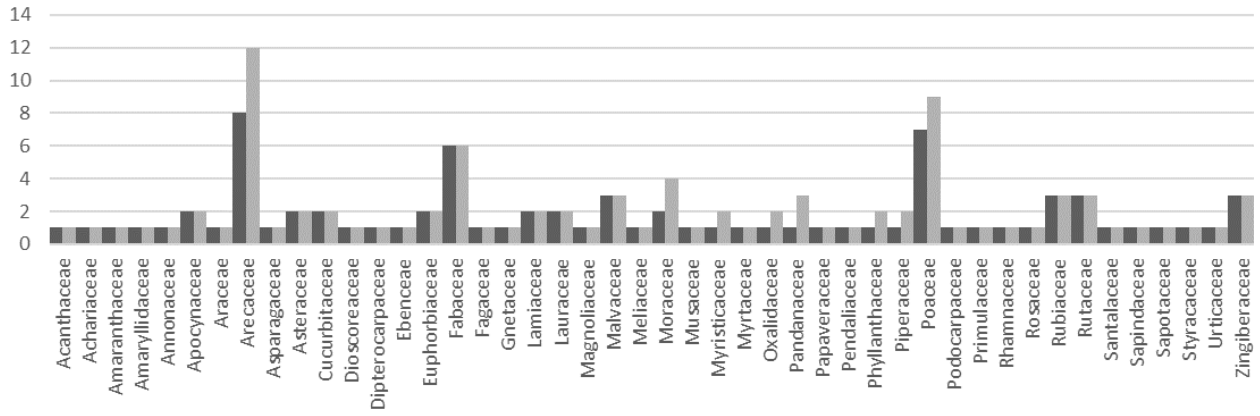


Fig. 1. Distribution of species and genera of plant families.

*nga odorata*), and *pucuk* (puchuk, *Saussurea costus*), were believed to exist in heaven together with precious goods made of gold and silver, as well as imported merchandise from Java, China, and India (lines 1692–1697). This highlighted the cultural significance attributed to these plant-based fragrances and their association with heavenly realms.

By considering both the plant species explicitly mentioned in the text and those indirectly included in plant-related glosses, a comprehensive tally showed a total of 93 distinct species and 57 genera from 44 different plant families (Fig. 1). Among these families, Arecaceae, Poaceae, and Fabaceae contained 12, nine, and six species, respectively. This indicated a remarkable botanical diversity associated with the topic, showcasing the significant presence and importance of various plant families and their species.

## DISCUSSION

In this study, several Old Sundanese glosses were interpreted differently compared to previous authors' interpretations. The meaning of term 'ngahingul' mentioned in Bujangga Manik line 112 and line 910, for example, was interpreted by Gunawan (2019) and West (2021) as "a pattern resembling a writhing fish" based on Sasmita's interpretation of the term 'hihinggulan', believed to be strongly linked to the Old Javanese word 'igul'. However, this interpretation appears imprecise for three reasons. First, in Sundanese grammatical patterns, morphological transformation from the noun 'igul' should be 'hihigulan' not 'hihinggulan'. Second, syntactic context of the verb 'ngahingul' in sentence "hurung beunang ngahingul" of line 112 is a specific colour of certain part of vessel interior because the term 'hurung' means 'fiery-red'. Thirdly, the term 'hingul' is a phytonym in several Sumatran languages, synonymous with 'suren' in Sundanese language, that refers to *Toona sureni* and/or *T. sinensis* (de Clercq, 1909; Heyne III, 1917; Endert, 1924; den Berger, 1926). These two species, like other members of mahogany family, are valuable timber trees. They are a source of high quality hardwoods used for highend furniture work, interior finishing, decorative paneling, and other wood crafts. The difference between two species is that the sapwood of *T. sureni* is white to pinkish or pale red, and the heartwood is light red or brown in colour. The sapwood of *T. sinensis* is cream-coloured to red, the heartwood is light brick -red when exposed, and becomes reddish brown when aging (Orwa *et al.*, 2009; Peng *et al.*, 2012).

Other authors (Noorduyn & Teeuw, 2006; West, 2021) interpret the term 'bayabon' as "a particular kind of cloth". In this study, the term refers to *Collocasia esculenta* based on information from old botanical works on Java (Teijsmann, 1866; Seed & Plants, 1909) as well as other sources (Pleyte, 1911; van Dapperen, 1934). In traditional textile production in Southeast Asia, the corm of this plant used to create a vibrant purple dye for fabrics (Mongkholrattanasit *et al.,* 2021).

Even though previous authors reviewed in depth toponyms mentioned in Bujangga Manik (Noorduyn, 1982; Noorduyn & Teeuw, 1999; Noorduyn & Teeuw, 2006; West, 2017; West 2021), the phytotoponyms were not specifically discussed. Phytotoponymy is a linguistic expression that highlights the significant role of plants, constituting crucial remnants of ancient languages. It forms an essential lexical domain within toponymy, providing relatively reliable evidence regarding the botanical landscape of the past (Sindik & Caric, 2016; Pinna *et al.*, 2017; Camarda, 2019;

Fagundez & Izco, 2016; Vidal-Luengo *et al.*, 2019; Al-Okashi, 2021; Khisamitdinova *et al.*, 2022). The use of society's vernacular phytonym in Bujangga Manik for naming geographical features such as mountains, rivers, and important places is evident. With other historical texts from pre-Columbian exchange period, it serve as a significant source for reconstructing the past distribution of plant communities and plant uses in western Java.

Most Old Sundanese phytonyms identified in this study are monolexemic and barefaced names (Berlin, 1992; Franco *et al.*, 2022; Hidayati *et al.*, 2022) that have no meaning other than their own. Some of them, such as 'jerinang' and 'hingul' for example, are clearly loanwords unknown in the modern Sundanese botanical vocabulary. There are only seven names are bilexemic in which their secondary name has another meaning and need another study to decode them and unclose the meaning.

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