J. Trop. Agric. and Fd. Sc. 40(2)(2012): 289–296

Short Communication

Leaf anatomical study of five *Macaranga* species (Euphorbiaceae)

[Kajian anatomi daun lima spesies *Macaranga* (Euphorbiaceae)]

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Keywords: comparative, leaf anatomy, *Macaranga*

Abstract

The anatomical variations in the leaves of *Macaranga tanarius, M. javanica, M. gigantea, M. hulletii* and *M. hypoleuca* were investigated so that they can be used as an additional tool for identification of the species. Characters such as the anticlinal wall pattern, types of stomata, vascular tissue pattern, shapes of the leaf margin, types of trichomes and types of leaf venation were identified. The adaxial and abaxial anticlinal walls are sinuous in *M. hulletii, M. tanarius, M. javanica* and *M. gigantea* but straight to wavy in *M. hypoleuca*. The paracytic stomata are present in all species except *M. javanica* which has the staurocytic type. All species except *M. gigantea* have open-type venation in the lamina and the incomplete-type at the margins. The closed vascular system and medullary vascular bundle were observed in all species. All five *Macaranga* species showed the presence of laticifer cells in their petiole and midrib. All have similar rounded-end shape leaf margin except for *M. hypoleuca* which has a fibrous cell at the end of the leaves. This unique characteristic will be useful in identification of that species. Simple unicellular trichomes were present in *M. tanarius, M. hulletii,* and *M. gigantea*, while glandular trichomes were found in *M. hypoleuca* and *M. javanica*. In *M. javanica*, the glandular trichomes were situated in crypts on the abaxial surface which can be a useful diagnostic character.

Introduction

There are 200 species of *Macaranga* (Euphorbiaceae; Tribe Acalyphae) in the world and 24 of them are common in Malaysia. *Macaranga* taxon includes species that have a symbiotic relationship with ants (Fiala et al. 1989). These species are mainly distributed in the primary lowland forest. Some species extend up to Burma and Indo China in the north, some as far as China and India. This genus is also evenly spread from Tropical Africa, Madagascar, Malesia, Australia and some places in the Pacific (Wagner et al. 1999).

Leaf morphological characters have been used for identification of the species (Ridley 1924; Corner 1952; Whitmore 1980). Ridley (1924) divided the genus into six sections based primarily on leaf morphology, while Airy (1973) divided the genus into eight sections also using leaf morphological characteristics.

Macaranga is often confused with *Mallotus*, a closely related species. Corner

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(1952) differentiated *Macaranga* from *Mallotus* based on the presence of red latex and its myrmecophytic habit, characters which are not shown in herbarium specimens. Leaf anatomy is useful in systematic studies such as in the genus *Euphorbia* where leaf anatomical evidence supports the division of the genus into genera or subgenera (Sehgal and Phaliwal 1974). Currently most of the research on this genus is concentrated on the relationship of plant anatomy, plant physiology and morphological structures of the species (Fiala et al. 1989; Ishida et al. 2005).

It is useful to study the anatomy of the plant species so that they can be easily identified for specific usage (Fahn 1974). The *Macaranga* species are useful in traditional medicines and has been used for treatment of various ailments. *Macaranga gigantea* is used in traditional practices for treatment of diarrhoea and dysentery by drinking the concentrated juice extracted from the leaves (Burkill 1929). This species has been clinically approved to have antibacterial property towards *Staphylococcus aureus, Saccharomyces cereviso* and *Fusarium oxysporum* (Lim et al. 2009).

Macaranga tanarius is said to have antimicrobial property towards *Staphylococcus* bacteria, and healing property for high fever (Lim et al. 2009). The concoction of the leaves of *M. hulletii* is said to be effective as a cleansing agent in the blood (Burkill 1929). The juice prepared from the leaves can also be taken internally for dysentery treatment (Burkill 1929). The juice from the bark and roots of *M. hypoleuca* is also used for treatment of dysentery and coughs as most of the leaves have a high tannin content (Attschul 1973).

This paper presents the results of a comparative study of the anatomy of leaves and petioles of five selected *Macaranga* species from Peninsular Malaysia. The anatomical variations in the leaves may be used as an additional tool for identification of the species. They can also be used to determine the diagnostic characters to differentiate the *Macaranga* species and also as additional data for comparison between its closely related species, *Mallotus* (Euphorbiaceae) (Khatijah et al. 1996).

Material and methods

Fresh *Macaranga* specimens used in this study were obtained from the fields at UKM Bangi Campus, Bandar Baru Bangi, Malacca, Kajang and Muar. The specimens consist of five species: *M. javanica* (Blume) Muell. Arg., *M. tanarius* (L.) Muell. Arg., *M. gigantea* (Rchbf and Zoll.) Muell. Arg., *M. hulletii* King ex. Hook.f. and *M. hypoleuca* (Rchbf and Zoll.) Muell. The voucher specimens were deposited at UKM Herbarium (UKMB) for future references. A total of 15 specimens from five species of *Macaranga* were collected (*Table 1*) and their morphological characteristics recorded.

Fresh leaves (lamina and petiole) of the *Macaranga* specimens were fixed immediately in AA (1 acetic acid: 3 alcohol), sectioned on the sliding microtome and stained in Safranin and Alcian Green solution. Fixation, embedding and sectioning were made following Sass (1940) with some modifications. Leaf specimens were sectioned with a sliding microtome at 20 – 30 µm thickness and stained in 1% Safranin in 50% alcohol and 1% Alcian Green solution in 100 ml purifying water with three drops of acetic acid.

Sections were made from the middle and marginal parts of the leaf lamina using a Leica sliding microtome. Epidermal peels were prepared by mechanical scraping and staining in Safranin. For venation studies, leaves were cleared in 70% alcohol with a drop of hydrochloric acid, dehydrated and stained in 1% basic Fuchsin in 6% potassium hydroxide. All slides were mounted in Euparal after dehydration. Photomicrographs of sections and epidermal peels were made using either a Leitz Diaplan polarizing microscope fitted with a JVC CCD camera or Reichert Polyvar 2 microscope fitted with a digital camera.

Table 1. List of *Macaranga* species used in slides preparation

Species	Code for herbarium	Locality
M. javanica	MNFG1	UKM
	MNFG2	Kajang
	MNFG3	Bangi
M. tanarius	MNFG4	Bangi
	MNFG5	Melaka
	MNFG6	Muar
M. gigantea	MNFG7	UKM
	MNFG8	UKM
	MNFG9	UKM
M. hulletii	MNFG10	UKM
	MNFG 11	UKM
	MNFG12	UKM
M. hypoleuca	MNFG13	UKM
	MNFG14	Bangi
	MNFG15	UKM

Images were processed using Analysis Document Software (soft-imaging system). All slides were deposited in the anatomy section at the Microtechnique Laboratory, Universiti Kebangsaan Malaysia, Bangi.

Results and discussion

Generic anatomical description

Results on the leaf anatomical study are shown in *Figure 1*. Cells on abaxial and adaxial leaf surfaces of *Macaranga* species varied from wavy to sinuous (*Figure 1A*). Paracytic stomata were found on the abaxial side of four species of *Macaranga*, with the exception of *M. javanica* which showed staurocytic stomata. Simple and unicellular glandular hairs were present on the abaxial surface of leaves of *M. gigantea* and *M. hypoleuca*. Drusses were present in mesophyll and parenchyma tissues in petioles transverse section of all *Macaranga* species.

Lamina transverse section

Transverse section of the leaf lamina of *Macaranga* species showed that the five specimens have a single layer of palisade cells of variable height. A hypodermal layer was not seen in any of the species. Papillae were present in adaxial surface of *M. hulletii* and abaxial surface of *M. javanica*. In mesophyll tissues of *M. hypoleuca*, sclereid cells were present in the palisade and spongy mesophyll cells. Glandular trichomes found inside the papilla holes or crypts were present in *M. javanica* and this can be used as a diagnostic characteristic for this species (*Figure 1J*).

Midrib transverse section

The main purpose in this midrib transverse section study was to differentiate the shape of the midribs within the species. In this study, almost all the midrib transverse sections of the *Macaranga* species were u-shaped or circular, while on the adaxial side the shapes were semi-circular or flat (*Figure 2*). Collenchyma cells were not seen in all species. While closed vascular tissues were observed in *M. javanica, M. tanarius, M. gigantea* and *M. hypoleuca*, open vascular tissues were observed in *M. hulletii* with u-shaped to circular abaxial and semi circular to flat adaxial. The bundle sheaths were schlerenchymatous with strands of fibres incompletely encircling the midrib complex.

Petiole transverse section

For petiole sectioning analysis, it is important to analyse the outer shape of the petiole for each species. From this study, it was observed that the outline shape of the petioles for all species is circular except for *M. tanarius* which is oval (*Figure 3A*). The epidermis has a cell thickness ratio of 1:1 (high:wide) with simple type trichomes. Druses were present in the parenchyma and phloem tissues. The vascular tissues were closed ring type with medulla bundle sheaths in the centre of the petioles (*Figure 3 A – E*).

Leaf anatomical variation

There was considerable anatomical variation between the leaves of the five species of

Macaranga. For petiole transverse section (TS), all species showed sub-rounded to rounded shape, except for *M. tanarius* which showed an oval shape (*Table 2*). All the *Macaranga* specimens showed closed main vascular tissue and medulla-type additional vascular tissue (*Figure 1K*). Druses crystals were observed in *M. hulletii, M. tanarius* and *M. javanica* but absent in *M. hypoleuca* and *M. gigantea*. In the vascular tissues, druses were usually present in the parenchyma cells of the midrib of the leaves

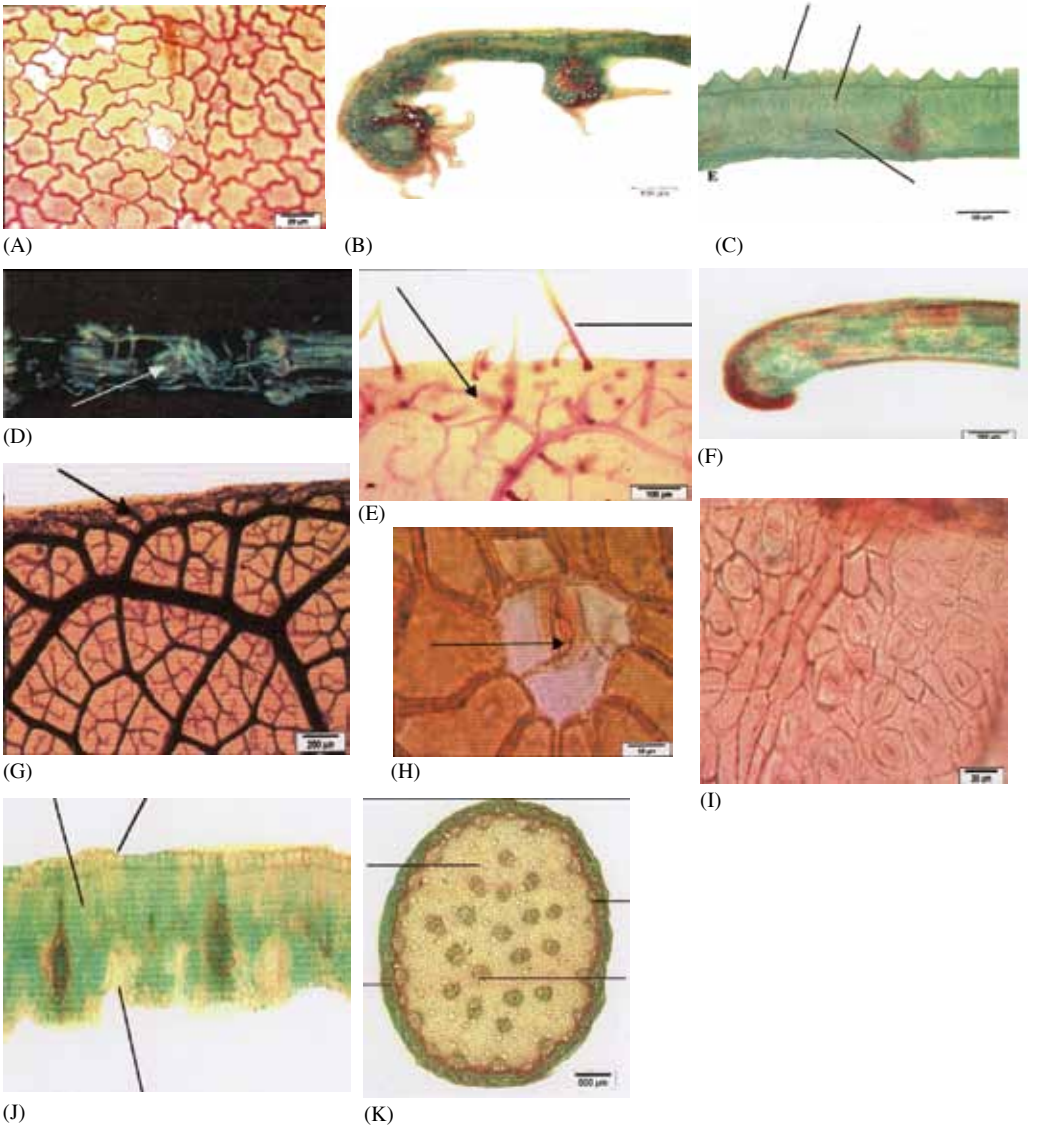

Figure 1. Leaf anatomical variations of five Macaranga species. A. Abaxial surface of ***M. javanica****, B. TS of leaf margin of* ***M. gigantea****, C. TS of leaf lamina of* ***M. hulletii****, D. Schelereid cell (arrow) in the TS of* ***M. hypoleuca****, E. Leaf venation of* ***M. tanarius****, F. Leaf tip of* ***M. tanarius****, G. Closed leaf venation of the leaves of* ***M. hypoleuca****, H. Staurocytic stomata in* ***M. javanica*** *abaxial surface, I. Adaxial surface of* ***M. hypoleuca,*** *J.* ***Macaranga javanica****: Glandular trichomes in crypt, K. TS of* ***M. tanarius*** *petiole*

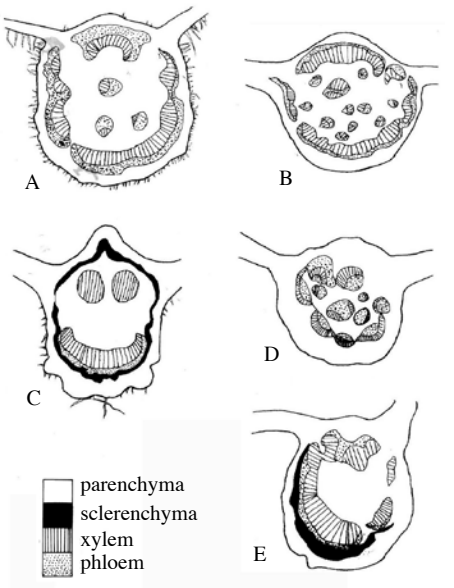

Figure 2. Transverse section of midrib of Macaranga species. (A). ***M. tanarius****, (B).* ***M. gigantea****, (C).* ***M. hulletii****, (D).* ***M. javanica*** *and (E).* ***M. hypoleuca***

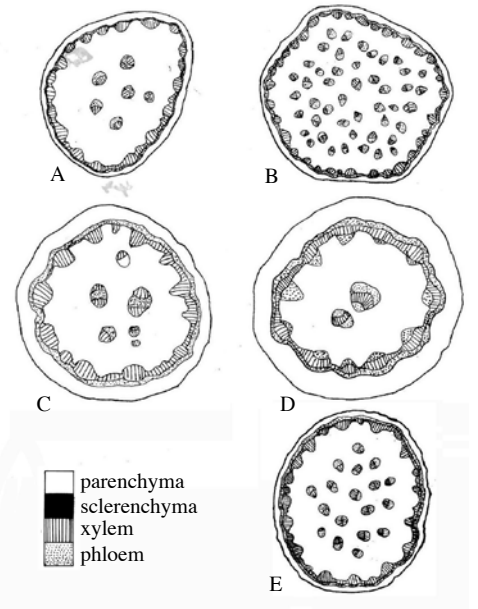

Figure 3. Transverse section of petiole of Macaranga species. (A). ***M. tanarius****, (B).* ***M. gigantea****, (C).* ***M. hullettii****, (D).* ***M. javanica*** *and (E).* ***M. hypoleuca***

Table 2. Anatomical characteristics of petioles of five *Macaranga* species

Species	Outer shape	Crystals
M. hulletii	Rounded	Druses
M. hypoleuca	Rounded	Absent
M. tanarius	Oval	Druses
M. javanica	Rounded	Druses
M. gigantea	Sub-rounded	Absent

and petioles as well as in the phloem cells. Trichomes were absent in all the *Macaranga* specimens studied.

The midrib transverse section for all species showed some variations between each species. The outline shape of the midrib in four *Macaranga* species (*M. tanarius, M. javanica, M. hulletii* and *M. gigantea*) were curved on the adaxial side and u-shaped on the abaxial side. Only *M. hypoleuca* has a straight shape on the adaxial and u-shaped on the abaxial side (*Table 3*). Simple and unicell trichomes were observed in *M. gigantea*, *M. hulletii* and *M. tanarius* but absent in *M. hypoleuca* and *M. javanica*. Fibrous cells were found encircling the vascular tissues in *M. hullettii, M. hypoleuca* and *M. javanica*.

Some variations were also found on the leaf margins of the various *Macaranga* species. *Macaranga hypoleuca* and *M. gigantea* have glandular trichomes while simple and unicell trichomes were found in *M. tanarius* (*Table 4*). Leaf across sections showed that leaves from seedlings, saplings, the adult tree and suckers mainly had only one palisade cell layer (Ishida et al. 2005). The TS of leaf margins of all *Macaranga* specimens showed a single palisade layer similar to the observation made by Ishida et al. (2005). Papillae were observed at the abaxial side of *M. hulletii* and at the adaxial side of *M. javanica*. Fibrous cells were observed at the leaf margin of *M. hypoleuca*. All specimens showed a rounded and curved down shape of leaf margins except *M. tanarius*. This feature can be used as a diagnostic character to confirm the species.

Paracytic stomata (*Figure 1I*) were observed for all species except *M. javanica*

Table 3. Anatomical characteristics of leaf midribs of five *Macaranga* species

Species	Outer shape	Vascular tissue	Trichomes	Crystals	Fibrous cells
M. hulletii	Adaxial: Curved Abaxial: U-shaped	Open	Simple, unicell	Absent	Encircling vascular tissue
M. hypoleuca	Adaxial: Straight Abaxial: U-shaped	Closed	Absent	Druses	Encircling vascular tissue
M. tanarius	Adaxial: Curved Abaxial: U-shaped	Closed	Simple, unicell	Absent	Absent
M. javanica	Adaxial: Curved Abaxial: U-shaped	Closed	Absent	Druses	Encircling vascular tissue
M. gigantea	Adaxial: Curved Abaxial: U-shaped	Closed	Simple, unicell	Absent	Absent

Table 4. Anatomical characteristics of leaf margins of five *Macaranga* species

Species	Trichomes	Papillae	Leaf margin	Fibrous cell
M. hulletii	Not seen	At abaxial	End: rounded, curved down	Absent
M. hypoleuca	Glandular	Not seen	End: rounded, curved down	At the tip
M. tanarius	Simple, unicel	Not seen	End: rounded, straight	Absent
M. javanica	Not seen	At adaxial	End: rounded, curved down	Absent
M. gigantea	Glandular	Not seen	End: rounded, curved down	Absent

Table 5. Anatomical characteristics of epidermis and leaf venation of five *Macaranga* species

Species	Epidermis			Leaf venation pattern	
	Stomata	Anticline wall Adaxial	Anticline wall Abaxial	Centre	Margin
M. hulletii	Paracytic	Sinuous	Sinuous	Open, branching at the end	Incomplete
M. hypoleuca	Paracytic	Straight-wavy	Wavy	Open, branching at the end	Incomplete
M. tanarius	Paracytic	Sinuous	Wavy	Open, branching at the end	Incomplete
M. javanica	Staurocytic	Sinuous	Sinuous	Open, branching at the end	Incomplete
M. gigantea	Paracytic	Sinuous	Sinuous	Closed, branching at the end	Circular

which had staurocytic stomata (*Figure 1H, Table 5*). The 'rubiaceous' or paracytic type characteristic for the tribe Acalyphae (Metcalfe and Chalk 1950) forms the basic type characteristic for the family, although the anomocytic type is more common in *Euphorbiaceae* species (Kakkar and Paliwal 1972). The centre venation of all the *Macaranga* species showed open type with branching at the end. Incomplete venation was observed at the margin of the leaves for all *Macaranga* species except *M. gigantea* which showed circular venation.

Diagnostic characters

Diagnostic characters which can be useful in quick identification of the species were detected in only four species of

Table 6. Diagnostic characteristics for four *Macaranga* species

Species	Diagnostic analysis
M. javanica	1. Abaxial surface with papilla 2. Glandular trichomes in crypt 3. Staurocytic stomata
M. hypoleuca	1. Fibrous cell at the end of leaves margin 2. Tannin hole in the petioles 3. Abaxial and adaxial wall straight-wavy
M. hulletii	1. Abaxial surface with papilla 2. Fibrous cell encircling main bundle sheath
M. gigantea	1. Lamina venation closed 2. Margin venation circular

Macaranga (*Table 6*). *Macaranga javanica* showed glandular trichomes in crypts, staurocytic stomata and its abaxial surface comes with papillae structure (*Table 6*). In *M. hypoleuca,* the diagnostic characters which can be observed were the tannin holes in the petioles, fibrous cells at the end of the leaf margin and straight-wavy type abaxial and adaxial walls. Closed lamina venation and circular margin venation were diagnostic characters of *M. gigantea*. *Macaranga hulletii* showed abaxial surface with papillae and fibrous cells encircling the main bundle sheath.

Taxonomic implication of characters

The anatomical characters are very useful in the identification of the *Macaranga* species. The results, however, did not show any clustering of characters which could be used to classify the genus into broad groups. However, the results of the study can be used to identify the five *Macaranga* species by detecting the variation among the species. Identification of the diagnostic characters can be used as a quick guide in identification of the various *Macaranga* species (*Table 6*).

Conclusion

This study showed that there are many differences and similarities within the *Macaranga* species. Anatomical characteristics can be used to differentiate the species which have similar morphological forms. The shape of the petiole and midrib transverse section, leaf margin, anticline wall pattern, trichomes, presence of crystals, venation pattern, presence of papillae, and many other features can be used as a guide in species identification. This study also showed that the five *Macaranga* species have similar anatomical features such as the closed vascular tissue system with medullary vascular bundle and the presence of druses and laticiferous secretory cells. As a conclusion, this brief study on the *Macaranga* species can be used as a guide in species identification and classification. However, there is a need to study the other *Macaranga* species in the future in order to complete the anatomical study on the genus, as the present study is still insufficient.

Acknowledgement

The authors wish to thank the staff of UKM Microtechnique Laboratory and UKM Botanical and Herbarium Department for their technical help.

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Abstrak

Variasi anatomi lima spesies daun *Macaranga* iaitu *M. tanarius, M. javanica, M. hypoleuca, M. hulletii* dan *M. gigantea* telah dikaji bagi membantu data sokongan pengecaman spesies. Ciri-ciri anatomi seperti corak dinding antiklin, jenis stomata, corak tisu vaskular, bentuk tepi daun, jenis trikom dan corak peruratan telah dikenal pasti sebagai ciri pembeza antara spesies. Dinding antiklin adaksial dan abaksial sinuous ditemui pada *M. hulletii, M. tanarius, M. javanica* dan *M. gigantean,* manakala dinding antiklin abaksial dan adaksial lurus dan berombak ditemui pada *M. hypoleuca*. Stomata jenis parasitik didapati pada semua spesies kecuali *M. javanica* yang mempunyai stomata jenis staurositik. Semua spesies mempunyai corak peruratan jenis terbuka di dalam lamina dan jenis yang tidak lengkap pada tepi daun kecuali *M. gigantea*. Sistem tisu vaskular tertutup dan kehadiran berkas vaskular medula didapati pada semua spesies. Kesemua spesies *Macaranga* menunjukkan kehadiran sel latisifer di dalam petiol dan tulang daun. Bentuk hujung daun kesemua spesies hampir serupa iaitu berbentuk bulat dan melengkung ke bawah kecuali *M. hypoleuca* yang mempunyai sel serabut pada hujung tepi daun. Ciri-ciri yang unik ini berguna sebagai panduan untuk mengenal pasti spesies tersebut. Trikom jenis ringkas dan unisel didapati dalam spesies *M. tanarius, M. hulletii* dan *M. gigantea* sementara trikom berkelenjar didapati pada *M. hypoleuca* dan *M. javanica*. Dalam daun *M. javanica,* trikom berkelenjar berada dalam krip pada permukaan abaksial dan boleh dijadikan ciri-ciri diagnostik yang berguna.

Accepted for publication on 2 August 2012