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IDENTIFICATION OF *TACCA CHANTRIERI* (DIOSCOREACEAE) THROUGH ANATOMICAL AND MORPHOLOGICAL CHARACTERS

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ABSTRACT

Anatomy and morphological characters were employed to identify four samples of Tacca sp. (Belimbing Tanah) from different localities in Perak. Anatomical characters including leaf epidermal characters, leaf venation, type of stomata and trichomes were investigated through transverse sections, epidermal peel and leaf clearing procedures. The outline and vascularization of the sections were also investigated. Result has shown that Tacca from four different localities in Perak were similar anatomically. However, morphologically these four species can be differentiated mostly based on their leaves, flowers and fruits. The leaf margins are slightly different of either entire or denticulate, and the colours of floral bracts are either purplish-black or purplish-black at the base gradually turns white to the apex. Eventhough they are slightly different morphologically, Tacca of Gunung Lang and Gunung Liang were suggested as T. chantrieri based on dichotomous key of Kalkman which emphasizes on the shape of leaves, shape of seed and position of involucral bracts. The reniform shape of the seed confirms that these Tacca belong to T. chantrieri. Tacca of Sungai Dara and Royal Belum State Park were also suggested as T. chantrieri based on their anatomical similarity to Tacca of Gunung Lang and Gunung Liang. T. chantrieri is not commonly recorded in Peninsular Malaysia as compared to T. Intergrifolia, it is therefore recommended for further investigation.

Keywords: Dioscoreaceae; Tacca chantrieri; anatomy; morphology; reniform seed.

1. INTRODUCTION

Belimbing Tanah or scientifically known as *Tacca* is well known with its medicinal values in treating various illnesses. *T. chantrieri* is claimed to have analgesic, antipyretic and anti-inflammatory effects (Keardrit, Rujjanawate, & Amorrnlerdpison, 2010) and Jiang, Yang, Wang, & Chen, (2014) has also proven that *Tacca* has anticancer effect. Despite being well employed in alternative traditional medicinal practices, *Tacca* has become increasingly popular in horticultural trade due to its unique and attractive floral display (Ling et al., 2005). The inflorescence is cymose umbellate with large prominent involucral bracts and long

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whisker-like filiform bracteoles. The floral colour is whitish to dark purple, brown or near black. *Tacca* was previously classified in Taccaceae family and through APG III (Angiosperm Phylogeny Group, 2009) the family was united together with Trichopodaceae in Dioscoreaceae the Yam family (Simpson, 2010). There are 10 species of acaulescent forest understory herbs (Ling et al., 2005). Kalkman, Kirkup, Nooteboom, Saw, & Stevens (1998) enumerated eight species of *Tacca* and constructed dicothomous key using the shape of the leaf, the fruit and the seed and arrangement of the floral bracts. Of these only four species were recorded in Peninsular Malaysia (Turner, 1995). The four species in Peninsular Malaysia are *T. chantrieri*, *T. intergrifolia*, *T. leontopetaloides* and *T. palmata*. The most common is *T. intergrifolia*.

Systematic of *Tacca* remains contraversial and species circumscriptions vary widely (Ling et al., 2011). In Peninsular Malaysia systematic treatment of *Tacca* is not comprehensive and outdated as recent studies were more than a decade ago, done by Turner (1995) and Kalkman et al. (1998). Furthermore, there is no sufficient anatomical description of *Tacca* has been published. Hence, *Tacca* should be investigated for its workable anatomical description together with morphological characters. Anatomical and micromorphological information of *Tacca* are important for species identification as the species are very much similar when they are sterile in field. On the other hand, both anatomical and micromorphological characters are crucial in standardization of the quality of a herbal medicine especially *Tacca* as it is widely accepted globally as an alternative medicine. In this current study, anatomical and morphological studies are performed to investigate the systematics significance of the characters for species identification.

2. MATERIALS AND METHOD

Tacca samples from four different phytogeographical areas were investigated anatomically and morphologically. Fresh samples of four Tacca species were collected from four different localities in Perak; Sungai Dara, Gunung Lang, Gunung Liang and Royal Belum State Park. The four species, each representing a different phytogeograpical area in Perak which are riverine forest, limestone forest, montane forest and lowland forest respectively. Fresh samples including the leaf, flower and fruit collected were fixed in FAA and studied anatomically subjected to the organs availability. Microtechnique procedures were conducted according to Johansen (1940) and Nor Nafizah (2006) and microimages were captured using Pixelink Leica DMLB image analyzer. Anatomical characters are described according to Metcalfe and Chalk (1979). Morphological study including field and herbarium study was done at Forest Research Institute of Malaysia, Kepong, Selangor and UPSI herbaria using Leica light and disecting microscopes. Voucher herbarium prepared were deposited at Universiti Pendidikan Sultan Idris herbaria and anatomical slides were kept at Plant Microtechnique laboratory, Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris for future references.

3. RESULT AND DISCUSSION

All *Tacca* samples from four localities collected show high similarity anatomically (Table 1). *Tacca* has open venation, free non-branching vein ending, lacking of areole and complete marginal vein (Figure 1). Trichomes observed are simple and unicellular. Adaxial epidermal anticlinal walls show slightly sinuous to sinous type (Figure 3 & 4) with no stomata present.

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All species observed are hypostomatic with anomocytic stomatal type (Figure 4) which confirms the previous findings by Watson and Dallwitz (1992) and Kubitzki (1998). However, hypostomatic is not typical in monocot plants. Lamina of *Tacca* is entire and the margin tips are rounded and incurved as described by Watson and Dallwitz, (1992) (Figure 5).

Table 1: Leaf Surface, Lamina and Margin Anatomical Characters of *Tacca* From Four Different Localities

Sample Localities	Adaxial	Abaxial	Stomatal Type	Venation	Trichome	Margin	Lamina
Tacca of Sungai Dara	Slightly sinuos anticlinal wall	Sinuous anticlinal wall	Anomocytic	Open, lacking areoles, complete marginal vein	Simple and uniseriate	Obtuse tip, incurved, Presence of secretory cell	Hypodermis not seen, mesophyll in 9-11 layers
Tacca of Gunung Lang	Slightly sinuos anticlinal wall	Sinuous anticlinal wall	Anomocytic	Open, lacking areoles, complete marginal vein	Simple and uniseriate	Obtuse tip, incurved, Presence of secretory cell	Hypodermis not seen, mesophyll in 9-11 layers
Tacca of Gunung Liang	Slightly sinuos anticlinal wall	Sinuous anticlinal wall	Anomocytic	Open, lacking areoles, complete marginal vein	Simple and uniseriate	Obtuse tip, incurved, Presence of secretory cell	Hypodermis not seen, mesophyll in 8-11 layers
Tacca of Royal Belum State Park	Slightly sinuos anticlinal wall	Sinuous anticlinal wall	Anomocytic	Open, lacking areoles, complete marginal vein	Simple and uniseriate	Obtuse tip, incurved, Presence of secretory cell	Hypodermis not seen, mesophyll in 17-19 layers

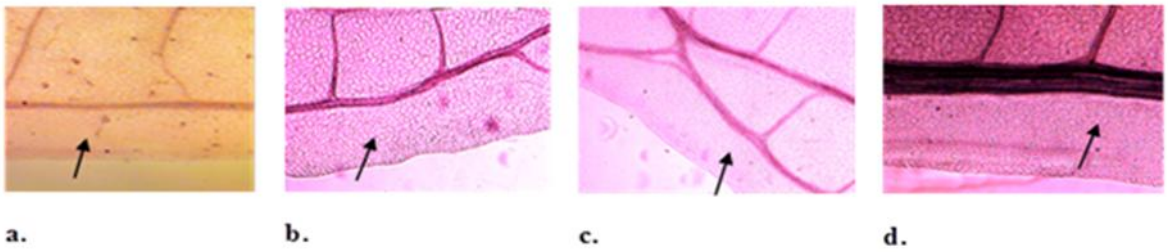

Figure 1: Complete Marginal Venation of *Tacca*. a) *Tacca* of Sungai Dara, b) *Tacca* of Gunung Lang, c) *Tacca* of Gunung Liang and d) *Tacca* of Royal Belum State Park. Arrows Show the Marginal Venation. (Magnification 5x10)

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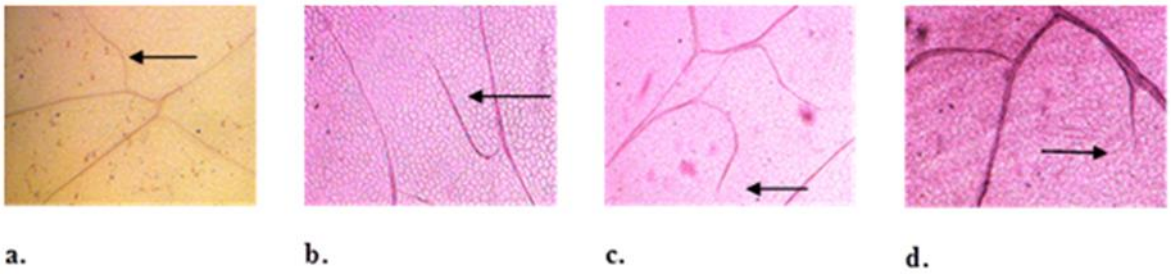

Figure 2: Venation of *Tacca* Lacking of Areoles With Open and Free Veinlets. a) *Tacca* of Sungai Dara, b) *Tacca* of Gunung Lang, c) *Tacca* of Gunung Liang, d) *Tacca* of Royal Belum State Park. Arrows Show Open Vienlets. (Magnification 5x10)

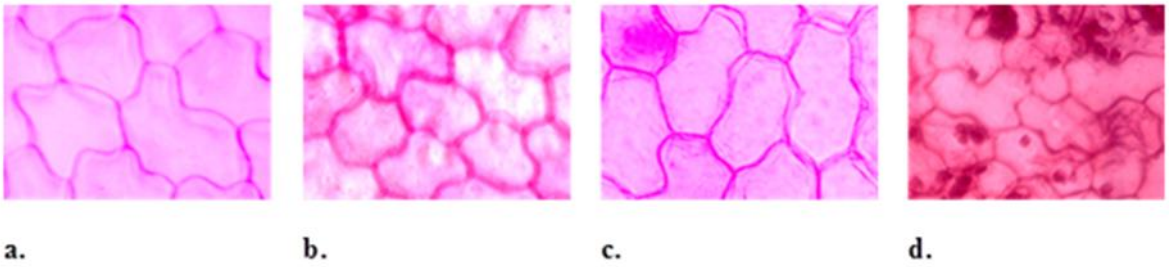

Figure 3: Sinuous Anticlinal Walls of Adaxial Epidermis. a) *Tacca* of Sungai Dara, b) *Tacca* of Gunung Lang, c) *Tacca* of Gunung Liang, d) *Tacca* of Royal Belum State Park. (Magnification 40x10)

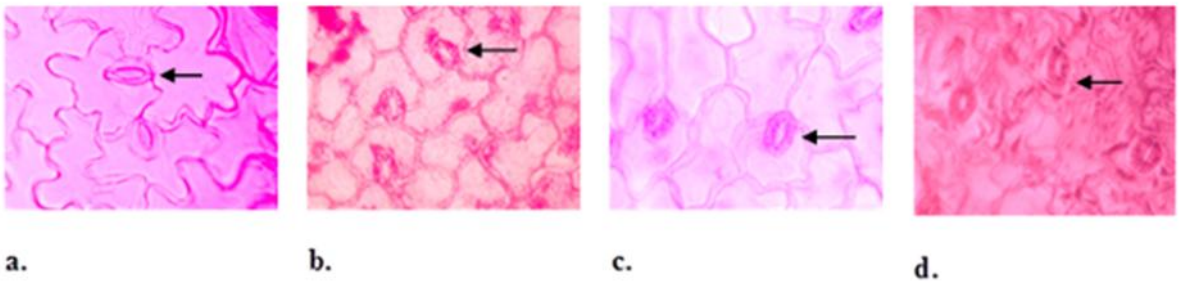

Figure 4: Sinuous Anticlinal Wall of Abaxial Epidermis and Anomocytics Stomata of *Tacca* a) *Tacca* of Sungai Dara, b) *Tacca* of Gunung Lang, c) *Tacca* of Gunung Liang, d) *Tacca* of Royal Belum State Park. Arrows Show Anomocytic Stomata. (Magnification 40x10)

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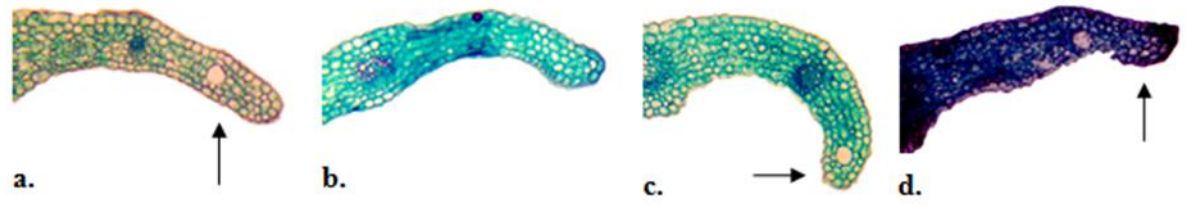

Figure 5: Margin Transverse Section of *Tacca.* a) *Tacca* of Sungai Dara, b) *Tacca* of Gunung Lang, c) *Tacca* of Gunung Liang, d) *Tacca* of Royal Belum State Park. Arrows Show Secretory Cells. (Magnification 10x10)

Midrib outlines are found to be concave and grooved in broad V-shaped adaxially (Figure 6) and U-shaped adaxially. Vascular bundles arrangement seen in midrib, bundle arcs are U to slightly V-shaped pertaining to the outlines (Figure 6). Vascular bundles of midrib and petiole are closed with conjoint collateral type. Petiole outlines are circular and concave to groove slightly at the adaxial (Figure 7). Both vasculature of petiole and midrib are similar, they have closed system bundle with absence of cambium, the vascular tissues are in typical arrangement where phloem and xylem lie together on the same radius in the position that xylem facing inwards and phloem to the outwards. Simple uniseriate trichomes present in midrib are the same type as seen in leaf lamina, however are absent in petiole transverse section. The whole leaf anatomical features of *Tacca* from four different localities collected are presented in Table 1 and Table 2. The morphological characters observed were summarized in Table 1- 3.

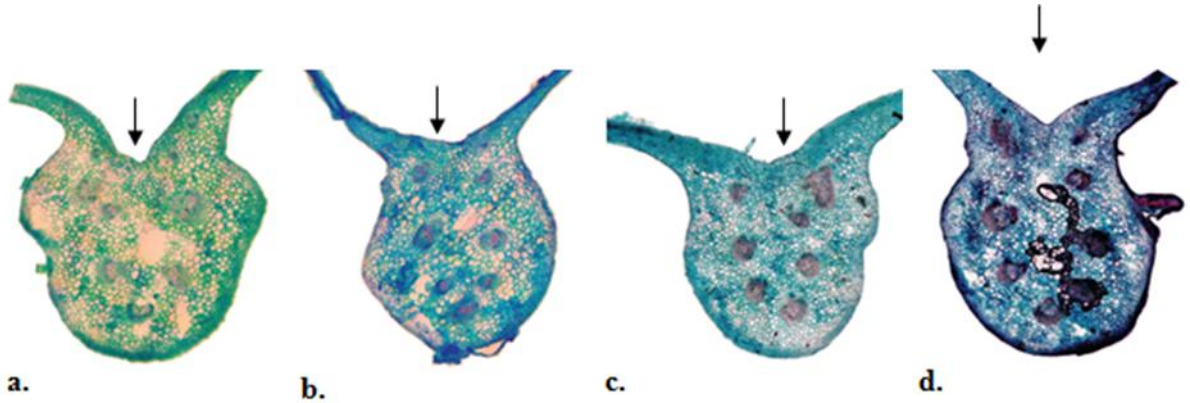

Figure 6: Midrib Transverse Section of Tacca. a) *Tacca* of Sungai Dara, b) *Tacca* of Gunung Lang c) *Tacca* of Gunung Liang, d) *Tacca* of Royal Belum State Park. (Magnification 10x10)

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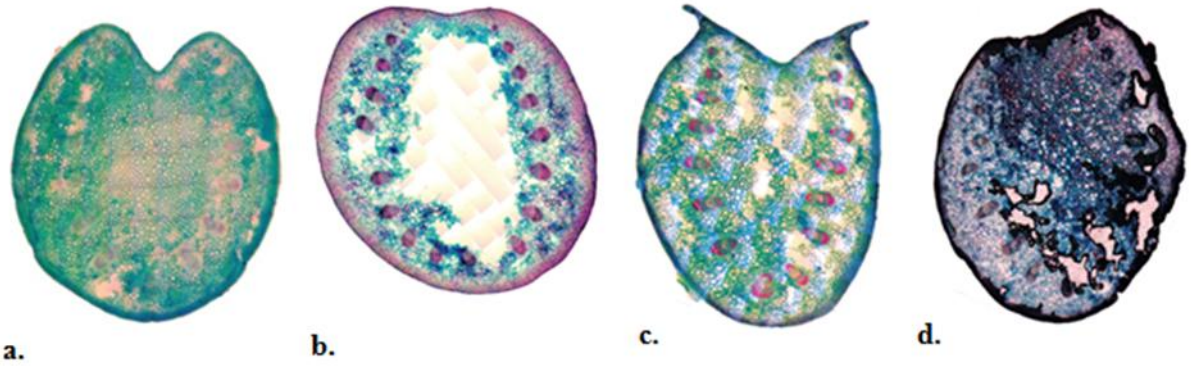

Figure 7: Petiole Transverse Section of *Tacca*. a) *Tacca* of Sungai Dara, b) *Tacca* of Gunung Lang, c) *Tacca* of Gunung Liang, d) *Tacca* of Royal Belum State Park. (Magnification 10x10)

Table 2: Midrib and Petiole Anatomical Characters of *Tacca* From Four Different Localities

Anatomical Characters	*Tacca* of Sungai Dara	*Tacca* of Gunung Lang	*Tacca* of Gunung Liang	*Tacca* of Royal Belum State Park
Midrib:				
Outline	Concave adaxially, U-shaped abaxially	Concave adaxially, U to – V-shaped abaxially	Concave adaxially, U-shaped abaxially	Concave adaxially, U-shaped abaxially
Ground Tissues	Collenchyma at 7-9 layers adaxially, absent abaxially; Parenchyma at 6-9 layers	Collenchyma not seen; Parenchyma at 5-8 layers	Collenchyma at 7-9 layers adaxially, absent abaxially; Parenchyma at 5-8 layers	Collenchyma not seen; Parenchyma multiple layers
Vascular tissues	V-shaped arc, closed bundle	V-shaped arc, closed bundle	U-V-shaped arc, closed bundle	U-shaped arc, closed bundle
Sclerenchyma	Present	Present	Present	Present
Trichome	Not seen	Not seen	Simple type	Simple type
Crystals	Not seen	Not Seen	Not Seen	Not seen
Petiole:				
Outline	Circular, gooved in V- shaped adaxially	Circular, slightly concave adaxially	Circular, grooved in V-shaped adaxially	Circular, slightly concave adaxially
Ground Tissues	Collenchyma absent; Parenchyma in 18-20 layers	Collenchyma absent; Parenchyma in 15-17 layers	Collenchyma absent adaxially, multiple layers abaxially; Parenchyma in 12-14 layers	Collenchyma absent; Parenchyma in 26-28 layers
Vascular tissues	Closed, U-shaped	Closed, U-shaped	Closed, U-shaped	Closed, U-shaped
Sclerenchyma	Present	Present	Present	Present
Trichome	Not seen	Not seen	Not seen	Not seen
Crystals	Not seen	Not seen	Not seen	Not seen

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Morphological observation also shows high similarity and homogeneity in the characters. All *Tacca* leaves are very much similar with oblong or elliptic shape with caudate apex and attenuate base in various sizes. The leaves are green in colour and having arcuate, reticulate, palmate, camptodromous and brochidodromous venation. Leaf margins show slight differences between them. All species show entire leaf margins as mentioned in Kalkman *et al*. (1998) except denticulate margin observed in *Tacca* of Gunung Lang.

Floral morphology vary in colours from purplish black-purple-whitish of bracts and the modified long drooping whisker-like filiform involucre bracteoles (Figure 8a & b). Umbel infloresences with eight florets in cluster and inferior ovary as reported by Keng (1986), Kubitzki (1998) and Kalkman et al. (1998). Fruits longitudinal section displays parietal placentation of the ovary and plenty of seeds fill the central part. Two types of seed found in Taceceae are as reported by Kubitzki (1998); in this study the seeds found to have reniform type (Figure 8c) therefore, confirm that the *Tacca* sp. collected from gunung Lang and Gunung Liang are *T. chantrieri*. According to Kalkman et al. (1998), *T. chantrieri* has reniform seeds while *T. integrifolia* has ovate to ovate-oblong seed type.

Figure 8: Reproductive Organs of *Tacca*. a) *Tacca* of Gunung Lang flower, b) *Tacca* of Gunung Liang flower, c) Reniform Seed of *Tacca*

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Table 3: Leaf and Reproductive Morphological Character of *Tacca*

Anatomical Characters	*Tacca* of Sungai Dara	*Tacca* of Gunung Lang	*Tacca* of Gunung Liang	*Tacca* of Royal Belum
Leaf:				
Apex	Caudate	Caudate	Caudate	Caudate
Venation	Arcuate, reticulate, palmate, camptodromous, brochidodromous	Arcuate, reticulate, palmate, camptodromous, brochidodromous	Arcuate, reticulate, palmate, camptodromous, brochidodromous	Arcuate, reticulate, palmate, camptodromous, brochidodromous
Shape	Oblong to slightly elliptic	Oblong to slightly elliptic	Oblong to slightly elliptic	Oblong to slightly elliptic
Base	Attenuate; some have asymmetry ending of base	Attenuate; some have asymmetry ending of base	Attenuate; some have asymmetry ending of base	Attenuate; some have asymmetry ending of base
Margin	Entire	Denticulate	Entire	Entire
Colour	Green	Green	Green	Green
Width	12.5-20.2cm	12.5-20.2cm	12.5-20.2cm	12.5-20.2cm
Length	36-59.7cm	36-59.7cm	36-59.7cm	36-59.7cm
Flower:				
Inflorescences	-	Umbellate, involucrate	Umbellate, involucrate	-
Number of flowers in clusters	-	8	8	-
Involucral bracts	-	4; purplish-black	4; purplish-black at base and followed by white to apex	-
Thread-like bracts	-	Purplish, Purplish followed by white ending	Purplish, Purplish followed by white ending	-
Outer bracts	-	Ovate shape; apex: Broadly ovate shape; apex: acute acute	Ovate shape; apex: Broadly ovate shape; apex: acute acute	-
Inner bracts	-	Broadly ovate shape; acute apex	Broadly ovate shape; acute apex	-
Number of stamens in corolla		6	6	
Fruit & Seed:				
Plancentation	-	Parietal	Parietal	-
Shape of seed	-	Reniform	Reniform	-

Note. Reproductive morphology was only observed on morphological characters species from Gunung Lang and Gunung Liang as subjected to availability.

Tacca of Gunung Liang and Gunung Lang are identified as *T. chantrieri* as suggested by Kalkman et al., (1998) dichotomous key of *Tacca* species. *T. chantrieri* has entire, elliptic or oblong leaves; reniform seeds and decussate involucral bracts. Sterile samples collected from Sungai Dara and Royal Belum State Park are also suggested to be *T. chantrieri* after extensive comparison made on the leaf morphological and anatomical characters. Identification are made based on anatomical similarities and partly of morphological similarities.

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4. CONCLUSION

Tacca species collected in the current study are suggested to be *T. chantrieri* after extensive observation made morphologically and anatomically referring to Kalkman et al. dichotomous key. Kalkman et al. dichotomous key which was published in 1998 is the only recent available reference to *Tacca* species. In this research some variations seen within the leaf margin and the floral colour but these two characters are not considered in Kalkman's dicotomous key, therefore they are not taken into account. All *Tacca* spesies studied are similar in anatomical characters thus, suggested that the other two samples from Sungai Dara and Royal Belum State Park are also *T. chantrieri*. Further investigation are very much encouraged to reconfirm that the species is *T. chantrieri* as it is not as common as *T. intergrifolia* recorded in Peninsular Malaysia. Reproductive organs of *Tacca* from Sg Dara and Royal Belum State Park are suggested to be examined in the future to support this research finding.

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