



On the morphological variation of *Rafflesia cantleyi* (*Rafflesiaceae*) on Pulau Tioman, Pahang, Peninsular Malaysia

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Key words

morphology
Pulau Tioman
Rafflesia cantleyi complex
variability

Abstract In Peninsular Malaysia, *Rafflesia* is represented by seven species of which *R. kerrii* (and *R. su-meiae*) stands out distinctly from the other five. The other five species, *R. azlanii*, *R. cantleyi*, *R. parvimaclata*, *R. sharifah-hapsahiae* and *R. tuanku-halimii*, are collectively close enough to each other to be referred to as the *R. cantleyi* complex after its first-described species, *R. cantleyi*. Pulau Tioman has a population of *R. cantleyi*, which, because of its island location, is isolated from the mainland complex. This study was conducted to determine morphological variation in a selected location in Pulau Tioman. Twelve flowers were studied with respect to characteristics such as wart (blotch) pattern on perianth lobes, warts (dots) on upper surface of the diaphragm, shape of the aperture, shape of processes and types of ramenta. These are the characters that have been used to define species in the *R. cantleyi* complex. The variation in the local Tioman population was compared with the variation in the *R. cantleyi* complex on the mainland, which is about the same magnitude. This supports the idea that *R. cantleyi* is a single highly polymorphic species and that the species that have been described in the *R. cantleyi* complex should be reduced to varieties.

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INTRODUCTION

Rafflesia R.Br. (*Rafflesiaceae*) is a genus of fleshy parasitic plants that comprises about 38 species distributed only in the tropical regions of Southeast Asia with twelve to thirteen species each in Indonesia, the Philippines and Malaysia (Nickrent et al. 1997 and onwards). The ranges of the majority of species are very small and some species have only been collected once. *Rafflesia* species are confined to secondary and primary rainforests where they grow on the stems and roots of *Tetrastigma* species (*Vitaceae*) and the young buds may be easily overlooked.

In Malaysia, currently, there are 12 species described: seven in Peninsular Malaysia and five in West Malaysia (Sabah and Sarawak). In Peninsular Malaysia, *Rafflesia* species are found in the states of Kedah, Perak, Kelantan, Pahang and Terengganu, in lowland dipterocarp to hill dipterocarp forests. *Rafflesia* was first documented for the Malay Peninsula in 1910 by Solms-Laubach's publication of *Rafflesia cantleyi* Solms based on a specimen collected by Cantley in Perak. The exact location was not indicated. The type specimen is in Kew. When Ridley (1915) published his account of *Rafflesia*, he was unaware of Solms-Laubach's publication and he assigned the Malayan specimens, including the Cantley specimen, to *Rafflesia hasseltii* Suring. *Rafflesia hasseltii* was, until then, only known from Sumatra. Following Ridley, all Malayan collections of *Rafflesia* were assigned to *R. hasseltii* (e.g., Ridley 1924, Henderson 1930, Keng 1969). In 1984, *R. cantleyi* was restored by Meijer, who, in a key to all the known species, distinguished *R. hasseltii* from *R. cantleyi* by differences in the pattern of warts on the perianth lobes. In *R. hasseltii*, white warts across the base of the perigone lobes are very large and number 4 or 5 only. In *R. cantleyi* the white warts are smaller and number 7–9. In

Meijer's (1997) account of *Rafflesia* for Flora Malesiana, three species were recognized for the Malay Peninsula, *R. cantleyi*, *R. hasseltii* and *R. kerrii* Meijer. In 2003, Latiff & Wong decided that *R. hasseltii* in the Malay Peninsula was not the same as *R. hasseltii* of Sumatra, but represented a new species, which they named *R. azlanii* Latiff & M.Wong. Since then, several more species of *Rafflesia* have been described.

Currently, there are seven species described in Peninsular Malaysia: *R. cantleyi*, *R. kerrii* (Meijer 1984); *R. azlanii* (Latiff & Wong 2003), *R. su-meiae* M.Wong, Nais & F.Gan (Wong et al. 2009), *R. sharifah-hapsahiae* J.H.Adam, R.Mohamed, Aizat-Juhari & K.L.Wan (Adam et al. 2013), *R. parvimaclata* Sofiyanti, K.Mat-Salleh, Khairil, Zuhailah, Mohd.Ros. & Burslem (Sofiyanti et al. 2016) and *R. tuanku-halimii* J.H.Adam, Aizat-Juhari, Azilah & K.L.Wan (Adam et al. 2016). In general, based on morphological patterns, all the species described in Peninsular Malaysia can be placed into two groups: the *R. cantleyi* complex and the *R. kerrii* complex (Fig. 1). The *Rafflesia kerrii* complex, which includes *R. su-meiae*, immediately stands out as something totally different from the *R. cantleyi* complex (the size, the type of warts on the perianth lobes and the ramenta type). However, all the other species appear to form part of the complex around *R. cantleyi*.

Pulau Tioman is the only island in Malaysia with *Rafflesia* individuals and it is the southernmost location for the genus in the Peninsula (Map 1). *Rafflesia* at Pulau Tioman was mentioned by Henderson (1930) at Sedagong at about 300 m asl. Then, as noted in Lee et al. (1977), a *Rafflesia* specimen was collected by Kadim & Nur from Sungai Ayer Besar. Both specimens were then known as *R. hasseltii*. However, they were later referred to *R. cantleyi* in the first published colour photographs by Bidin (1991), showing *Rafflesia* flowers on the trail to the peak of Gunung Nenek Semukut. Mahdini (2006) studied *Rafflesia* in Pulau Tioman and noted that the species there has characters similar to *R. cantleyi*. In 2012, the first author inventoried the *Rafflesia* population along the trail up to Gunung Kajang and

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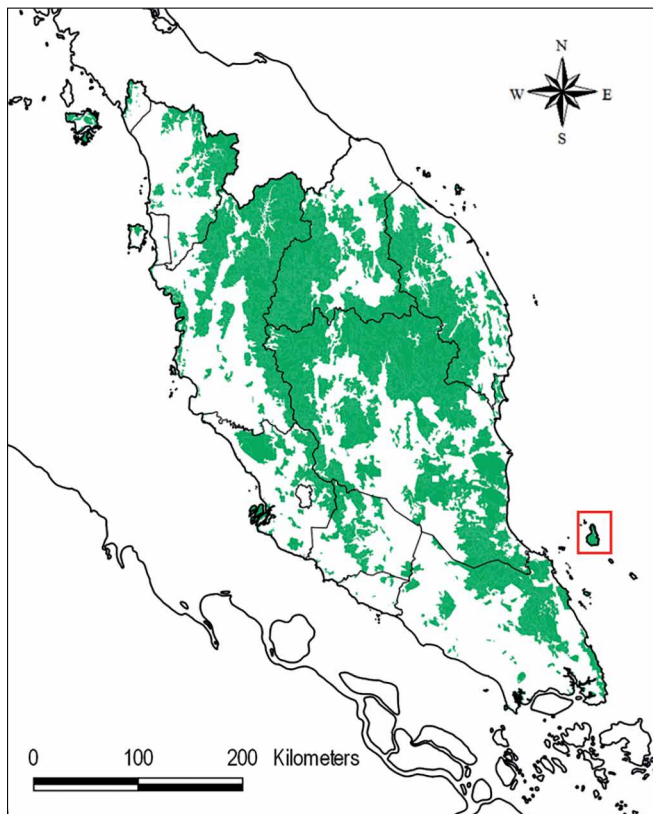
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Table 1 Comparative morphology of 12 flowers of *Rafflesia cantleyi*.

Host plant	Flower number (date observed)	Identification of variety/form within the <i>R. cantleyi</i> complex	Form of large blotches on perigone lobes	Dotted warts on diaphragm	Aperture shape	Processes		Ramenta Stem (stalk); apex			
						amount	stem	apex	upper	middle	lower
T1	R1 (03 May 2012)	var. <i>cantleyi</i> /var. <i>parvimaclulata</i>	small, < 5 cm (discrete)	many (> 50–100) c. 89	circular	28	cylindrical	falcate (smooth pointed)	stems erect cylindrical and 'loadstool' rounded	long, slender; toadstool with short branches	long, slender; thin; simple swollen or branching
T2	R3 (12 January 2014)	var. <i>cantleyi</i> /var. <i>parvimaclulata</i>	small, < 5 cm (discrete)	many (> 50–100) c. 60	circular	21	cylindrical	expanded warty	stems erect cylindrical and 'loadstool' rounded	long, slender; toadstool with long branches	long, slender, thin; simple swollen or branching
T3	R2 (06 July 2013)	var. <i>cantleyi</i> /var. <i>azlanii</i>	(all) average to big, > 5 cm (discrete to coalesced)	few (< 20) (all) unclear (fake dotted)	circular;	16	(all) trigonal	(all) smooth-rounded	(all) stems erect cylindrical and 'loadstool' rounded	(all) long, slender; toadstool with short branches	(all) long, slender, thin; simple swollen
	R7 (08 September 2014)				circular	19					
	R8 (07 June 2015)				circular	7					
	R12 (29 May 2017)				angular	17					
T4	R4 (26 February 2014)	var. <i>cantleyi</i> /var. <i>parvimaclulata</i>	small, < 5 cm (discrete);	average (< 50); c. 25	circular	25	(all) cylindrical	(all) smooth-angular	(all) short- or long-stemmed, and the 'loadstool' with a brown depression in its centre	(all) long, slender; toadstool unbranched	(all) long, slender, thin; simple swollen
	R5 (16 March 2014)		small, < 5 cm (discrete)	average (< 50); c. 40	circular	26					
T5	R6 (04 July 2014)	var. <i>azlanii</i> /var. <i>tuanku halimii</i>	average to big, > 5 cm (more coalesced)	average (< 50) c. 44	angular	21	cylindrical	expanded warty	short-stemmed with a flat-topped 'loadstool'	long, slender; toadstool with long branches	long, slender, thin; simple swollen
T6	R11 (07 May 2017)	var. <i>cantleyi</i>	small, < 5 cm (discrete)	many (> 50–100) c. 65	lobed	22	cylindrical	expanded warty	short (erect); flat-topped 'loadstool'	long, slender; toadstool unbranched	long, slender, thin; simple swollen
T7	R9 (16 June 2015)	var. <i>cantleyi</i> /var. <i>azlanii</i>	(all) average to big, > 5 cm (discrete to coalesced)	(all) average (< 50); c. 35	circular	19	(all) cylindrical	(all) smooth-angular	(all) short-stemmed with a flat-topped 'loadstool'	(all) similar to upper or lower	(all) erect, white toadstool
	R10 (23 June 2015)			c. 45	circular	19					



Fig. 1 Two specimens representative of the two complexes in Peninsular Malaysia. a. *Rafflesia kerrii*, from Lojing Highland, Kelantan; b. *Rafflesia cantleyi*, from Gerik, Perak (M.Y. Siti Munirah).



Map 1 Peninsular Malaysia with Pulau Tioman in the red box (with NFI III Courtesy of the Forest Department Peninsular Malaysia).

visited a population near the Kampung Juara forest (Siti Munirah 2013). Since then, in collaboration with the second and third author, as well as a local guide and a naturalist (M.S. Razelan and A. Salamah, respectively), blooming *Rafflesia* data have been systematically documented.

Rafflesia has been one of the important iconic plants for Pulau Tioman tourism for many years. The habitat is lowland to hill dipterocarp forest. The host plant of *Rafflesia* is distributed all over the forests of Pulau Tioman. Most of the *Rafflesia* populations in south Pulau Tioman have been seen in the vicinity of the forest along the road between Tekek to Juara, and are therefore accessible. However, the population in Gunung Kajang, via the Gunung Kajang trail, is more difficult to access. At the north side of Pulau Tioman, *Rafflesia* has been recorded

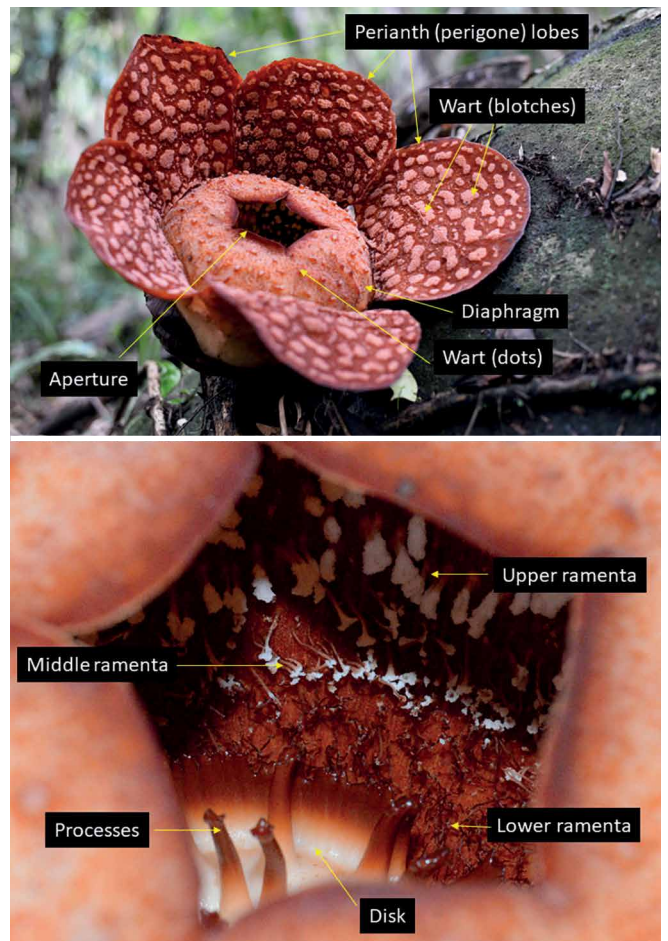


Fig. 2 *Rafflesia* characters observed and documented for this study (M.Y. Siti Munirah).

from Gunung Mukut. The host plant of *Rafflesia* in Tioman has been identified as *Tetrastigma rafflesiae* Planch.

The taxonomy of *Rafflesia* is based on the morphology of the flowers with most emphasis on the outer appearance. In the Malaysian species, the characters used to distinguish between species in the *R. cantleyi* complex include the pattern of the warts on perianth lobes (*R. azlanii*, *R. parvimaculata*, *R. sharifah-hapsahiae*), number and arrangement of warts on the diaphragm surface (*R. sharifah-hapsahiae*), pattern of white spots (windows) (*R. tuanku-halimii*), the absence of a marginal ring

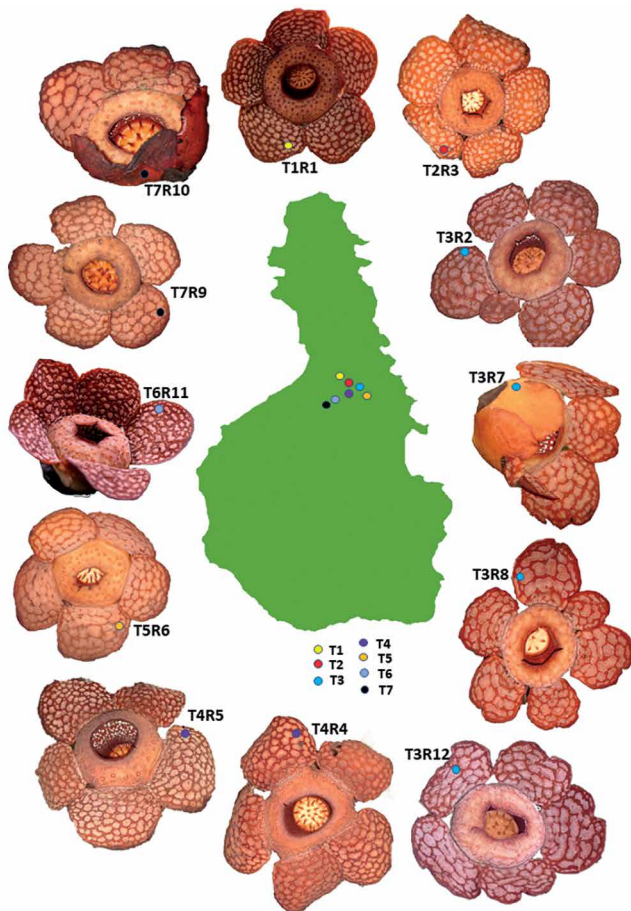


Fig. 3 *Rafflesia cantleyi* flowers in Pulau Tioman. The T numbers refer to the *Tetrastigma* host specimens, the R numbers to the *Rafflesia* parasites (M.Y. Siti Munirah, A. Salamah & M.S. Razelan).

(*R. sharifah-hapsahiae*), structure of ramentas on the perianth tube (*R. azlanii*, *R. su-meiae*). The question arises whether these differences are adequate for species delimitation or not. To evaluate these characters, we studied how they vary within the compact and well-defined population in Tioman Island. The terminology used for this study is provided in Fig. 2.

MATERIALS AND METHODS

Twelve flowers (Fig. 3) were studied in detail over a period of five years. They were observed on seven host plants in a population in Hutan Rezab Hidupan Liar Pulau Tioman. Since this is a tourist area, all individuals found were only documented in a non-destructive way by taking measurements and describing the morphological features in the field, supported by photographic images without making any herbarium specimens. The observed characters include the morphology of outer surfaces such as the large wart (blotch) pattern on perianth lobes, dotted warts (dots) on upper surface of diaphragm, shape of diaphragm aperture, shape of processes and types of ramenta (Fig. 2).

RESULTS

The morphological characters of the 12 flowers are discussed below and the individual observations are provided for comparison in Table 1.

Perianth (perigone) lobes — Fig. 4

Abaxial surface (undersurface) smooth or glabrous, reddish orangish with whitish tiny dots; adaxial (upper) surface glabrous, reddish orangish, covered with warts (blotches). The warts whitish to pale reddish and may as discrete rounded patches or as bands of coalescent patches. The warts may be: (a) small, < 5 cm, discrete (Fig. 4a: R1, R3, R4, R5, R11); (b) average



Fig. 4 Form of perianth lobes. a. Small and discrete; b. average to big and discrete to slightly coalesced; c. average to big and strongly coalesced. — Photos by a: M.Y. Siti Munirah; b–c: A. Salamah & M.S. Razelan.

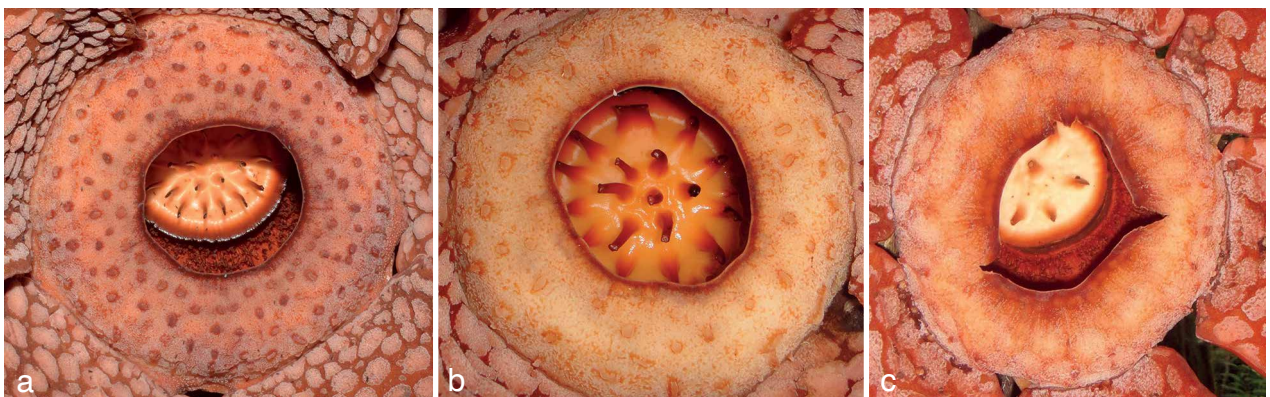


Fig. 5 Warts (dots) on the diaphragm. a. Many warts; b. < 50 warts in 2 or 3 rings; c. < 20 warts. — Photos by a: M.Y. Siti Munirah; b–c: A. Salamah & M.S. Razelan.

to big, > 5 cm, discrete to slightly coalesced (Fig.4b: R2, R7, R8, R9, R10, R12); and (c) average to big, > 5 cm, strongly coalesced (Fig. 4c: R6).

Diaphragm — Fig. 5

The diaphragm upper surface is glabrous and generally smooth (R4, R5, R9), but sometimes indented by impressions made by the warts of the perianth lobes (R2, R7, R8, R12).

Warts (dots) — Fig. 5

Small dotted warts cover the upper surface of the diaphragm. The dotted warts are of irregular shape, orangish in colour or yellowish in the middle with a darker ring. All the dotted warts are arranged in circular ring or are well distributed. The number can be up to more than 100 dots.

Three types of wart disposition are observed: (a) many (> 50–100; Fig. 5a) evenly distributed (R1, R3, R11); (b) average (< 50; Fig. 5b) arranged in 2 or 3 rings (R4, R5, R6, R9, R10); or (c) few (< 20; Fig. 5c) well-spaced (R2, R7, R8, R12) and sometimes unclear.

The warts on the lower surface of the diaphragm, known as windows, are whitish, of irregular shape and either discrete or coalesced. However, this character is not observed in this study, because they are at the underside of the diaphragm and cannot be fully seen without dissection.

Aperture — Fig. 6

The aperture is the opening in the centre of the diaphragm. It can be: (a) circular (Fig. 6a: R1, R2, R3, R4, R5, R7, R8, R9, R10); (b) lobed and star-shaped (Fig. 6b: R11); or (c) irregularly shaped (angular) (Fig. 6c: R6, R12). The margin is glabrous and the colour normally darker than the rest of the upper surface colour of the diaphragm.

Disk — Fig. 7

The disk is located in the central part of the flower. Its surface is flat, or sunken (concave) in the staminate flower (Fig. 7a: R1, R6) and convex in the pistillate flowers (Fig. 7b–d: R2, R3, R4, R5, R7, R8, R9, R10, R11, R12).

Processes — Fig. 8

The erect processes on the disk surface vary in number. The stem is cylindrical (Fig. 8a, b, d: R1, R3, R4, R5, R6, R9, R10, R11), or trigonal (Fig. 8c: R2, R7, R8, R12), with the base wider than the apex; the apex (tip) can be expanded warty (Fig. 8a: R3, R6, R11) or smooth-angular (Fig. 8b: R4, R5, R9, R10), smooth-rounded (Fig. 8c: R2, R7, R8, R12) or smooth-pointed, falcate (Fig. 8d: R1). The colours range from pale creamy yellow to orangish and darker.



Fig. 6 Form of the aperture. a. Circular, b. lobes and star-shaped, c. irregular. — Photos by a–b: M.Y. Siti Munirah; c: A. Salamah & M.S. Razelan.

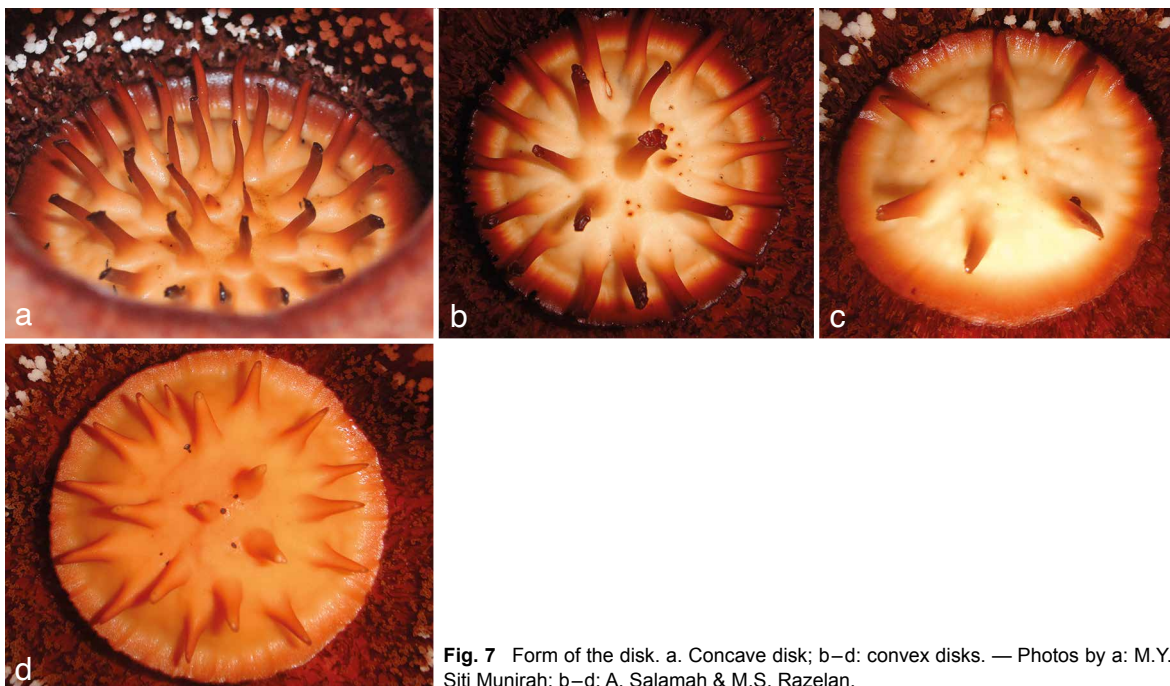


Fig. 7 Form of the disk. a. Concave disk; b–d: convex disks. — Photos by a: M.Y. Siti Munirah; b–d: A. Salamah & M.S. Razelan.

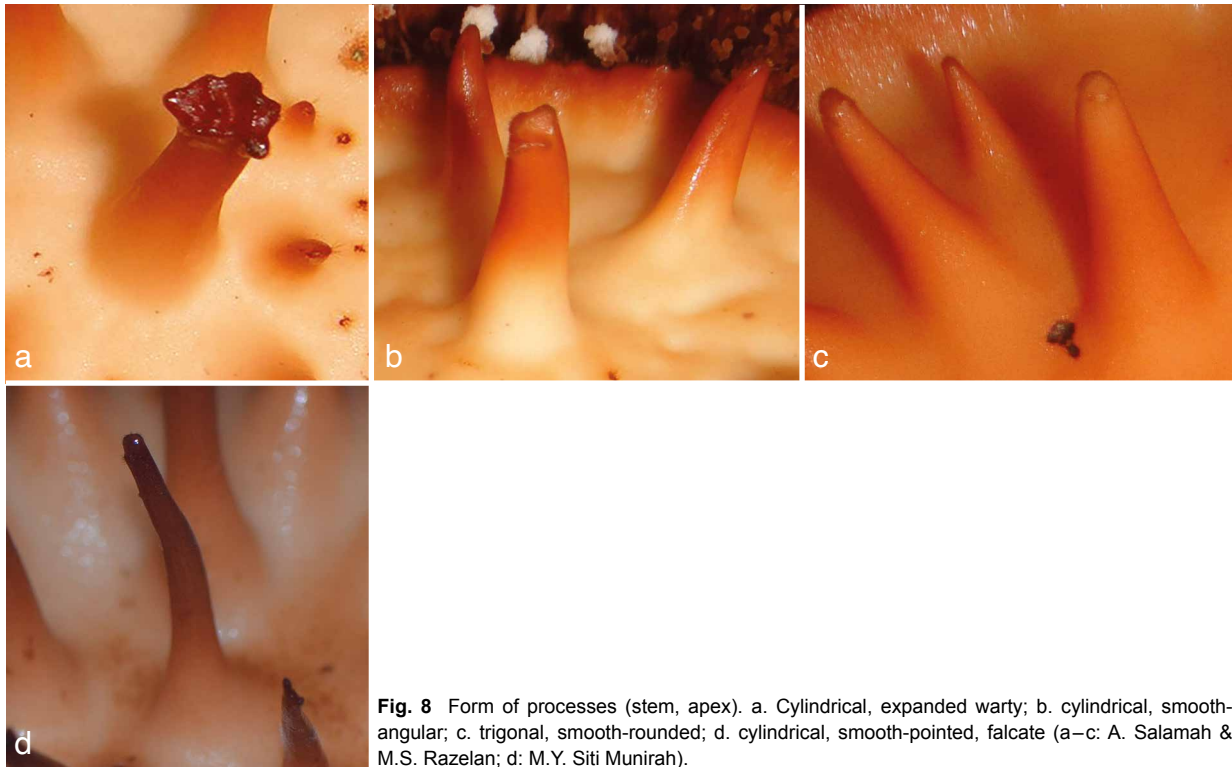


Fig. 8 Form of processes (stem, apex). a. Cylindrical, expanded warty; b. cylindrical, smooth-angular; c. trigonal, smooth-rounded; d. cylindrical, smooth-pointed, falcate (a–c: A. Salamah & M.S. Razelan; d: M.Y. Siti Munirah).

Ramenta — Fig. 9

The ramenta cover the adaxial (inner) surface of the perianth tube and are differentiated into two (upper and lower; Fig. 9a: R9, R10) or three zones (upper, middle and lower; Fig. 9b: R1, R2, R3, R4, R5, R6, R7, R8, R11, R12).

Upper ramenta — Fig. 10

The upper ramenta have short erect stems and an expanded white 'toadstool' apex. Three types may be distinguished: (a) short-stemmed with a flat-topped 'toadstool' (Fig. 10a: R6, R9, R10, R11); (b) short- or long-stemmed, and the 'toadstool' with a brown depression in its centre (Fig. 10b: R4, R5); or (c) stems erect cylindrical and 'toadstool' rounded (Fig. 10c: R1, R2, R3, R7, R8, R12).

Middle ramenta — Fig. 11

The middle ramenta of all specimens has slender stalks with a terminal white 'toadstool', which may have: (a) short branches (Fig. 11a: R1, R2, R7, R8, R12); (b) long branches (Fig. 11b: R3, R6); or (c) is unbranched (Fig. 11c: R4, R5, R11).

Lower ramenta — Fig. 12

In the lower part of the perianth tube the ramenta are: (a) mostly uniformly reddish maroon (simple swollen or branching apex; Fig. 12a: R1, R2, R3, R4, R5, R6, R7, R8, R11, R12); or (b) sometimes bearing a white rounded toadstool (Fig. 12b: R9, R10).

DISCUSSION

Most character states present in Tioman are also found in the *R. cantleyi* complex in mainland Peninsular Malaysia. For example, the wart pattern on the perianth lobes has been used to differentiate *R. cantleyi* (small and discrete, Fig. 4a, b) from *R. hasseltii* (big and coalesced) (Solms-Laubach 1910) and also to differentiate *R. azlanii* (coalesced) from *R. cantleyi* (discrete) (Latiff & Wong 2003), but within the population of Tioman, both discrete and coalesced warts can be found. In general,

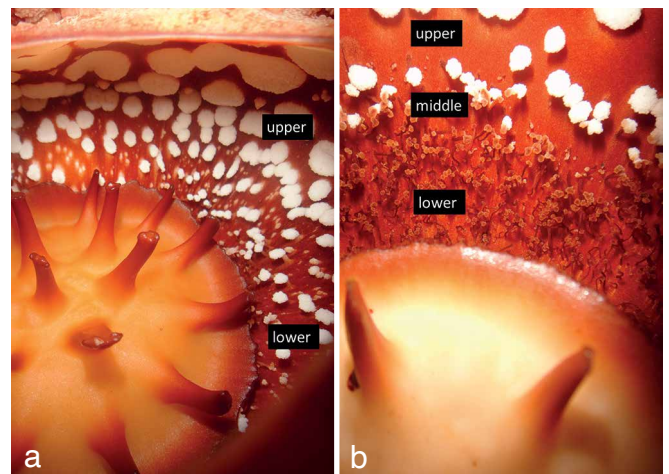


Fig. 9 Distribution of ramenta. a. Consisting of two layers; b. consisting of three layers (A. Salamah & M.S. Razelan).

the range of variation in the morphology of the processes, the pattern of dotted warts on the diaphragm upper surface and the variation in ramenta structures in Tioman resembles that of the mainland. This is based on information from all the original protologues of *Rafflesia* species described from mainland Peninsular Malaysia and also personal observations and data documentation in the wild by the first author while studying *Rafflesia* species in Peninsular Malaysia.

Based on Table 1, the morphological data are mostly consistent within the flowers on the same host but differ from flowers on different hosts. Therefore, there are a few characters observed in Tioman, which have not been recorded for mainland Peninsular Malaysia. These include the lobed (star-like) shape of the aperture (T6R11; Fig. 6b), number of processes fewer than 10 (T3R8; Fig. 7c) and ramenta with white 'toadstool' apex with brown central depression (T4R4, T4R5; Fig. 10b). Although the ramenta character is used to differentiate between species (Susatya et al. 2017), we found that its variability to be really high within the flowers examined on Pulau Tioman populations.

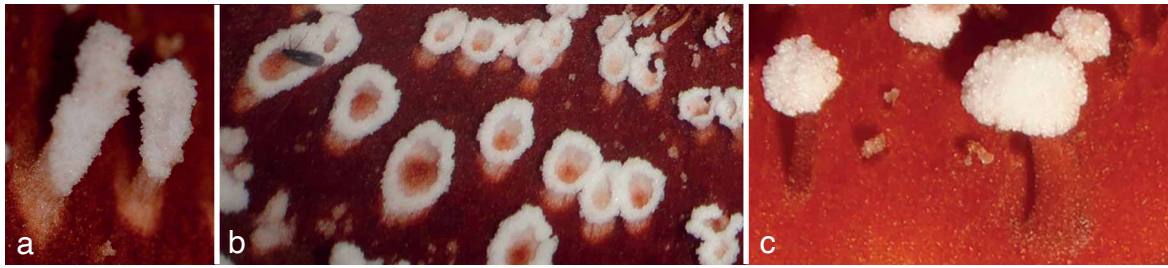


Fig. 10 Forms of upper ramata: a. short-stemmed with a flat-topped 'toadstool'; b. short- or long-stemmed, and the 'toadstool' with a brown depression in its centre; c. stems erect cylindrical and 'toadstool' rounded (a: M.Y. Siti Munirah; b–c: A. Salamah & M.S. Razelan).

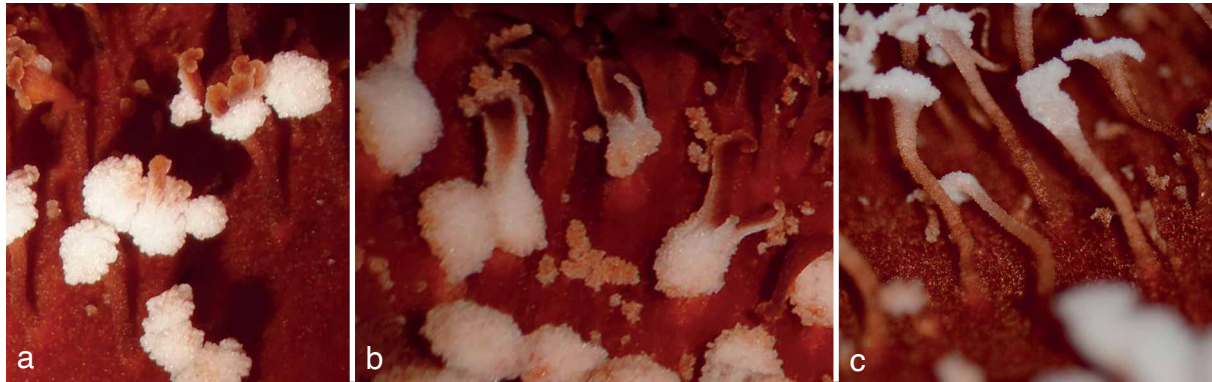


Fig. 11 Form of middle ramata: a. short branches; b. long branches; c. unbranched (a–b: A. Salamah & M.S. Razelan; c: M.Y. Siti Munirah).

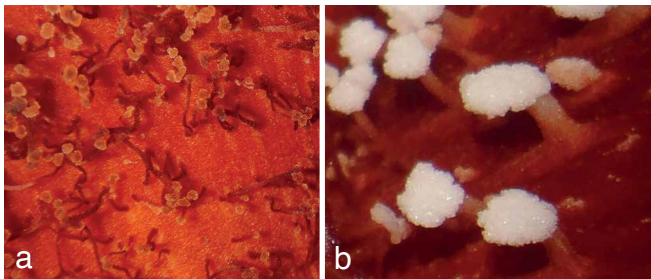


Fig. 12 Form of lower ramata. a. Uniformly reddish maroon with simple swollen apex; b. with white rounded toadstool (a: M.Y. Siti Munirah; b: A. Salamah & M.S. Razelan).

The differences within the Tioman population could be used to describe 'new taxa', but this would be unjustified, because the population is small, compact and isolated and, therefore, the differences are only typical for a few specimens, while most others matched with the majority of the characters within the *R. cantleyi* complex. This is more typical for a single species. However, a further examination of molecular data or other taxonomically important data, may shed more light on the species delimitation in this complex.

On the mainland, three species were newly described based on specimens from within the same forest reserve, the Benom Forest Reserve in Pahang: *Rafflesia sharifah-hapsahiae* (Adam et al. 2013), *R. parvimaculata* (Sofiyanti et al. 2016) and *R. tuanku-halimii* (Adam et al. 2016). Their morphological differences fall within the variation seen within the *R. cantleyi* complex and that makes the status of these species doubtful. Our study on the Tioman population suggests that the Benom FR population is similarly polymorphic and it is not realistic to describe each form as a separate species. On a Peninsula-wide basis, it would make sense to treat all currently recognised species in the *R. cantleyi* complex as varieties of one species, *R. cantleyi*.

The high degree of morphological variation within *Rafflesia* indicates a high genetic diversity and supports the work of Barkman et al. (2017), who show that the destruction of a single

infected host vine could result in large genetic losses. Therefore, further work on the genetic diversity and structure of the *R. cantleyi* complex in Peninsular Malaysia is crucially needed. It can be fashioned as Pelser et al. (2017) did in their study on the *Rafflesia lagascae* complex.

Concerning the future of *Rafflesia* in Tioman, the status of the reserve Hutan Rezab Hidupan Liar Pulau Tioman should be maintained as a totally protected area in order to safeguard the genetic diversity and population richness of *Rafflesia*, which is shown by its high morphological variability.

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