

Rafflesia tunku-azizahiae (Rafflesiaceae), a New Species from Pahang, Malaysia (*Rafflesia tunku-azizahiae* (Rafflesiaceae), Spesies Baharu dari Pahang, Malaysia)

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ABSTRACT

A new species of *Rafflesia* (Rafflesiaceae) from Mt. Benom Forest Reserve, Ulu Dong, Raub, Pahang, *Rafflesia tunku-azizahiae* Adam, Aizat-Juhari & Wan is described and illustrated. Cluster analysis and principal component analysis showed that *R. tunku-azizahiae* and *R. tuanku-halimii* are closely related morphologically, characterized by perigone lobes covered by rows of very closely spaced coalesced white warts, and windows wholly covered by very closely rings of coalesced white warts. Score plot and bi-plot showed that *R. tunku-azizahiae* differs from *R. tuanku-halimii* by larger flower diameter, broader diaphragm and disc diameter, larger aperture diameter and longer ramenta.

Keywords: Cluster analysis; Peninsular Malaysia; principal component analysis; *Rafflesia*; taxonomy; *Tetrastigma*

ABSTRAK

Suatu spesies baharu *Rafflesia* (Rafflesiaceae), dari Hutan Simpan Gunung Benom, Ulu Dong, Raub, Pahang, *Rafflesia tunku-azizahiae* Adam, Aizat-Juhari & Wan telah dihurai dan diilustrasikan. Analisis kelompok dan analisis komponen utama menunjukkan *R. tunku-azizahiae* dan *R. tuanku-halimii* mempunyai pertalian morfologi yang rapat; dua spesies tersebut mempunyai barisan bintil putih yang rapat bersambung pada permukaan cuping perigon dan seluruh tettingkap ditutupi oleh lingkaran tersusun rapat oleh bintil putih bersambung. Plot skor dan plot bi menunjukkan *R. tunku-azizahiae* berbeza daripada *R. tuanku-halimii* dengan saiz diameter bunga yang lebih besar, diameter diafragma dan cakera yang lebih lebar, diameter apertur yang lebih besar dan ramenta yang lebih panjang.

Kata kunci: Analisis kelompok; analisis komponen utama; *Rafflesia*; Semenanjung Malaysia; taksonomi; *Tetrastigma*

INTRODUCTION

Rafflesia R. Br. (Rafflesiaceae) is a genus of holoparasitic flowering plants, globally known for its large and beautiful flowers (Beaman & Adam 1983; Kuijt 1969; Meijer 1997, 1985; Nais 2004), rarity and unique mode of nutrition (Adam & Masni 1984). In Malaysia, *R. tengku-adlinii* has been reported to produce the smallest flower with a diameter of ca. 20 cm (Mat-Salleh & Latiff 1989), while a 24 cm diameter male flower of

R. tengku-adlinii from the foothill of Mt. Trus Madi, Sabah has been previously recorded (Afiq Aizat-Juhari, unpublished data). Peninsular Malaysia is one of the centers of diversity of the genus (Adam et al. 2016, 2013; Sofiyanti et al. 2016), with nine species currently recorded, including this new species. Of all the known Peninsular Malaysian species, four were recorded from the study area, viz., *R. cantleyi* Solms-Laubach from Lata Jarum and Bukit Laha; *R. parvimaclulata* Sofiyanti, Mat-Salleh,

Khairil, Zuhailah, Mohd Ros & Burslem (Sofiyanti et al. 2016) from Lata Jarum; *R. sharifah-hapsahiae* Adam, Mohamed, Aizat-Juhari & Wan (Adam et al. 2013) from Celik Ara; and *R. tunku-azizahiae* Adam, Aizat-Juhari & Wan from Lata Tinggi. All these localities are located within Gunung Benom Forest Reserve, Ulu Dong, Raub, Pahang (Figure 1). Five other species recorded elsewhere in Peninsular Malaysia include *R. azlanii* Latiff & Wong (Latiff & Wong 2003), *R. kerrii* Meijer (Meijer, 1984), *R. su-meiae* Wong, Nais & Gan (Wong, Nais & Gan 2009), *R. tuanku-halimii* Adam, Aizat-Juhari, Azilah & Wan

(Adam et al. 2016) and *R. tiomanensis* Siti-Munirah, Salamah & Razelan (Siti Munirah, Salamah & Razelan 2021). Morphological characteristics that differentiate the *Rafflesia* species include i) flower size, ii) pattern warts on the perigone lobes, iii) size of diaphragm, iv) pattern of window, v) the dimensions of aperture, vi) number of processes, vii) characters of ramenta, and viii) number of anthers (Adam et al. 2013; Meijer 1997; Nais 2001). In this paper, we describe *R. tunku-azizahiae*, the fourth species from the area of Gunung Benom, the ninth for Peninsular Malaysia and the fourteenth for Malaysia.

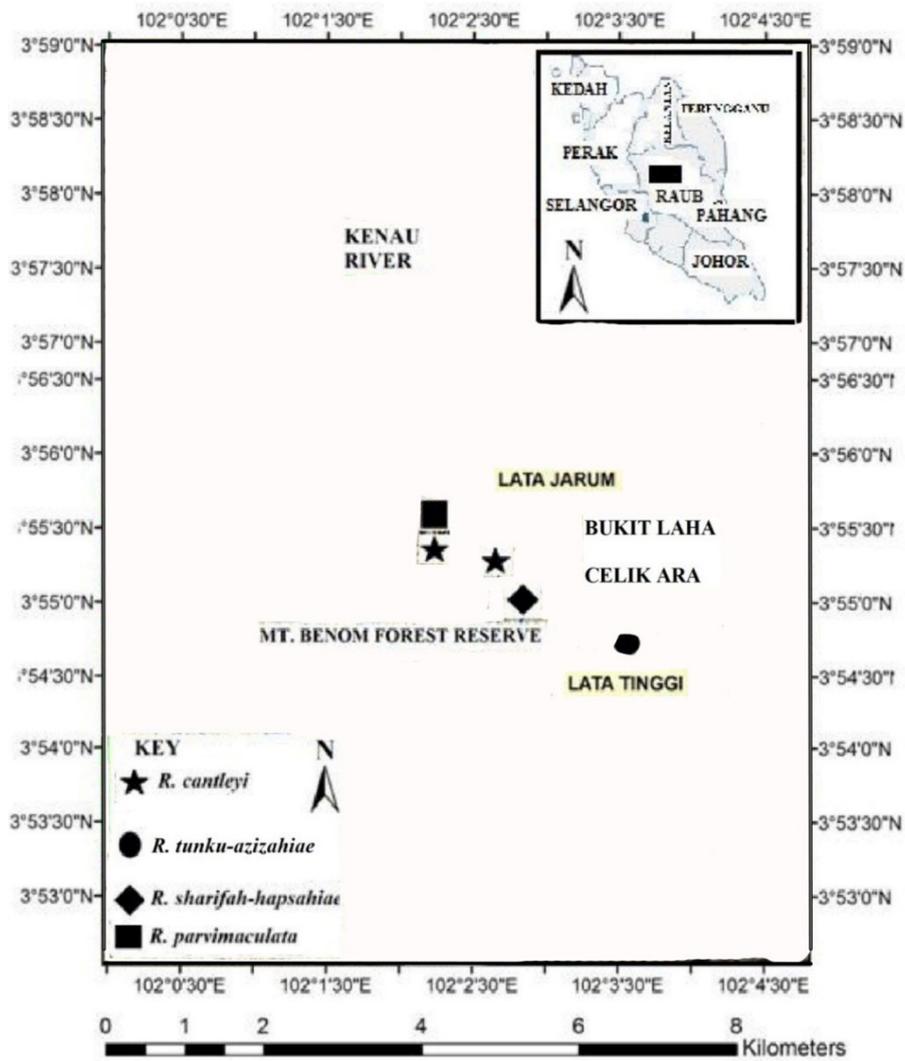


FIGURE 1. Distribution of the four species of *Rafflesia* from Mt. Benom Forest Reserve, Ulu Dong, Raub, Pahang, Peninsular Malaysia, including the new species, *R. tunku-azizahiae*

MATERIALS AND METHODS

Statistical analyses were conducted on eleven *Rafflesia* specimens with 26 morphological features using the Minitab 19 application (Table 1). The data of eleven *Rafflesia* specimens and 26 characters were subjected to statistical multivariate analysis. For cluster analysis, complete linkage method was applied for clustering of the specimens whereas the Euclidean distance was used as the distance measure. The same data set were subjected to multivariate analysis and principal component analysis (PCA). The advantage of PCA is its ability to identify a smaller number of uncorrelated variables or principal components (PCs) in a multivariable database, and to identify new meaningful underlying variables. PCA also explains the maximum amount of variance with the fewest number of principal components (Esposito et al. 2007; Klaassen, Kwembeya & Maass 2009; Yasmin & Roslan 2015). In this study, statistical calculation of PCA was carried out using MINITAB VER.19 to obtain Eigen analysis of correlation matrix and eigenvectors and to produce PCA plots of the first two PCs namely score plot, loading plot and bi-plot. The lower number of PCs that explained the majority of the variation in the original data can be determined using a scree plot or eigenvalues; score plot identifies clusters of the first two PCs, loading plot can visually interpret the first two PCs, and bi-plot can identify clusters through the interpretation of the first two PCs.

NEW SPECIES

Rafflesia tunku-azizahiae Adam, Aizat-Juhari & Wan, sp. nov., Figures 2 and 3.

R. tunku-azizahiae is similar to *R. tuanku-halimii* in having similar perigone lobes and window pattern but differs from it by larger flower size, broader diaphragm and disc diameter, large aperture with the diameter 14 cm (Figure 2(E)) and the inner surface of the perigone tube covered by simple, branched and long ramenta, 14-18 mm in length (Figures 2(J), 2(K), 2(L), 3(E) & 3(F)). On the other hand, *R. tuanku-halimii* has smaller flower size, narrower diaphragm and disc diameter, smaller aperture, 9-9.5 cm in diameter (Figure 4(B); Table 1), and simple and shorter ramenta, 4-7 mm long (Figure 4(C)).

Type: Malaysia, Pahang, Raub, Ulu Dong, Mt. Benom Forest Reserve, Lata Tinggi, N03°54'46.8" & E102°03'33.5", 624 m elevation, ♀ flower, 20 May 2011, Nik Nadira Farhana, Jumaat Adam, Mohd Afiq

Aizat & Siti Norhafizah, LTP2B6, Parasitic on woody climber *Tetrastigma rafflesiae* (Miq.) Planch. (Vitaceae) (Holotype UKMB).

Description Holoparasite. Male bud and flower unknown. Female mature bud 25-28 cm in diameter, yellowish red, surface smooth with no pattern (Figure 2(B)). Bract black and brown, thin and scaly, innermost largest, outermost smallest (Figure 2(A)). Female flowers diameter 63-80 cm when fully opened. Perigone lobes five, vary in sizes, 16.5-18 cm long, 22-24 cm wide. Perigone lobes upper surface covered by 6-8 rows of closely spaced coalesced white warts, 2-8 cm long, 2-3 cm thick; density of warts on each perigone lobe varies, 19-58. Diaphragms 33-38 cm wide, creamy white, 60-71 white spots with red margin, randomly distributed in 3 concentric rings. Apertures circular to ovate, 14 cm in diameter (Figures 2(D), 2(E), 3(A), 3(B) & 3(C)). Windows wholly covered by 3-4 very closely spaced concentric rings of thick and long white warts towards the aperture, rings 11-27 mm thick (Figures 2(D), 2(E) & 3(C)). Discs 12-14 cm in diameter, 2.5 cm thick, margin with raised rim and bearing 24-25 processes. Processes 2-3 cm high, black tip, wider at the base 4-6 mm wide, narrower towards tip, 2-3 mm wide. Perigone tubes 14-15 cm high from base of tube towards aperture; inner surface wholly covered by stout and simple toadstool-like ramenta towards the base of perigone lobes (Figure 2(I)) and ramenta in the middle and towards perigone tube base, slender, simple and branched, 14-18 mm long (Figures 2(J), 2(K), 2(L), 3(E) & 3(F)). Ovary 1-locular, bearing numerous J-shaped ovules (Figure 3(D)).

Distribution Peninsular Malaysia. *R. tunku-azizahiae* is currently known from Lata Tinggi, at the foothill of Gunung Benom, in Mt. Benom Forest Reserve, Ulu Dong, Raub, Pahang. It is an allopatric species, growing about 3-5 km from three other species (Figure 1).

Habitat and ecology This species was found growing in logged-over lowland dipterocarp forest at about 624 m above sea level. It is a parasite on the stem of *T. rafflesiae* (Miq.) Planch. (Vitaceae), which grows together with clusters of bamboos and remnant of small trees of dipterocarp (Figure 6). The density of buds of the new species recorded during the research period (2011-2012) was found to be low. We counted a total of nine buds. Four of these buds died; only three bloomed successfully, and two are still alive. All of these flowers were found to be female.

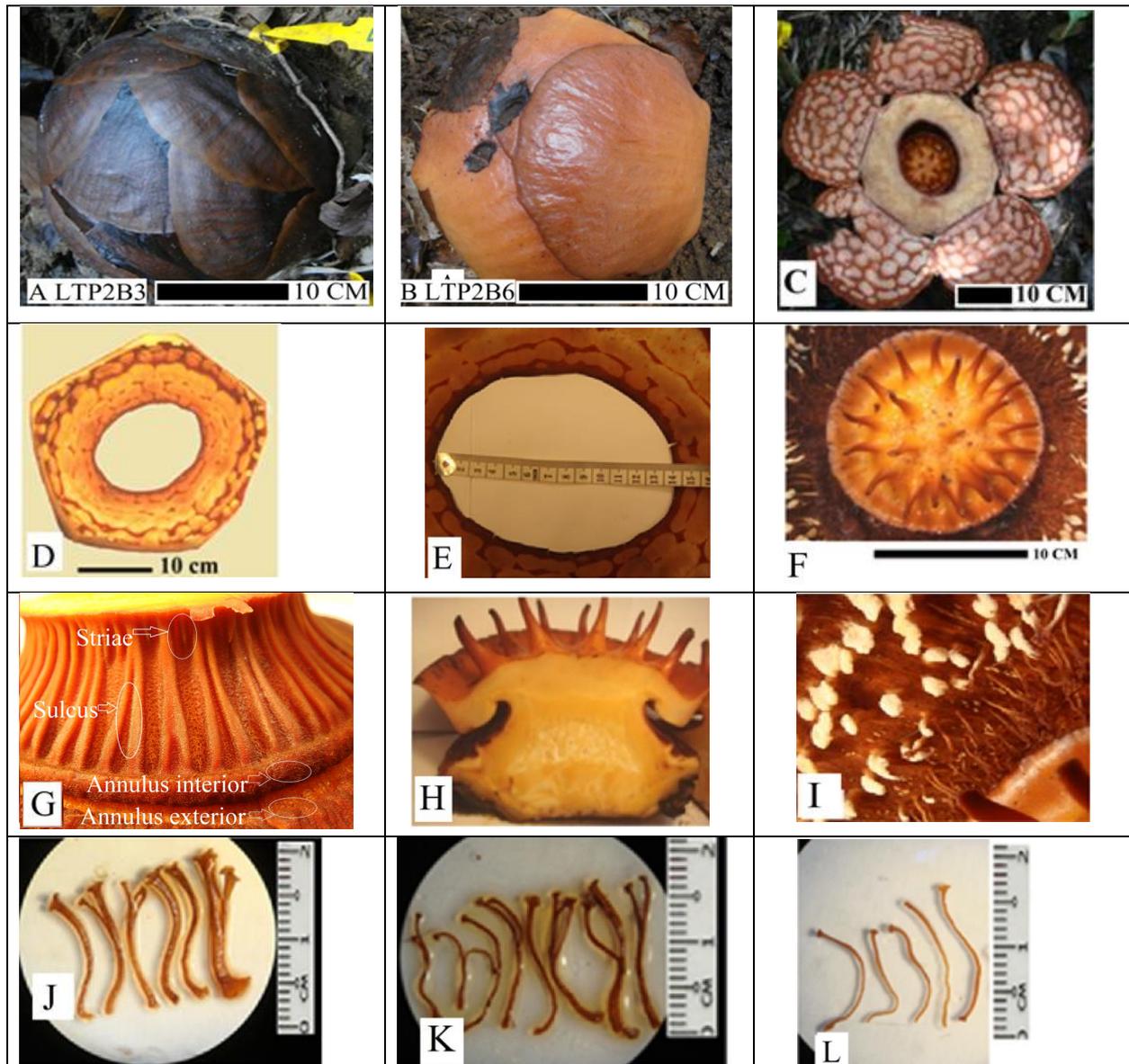


FIGURE 2. *R. tunku-azizahiae* LTP2B6♀ (holotype). A & B. Matured bud; C. Open flower; D. Window pattern; E. Aperture; F. Disc; G. Central column with annulus rings, striae and sulcus; H. Cross section of ovary; I. Distribution of ramenta on inner surface of perigone tube. J-L. Ramenta

Additional specimens examined Malaysia, Pahang, Raub, Ulu Dong, Mt. Benom Forest Reserve, Lata Tinggi, N 03° 54' 46.8" & E 102° 03' 33.5", 624 m elevation, ♀ flower, Nik Nadira Farhana, Jumaat, Mohd Afiq Aizat & Siti Norhafizah, LTP2B3 -13 July 2011, LTP2B1- 22 March 2011.

Etymology The specific epithet of this new species is named in honour of Her Majesty Seri Paduka Baginda the Raja Permaisuri Agong Tunku Hajah Azizah

Aminah Maimunah Iskandariah Binti Almarhum Al-Mutawakkil Alallah Sultan Iskandar Al-Haj, the Raja Permaisuri Agong of Malaysia and the Tengku Ampuan of Pahang for her active role in conserving *Rafflesia* in Pahang.

CONSERVATION STATUS

All species of *Rafflesia* are totally protected species in Malaysia due to its rarity, endemicity and endangered

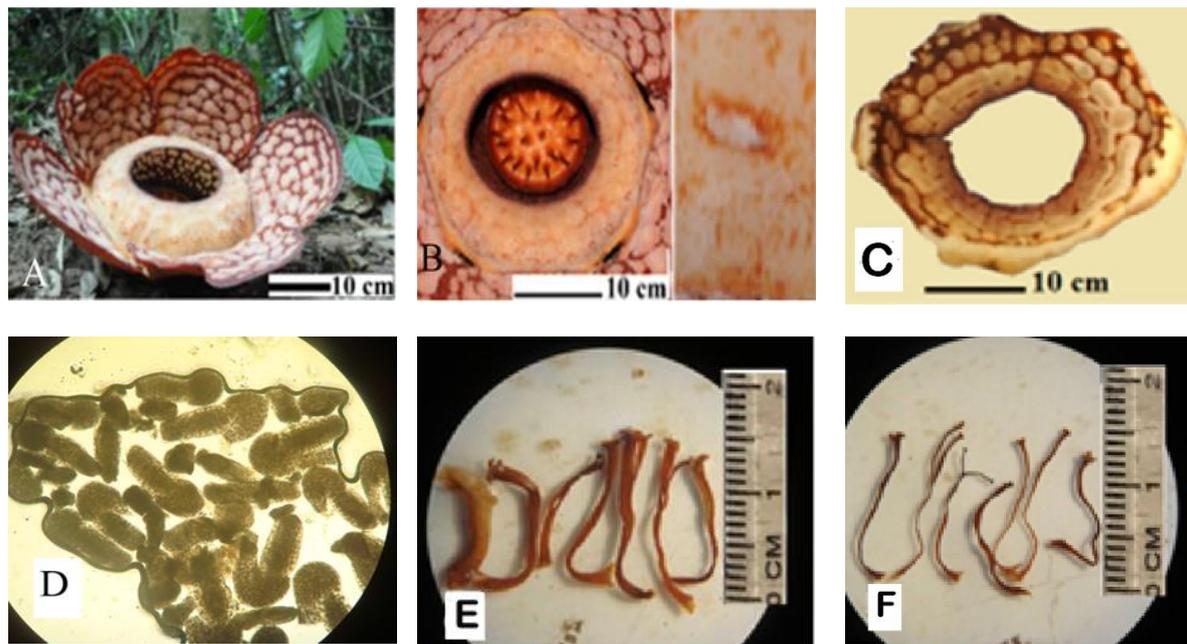


FIGURE 3. *R. tunku-azizahiae* LTP2B3♀. A. ♀ flower, perigone lobes covered by coalesced warts; B. Diaphragm, white spot with red margin on diaphragm; C. Window pattern; D. Ovules; E & F. Ramenta on inner surface of perigone tube

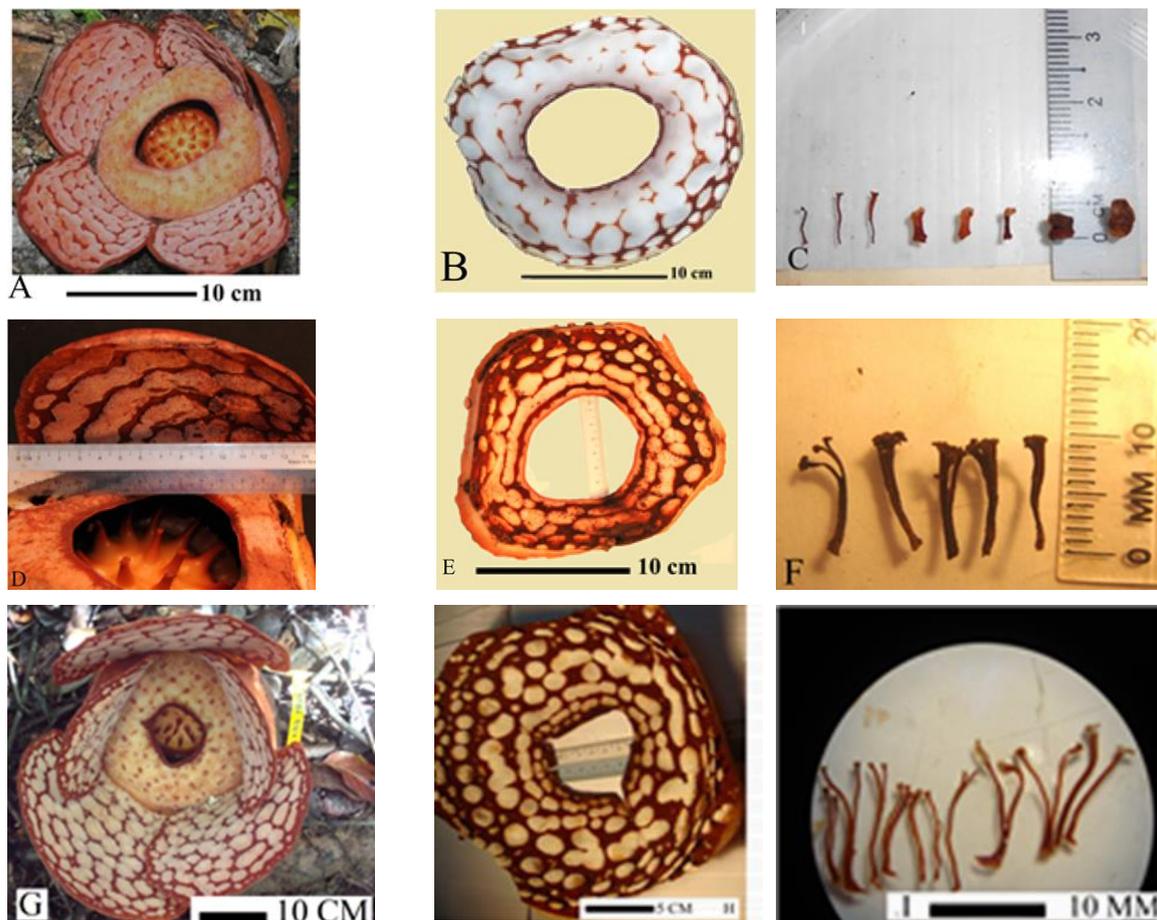


FIGURE 4. A-C. *R. tuanku-halimii* SMP7B1♀ (holotype), Matau; A. ♀ flower: perigone lobes covered by coalesced warts; B. Thick rings of inter coalescing white warts and wholly covered the surface of the window; C. Short and simple ramenta on inner surface of perigone tube; D-F. *R. azlanii* Akmal 01♀, Bersia: D. ♀ flower; E. Window pattern; F. Ramenta on inner surface of perigone tube; G-I. *R. sharifah-hapsahiae* CAP1B6♂ (holotype), Celik Ara; G. Male flower fully open; H. Window pattern; I. Ramenta on inner surface of perigone tubes

status. The only known locality of *R. tunku-azizahiae* is from its type locality, Lata Tinggi, Pahang, Peninsular Malaysia within Mt. Benom Forest Reserve where logging and other human activities are currently prohibited. Following the IUCN Categories and Criteria (IUCN 2012), this species is regarded as Critically Endangered. The density of buds of the new species recorded during the research period was found to be low. Nine buds in all were found. Only three of these buds bloomed successfully, and two are still living. Four of the buds perished. It was discovered that all of these blossoms are female. We also have encountered several

dormant and one infected host plants, located about 200 m from our plot. The host plants, *T. rafflesiae* are facing normal competition with other plants, especially clusters of bamboo, other woody lianas, potential attack by termites, age and accidental cuttings of host plants by locals. Efforts to involve local community in conservation strategies need to be continued as well as logistical and funding support for carefully planned ecotourism. The campaign of public awareness of *Rafflesia* and its host plant should always be conducted to increase the understanding and importance of *Rafflesia* to the environment.

Key to the species of *Rafflesia* in Peninsular Malaysia

- | | | |
|------|---|------------------------------|
| 1 a. | Perigone lobes covered by minute, small & distinct white warts | 2 |
| b. | Perigone lobes covered by coalesced white warts | 6 |
| 2 a. | Aperture variably lobed | 3 |
| b. | Aperture not lobed | 4 |
| 3 a. | Discs 8 cm diameter, lack of processes; anthers 26..... | <i>R. tiomanensis</i> |
| b. | Discs 20 cm in diameter, bearing 55-62 processes; anthers 48 | <i>R. su-meiae</i> |
| 4 a. | Flower large, 61-74 cm; disc with 34-56 processes, distributed into 4 rings; aperture 11-14 cm in diameter; numerous red-ringed white spots on diaphragm, 65-198; anthers 31-37..... | <i>R. kerrii</i> |
| b. | Flower medium, 41-47 cm; discs with 17-25 processes, distributed into 3 rings; aperture 7-13 cm in diameter; white spots on diaphragm 67-95; anthers 24-29... | 5 |
| 5 a. | Flower 41 cm; discs with 25 processes; aperture 7-7.5 cm in diameter; 67 red-ringed white spots on diaphragm; anthers 24..... | <i>R. parvimaiculata</i> |
| b. | Flower 47 cm; discs with 17-24 processes; aperture 8.8-13 cm in diameter; 95 red-ringed white spots on diaphragm; anthers 26-29 | <i>R. cantleyi</i> |
| 6 a. | Presence of continuous distinctive white ring on window rim or marginal white ring; discs with 18-18 processes, distributed into 2 concentric rings; diaphragm with 19-25 red-ringed white spots, distributed into 1 ring | <i>R. azlanii</i> |
| b. | Absence of distinctive white ring on window rim; discs with 21-28 processes and distributed into 3 concentric rings; diaphragm with 60-94 red-ringed white spots and distributed into 3-4 ring | 7 |
| 7 a. | Window covered by 5-7 closely spaced white rings of long coalesced and distinct white warts; diaphragm with 79-94 distinctive red-ringed white spots and distributed into 4 rings | <i>R. sharifah-hapsahiae</i> |
| b. | Window almost wholly covered by 3 very closely spaced white rings of wide and continuous warts; diaphragm with 60-87 paler red-ringed white spots and distributed into 3-4 rings | 8 |
| 8 a. | Flower medium in size, 43-60 cm in diameter; small aperture, 9 cm in diameter; diaphragm with 85-87 paler red-ringed white spots, distributed into 3-4 rings; disc 10-11 cm in diameter; ramenta simple and shorter, 4- 8 mm long.... | <i>R. tuanku-halimii</i> |
| b. | Flower large, 63-80 cm in diameter; large aperture 12-14 cm in diameter; diaphragm with 60-71 paler red-ringed white spots, distributed into 3 ring; disc 12-14 cm in diameter; ramenta simple and branching, 14-18 mm long..... | <i>R. tunku-azizahiae</i> |

TABLE 1. Flower morphology of *R. tunku-azizahiae* (RTA), *R. tuanku-halimii* (RTH), *R. azlanii* (RAZ), *R. sharifah-hapsahiae* (RSH), *R. cantleyi* (RC) and *R. parvimaculata* (RP) from Peninsular Malaysia

	Flower Characters	RTA	RTH	RAZ	RSH	RC	RP
C3	Flower diameter (cm)	63-80	43-60	48-50	44-45	47	41
C4	Length of longest PL (cm)	16.5-18	12.5-15	9.5-12	14.5-19.5	12-17.5	15-21
C5	Width of widest PL (cm)	22-24	11-22	12-16	11-24	10-24	10-15
C6	Pattern of warts on PL	CW	CW	CW	CW	DW	DW
C7	Spatial arrangement CW on PL	VCS	VCs	CS	VCS	WS	WS
C8	No. CW on each PL	19-58	17-27	6-21	10-33	0	0
C9	No. rows of CW on PL	6-8	5-8	4-6	7-9	0	0
C10	No. DW on each PL	0	0	0	0	46-82	84-91
C11	No. rows of DW on PL	0	0	0	0	6-7	9-10
C12	Diaphragm diameter (cm)	33-38	21-28	16-18	22.5-23	21-26.5	15
C13	No. spots on diaphragm	60-71	85-87	19-25	79-94	95	67
C14	No. rings of spots on diaphragm	3	3-4	1	4	5-6	4
C15	Aperture diameter ((cm)	14	9	5.5-8	7.5-9.5	8.8-13	7-7.5
C16	Longest ramenta on PT (mm)	14-18	8	6-9	11-13	13-14	11
C17	Structure of ramenta	SB	S	SB	SB	SB	S
C18	Disc diameter (cm)	12-14	10-11	10-10.3	10	9-10.8	10
C19	No. processes	24-25	21-26	16-18	26-28	17-24	25
C20	No. rings of processes	3	3	2	3	3	3
C21	Pattern of warts on window	CW	CW	CW	CW	DW	DW
C22	No. of CW on window	12-18	3	5-38	39-59	0	0
C23	No. of DW on window	0	0	0	0	120-131	66
C24	No. rings of CW on window	3-4	3	2-3	5-7	0	0
C25	No. rings of DW on window	0	0	0	0	5-6	7
C26	SBR of CW/ DW on WDW	VCS	VCS	CS	WS	WS	WS
C27	Coverage of CW /DW on WDW	Wholly	Wholly	Partly	Partly	Partly	Partly
C28	MWR on WDW	Absent	Absent	Present	-	-	
	Specimens	LTP2B6 LTP2B3	SMP7B1 SKP5B5	AKMAL01Wong 5	CAP1B6 CAP1B14	SMP5B1 BLP2B3	LJ4 et al

C: Character; CW: Coalesced warts; DW: Distinct warts; MWR: Distinct marginal white Ring; PL: Perigone lobes; PT: Perigone tube; WDW: Window; SBR: spacing between rows; CS: Closely space; VCS: Very closely space; WS: Well-spaced; S: Simple; SB: Simple & branching

DISCUSSION

Cluster analysis categorized eleven specimens of *Rafflesia* into two main clusters, A and B with similarity level (SL) <30.0975 with the distance level (DL) at >6.55744 (Figure 5 & Table 2). Cluster B (SL=45.6443; DL= 5.09902) contained specimens of *R. cantleyi* (I) and *R. parvimaculata* (J). Examination on flower morphology of the two species in Cluster B showed that their perigone lobes are covered by well-spaced and distinct white warts and their windows covered by rings of well-spaced distinct white warts.

On the other hand, Cluster A (SL=30.0975; DL= 6.55744) contained clusters of *R. tunku-azizahiae* (G), *R. tuanku-halimii* (H), *R. sharifah-hapsahiae* (F) and *R. azlanii* (D). All the species in Cluster A have their perigone lobes covered by rows of coalesced white warts

(Figures 2(C), 3(A), 4(A), 4(D) & 4(G)) and windows covered by rings of coalesced white warts (Figures 2(D), 2(E), 3(C), 4(B), 4(E) & 4(H)).

The dendrogram also demonstrated that *R. tunku-azizahiae* (G) and *R. tuanku-halimii* (H) are closely related morphologically with SL at 58.7139 and DL at 3.87298. *R. tunku-azizahiae* (Figures 2(A), 2(B), 3(A), 3(C) & 3(F)) resembles *R. tuanku-halimii* (Figures 4(A) & 4(B)) by similar window and perigone lobes pattern but differ in having larger aperture (Figures 2(D), 2(E), 3(B) & 3(C)) and longer ramenta (Figures 2(J), 2(K), 2(L), 3(E) & 3(F)) covering the inner surface of perigone tube. The dendrogram also showed that Cluster G-Cluster H is more closely related to Cluster F (*R. sharifah-hapsahiae*; SL= 45.6443; DL=5.09902) than Cluster D (*R. azlanii*; SL= 30.0975; DL= 6.55744).

TABLE 2. Similarity and distance matrix of eleven *Rafflesia* specimens analyzed by complete linkage method and Euclidean distance

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	10	89.3400	1.00000	1	2	1	2
2	9	81.5363	1.73205	3	4	3	2
3	8	73.8884	2.44949	5	6	5	2
4	7	71.7962	2.64575	9	10	9	2
5	6	60.1138	3.74166	7	8	7	2
6	5	58.7139	3.87298	1	3	1	4
7	4	45.6443	5.09902	9	11	9	3
8	3	45.6443	5.09902	1	7	1	6
9	2	30.0975	6.55744	1	5	1	8
10	1	0.0000	9.38083	1	9	1	11

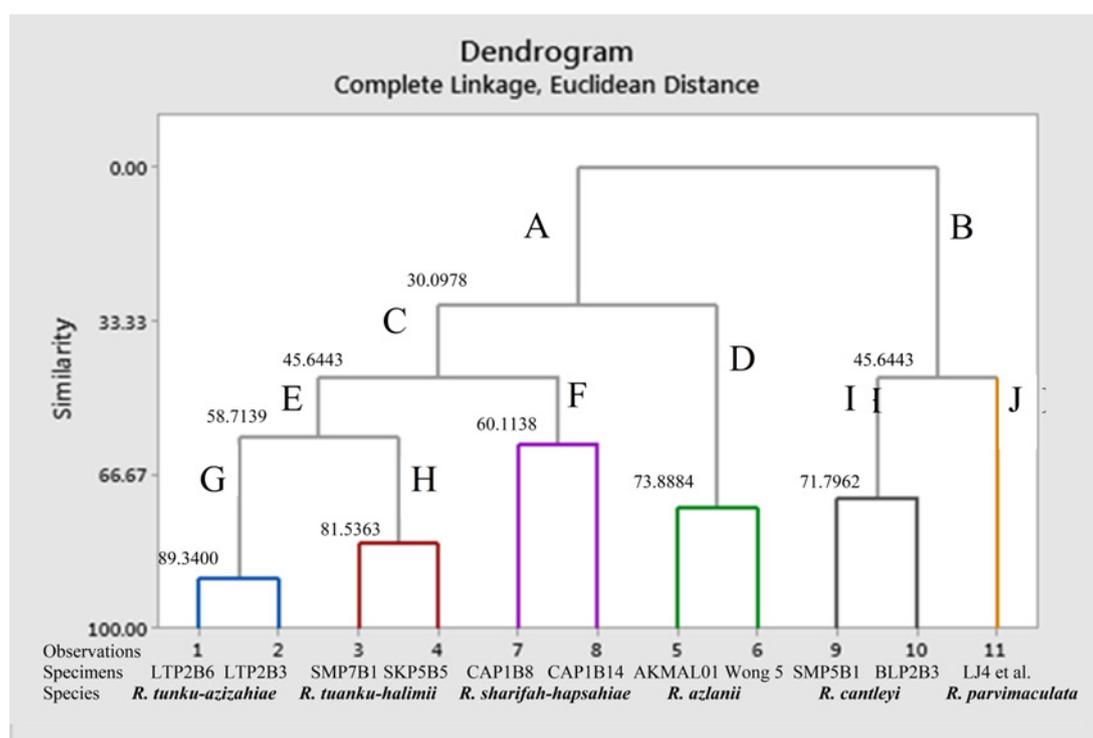


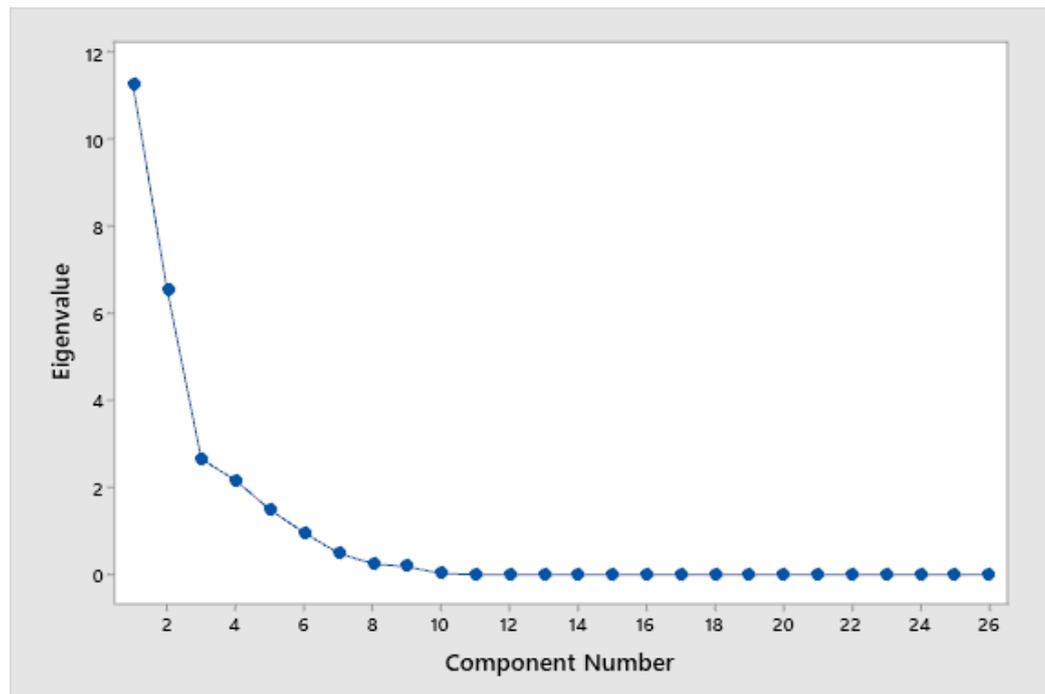
FIGURE 5. Dendrogram of eleven *Rafflesia* specimens from Peninsular Malaysia

The PCA was carried out by MINITAB VER. 19. Using the Kaiser criterion, the first five PCs are considered important, which contribute 92.7% of the total variation of the original data observed among eleven *Rafflesia*

specimens (Table 3). However, based on the scree plot, shown in Figure 6, we decided to choose only the first three PCs that explained approximately 78.7% of the total variation of the original data.

TABLE 3. Eigen analysis of the Correlation Matrix of eleven *Rafflesia* specimens from Peninsular Malaysia

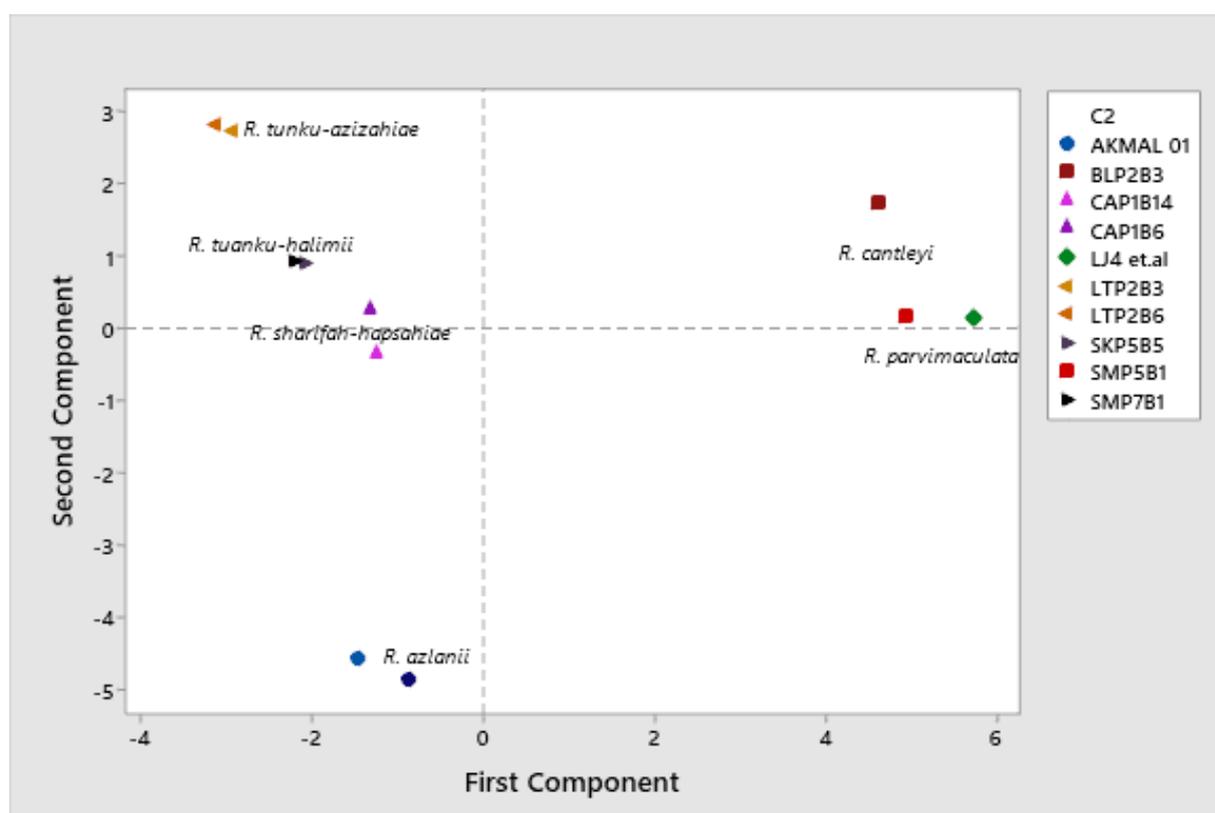
PC	Eigenvalue	Proportion	Cumulative	PC	Eigenvalue	Proportion	Cumulative	PC	Eigenvalue	Proportion	Cumulative
1	11.262	0.433	0.433	10	0.024	0.001	1.000	19	-0.000	-0.000	1.000
2	6.543	0.252	0.685	11	0.000	0.000	1.000	20	-0.000	-0.000	1.000
3	2.648	0.102	0.787	12	0.000	0.000	1.000	21	-0.000	-0.000	1.000
4	2.159	0.083	0.870	13	0.000	0.000	1.000	22	-0.000	-0.000	1.000
5	1.489	0.057	0.927	14	0.000	0.000	1.000	23	-0.000	-0.000	1.000
6	0.957	0.037	0.964	15	0.000	0.000	1.000	24	-0.000	-0.000	1.000
7	0.491	0.019	0.983	16	0.000	0.000	1.000	25	-0.000	-0.000	1.000
8	0.248	0.010	0.992	17	-0.000	-0.000	1.000	26	-0.000	-0.000	1.000
9	0.177	0.007	0.999	18	-0.000	-0.000	1.000				

FIGURE 6. Scree plot of flower characters of *Rafflesia*TABLE 4. Eigenvectors of principal components (PCs) of eleven *Rafflesia* specimens from Peninsular Malaysia

Variable	PC1	PC2	PC3	PC4	PC5
C3	-0.133	0.211	0.254	-0.266	-0.326
C4	0.170	0.183	-0.187	-0.315	-0.307
C5	-0.145	0.149	0.174	0.026	0.080
C6	0.254	0.039	-0.100	0.058	-0.387
C7	0.277	-0.089	0.165	-0.020	-0.048
C8	-0.269	0.108	0.030	-0.133	-0.144
C9	-0.285	0.011	-0.125	-0.008	0.018
C10	0.253	0.085	0.191	-0.037	0.259
C11	0.291	0.068	0.075	-0.002	-0.005
C12	-0.120	0.325	0.125	-0.169	0.124
C13	0.074	0.301	-0.081	0.233	0.238
C14	0.142	0.234	-0.330	0.022	0.245
C15	-0.070	0.161	0.314	0.075	0.383
C16	0.014	0.305	-0.000	-0.385	-0.153
C17	-0.027	-0.066	0.186	-0.589	0.262
C18	-0.064	0.251	0.340	0.013	-0.054
C19	-0.050	0.304	-0.247	0.076	-0.099
C20	0.051	0.357	-0.196	0.041	0.096
C21	0.291	0.068	0.075	-0.002	-0.005
C22	-0.194	-0.115	-0.303	-0.234	0.174
C23	0.277	0.039	0.170	-0.024	0.164
C24	-0.261	0.010	-0.201	-0.022	0.071
C25	0.288	0.059	0.005	0.023	-0.165
C26	0.217	-0.088	-0.254	-0.280	0.199
C27	0.183	-0.226	-0.159	-0.278	0.148
C28	-0.051	-0.357	0.196	-0.041	-0.096

The first PC is associated (abs (eigenvector) ≥ 0.2) with variables C6, C7, C8, C9, C10, C11, C21, C23, C24, C25 and C26, which are related to the CW and DW of the flower specimens. Meanwhile, variables C12, C13, C16, C19, C20 and C28 highly contributed towards the second PC (abs (eigenvector) ≥ 0.3). This means that the second PC explains more on the diaphragm, length of ramenta, processes and marginal white ring on the window. The third PC is associated with variables C14, C15, C18 and C22, which are related to the number of rings on diaphragm, diameter of aperture, diameter of disc and number of coalesced warts on window.

The score plot of the two first PCs showed grouping of eleven *Rafflesia* specimens into two main clusters, as shown in Figure 7. The first cluster comprised of *R. cantleyi* and *R. parvimaclata* on component 1; and the second cluster contained *R. tunku-azizahiae*, *R. tuanku-halimii*, *R. sharifah-hapsahiae*, and *R. azlanii* on component 2. The score plot also showed that *R. tunku-azizahiae* is closely related to *R. tuanku-halimii* and *R. sharifah-hapsahiae*, while *R. azlanii* is distantly related to the other first cluster members.



Coloured symbols represent different specimens

FIGURE 7. Score plot of characters of *Rafflesia*

The bi-plot (Figure 8) showed that C7, C10, C11, C21, C23, C25 and C26 are positively associated with the first PC (Table 4), indicating that *R. cantleyi* and *R. parvimaclata* have their perigone lobes covered by well-spaced rows of numerous distinct white warts and their windows covered by well-spaced rings of numerous distinct white warts, compared to the other specimens. On the other hand, C8, C9 and C24 are negatively

associated with the first PC, indicating *R. tunku-azizahiae*, *R. tuanku-halimii*, *R. sharifah-hapsahiae*, and *R. azlanii* have their perigone lobes covered by rows of coalesced white warts and their windows by rings of coalesced white warts. The bi-plot also showed that C3, C12, C15, C16, C18 and C19 are positively associated with the second PC, indicating that *R. tunku-azizahiae* have large flower, broader disc and diaphragm diameter, and longer ramenta.

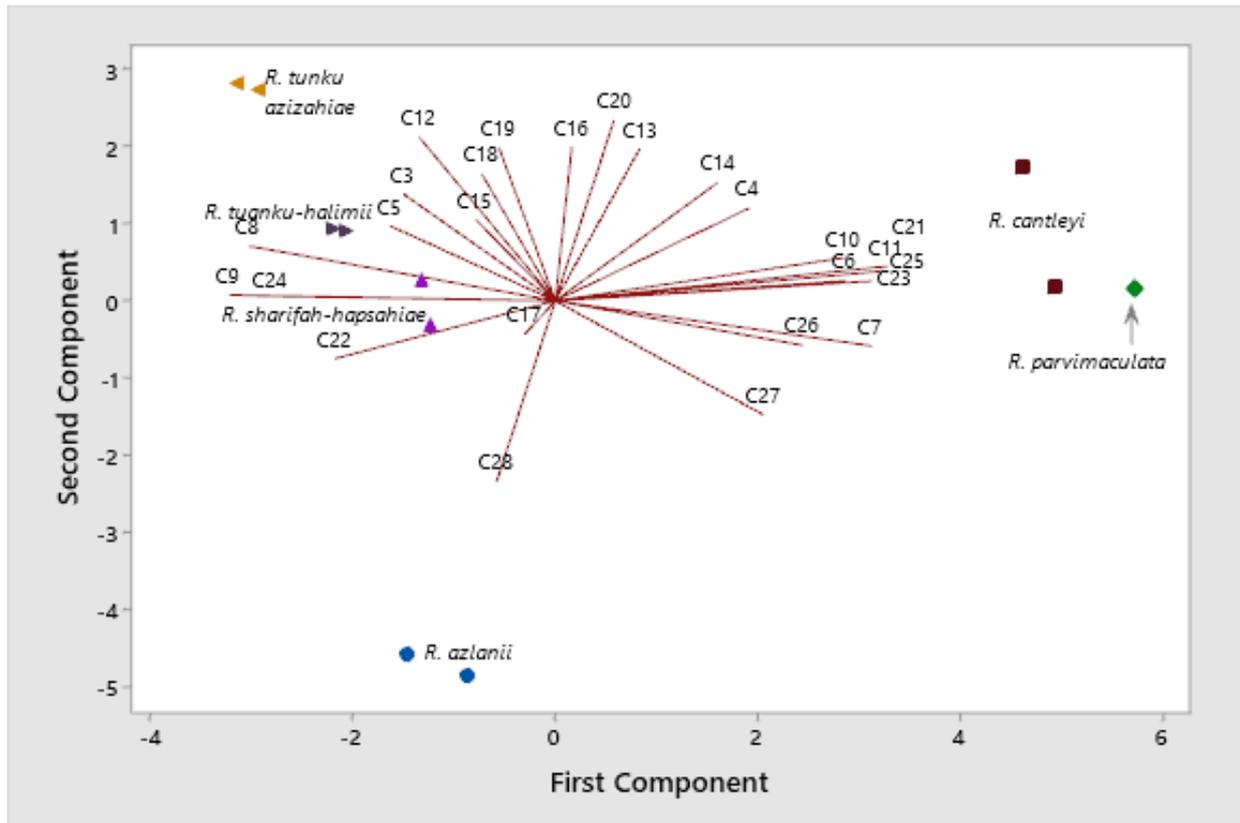


FIGURE 8. Bi-plot of characters of *Rafflesia*

Based on the bi-plot, it is shown that *R. tunku-azizahiae* and *R. tuanku-halimii* are related in having their windows wholly covered by very closely spaced rings of coalesced warts but *R. tunku-azizahiae* differs from *R. tuanku-halimii* by larger flower diameter, broader diaphragm and disc diameter, longer ramenta. PC2 and PC3 showed *R. tunku-azizahiae* has broader diaphragm and disc diameter, higher number of spots on the diaphragm, longer ramenta and larger flower and aperture. These characters distinguished *R. tunku-azizahiae* from *R. tuanku-halimii*, *R. sharifah-hapsahiae* and *R. azlanii*.

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