

**MORPHOLOGY OF OVULE, SEED AND POLLEN GRAIN OF *RAFFLESIA* R.  
BR. (RAFFLESiaceae)**

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*Keywords: Rafflesia; Ovule; Seed; Pollen grain.*

**Abstract**

The ovules, seeds and pollen grains of 24 *Rafflesia* specimens from nine species were examined. The ovules and seeds of all specimens showed similar shapes and structures. *Rafflesia* ovules are J-shaped and incompletely anatropous, while the seeds are chestnut-shaped. The pollen grains are small (10-25 µm in diameter), prolate-spheroidal and subprolate, monoporate, with a smooth surface and no ornamentation.

**Introduction**

*Rafflesia* R. Br. is a parasitic genus comprising 33 species (summarized from Nais, 2001; Susatya *et al.*, 2006; Mat-Salleh *et al.*, 2010; Wiriadinata, 2010; Barcelona *et al.*, 2011). This genus grows in limited localities in the tropical rainforest of Southeast Asia (Wong, 1992; Hidayati *et al.*, 2000; Balete *et al.*, 2010; Barcelona *et al.*, 2011) including Sumatera, Java, Borneo, Peninsular Malaysia, Southern Thailand and the Philippines (Barcelona *et al.*, 2006, 2007; Ghazally *et al.*, 1988). As a holoparasitic plant, no members of *Rafflesia* have vegetative part, the only visible part is the reproductive part that emerges from its specific host, *Tetrastigma* (Vitaceae) (Meijer, 1997; Nais, 2001). *Rafflesia* is characterized by five perigone lobes which are orange to reddish in colour (Nais, 2001) and thought to mimic rotting flesh flower (Barkman *et al.*, 2008).

Since the discovery of first species in Bengkulu, Sumatera (Brown, 1821), *Rafflesia* has astounded the scientific community and caused a sensation in the botanical world (Banzinger, 1991). This genus includes the largest flower in the world, and according to Barkman *et al.* (2008) the flower diameter of *Rafflesia* is 10 to 100 times larger than those of most other flowering plant genera. This plant with gigantic flower has fascinated and baffled researchers, and become a public interest.

As other Rafflesiaceae genera, the flowers of *Rafflesia* are very rare (Banzinger, 2004). Some reasons for their rarity are: 1. The parasitic way of life, it needs a specific host (*Tetrastigma*); 2. most of the species are dioecious (male and female flowers do not usually simultaneously bloom); 3. imbalanced sex ratio (the overall male and female flower ratio is 7:1); 4. fruit set percentage among female is low, 35.71% (Nais and Wilcock, 1998); 5. high bud mortality, 40-91% in some cases up to 100 % (Nais, 2001) and 6. long life cycle up to five years from seed to seed in *Rafflesia arnoldii* (Meijer, 1997). Therefore, the biology of *Rafflesia* remains largely unknown.

The study of ovule, seed and pollen morphology is limited in *Rafflesia*. Very few studies had been conducted on micromorphological aspects (Brown, 1834; Olah, 1960; Takhtajan *et al.*, 1985; Bouman and Meijer, 1994). Most of these studies deal with a single or few species only. Here, we describe the morphology of the ovules, seeds and pollen grains of nine species of *Rafflesia* from

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Pahang, Perak (Peninsular Malaysia), Sabah (East Malaysia), North Sumatera, Bengkulu and Riau (Indonesia).

### Materials and Methods

Table 1 shows the materials examined in this study. This small sample size is due to the rarity of *Rafflesia*. Moreover, studying micromorphology of this genus is very destructive. All materials were observed using a Scanning Electron Microscope (SEM). The specimens were fixated in 2.5% glutaraldehyde, followed by a series of ethanol dehydration. A Critical Point Drier 7501 was used to dry the dehydrated tissues. The samples were then sputter-coated with gold-palladium for 5 minutes using Thermo UG Scientific Polaron.

The measurement and morphological observations were made with a Phillips XL 30 microscope, based on 20 samples per specimen to perform statistical analyses. For ovules and seeds, we measured the following: microphyle diameter (MD), micropylar part length (MPL), micropylar part width (MPW), funiculus width (FW), funiculus length (FL), raphal width (RW), and raphal length (RL). Some pollen grains were also observed using light microscope (LM) Meiji MX microscope assisted with Olympus E 330 camera in unstained glycerin jelly. The terminology of pollen used in this study is based on Erdtman (1972).

### Results and Discussion

#### *Ovules and seeds*

The ovules and seeds of all specimens examined (Fig. 1) have similar shapes and structure but differ in their sizes. Therefore, due to this morphological homogeneity, the descriptions of ovules and seeds given below are based on the summary of all specimens examined, while detailed measurements are presented in Table 2.

#### *Description of ovule*

White when fresh, J-shaped and irregularly attached to the placenta. Placenta has a polygonal cell wall. The structure can be divided into three parts, the funiculus, raphal and micropylar parts. *Funiculus*: Cross-section is rose-shaped, has rudimentary cells at outer integument; *Raphal part*: Irregularly shaped, rudimentary cells at outer integument, located at the middle part of ovule, no clear distinction with funiculus. *Micropylar part*: Outer wall presents irregular rows, the width is almost the same from tip to base, the tip is donut-shaped.

Bouman and Meijer (1994) indicated that species with wider flower diameter usually have bigger ovules. Fig. 1 presents the SEM photographs of ovules of *R. azlanii* (Fig. 1A-C), *R. kerrii* (Fig. 1D-E), *R. hasseltii* (Fig. 1F), *R. cantleyi* (Fig. 1G), and *R. tuan-mudae* (Fig. 1H-J). In this study, *R. kerrii* had the biggest flower while *R. tuan-mudae* had the smallest flower among the taxa examined. According to Meijer (1997) and Nais (2001) the open flower diameter of *R. kerrii* ranges from 50 to 70 cm. Mat Ros (pers. comm.) recorded the widest flower of *R. kerrii* from Peninsular Malaysia, up to 111 cm. However, the ovule of *R. kerrii* examined in this study was smaller than *R. azlanii*, *R. hasseltii* and *R. cantleyi* as presented in Table 2. This may be due to the ovule of *R. kerrii* used in this study was collected from female bud, and hence the size was smaller because the ovule is not yet well-developed. The probably size of the matured ovules should be greater than ovules of *R. cantleyi* examined in this study. The flower diameter of *R. tuan-mudae* according to Nais (2001) is 26-33 cm, smaller than *R. hasseltii*, *R. cantleyi* and *R. azlani*. Its ovules are also smaller than these three species. But surprisingly, the microphyle diameter of *R. tuan-mudae* (Fig. 1I) is the widest among the species examined ( $46.22 \pm 04.01 \mu\text{m}$ ) and quite unique.

Table 1. List of *Rafflesia* species examined.

Material	Species	Flower Diameter (cm)*	Collection Note
Ovule	1. <i>R. azlanii</i> Latiff & Wong	31	AZLO-1; Peninsular Malaysia, Perak, Sungai Bersia; 8 <sup>th</sup> population near the valley; Collector- Sofiyanti, Mat Salleh, Zuhailah
	2. <i>R. cantleyi</i> Solms-Laubach	41	CANO-1; Peninsular Malaysia, Pahang, Lata Berembun; 1 <sup>st</sup> population, 1 m from the river; Collector- Sofiyanti, Mat-Ros, Mat Salleh
	3. <i>R. hasseltii</i> Suringar	30	HASO-1; Indonesia, TNBT Riau; Collector- Sofiyanti
	4. <i>R. kerrii</i> Meijer	Not applicable	KERO-1-KMS; Peninsular Malaysia, Perak, Gua Musang; Collector-Mat-Ros, Mat Salleh; flower was not available therefore ovules were collected from bud (21 cm in diameter).
	5. <i>R. tuan-mudae</i> Becc.	26	TUMO-KMS; East Malaysia, Sarawak; Collector- Mat Salleh
Seed	1. <i>R. azlanii</i>	25-50*	AZLS-1; Peninsular Malaysia, Perak, Sungai Bersia; Collector- Sofiyanti, Mat Salleh, Zuhailah
	2. <i>R. cantleyi</i> Solms-Laubach	30-55*	CANS-1; Peninsular Malaysia, Pahang, Lata Berembun; C-collector Sofiyanti, Mat-Ros, Mat Salleh
	3. <i>R. kerrii</i> Meijer	50-70*	KERS-1-KMS; Peninsular Malaysia, Perak, Gua Musang; Collector- Mat-Ros, Mat Salleh
	4. <i>R. pricei</i>	16-45*	PRIS-1-KMS; East Malaysia, Sabah; Collector- Mat Salleh
	5. <i>R. arnoldii</i> R.Br.	70-150*	ARNS-AGU; Indonesia, Bengkulu, TNKS; Collector- Susatya
Pollen	1. <i>R. azlanii</i>	27 (AZLP-1), 28 (AZLP-2)	AZLP-1, AZLP-2; Peninsular Malaysia, Pahang, Sungai Bersia; Collector- Sofiyanti, Zuhailah, Mat-Ros, Mat Salleh
	2. <i>R. cantleyi</i>	38 (CANP-1), 28 (CANP-2), 27 (CANP-3), 43 (CANP-4), 25 (CANP-5), 36 (CANP-6)	CANP-1,2,3; Peninsular Malaysia, Perak, Sungai Bersia; Collector- Nery, Mat Salleh, Zuhailah, Mat Ros, Faizi. CANP-4 and 5; Peninsular Malaysia, Perak, bungor; Collector Mat Salleh. CANP-6; Peninsular Malaysia, Pahang, Lata Jarum; Collector- Sofiyanti, Zuhailah, Mat Ros, Faizi.
	3. <i>R. hasseltii</i>	36	HASP-1; Indonesia, TNBT Riau, Datai Atas; Collector- Sofiyanti
	4. <i>R. keithii</i> Meijer	80	KEIP-1; Peninsular Malaysia, Perak; Collector- Mat Ros
	5. <i>R. kerrii</i>	81 (KERP-1), 92 (KERP-2)	KERP-1, KERP-2; Peninsular Malaysia, Perak; Collector- Mat Ros
	6. <i>R. lawangensis</i> Mat-Salleh, Mahyuni, Susatya Veldkamp	63	ATJP-RW; Indonesia, North Sumatra, Bukit Lawang; Collector- R. Mahyuni
	7. <i>R. tuan-mudae</i>	33	TUMP-KMS; East Malaysia, Sabah; Collector- Mat Salleh.

Note : \* Based on Nais (2001)

Table 2. Ovule and seed measurements (mean  $\pm$  standard deviation) of *Rafflesia* species examined.

Species	MD ( $\mu\text{m}$ )	MPW ( $\mu\text{m}$ )	MPL ( $\mu\text{m}$ )	RW ( $\mu\text{m}$ )	RL ( $\mu\text{m}$ )	FW ( $\mu\text{m}$ )	FL ( $\mu\text{m}$ )
<b>A. Ovule</b>							
1. <i>R. azlanii</i>	35.05 $\pm$ 4.62	153.91 $\pm$ 17.65	294.63 $\pm$ 17.86	149.15 $\pm$ 19.86	252.34 $\pm$ 34.54	73.12 $\pm$ 4.19	209.35 $\pm$ 34.04
2. <i>R. cantleyi</i>	42.12 $\pm$ 11.41	264.62 $\pm$ 24.62	313.85 $\pm$ 44.16	215.38 $\pm$ 13.76	245.76 $\pm$ 26.73	56.00 $\pm$ 7.45	228.81 $\pm$ 35.53
3. <i>R. hasseltii</i>	26.04 $\pm$ 4.46	127.09 $\pm$ 9.80	207.79 $\pm$ 39.64	120.77 $\pm$ 26.69	131.08 $\pm$ 29.00	71.68 $\pm$ 11.46	92.56 $\pm$ 16.54
4. <i>R. kerrii</i>	30.76 $\pm$ 5.24	134.68 $\pm$ 22.68	191.67 $\pm$ 25.57	145.06 $\pm$ 18.42	159.76 $\pm$ 36.86	64.63 $\pm$ 26.66	116.26 $\pm$ 18.85
5. <i>R. tuan-mudae</i>	46.22 $\pm$ 4.01	81.02 $\pm$ 12.58	129.81 $\pm$ 43.08	75.09 $\pm$ 10.64	111.09 $\pm$ 5.11	42.13 $\pm$ 25.53	102.04 $\pm$ 23.65
<b>B. seeds</b>							
1. <i>R. arnoldii</i>	158.00 $\pm$ 48.68	404.00 $\pm$ 99.40	608.20 $\pm$ 30.68	267.20 $\pm$ 18.14	453.40 $\pm$ 37.55	95.60 $\pm$ 8.65	280.20 $\pm$ 15.27
2. <i>R. azlanii</i>	119.47 $\pm$ 30.44	316.25 $\pm$ 98.57	465.16 $\pm$ 79.88	280.37 $\pm$ 64.29	352.32 $\pm$ 80.04	62.31 $\pm$ 14.94	280.71 $\pm$ 127.09
3. <i>R. cantleyi</i>	104.08 $\pm$ 18.62	338.40 $\pm$ 25.76	458.20 $\pm$ 55.60	235.00 $\pm$ 23.24	263.60 $\pm$ 53.78	58.80 $\pm$ 15.71	250.20 $\pm$ 39.88
4. <i>R. kerrii</i>	178.33 $\pm$ 11.25	289.17 $\pm$ 24.98	477.00 $\pm$ 27.81	321.83 $\pm$ 41.95	377.50 $\pm$ 39.08	85.33 $\pm$ 6.65	368.67 $\pm$ 81.65
5. <i>R. pricei</i>	101.74 $\pm$ 2.46	280.14 $\pm$ 9.63	448.41 $\pm$ 6.97	272.61 $\pm$ 10.45	412.32 $\pm$ 111.70	50.43 $\pm$ 14.76	220.14 $\pm$ 94.49

Note : MD (microphyle diameter), MPW (microphyllar part width), MPL (microphyllar part length), RW (raphal width), RL (raphal length), FW (funiculus width), FL (funiculus length)

Table 3. Pollen characters of the *Rafflesia* species studied.

Species	E ( $\mu\text{m}$ )	P ( $\mu\text{m}$ )	P/E	Shape	Pore size ( $\mu\text{m}$ )	Pore Number	Pollen surface
<i>R. azlanii</i> (AZLP-1)	11.67 $\pm$ 0.98	12.34 $\pm$ 0.91	1.03	Prolate-spheroidal	3.40 - 4.30	monoporate	Smooth
<i>R. azlanii</i> (AZLP-2)	11.98 $\pm$ 1.69	13.04 $\pm$ 1.69	1.02	Prolate-spheroidal	3.40 - 4.20	monoporate	Smooth
<i>R. cantleyi</i> (CANP-1)	16.68 $\pm$ 2.56	17.51 $\pm$ 2.55	1.05	Prolate-spheroidal	1.09 - 1.18	monoporate	Smooth
<i>R. cantleyi</i> (CANP-2)	14.87 $\pm$ 1.12	15.37 $\pm$ 0.78	1.03	Prolate-spheroidal	1.07 - 1.12	monoporate	Smooth
<i>R. cantleyi</i> (CANP-3)	12.23 $\pm$ 0.85	11.03 $\pm$ 0.69	1.11	Prolate-spheroidal	1.03 - 1.08	monoporate	Smooth
<i>R. cantleyi</i> (CANP-4)	18.21 $\pm$ 1.66	19.09 $\pm$ 2.06	1.05	Prolate-spheroidal	1.06 - 1.17	monoporate	Smooth
<i>R. cantleyi</i> (CANP-5)	10.97 $\pm$ 0.93	12.2 $\pm$ 1.18	1.12	Prolate-spheroidal	1.03 - 1.08	monoporate	Smooth
<i>R. cantleyi</i> (CANP-6)	16.71 $\pm$ 2.44	14.28 $\pm$ 1.10	1.17	Subprolate	1.06 - 1.12	monoporate	Smooth
<i>R. hasseltii</i>	13.74 $\pm$ 1.88	16.80 $\pm$ 2.49	1.22	Subprolate	3.80 - 4.90	monoporate	Smooth
<i>R. keithii</i>	18.77 $\pm$ 1.70	19.27 $\pm$ 1.42	1.03	Prolate-spheroidal	4.90 - 6.10	monoporate	Smooth
<i>R. kerrii</i> (KERP-1)	18.23 $\pm$ 1.36	19.71 $\pm$ 1.42	1.08	Prolate-spheroidal	5.00 - 6.00	monoporate	Smooth
<i>R. kerrii</i> (KERP-2)	21.88 $\pm$ 3.16	22.20 $\pm$ 3.29	1.08	Prolate-spheroidal	5.50 - 6.20	monoporate	Smooth
<i>R. lawangensis</i>	23.41 $\pm$ 1.63	25.71 $\pm$ 1.96	1.09	Prolate-spheroidal	5.10 - 6.30	monoporate	Smooth
<i>R. tuan-mudae</i>	12.99 $\pm$ 1.46	15.73 $\pm$ 2.63	1.21	Subprolate	3.50 - 4.60	monoporate	Smooth

Note : P = Polar axis length, E = Equatorial diameter

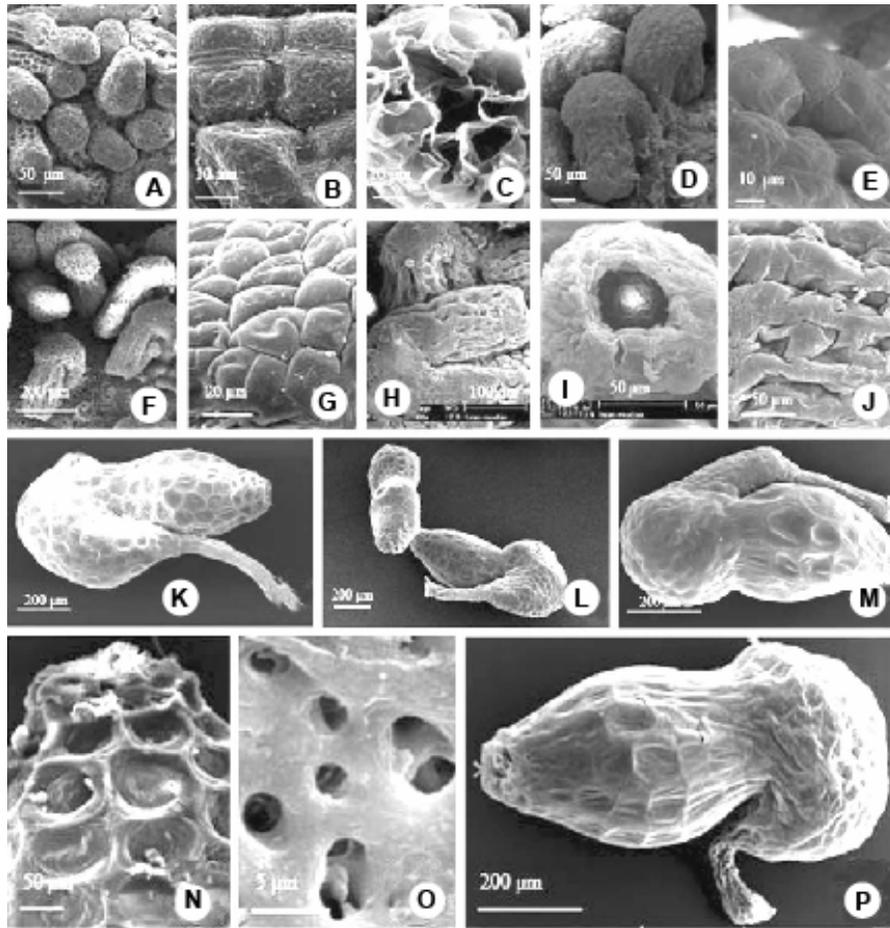


Fig. 1. SEM photographs of *Rafflesia* ovules (A-J) and seeds (K-P). A - C. *R. azlanii*, B. Outer integument, C. cross section of funiculus, D-E. *R. kerrii*, E. microphyle, F. *R. hasseltii*, G. placenta wall of *R. cantleyi*, H-J. *R. tuan-mudae*, I. microphyle, J. outer integument of microphylar part, K. *R. azlanii*, L. *R. kerrii*, M. *R. pricei*, N-O. *R. arnoldii*, N. microphylar part, O. inner integument with pores, P. *R. cantleyi*.

*Rafflesia* has an incomplete anatropous ovules with the microphyle facing downward and situated near the base of funiculus, but the apical part is not bound to the raphe. Sometimes the distinction between raphal and funiculus is not clear, because the funiculus of ovule was not well developed, and its width was almost the same as raphal width. The placenta of *Rafflesia* consisted of polygonal cell walls (as seen in Fig. 1G). The cross-section of the funiculus showed the empty cell (Fig. 1C). The ovules of all the species studied here were unitegmic. This result was in accordance to that of Bouman and Meijer (1994), who observed the same type of ovules in all the genera of Rafflesiaceae (*Rafflesia*, *Rhizanthus* and *Sapria*). According to Takhtajan (2009), the unitegmic ovule could have resulted from the congenital fusion of inner and outer integuments or due to the abortion of one of the integuments. The embryo sac of *Rafflesia* is a monosporic type as the single surviving megaspore undergoes three rounds of mitotic nuclear division without any wall formation. The type of embryo sac of *Rafflesia* is *Polygonum* type, which is found in 81% of the Angiospermae (Mauseth, 1988).

*Rafflesia* seeds are usually chestnut-shaped, brown when fresh. The funiculus shows a different outer integument from raphal and micropylar part. This part can be easily distinguished from the raphal part. Raphal part has a curvature, unequal shape and swollen at the middle. The micropylar part is located near the funiculus due to the curvature of raphal part, the middle portion has the largest integument cell, whereas the rounded microphyle is located on the tip. The outer integument is reduced, visible only as a broad, insignificant rim proximal to the base of the inner integument and consists of polygonal cell wall. The inner integument is U-shaped in cross section and has many pores as seen in *R. arnoldii* seed (Fig. 1O). Figure 1 K-P present the seeds of all species studied, while Table 2 shows the measurements of the different seed parts. The highest values of total measurement were observed in *R. arnoldii*. This species is known as the widest flower in the world (Nais, 2001) with open flower diameter ranging at 70-150 cm.

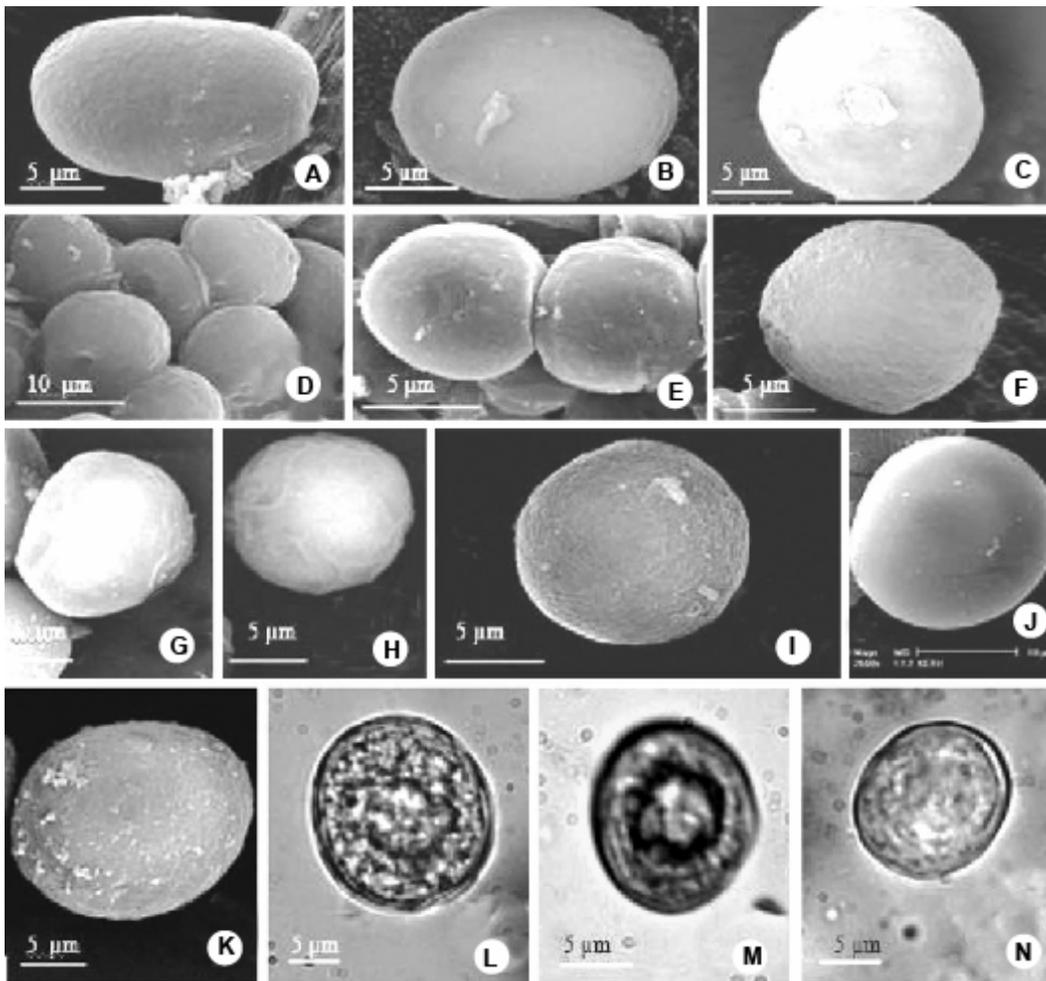


Fig. 2. SEM (A-K) and LM (L-N) photographs of *Rafflesia* pollen grains. A. *R. tuan-mudae*, B. *R. hasseltii*, C. and D. *R. azlanii* (C. AZLP-1, D. AZLP-2), E-I. *R. cantleyi* (E. CANP-1, F. CANP-2, G. CANP-3, H. CANP-4, I. CANP-6), J. *R. keithii*, K. *R. kerrii* (KERP-1), L. *R. lawangensis*, M and N. *R. cantleyi* (CANP-5).

### Pollen

*Rafflesia* pollen is not released as a single grain, but adhered to each other due to a sticky yellow mush. SEM and LM photographs of pollen are shown in Fig. 2. The pollen grains of all species observed in this study have one pore (monoporate pollen) and a smooth surface without ornamentation. However, two distinct shapes were observed, subprolate and prolate-spheroidal. Subprolate shape is describing the shape of a pollen grain in which the ratio between the polar axis and the equatorial diameter (P/E ratio) is 1.14-1.33, while prolate-spheroidal shape has P/E ratio ranging from 1.00 to 1.14 (Erdmant, 1972). Subprolate pollen was found in *R. tuan-mudae* (Fig. 2A), *R. hasseltii* (Fig. 2B), and one *R. cantleyi* specimen (CAN 6). But the common shape is prolate-spheroidal, which is found in *R. azlanii* (Fig. 2C and D), *R. cantleyi* (CAN 1-5) (Fig. 2E-H and M-N), *R. keithii* (Fig. 2J), *R. kerii* (Fig. 2K) and *R. lawangensis* (Fig. 2L). Prolate-spheroidal pollen grains in this study have P/E ratio ranging from 1.02 to 1.12, and the P/E ratio for subprolate is from 1.17 to 1.22. Usually species with wider flower diameter has bigger pollen grains, however in this study we find an interesting result, *R. lawangensis* from North Sumatera (Indonesia) shows the bigger pollen size than *R. keithii* and *R. kerrii* eventhough its flower diameter is smaller (63 cm) than both species (Table 1).

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