Examining the Anatomical Characteristics of *Rafflesia kerrii* in Lojing Highlands, Peninsular Malaysia

To cite this article: I Asfarina *et al* 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **1102** 012068

Examining the Anatomical Characteristics of *Rafflesia kerrii* in Lojing Highlands, Peninsular Malaysia

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Abstract. *Rafflesia kerrii* is an endemic species to Peninsular Malaysia and Southern part of Thailand. The genus grows without the vegetative components such as, leaves, stems and true roots, where the only visible structure is the flower itself. It is easy to identify *R. kerrii* by its large dull red flowers, perigones covered by numerous small white pinkish warts and a wide diaphragm opening. However, there is a scarcity of knowledge about the species's anatomical traits. Herein, this study is aimed to evaluate the anatomical characteristics of *R. kerrii* in Lojing Highlands by using micro techniques analysis. The results were compared to those of a similar species found in Thailand. Four main components of flower were analysed, these are; the perigone lobe, window, processes and ramenta. The anatomical analysis of *R. kerrii* collected in Lojing Highlands have revealed that all the four components had simple and uncomplicated structures. Both upper and bottom layers of the plant have epidermal cell layers. The ground tissues also have an undeveloped vascular bundle. Based on the findings, the anatomical characteristics of *R. kerrii* found in Lojing Highlands and in Khao Sok National Park in Thailand were identical and shared similar types of main tissues. However, there are minor differences such as in the presence of starch grains, distance between vascular bundles in perigone lobe, and the presence of trichome in the compartment of windows.

1. Introduction

Rafflesia, a parasitic flowering plant genus in Rafflesiaceae, is one of the most astonishing flowers ever discovered in the botanical world [1]. Sir Thomas Stamford Raffles and Dr. Joseph Arnold made the first discovery of the species (*Rafflesia arnoldii*) in Bengkulu, Sumatera, Indonesia, 204 years ago. The finding of the *Rafflesia* was expanded to encompass Brunei, the Philippines, Thailand and Malaysia. The genus was discovered to grow in lowland and hill primary, over-logged, and old secondary forests at altitudes ranging from 300 to 1600 m a.s.l [2]. To date, the total number of *Rafflesia* species in Malaysia is 13 species. *R. kerrii* was noted as the second largest species after *R. arnoldii* [3].

R. kerrii was first described in 1984 by William Meijer, based on a holotype deposited in KEW, collected in 1929 by A.F.G. Kerr (specimen: K000854507) from Kho Pawra Luang Keo, Ranong Province, Thailand [4,2,5]. In Peninsular Malaysia, the species was discovered at Bukit Tepuh (Kelantan – Thailand border) in 1935. More habitat for *R. kerrii* was discovered later, including the Betis River Forest Permanent Reserve in Kelantan and Pengkalan Hulu in Perak, as well as Mount

Tepuh, Mt. Chamah, Mt. Stong, Mt. Basor, and the Lojing Highlands [6, 2]. According to various data reports, Lojing Highlands is a well-known location with the most *R. kerrii* population in Peninsular Malaysia [7, 8, 9, 10, 11].

Rafflesia is an endoholoparasite flowering plant that grows without leaves, stems, or true roots, with the flower being the sole apparent structure [12]. Many researchers have explored the morphological study of *Rafflesia* [13, 14, 2, 15]. Nonetheless, there is a scarcity of research on the anatomy of *Rafflesia*, particularly in Malaysia. The anatomy of *Rafflesia* in Thailand was discreetly elaborated by [16]. The pieces were unfortunately written in Thailand language. [17], [18], [19] and [20] all actively undertook anatomical studies on *R. patma* from Indonesia. The origin of *Rafflesia*, according to [21] and [22], begins with the production of uniserate strands and cell clusters inside the cambium of the host plant, *Tetrastigma* sp. Recent updates by [23] on the anatomical studies of *R. azlanii* and *R. cantleyi* with the host plant, *Tetrastigma rafflesiae* in the Belum-Temenggor Forest Complex, Perak, Malaysia, provided some information on host-parasitic plant relationships.

The greatest strategy to protect *Rafflesia*, according to [24], is to keep it in its natural environment. In-situ approaches, need a thorough understanding on the biological and ecological requirements of the species in order to preserve it from extinction. Thus, the anatomical research of *R. kerrii* is crucial. *Rafflesia*'s extinction rate may be accelerated by environmental changes. It may also have an impact on the anatomical structures of *Rafflesia*. Immediate conservation efforts for *Rafflesia* must be done at once in order to prepare most updated documentation on this endangered species and conserving them from perished locally. Furthermore, the information gathered provides fundamental information on the differences between *R. kerrii* in Kelantan and in Southern Thailand which influenced by environmental factors. The key components of the investigation were the perigone lobe, window, processes and ramenta.

2. Materials and method

2.1. Plant material collections and sample preparation

The fresh specimen of *R. kerrii* was collected in an upper hill dipterocarp forest, near to Kg. Mendrop, Lojing Highlands (latitude 4º32’ to 4º47’ N and longitude 101º20’ to 101º34’ E) in February, 2015. The collected specimen was preserved in 70% ethanol for long-term storage. Additional observations were also made on freshly collected sample.

2.2. Chemicals and reagents

Safranin, Alcian green, sodium hypochlorite, acetic acid, 50% ethanol, 70% ethanol, 95% ethanol, 100% ethanol, glue (Euphral), methylated spirit solution (95% v/v), concentrated hydrochloric acid and distilled water.

2.3. Assessment on anatomical structures

The anatomical structures of perigone lobe, window, processes and ramenta were investigated to describe the cell and tissue structures. The protocols were conducted as described by [25] with some modifications. For the perigone lobe, the structure was cut into three parts (top, middle and bottom), two parts of window (top and bottom), the whole structure of processes and ramenta (Figure 1.A). Each part was prepared in triplicates. Before slicing, the specimens were preserved in methylated spirit solution and 70% ethanol for overnight (Figure 1.B). Then, the specimens were sectioned by using sliding microtome (Thermo HM4-30) at a thickness of 70-100 µm. For staining process, the samples were stained with safranin and Alcian green, then rinsed with distilled water. Series of different ethanol concentration (50%, 70%, 95% and 100%) were used in dewatering process. Later, the samples were mounted on glass slides using Euphral and dried in the oven at 50 ℃ for a week (Figure 1.C). The anatomical structures of the prepared samples (Figure 1.D) were observed and captured using light microscope under 4x, 10x and 25x magnifications (Leica Diaplan microscope) attached with digital camera. Voucher specimens were deposited in the Herbarium of Forest Research Institutes Malaysia (KEP).

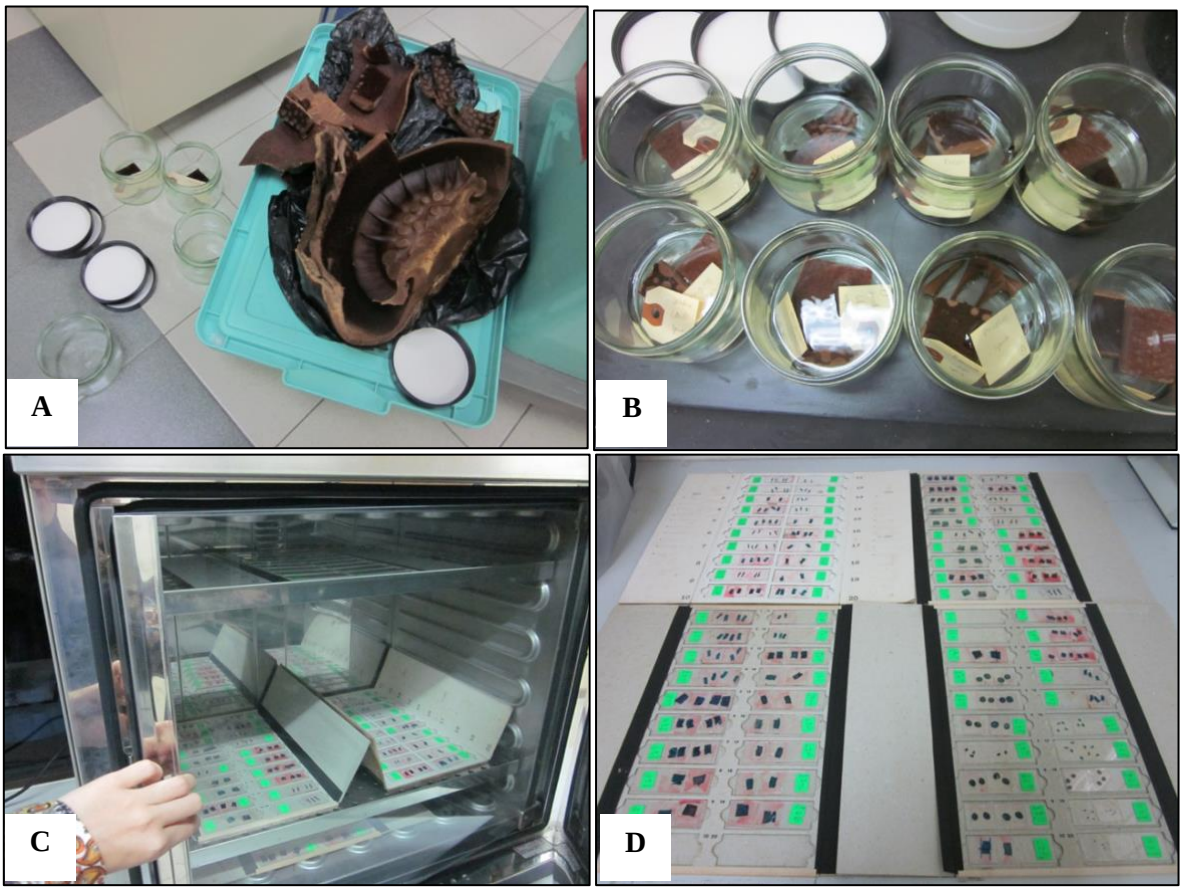


Figure 1. (A) The collected samples were preserved in 70% ethanol ; (B) The cut samples were soaked in spirit and ethanol solution; (C) The samples were dried in the oven at 50 ℃ for a week; (D) The prepared samples ready to be observed under light microscope.

3. Result and discussion

The results show that, the anatomical characteristics of *R. kerrii* flower are simple and uncomplicated. Figure 2 (A-D) are the images of the top part of perigone lobes, Figure 2 (E-H) are the images of the middle part of perigone lobes, whereas Figure 2 (I-L) are the anatomical images of the bottom part of perigone lobes. It can be observed in Figure 2 (A, E, I) by using 4x magnification, the presence of epidermal cells on the upper and lower part of the flower. The cells are arranged closely to each other. Figure 2 (B, F, J) shows the cell wall consist of thickened cells by 10x magnification. The ground tissues located in between of the epidermal cell layers and mainly composed of parenchyma cells. Figure 2 (C, G, K) shows the various shape of parenchyma cells, consisting of oval, bluntly cornered and largely irregular shapes. Whereas, Figure 2 (D, H, L) shows the images of undeveloped vascular bundles. According to [26], the vascular bundles in all parasitic plants were less developed, with poorly formed phloem but highly developed xylem. The phloem and xylem assist in the transporting water, minerals and sugar to various part of plants. Thus, *R. kerrii* is obligated on the food supply provided by the host plant. The findings were supported by [2] which stated that *Rafflesia,* as parasitic plant, must rely on *Tetrastigma* for sustenance.

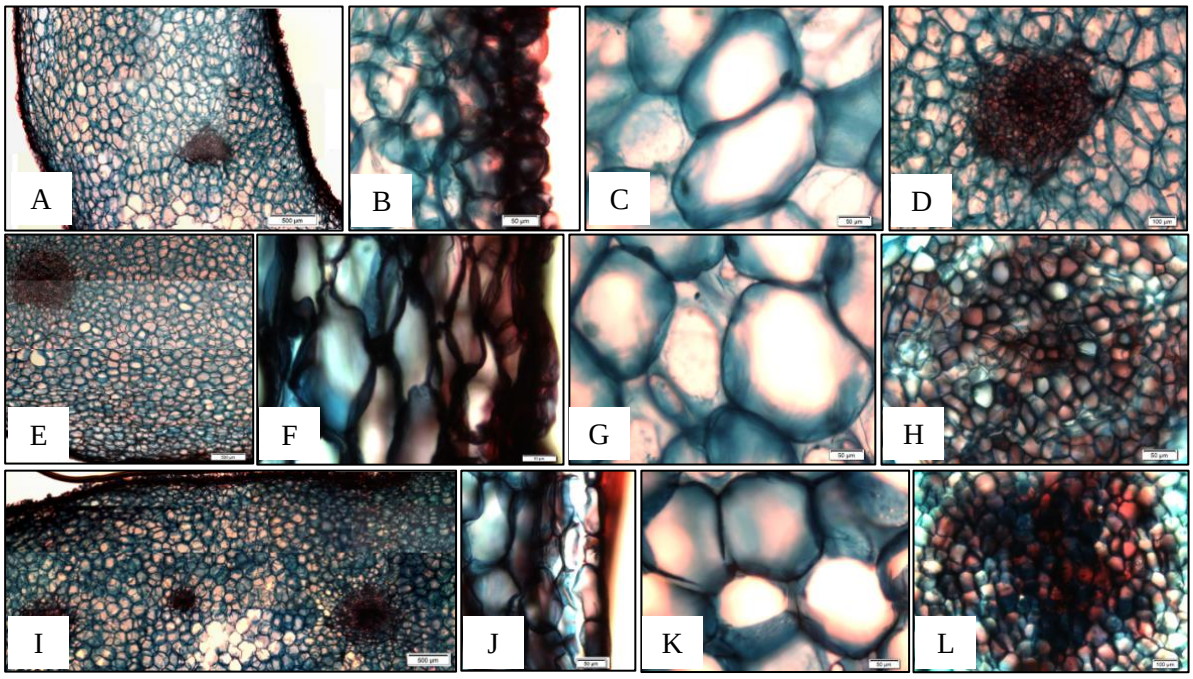
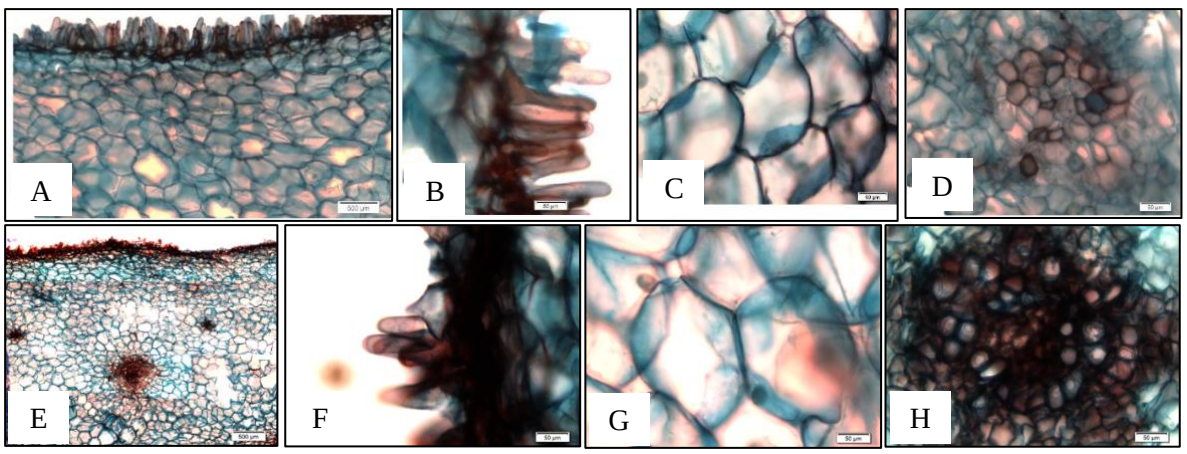


Figure 2. Perigone lobe (top part: A-D; middle part: E-H; bottom part: I-L, the scale used for the image is 500 µm) of *R. kerrii*; (A, E, I) The irregular shape of parenchyma cells that were closely arranged to each other (4x), (B, F, J) Cell wall consists of thickened cell (10x), (C, G, K) Irregular shape of parenchyma cells (10x), (D, H, L) Undeveloped vascular bundle (10x).

Figure 3 (A-D) are the images of the top part of window, whereas (E-H) are the images of the bottom part of the window. The existence of epidermal layer and parenchyma cells may be seen in the internal part (Figure 3.A & E). The irregular shaped of parenchyma appears to be closely packed. Figure 3 (B & F) are the images of the thickened cell wall, whereas Figure 3 (C & G) shows the irregular shape of parenchyma cell under 10x magnification. Figure 3 (D & H) are the close-up images of undeveloped vascular bundles. The phloem and xylems are difficult to differentiate since they are mixed together. Figure 3 (I & J) are the images of trichome papillus from the white warts on the exterior part of the window. Trichome papillus is absent in *R. kerrii* found in Thailand. According to [27], floral induction causes trichome loss, which is accelerated by terminal flower 1-10, a mutation that accelerates inflorescence growth.



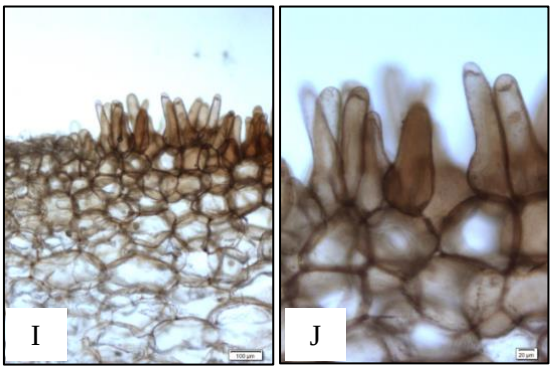


Figure 3. Window (top part A-D, bottom part E-J, the scale used for the images is 500 µm) of *R. kerrii*; (A & E) The irregular shape of parenchyma cells is closely arranged to each other (4x magnification), (B & F) Cell wall consists of thickened cell (10x), (C & G) Irregular shape of the parenchyma cells (10x), (D & H) Undeveloped vascular bundle (10x), (I) Trichome papillus on the outer side of window (4x), (J) Trichome papillus (10x).

Figure 4.A shows the anatomical structures of processes under 4x magnifications, with the scale 200 µm. The processes of *R. kerrii* composed of the epidermal layer on the cell wall and parenchyma cells at the internal. Figure 4.B clearly shows the cell in perpendicular and arranged closely to each other. Figure 4.C shows the structure of parenchyma cells that were round to oval shapes and arranged closely to each other. According to the findings, no starch formation was observed in *R. kerrii* of Lojing Highlands, despite [16] claiming the presence of starch in Thailand's *R. kerrii*. The presence of starch in *R. kerrii* of the Lojing Highlands was investigated using pole filter accessible on a light microscope, and none of the tissues were found to reflect the light, indicating that there is no starch within the tissues. According to [28], the presence of starch is greatly controlled by environmental factors. The rate and length of starch growth and protein accumulation are influenced by temperature and water availability. Thus, the lack of starch in *R. kerrii* from the Lojing Highlands believed was influenced by these environmental factors.

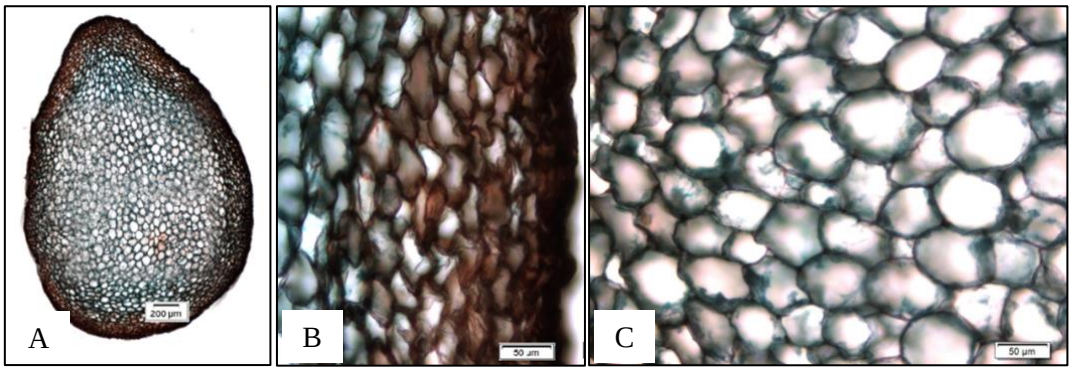


Figure 4. Processes of *R. kerrii* (The scale used for the image is 200 µm); (A) The irregular shape parenchyma cells are closely arranged to each other (4x), (B) Cell wall consists of thickened cell (10x), (C) Irregular shape of the parenchyma cells (10x).

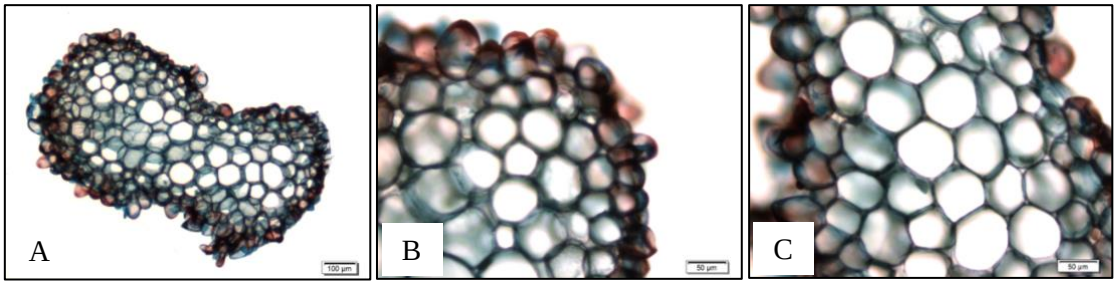


Figure 5. Ramenta of *R. kerrii* (The scale used for the image is 100 µm); (A) The irregular shape parenchyma cells are closely arrange to each other (4x), (B) Cell wall consists of thickened cell (25x), (C) Irregular shape of the parenchyma cells (25x).

Figure 5.A is the anatomical image of ramenta under 10x magnification, with the scale 100 µm. The present cells, in general, are comparable to the other structural parts (perigone lobe, window and processes), in that they have an epidermal layer on the cell wall and parenchyma cells at the internal part. The ground tissues that were discovered were mostly made up of parenchyma cells. Furthermore, mucilage cells can be found within the ground tissues of *R. kerrii* from Lojing Highlands and absent in *R. kerrii* of Thailand. The mucilage cells can be distinguished by their floral shape, in which one of the cells is larger than the others. According to [29], continuous exposure to pollutants causes parenchyma cells to flatten and fail to generate mucilage cells. Figure 5.B shows the thickened epidermal layer and Figure 5.C is the close-up image of parenchyma cells. No vascular bundle was observed in the ramenta part. Table 1 shows summarization on the anatomical characteristics of *R. kerrii* found in Lojing Highlands to the same species found in Thailand.

Table 1. Comparative anatomical characteristics of *Rafflesia kerrii* in Lojing Highlands, Peninsular Malaysia with *Rafflesia kerrii* from Khao Sok, Thailand

Rafflesia kerrii structural part	Lojing Highlands	Thailand
Perigone lobe	• Epidermal layer on the cell wall is present • Parenchyma cells is present in the internal part • Undeveloped vascular bundle • Irregular size of vascular bundle	• Epidermal layer on the cell wall is present • Parenchyma cells is present in the internal part • Undeveloped vascular bundle • Regular size of vascular bundle
Window	• Epidermal layer on the cell wall is present • Parenchyma cells is present in the internal part • Vascular bundle is present • Trichome papillus is present	• Epidermal layer on the cell wall is present • Parenchyma cells is present in the internal part • Vascular bundle is absent • No trichome is present
Processes	• Epidermal layer on the cell wall is present • Parenchyma cells is present in the internal part • No vascular bundle • Starch grains are absent	• Epidermal layer on the cell wall is present • Parenchyma cells is present in the internal part • No vascular bundle • Starch grains are present
Ramenta	• Epidermal layer on the cell	• Epidermal layer on the cell

	wall is present • Parenchyma cells is in the internal part • Mucilage cell is present among the ground tissues	wall is present • Parenchyma cells is present in the internal part • No mucilage cell was detected.

4. Conclusion

As a conclusion, the internal structures of *R. kerrii* were mainly composed of these followings: perigone lobes were made up of epidermal layer on cell wall, parenchyma cells at internal part and undeveloped vascular bundle; window parts were made up of epidermal layer, parenchyma cells, vascular bundles and trichome papillus; processes were made up of epidermal layer and parenchyma cells; and ramenta were made up of epidermal layer, parenchyma cells and presence of mucilage cell within the ground tissues. *R. kerrii* from Lojing Highlands has comparable anatomical features to *R. kerrii* from Thailand, with the exception of the presence of trichome papillus in the window part, mucilage cells in the ramenta and the lack of starch synthesis in the interior tissues. The differences occur believed due to the environmental factors, influenced the cell and tissues formation. Further studies are need to be conducted in order to determine the environmental factors which may contributes these said differences. This anatomical information provides insight into *Rafflesia*'s biology, allowing us to understand how this flower may persist as parasite.

Acknowledgement

The author would like to thank Universiti Malaysia Kelantan (UMK), Forest Research Institutes Malaysia (FRIM), Kelantan State Department, Gua Musang District Council (MDGM) and Lojing Highlands sub-district council for giving the opportunities to conduct the study. A special thanks dedicated to *Orang Asli* from *Temiar* ethnic for their assistance throughout the research.

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