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Growth Rate, Mortality Rate and Life Cycle of *Rafflesia azlanii* and *R. cantleyi* in Belum-Temenggor Forest Complex, Perak, Malaysia

(Kadar Pertumbuhan, Kadar Kematian dan Kitaran Hidup *Rafflesia azlanii* dan *R. cantleyi* di Kompleks Hutan Belum-Temenggor, Perak, Malaysia)

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ABSTRACT

The life cycle of *Rafflesia* is very complex because it involves the invisible and visible parts of its growth. The invisible part refers to its penetration, inoculation of the seeds into the host's tissue and seed formation whereas the visible part is the floral structure that is exposed to the external environments. Currently, there is little information on the life cycle of *Rafflesia*, therefore knowledge regarding its life cycle is crucial for a successful monitoring of the species as part of conservation efforts. The objectives of this study were to analyse the growth of flower buds at various developmental stages and mortality rates of *Rafflesia azlanii* and *R. cantleyi*, and to observe the life cycle of the two species. The growth rate of *R. azlanii* and *R. cantleyi* were recorded monthly for five months beginning from an emergence of buds to full bloom in the study sites of Belum-Temenggor Forest Complex, Perak. The buds were measured and vertically photographed for every observation and categorized into eight stages. *R. azlanii* buds grew at the mean rate of 0.20 cm/day and *R. cantleyi* was at 0.26 cm/day. This suggests that *R. azlanii* exhibited a slower growth rate than *R. cantleyi*. In addition, the mortality rate of *R. azlanii* (28%) was lower than *R. cantley*i (45%). The high mortality rate could be due to two factors; an exposure of the plants to direct sunlight and damage caused by pests. Meanwhile, the life cycle for the visible stage for *R. azlanii* was 14 months and *R. cantleyi* was 11 months. The information gathered from this study is useful for field monitoring and observation of the plants for conservation purposes.

Keywords: Bud growth rate; life cycle; mortality; *Rafflesia azlanii; Rafflesia cantleyi*

ABSTRAK

Kitaran hidup *Rafflesia* sangat kompleks kerana melibatkan bahagian pertumbuhan yang tidak kelihatan. Bahagian yang tidak kelihatan merujuk kepada penembusan, inokulasi benih ke dalam tisu inang dan pembentukan benih manakala bahagian kelihatan adalah struktur bunga yang terdedah ke persekitaran luaran. Pada masa ini, terdapat sedikit maklumat mengenai kitaran hidup *Rafflesia*, oleh itu pengetahuan mengenai kitaran hidupnya sangat penting untuk pemantauan dan sebahagian daripada usaha pemuliharaan. Objektif kajian ini adalah untuk menganalisis pertumbuhan bunga pada pelbagai tahap perkembangan dan kadar kematian *Rafflesia azlanii* dan *R. cantleyi* dan mencerap kitaran hidup untuk kedua-dua spesies tersebut. Kadar pertumbuhan *R. azlanii* dan *R. cantleyi* direkodkan setiap bulan selama lima bulan daripada kemunculan hingga bunga mekar di lokasi kajian Kompleks Hutan Belum-Temenggor, Perak. Tunas diukur dan difotograf secara menegak untuk setiap pemerhatian, dan ia dikategorikan kepada lapan tahap. Tunas

untuk *R. azlanii* telah tumbuh pada kadar 0.20 cm/hari dan *R. cantleyi* pula pada 0.26 cm/hari. Ini menunjukkan bahawa *R. azlanii* mempunyai kadar pertumbuhan yang lebih perlahan daripada *R. cantleyi*. Kadar kematian *R. azlanii* (28%) adalah lebih rendah daripada *R. cantleyi* (45%). Kadar kematian yang tinggi mungkin disebabkan oleh dua faktor; pendedahan tumbuhan kepada cahaya matahari secara langsung dan kerosakan oleh serangga perosak. Sementara itu, kitaran hidup untuk tahap yang dapat dilihat untuk *R. azlanii* adalah 14 bulan dan *R. cantleyi* adalah 11 bulan. Maklumat yang dikumpulkan daripada kajian ini berguna untuk pemantauan dan pemerhatian di hutan untuk tujuan pemuliharaan tumbuhan ini.

Kata kunci: Kadar pertumbuhan tunas; kematian; kitaran hidup; *Rafflesia azlanii; Rafflesia cantleyi*

Introduction

Rafflesia becomes a major tourists' attraction because of its uniqueness, beautiful, gigantic flower, rarity, and can only be found in Southeast Asia (Mat Salleh & Latiff 1989; Peters & Ting 2016). The occurrence of this rare species not only can help the economy of the country, but also can make the world to recognise this valuable species as the incredible inspiring works of nature (Kendall 2013). Recently, however, the population of *Rafflesia* is declining as a result of bud collection, natural disasters due to the monsoon season, shifting cultivation and deforestation (Hidayati & Walck 2016). The plant has widely been used by indigenous people for its purported medicinal attributes that in the belief it can help restore health and strength (Latiff 2018). Susatya (2011) reported that the mortality of *Rafflesia* is quite high due to various factors, such as extreme climate condition, small size of population, diameter of the buds, predation, the condition of the host plants and the number of buds in one host vine at the same time. A few studies reported that some of bud populations had 100% of buds dying before flowering as shown in *R. bengkuluensis* in Talang Tais, Indonesia with mortality rate up to 100% in two-month period (Susatya 2011; Susatya et al. 2017).

The declining trend of *Rafflesia* population indicates that a critical issue needs to be addressed in order to protect this valuable species from an extinction threat. To conserve *Rafflesia*, one has to have an understanding on various aspects of the species such as population size, life cycle and habitat management. Unfortunately, only six species (*R. patma*, *R. keithii*, *R. pricei*, *R. tengku-adlinii R. arnoldii,* and *R. consueloae*) were reported to have a detailed life history (Susatya 2020; Tolod et al. 2020).

The life cycle of the species is complex which is divided into two parts, invisible and visible parts (Hidayati et al. 2000; Susatya 2020). The invisible part includes the seed inoculation and seeds germination occurring inside the host plant which can take from 2 to 3 years whereas the visible part is the emergence of the bud on the host plant. Meanwhile, the growth of *Rafflesia* flowers according to Nais (2001) involved eight stages in the life cycle including pollination, seed dispersal, seed germination, emergence of flower bud, mature bud and anthesis stages. The different bud sizes represent different growth rates (Hidayati et al. 2000) and mortality rate was reported to be due to the combination of the exposure of various external environments and various bud sizes (Susatya et al. 2017). By focusing on the visible part of the species, this study aimed to determine the growth of flower buds at various development stages and mortality rates of *R. azlanii* and *R. cantleyi* and analyse the life cycle of these two species in Belum-Temenggor Forest Complex (BTFC), Perak, Malaysia.

Materials and Methods

This study was conducted in two areas in BTFC – Kg. Sg. Raba and Kg. Bongor, Perak (5° 20' 0" North, 101° 22' 0" East). BTFC is the biggest continuous forest complex in Peninsular Malaysia (Razak et al. 2015). BTFC consists of Royal Belum State Park, Gerik Forest Reserve, Banding Forest Reserve, Amanjaya Forest Reserve and Temenggor Forest Reserve (Malaysian Nature Society 2013). In this study the area consisted only of Gerik Forest Reserve (Kg. Bongor) and Banding Forest Reserve (Kg. Sg. Raba) (Figure 1). BTFC has a tropical climate mostly dominated by dipterocarp forests, with annual rainfall reaching 3,000 mm per year and an average temperature throughout the year ranging from 24 to 29.9 °C (Aiman Hanis et al. 2014). The humidity of the area ranges from 70 to 98% with high rainfall during the months of April and October and low rainfall in February and July (Aiman Hanis et al. 2014).

Field Data Collection

Two *Rafflesia* species (*R. cantleyi* and *R. azlanii*) were studied in Kg. Bongor and Kg. Sg. Raba, respectively. They were monitored and the measurements of the plant

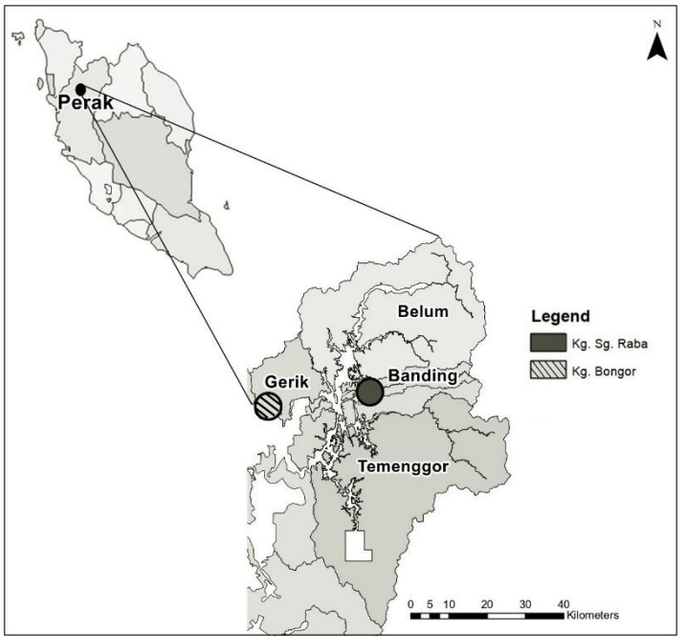

FIGURE 1. Location of study area in Belum-Temenggor Forest Complex. The circles represent the study sites

buds and flowers were recorded every month for five months in both study sites beginning from June 2019 to October 2019. The species identification of *Rafflesia* and *Tetrastigma* were performed by a taxonomist with the aid of herbarium specimens at Universiti Kebangsaan Malaysia (UKM). Overall, the samples were located in eight study areas in BTFC within different forest types such as hill dipterocarp forests with the elevation ranging at 314-355 m above sea level (asl) and extending to riparian forests. The diameters of *Rafflesia* buds and flowers were measured by the widest diameter length. The diameter increment was determined by calculating the increment from the first month to the second month and further followed by the subsequent months. The data involved for increment analysis only consisted of surviving buds during the entire five months' observation period (24 and 42 buds for *R. azlanii* and *R. cantleyi*, respectively). The life cycle for both species was measured according to the total period of eight stages as shown in Figure 2 with the invisible phase according to Hidayati et al. (2000). The mortality rate of the plants was calculated using the following equation (Nowak et al. 2004):

$$Mortality\ rate = \frac{Total\ number\ of\ dead\ bud}{Total\ number\ of\ bud} \text{ x } 100$$

In this study, the observation was limited to the visible structure of bud and flower developmental stages of *R. azlanii* and *R. cantleyi*. The measured buds were categorized into eight stages (Figure 2). The first is the swollen host stage which is referred to as the presence of a bulge on the host's stem. The second stage is the cupule stage, whereas the third stage is cupule-bract transition stage (CBT). In CBT, the bracts are present when the cupule parts (host tissue) are visible and gradually replaced by bracts. The fourth stage is the bract stage which refers to visible buds fully enclosed by the bracts in which the host tissues are no longer visible on top of the buds. The fifth stage is known as whitish perigone stage indicating the presence of whitish colour of perigone lobes as a result of abscission of bracts. The sixth stage is pinkish perigone stage in which the whitish colour turned to pinkish on perigone lobes, and seventh stage refers to when perigone lobes are exposed fully and the bracts can no longer be seen on top of perigone lobes. Finally, the eighth stage is the anthesis stage when the flower is in full bloom. In order to assist in visual observation, the buds were vertically photographed in order to categorize them accurately into the respective developmental stages. The stages were interpreted based on 80-100% of the images of vertically photographed buds covered by cupules, bracts and perigone lobes.

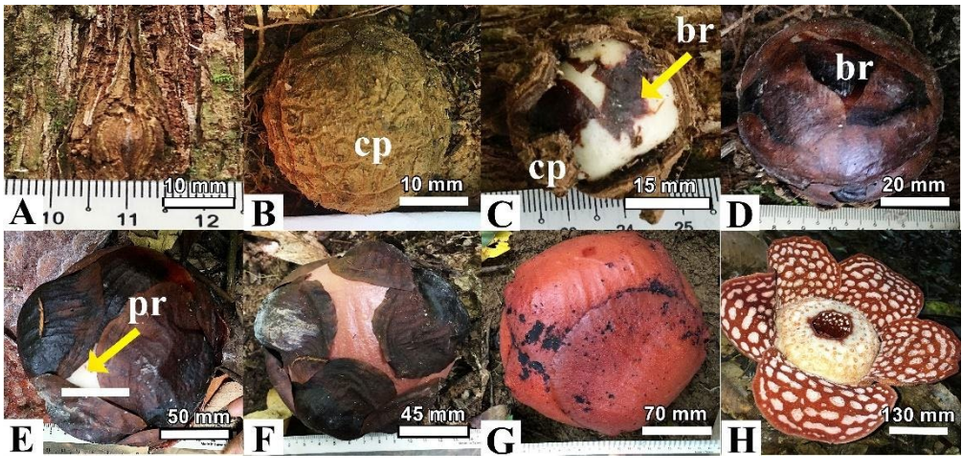

FIGURE 2. The flower developmental stages of *Rafflesia*. A) swollen host stage, B) cupule (cp) stage, C) cupule-bract transition stage, CBT, D) bract (br) stage, E) whitish perigone (pr) stage, F) pinkish perigone stage, G) perigone stage, and H) anthesis stage

DATA ANALYSIS

Analysis of Variance was used to analyse the mean of measured variables for each species in each study site. When significant differences were detected, a post-hoc Duncan's multiple comparison test was performed to determine which of the means are different. An independent Student's *t*-test was performed to test differences in the bud diameter increment between the two *Rafflesia* species. All statistical analyses were performed using Statistical Analysis Software (SAS) version 9.3 (SAS Institute Inc. 2018).

RESULTS AND DISCUSSION

The morphology and bud growth rate of *R. azlanii* in Kg. Sg. Raba and *R. cantleyi* in Kg. Bongor were monitored for five months beginning June 2019 until October 2019. The developmental stages were categorized and monitored from the newly emerged buds to fully bloom of flower.

GROWTH RATE

The growth of *R. azlanii* and *R. cantleyi* was analysed based on the diameter size with an additional analysis on the diameter increment for both species. Table 1 shows the descriptive statistics for bud and flower diameters for *R. azlanii* and *R. cantleyi.* During the first month, a total of 34 and 88 buds were sampled for *R. azlanii* and *R. cantleyi*, respectively. This suggests that Kg. Bongor consisted of a larger population size of *R. cantleyi* than Kg. Sg. Raba for *R. azlanii*. The number of samples was consistently reduced after the first measurement (Table 1). The reasons for the decline are discussed below later. The descriptive statistics of bud diameter increment for both *Rafflesia* species is presented in Table 2.

Analysis of Variance was performed to analyse differences in the mean diameter of buds during the five measurements for both species. From the analysis, it was found that there were no significant differences in the mean diameter of buds for *R. cantleyi* (P=0.2555) and *R. azlanii* (P=0.7131) throughout the five-month growth period. This suggests that the diameter growth rate is uniform for the *Rafflesia* buds and flowers in both areas. This could be due to the uniformity of climate in those areas. The research site was in the district of Gerik, Perak which harbours tropical rainforest with relatively constant temperature, relative humidity, rainfall and day length throughout the studied year. Table 3 shows the mean temperature, mean relative humidity and the total amount of rainfall per month for the five months' period from Bersia Power Station, the nearest power station to the research areas. The mean temperature in each month did not differ much. However, the mean for relative humidity was slightly different, the highest was recorded in the month June and the lowest was in September. The relative humidity increased until the end of the year due to the monsoon season. Slight difference in terms of the temperature in the research areas might have caused the uniformity in growth for the *Rafflesia* species in both areas. This condition makes the plants

tend to receive relatively similar rainfall, light intensity and temperature.

Figures 3 and 4 show the diameter measurements of *R. azlanii* in Kg. Sg. Raba and *R. cantleyi* in Kg. Bongor for five months' observation period. These figures present the results from Duncan's multiple comparison tests following the Analysis of Variance. Despite an increasing trend was observed in the bud growth of *R. azlanii* throughout five consecutive measurements, no significant differences were detected between them ($P \geq 0.05$) (Figure 3).

A slightly different trend was observed, however, in the case of the bud size of *R. cantleyi* in Kg. Bongor from a multiple comparison analysis. Significant differences in the mean of bud size between the first two measurements (June and July) and the third and fifth measurements (August and October) ($P \leq 0.05$) were observed. However, no significant differences were apparent in the mean size of buds during the last three measurements (August, September and October) ($P \geq 0.05$) (Figure 4). The variation of the means of bud diameter for *R. cantleyi* could be due the transition of the bud from one stage to another, i.e., from cupule-bract transition stage to bract stage. As the bud develops, it will also show different growth rate and mortality rate (Hidayati et al. 2000; Susatya et al. 2017).

An independent Student's *t*-test was performed to study the differences in the bud diameter increment between *R. azlanii* and *R. cantleyi*. From the analysis, it was found that there was a significant difference in the mean diameter increment between *R. azlanii* and *R. cantleyi* ($P=0.0294$). This suggests that the mean diameter increment or the diameter growth rate for *R. cantleyi* is significantly greater than *R. azlanii*.

Figure 5 shows the mean diameter increment for *R. azlanii* and *R. cantleyi* buds during the five observation months. The buds selected for increment analysis were from live buds during the entire five months which comprised of 24 buds for *R. azlanii* and 42 buds for *R. cantleyi*. The diameter increment of *R. cantleyi* buds was slightly higher than *R. azlanii* buds during the early growth stages (Figure 5). The difference became apparent towards the later growth stages. This was attributed to the difference in size between the buds in which *R. cantleyi* buds were larger in size than *R. azlanii* buds (Figure 5).

It was reported by Hidayati et al. (2000) that the larger buds exhibited a faster rate of increase in diameter compared to the smaller buds. It was concluded that the larger buds have faster growth rate with a wider diameter compared to the smaller buds. Nevertheless, the diameter increment of *R. azlanii* buds was decreased during the later growth stages, and it was during the last measurement month where the monsoon season started. The *R. azlanii* buds found dead mostly were at the cupule stage with a mean of 3.08 cm. Susatya et al. (2017) reported that the bud diameter ranging from 3 to 6 cm showed the highest mortality rate of 44%, followed by smaller diameter ranging from 1 to 3 cm (30%) and 12 to 15 cm (27%) due to the insufficient nutrients needed by the host.

Table 4 shows the number of the buds and flowers according to the stages for *R. azlanii* and *R. cantleyi*. The means of growth rate (cm/day) of buds at different developmental stages are tabulated in Table 5. The values were calculated by measuring the diameter differences between developmental stages and divided by the number of days. The visible buds were categorized into eight developmental stages (Table 5). The mean growth rate of bud diameter varied across the stages with the lowest rate in the cupule stage for both *R. azlanii* (1.04-2.1 cm/day) and *R. cantleyi* (0.91-2.3 cm/day). Meanwhile, the anthesis indicated the highest value for both species, *R. azlanii* was 39.64-43.56 cm and *R. cantleyi* was 44.0-46.66 cm. In a study conducted by Susatya (2020) in Sumatra, the perigone stage of *R. arnoldii* grew 12 times faster than the bract and cupule stages. In this study, the perigone stage of *R. azlanii* grew seven times faster than the cupule stage while the perigone stage of *R. cantleyii* grew five times faster than the cupule stage. This might be due to the larger size of *R. arnoldii* compared to other *Rafflesia* species as reported by Susatya (2020). Hidayati et al. (2000) reported that large buds exhibited a faster rate of increase in diameter compared to smaller buds, which is in agreement with the results obtained in this study (Table 5). Rapid growth of large buds was reported in several studies elsewhere for other *Rafflesia* species such as *R. arnoldii* (Susatya 2020), *R. patma* (Hidayati et al. 2000), *R. keithii, R. pricei*, and *R. tengku-adlinii* (Nais 2001), and *R. consueloae* (Tolod et al. 2020).

The bud growth rate for *R. azlanii* was 0.20 cm/day as compared to that of *R. cantleyi* at 0.26 cm/day, suggesting that the buds of *R. cantleyi* grew faster than *R. azlanii*. Meanwhile, the perigone lobes were fully exposed for *R. azlanii* when the buds reached at 21.5 cm in mean diameter size as compared to 17 cm for *R. cantleyi*. From the total of *R. azlanii* buds observed, only one bud attained maturity, and unfortunately it was found dead before blooming. Similarly, only one *R. cantleyi* bud

achieved maturity and was found dead during the full bloom (Figure 6). During the first month of observation, one dead *R. azlanii* flower was found in Kg. Sg. Raba. The flower began to bloom upon reaching ca. 20 cm diameter where the length of time from bud emergence to bloom for *R. azlanii* was from 13 to 15 months. However, *R. cantleyi* flower began to bloom when it reached ca. 17 cm where the time span from bud emergence to bloom was from 11 to 13 months. The mean diameters of the flowers were 41.8 and 48.0 cm for *R. azlanii* and *R. cantleyi,* respectively.

TABLE 1. The descriptive statistics of bud and flower diameters for five months

Location	Month	n	Mean (cm)	Std. deviation	Minimum	Maximum
Kg. Sg. Raba (*R. azlanii*)	June	34	3.88	3.05	1.00	13.0
	July	35	4.03	3.33	1.20	16.2
	August	32	4.4	3.84	1.20	21.5
	Sept	30	4.38	2.6	1.40	11.3
	Oct	28	4.97	2.87	1.60	12.0
Kg. Bongor (*R. cantleyi*)	June	88	3.71	3.75	0.50	34.0
	July	89	4.55	5.00	0.85	44.7
	August	87	4.47	3.12	0.60	19.2
	Sept	80	4.5	3.24	0.80	17.2
	Oct	70	5.26	5.63	0.80	38.7

TABLE 2. The descriptive statistics of bud diameter increment

Location	Increment	n	Mean (cm)	Std. deviation	Minimum	Maximum
Kg. Sg. Raba (*R. azlanii*)	1st	24	0.51	0.34	0.10	1.30
	2nd	24	0.55	0.36	0.05	1.30
	3rd	24	0.63	0.40	0.05	1.50
	4th	24	0.48	0.38	0.05	1.90
Kg. Bongor (*R. cantleyi*)	1st	42	0.66	0.45	0.10	1.80
	2nd	42	0.70	0.56	0.05	2.25
	3rd	42	1.32	1.85	0.10	7.90
	4th	42	1.49	3.37	0.10	21.50

TABLE 3. Mean temperature, relative humidity and rainfall from Bersia Power Station in year 2019 (Malaysian Meteorological Department 2019)

Month	Mean temperature (°C)	Mean relative humidity (%)	Rainfall (mm)
June	26.5	78.3	37.5
July	26.3	76.5	24.3
August	26.7	72.4	57.4
September	26.6	71.2	13.8
October	26.7	75.6	158.0

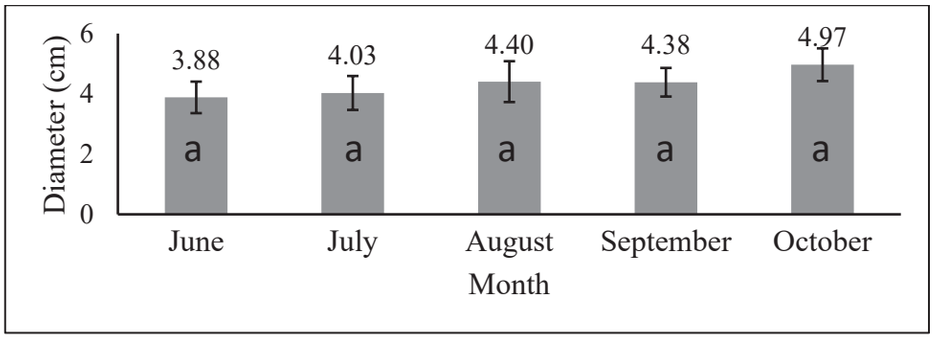

FIGURE 3. Means of *R. azlanii* diameter in Kg. Sg. Raba. Means with the same letter are not significantly different (P≥0.05)

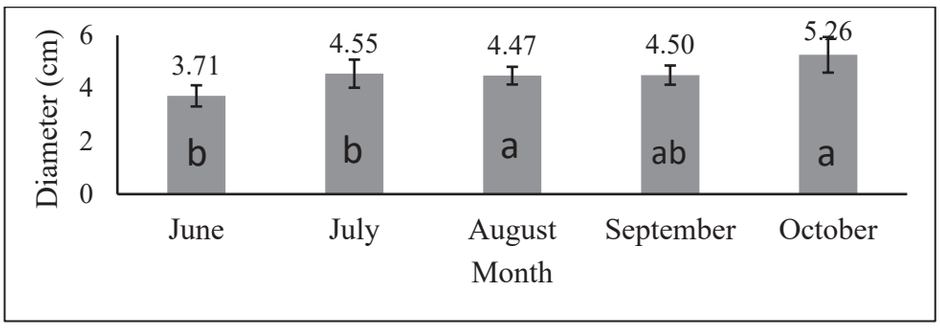

FIGURE 4. Means of *R. cantleyi* diameter in Kg. Bongor. Means with the same letter are not significantly different (P≥0.05)

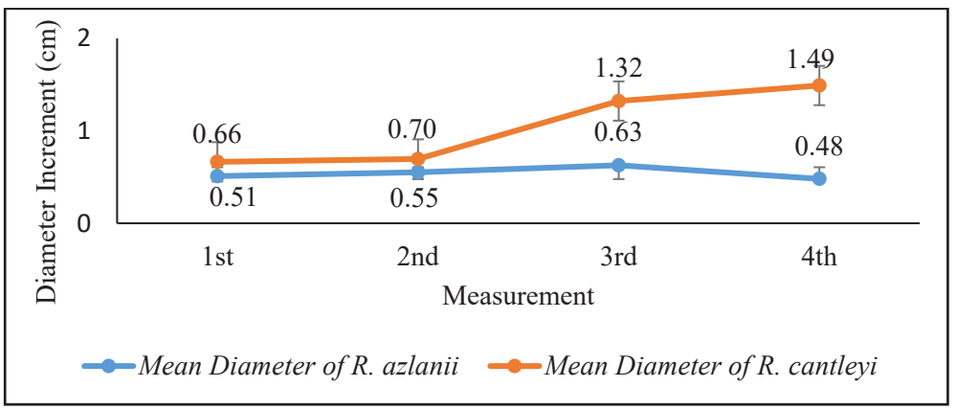

FIGURE 5. Average diameter increment of *R. azlanii* and *R. cantleyi* during five months of observation

TABLE 4. The number of buds and flowers according to it stages

Stage name	No. of buds and flowers	
	R. azlanii	*R. cantleyi*
Swollen host	16	82
Cupule	59	113
Cupule-bract transition	33	70
Bract	39	132
Whitish perigone	6	4
Pinkish perigone	3	8
Perigone	1	3
Anthesis	2	2
Total	159	414

TABLE 5. The mean of growth of the bud diameter according to its development stage

R. azlanii		*R. cantleyi*	
Stage name (range of buds diameter)	Growth mean (cm/day)	Stage name (range of buds diameter)	Growth mean (cm/day)
Swollen host (1.04-2.1 cm)	-	Swollen host (0.91-2.3 cm)	-
Cupule (1.54-4.62cm)	0.04	Cupule (1.53-4.1 cm)	0.03
Cupule-bract transition (3.03-5.1 cm)	0.02	Cupule-bract transition (2.89-5.45 cm)	0.02
Bract (4.73-11.83 cm)	0.02	Bract (4.36-11.18 cm)	0.04
Whitish perigone (7.6-11.59 cm)	0.03	Whitish perigone (10.91-14.3 cm)	0.07
Pinkish perigone (9.92-18.3 cm)	0.11	Pinkish perigone (9.9-18.85 cm)	0.04
Perigone (21.66 cm)	0.31	Perigone (16.09-18.34 cm)	0.16
Anthesis (39.64-43.56 cm)	0.89	Anthesis (44.0-46.66 cm)	1.46

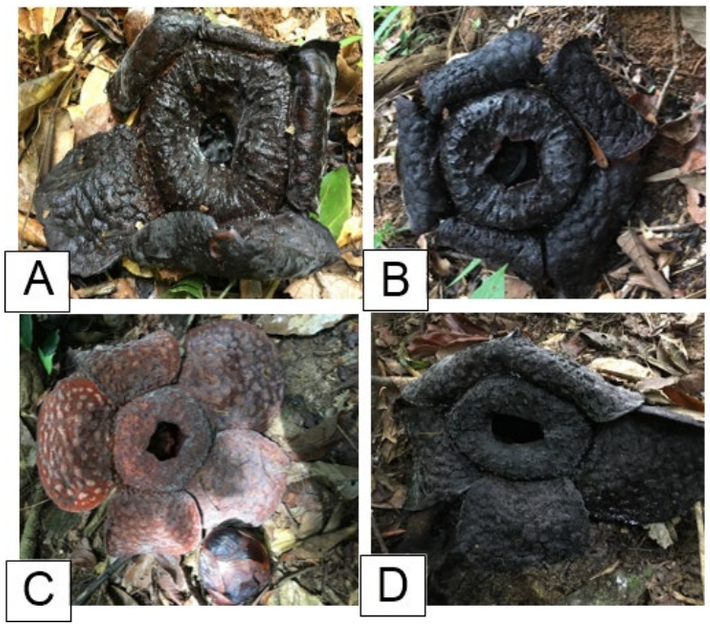

FIGURE 6. Full bloom of dead *R. azlanii* (A and B) and *R. cantleyi* (C and D) flowers found in the study areas

MORTALITY

Table 6 shows the information of the plots, coordinates, elevation (asl), canopy cover, distance from river and mean of soil temperature for five months monitoring periods in Kg. Sg. Raba and Kg. Bongor. Data were recorded in eight sites within the BTFC area at elevations ranging at 314-355 m asl in each of the forest sections and nearby riverbank (2.7-29.0 m). The visual method was used for estimating the canopy cover using the visual guide of tree canopy cover (Anderson 1986).

The elevation and soil pH affected the growth of *T. rafflesiae* and *R. kerrii* as the highest number of buds found located at 1200 m asl (Mokhtar et al. 2016). According to various studies on the *Rafflesia* host (Afiq Aizat 2018; Mokhtar et al. 2016; Syamsurina 2018), the *Tetrastigma* species grow in acidic condition. The weak acid in the soil was caused by the amount of the rainfall in the area where more rainfall can cause the acidity level of the soil decreases (Afiq Aizat 2018). *R. azlanii* recorded a lower mortality rate (28%) as compared to *R. cantleyi* (45%) (Table 7). During the five months of monitoring period, two full bloom flowers of *R. azlanii* were found dead in Kg. Sg. Raba whereas two full blooms with one dead flower of *R. cantleyi* were found in Kg. Bongor (Figure 8). It was observed that during these periods the buds were exposed directly under sunlight which might have affected the growth and eventually mortality after a few months. This could be due to the nature of *Rafflesia* plants that grow better under the shade and nearby the water sources (Patiño et al. 2002). It was also observed that the small buds had a high mortality rate as compared to the larger buds. According to Wunder et al. (2008), the larger plants or advanced development stages tend to have lower mortality and the smaller plants face inability to cope with the environment pressures leading to the failure of surviving.

Table 8 summarizes the data and description of the morphological characters of one of the *R. azlanii* buds consisting of different morphological stages nearly reaching the flower bloom which are associated with the figures of its morphological growth (Figure 7). During the fourth-month monitoring periods, the buds were unfortunately found dead just before blooming (Figure 7).

Morphological character description of one of *R. cantleyi* buds is summarized in Table 9. The bud selected was the one that reached the flower blooming stage and consisted of variation of bud stages during observation period. The morphology of the bud was observed when it was covered with bracts until the full blooming of the dead rotten flower (Figure 8(D)).

Figure 9 shows the differences of buds growing condition between *R. azlanii* and *R. cantleyi*. *R. azlanii* buds grew on the ground covering with soil (Figure 9(A)) as compared to *R. cantleyi* buds grew above the ground on the host vine (Figure 9(B)). According to Pranata et al. (2020), the buds grew on the ground tend to be damaged and this was one of the factors causing the death of *R. arnoldii* buds in West Sumatra, Indonesia. The finding from this study is in agreement with the finding of Triana et al. (2017), where the *R. patma* buds ranging from 1 to 10 cm diameter were found dead as their growing location was located at the host root and buried by the soil. It was reported that the buds covered with the soil had the potential to experience higher damage and death due to the presence of microorganisms or fungi (Triana et al. 2017). This could be the possible cause on the mortality of *Rafflesia* buds growing on the ground as observed in this study.

From the field observation, it is apparent that mortality of *Rafflesia* was caused by various factors such as the fungal attack, possibly damage by animals, natural disaster, exposure to extreme sunlight, bud grown in unfavourable conditions and bud grown on the ground covering with the soil (Figure 10). Wild pig, deer and squirrels also affected the growth of *Rafflesia* and host trees since the roots of host plants are easily injured by the footprints and nails of animals (Supartono & Herlina 2018). In Kg. Bongor, a few of *Rafflesia* fruits were found to be possibly attacked by pests (Figure 10(C) & 10(D)).

TABLE 6. Data collected in Kg. Sg. Raba and Kg. Bongor

Area	Plot	Coordinate	Elevation (m)	Canopy cover (%)	Distance from river (m)	Mean of soil temperature (°C)
Kg. Sg. Raba (*R. azlanii*)	A	N 05◦33. 132' E 101◦25.696'	325	55	18.0	23.4
	B	N 05◦33. 132' E 101◦25.696'	325	55	18.0	23.3
	C	N 05◦33. 181' E 101◦25.540'	314	45	10.5	23.5
	D	N 05◦33. 185' E 101◦25.544'	314	55	10.5	23.4
Kg. Bongor (*R. cantleyi*)	E	N 05◦32. 146' E 101◦12.317'	355	45	44.0	24.5
	F	N 05◦32. 147' E 101◦12.318'	355	35	44.0	24.6
	G	N 05◦32. 091' E 101◦12.346'	346	65	6.9	24.1
	H	N 05◦32. 126' E 101◦12.328'	348	65	2.7	24.2

TABLE 7. Mortality rate based on the number of surviving buds for *R. azlanii* and *R. cantleyi*

Location	Species	Average no. of total bud in each plot	Average no. of dead plants	Mortality rate
Kg. Sg. Raba	*R. azlanii*	10	3	28%
Kg. Bongor	*R. cantleyi*	32	14	45%

TABLE 8. Observation of morphological characters of one of the *R. azlanii* buds

Month observation	Diameter (cm)	Morphological observation
June	13.00	Buds covered fully with brown black bract without the presence of the wood caps (Figure (7A))
July	16.20	Matured buds with pinkish perigone lobes present (Figure (7B))
August	21.50	Fully perigone lobes exposed where the bracts were present below the perigones (Figure (7C))
September	19.6	Buds found rotten dead and shrunk (Figure (7D))

TABLE 9. Morphological characters of one of *R. cantleyi* buds

Month observation	Diameter (cm)	Morphological observation
June	7.20	Bud is fully covered by the bracts (Figure 8(A))
July	17.40	Matured bud with pinkish perigone lobes (Figure 8(B))
August	19.20	Perigone lobes are halfway open and the warts can be seen inside (Figure 8(C))
September	46.80	Full blooming of dead rotten flower (Figure 8(D))

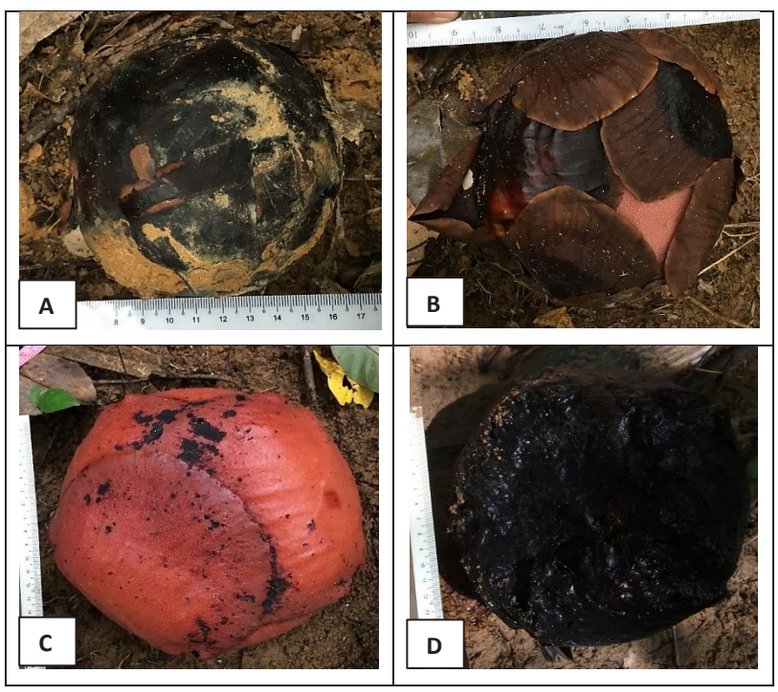

FIGURE 7. Morphological growth of one of the *R. azlanii* buds found in Kg. Sg. Raba

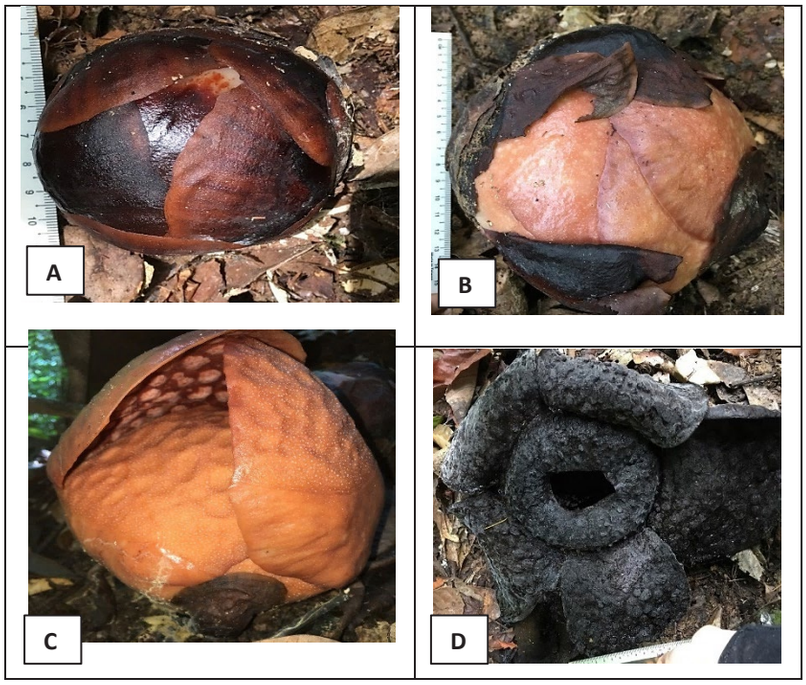

FIGURE 8. Morphological growth of one of the *R. cantleyi* buds found in Kg. Sg. Raba

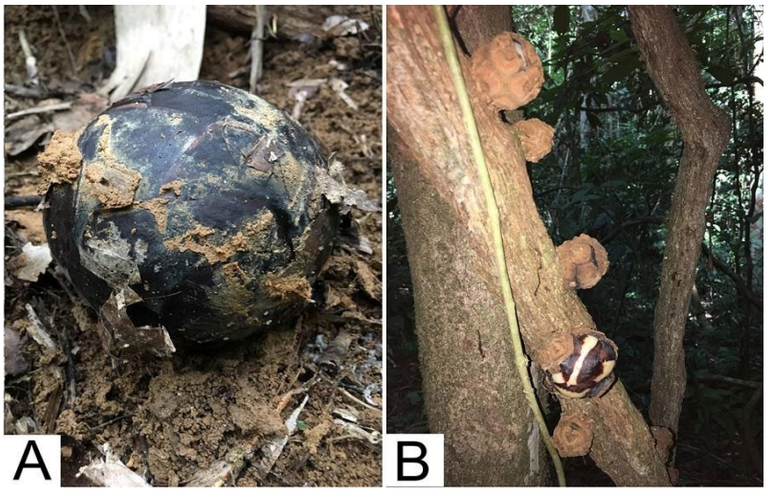

FIGURE 9. Growing location of the buds of *R. azlanii* (A) and *R. cantleyi* (B)

LIFE CYCLE

Based on the data obtained during the five months' period, the bud diameters of *R. azlanii* and *R. cantleyi* were measured (Table 2). The buds were categorized into eight developmental stages (Figure 11). The time for the Rafflesia parasite began to swell inside the host tissue cannot be estimated due to the time constraint in which the first observation began only when the stem of the host started to swell. Based on Hidayati et al. (2000), the invisible stage of *Rafflesia* was the growth of the seed

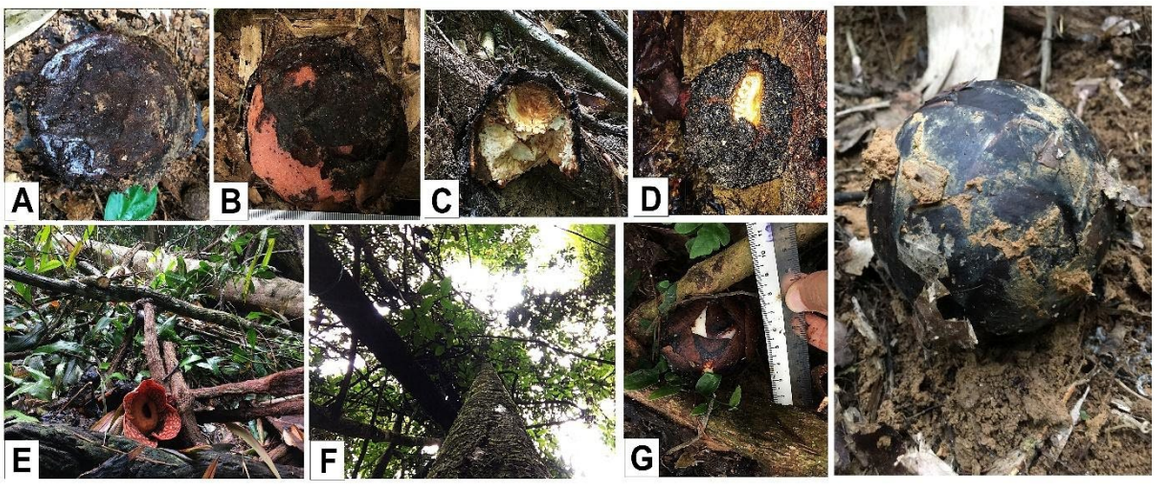

FIGURE 10. The threats to the Rafflesia population. Fungal attack (A), possibly damaged by animal (B-D), natural disaster (E), exposure to extreme of sunlight (F), bud grown in unfavourable condition (G) and bud grown on the ground covering with the soil (H)

inside the host which can take 2 to 3 years. The first stage of visible development of a bud called swollen host took about 30 days for *R. azlanii* and *R. cantleyi*. The second stage is a cupule stage where the bud is covered by host tissue (cupule) and starts to form a big bulge on the host stem. The second to the third stage took 90-120 days for *R. azlanii* and 60-90 days for *R. cantleyi*.

The third stage is the cupule-bract transition that took 90-120 days for *R. azlanii* and 60-75 days for *R. cantleyi* to reach the fourth stage. This was when the top of the bud was fully covered with the bracts. The fourth stage took 90-120 days for *R. azlanii* and 60-80 days for *R. cantleyi*. Stage five is when the bud reaches maturity, and the bract abscission takes place exposing the whitish perigone lobes which took 1-2 months for *R. azlanii* and 25-45 days for *R. cantleyi*. The sixth stage occurs when the presence of the pinkish perigone lobes is observed, and it took 15-30 days for *R. azlanii* and

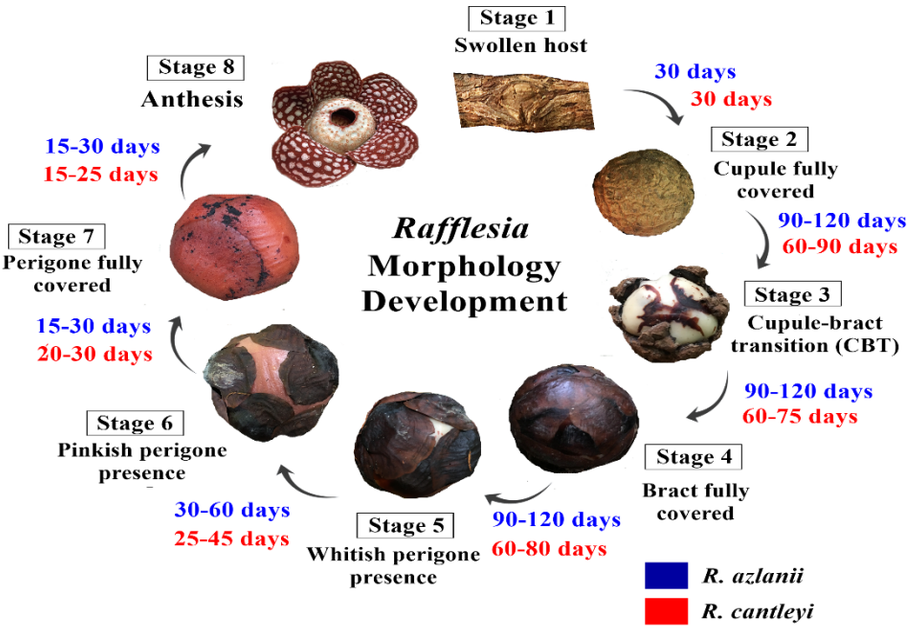

FIGURE 11. *Rafflesia* morphology development for *R. azlanii* and *R. cantleyi*

20-30 days for *R. cantleyi* to reach seventh stage when the perigone lobes were fully exposed with the reddish orange colour. The seventh to eighth stages are when the flowers have full bloom, and it took 15-30 days for *R. azlanii* and 15-25 days for *R. cantleyi*.

A study conducted by Mohamed and Mohd Noor (2016) in Sungkai, Perak found that the developmental stage from the newly emerged bud to fully opened flower for *R. azlanii* took 40–50 days which was shorter compared to the result obtained in this study. This might be due to the differences in the microclimatic and edaphic factors between the study sites. In another study conducted by Susatya (2020), it was found that *R. arnoldii* required 3.5 to 5 years to complete the life cycle with 16 months from the cupule to anthesis stage. Overall, it was observed that the duration for the visible stage from swollen host to flower bloom was 14 months for *R. azlanii* and 11 months for *R. cantleyi*. This is slightly similar to *R. consueloae* in Luzon Island, Philippine where it took roughly a year from swelling host to anthesis stage (Tolod et al. 2020). Meanwhile, the average time taken for seed inoculation was about two years and five months (Hidayati et al. 2000). Thus, to complete the life history including the invisible phase, *R. azlanii* might require three years and *R. cantleyi* might require three years and four months.

Conclusion

The development of life history of *R. azlanii* and *R. cantleyi* were 3.7 and 3.4 years, respectively. The developmental growth of *R. cantleyi* was found to be faster than *R. azlanii,* and the mortality rate of *R. cantleyi* was slightly higher than *R. azlanii.* Both *R. azlanii* and *R. cantleyi* required at least a year to develop from early emerging bud to fully flower blooming. The findings from this study are useful in enhancing our understanding of these rare parasitic plants that are becoming vulnerable, especially in terms of documenting scientific-based information that complement the efforts of its conservation strategies. Additionally, the information of the growth rate and the length time from emergence to flower blooming of *Rafflesia* will be useful in scheduling of visits for the tourism industry.

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