

Flower and Fruit Development and Life History of *Rafflesia consueloae* (Rafflesiaceae)

Janine R. Tolod^{1,2*}, John Michael M. Galindon³, Russel R. Atienza^{1,2},
Melizar V. Duya^{1,2}, Edwino S. Fernando^{1,4}, and Perry S. Ong^{1,2}

¹Institute of Biology, College of Science

University of the Philippines Diliman, Quezon City 1101 Philippines

²Diliman Science Research Foundation, Diliman, Quezon City 1101 Philippines

³National Museum, Padre Burgos Drive, Ermita, Manila 1000 Philippines

⁴Department of Forest Biological Sciences, College of Forestry and Natural Resources
University of the Philippines Los Baños, College, Laguna 4031 Philippines

Flower and fruit development of *Rafflesia consueloae* were studied between February 2014 and April 2016 in Pantabangan, Nueva Ecija, Philippines. Flower development was divided into five distinct phases: (1) emergence, (2) post-emergence, (3) bract, (4) perigone, and (5) anthesis. Fruit development was monitored from flower senescence until fruiting and maturation. A total of 512 individual buds were monitored – discovered at different stages of bud development. Only nine buds were monitored from post-emergence until the perigone phase. A bloom rate of 19.73% and an overall mortality rate of 77.34% were recorded. Mortality was highest during the early phases (post-emergence and bract) and lowest at the perigone phase. *R. consueloae* exhibited nocturnal flowering; wherein anthesis usually begins at dusk, signaled by the detachment of the first lobe, and from there on, full bloom took 15 ± 5.85 h to complete. Flowering was at its highest during the coldest and driest months of the year – between December and April. The growth rate is exponential, with an overall diameter growth of 1.80% every 4 d. The highest growth rate was recorded during the perigone phase. Six fruits, considered as dehiscent berries, ripened mostly in July – within 145 ± 11.03 d from anthesis.

Keywords: bud development, dehiscent berry, emergence, holoparasite, nocturnal anthesis, Rafflesiaceae

INTRODUCTION

Two centuries after its discovery in Sumatra, the biology and evolutionary characteristics of *Rafflesia* remain obscure and elusive to science. With the absence of recognizable plant structures and its cryptic parasitic behavior, along with the rarity of large populations, *Rafflesia* has been a challenging target organism in research. While more than 30 species of *Rafflesia* have been described to date

(Nikolov and Davis 2017), comprehensive studies on the parasite's growth and development are still relatively scarce, possibly because of the rarity of a large population at different stages of development. For instance, the average population of *R. arnoldii* is only 12.5 buds and the smallest population recorded for a *Rafflesia* species was *R. bengkulensis*, which had four subpopulations, each having only 3–8 buds (Susatya *et al.* 2017).

Barkman and co-authors (2017) reported the infection of host *Tetrastigma* by numerous individuals of *Rafflesia*.

*Corresponding Author: jrtolod@gmail.com

According to Nikolov and co-authors (2014), this chronic infection is responsible for consistent blooming of *Rafflesia* even after a period of long development inside the host. The latter contributed significant knowledge on the development of *Rafflesia* prior to visible emergence from the host, revealing that the vegetative stage developed directly from an undifferentiated endophyte, which started as a proembryo. The endophyte also appears to move from the initial location through the division of host cells, resulting in its spread throughout the host, usually towards the younger tissues (Wicaksono *et al.* 2017; Mursidawati *et al.* 2019). Nais (1997, 2001) undertook the most intensive study on the visible stages of bud growth and development wherein 1,110 buds from three different *Rafflesia* species were visited monthly for 13–29 mo. The accounts described the bud, flower, and fruit morphology – as well as population structure, reproductive ecology, life cycle, and distribution. However, growth and developmental stages were only monitored once a month, which might have resulted in a number of substantial undocumented observations. Furthermore, Hidayati and co-authors (2000), Mursidawati and co-authors (2014), Diway (2016), and Susatya (2020) made significant contributions regarding the growth, development, and life cycle of *Rafflesia*, although only very small populations in short periods of time were documented.

In 2016, *Rafflesia consueloae* became the most recently described Rafflesiaceae species in the Philippines. It is the sixth species of *Rafflesia* found in Luzon Island and was described to be the “smallest among the giants,” with its average flower diameter of 9.73 cm (Galindon *et al.* 2016). In addition to its relatively small size, this species is distinct from other *Rafflesia* species because of its cream-white disk surface that is often devoid of processes. In this study, bud development and flower phenology, as well as fruiting events were comprehensively monitored to account for their importance to the life history of the species. The combination of two factors – the availability of a large population of a single species and its accessibility – allowed for comprehensive monitoring (frequent visitation), conditions which were not available in the studies of other *Rafflesia* species. The study aims to characterize in terms of morphology the distinct stages of the development of *R. consueloae* from bud emergence until anthesis. Moreover, this will also provide further information on its flowering phenology and fruit development. Ultimately, this study aims to contribute to the very limited knowledge of the life history of the *Rafflesia* species in general.

METHODS

The study was conducted between February 2014 and April 2016 in Brgy. Fatima, Mount Balukbok, Pantabangan, Nueva Ecija, Luzon Island, Philippines

(see Appendix I for a map of the study site). The study area is located at 15°50'17.30" N, 121°05'21.60 E, and at 325 m above sea level. Pantabangan largely falls under the Philippine Climatic Type I with two pronounced seasons – namely, dry from December–April and wet during the rest of the year. Other descriptions of the study area are provided in Galindon *et al.* (2016). Three sites were used as study areas. Sites 1 and 2 were discovered in February 2014, followed by Site 3 a month later. Each site had its own distinct *Tetrastigma* host, making each population isolated from one another. Unfortunately, due to the very rare flowering of the *Tetrastigma* as well as the inaccessibility of the flowers (*i.e.* situated atop a tree that was unsafe to climb), the species of the host vine was not identified. In the whole duration of the study, 230 buds were documented in Site 1, 153 buds in Site 2, and 129 buds in Site 3. Site 1 is approximately 250 m away from Site 2 while Site 3 is approximately 210 m and 75 m away from Sites 1 and 2, respectively. The average circumferences of the *Tetrastigma* roots in Sites 1–3 are 1.22 cm, 1.55 cm, and 1.40 cm, respectively.

A series of replicated measurements were done on each of the 512 buds from discovery to death or anthesis – from 18 Feb 2014 to 30 Apr 2016. Using a Vernier caliper up to the hundredth decimal point, the longer diameter of each *R. consueloae* bud was measured with a three-day interval (*i.e.* on Day 1, buds on Site 1 were measured, followed by the measurement of buds on Site 2 on the second day, and Site 3 on the third day, and no measurement on the fourth day) between two consecutive measurements. There was no activity on the fourth day to reduce any potential stress that the buds may experience from mechanical disturbance associated with measurements. Male flowers were no longer monitored after bloom since no fruits were expected. Female flowers, on the other hand, were continuously monitored until senescence or until the formation and maturation of fruits. The growth rate was calculated using consecutive measurements of each bud that completed a specific phase. For over-all growth rate, only the buds that were monitored from post-emergence until the anthesis stage were used.

Top-view photographs of each bud were taken during every measurement and were later used to determine different bud developmental stages. The process included the following steps: 1) determining the distinct visual characteristics throughout the course of development of the buds (from discovery to death), 2) characterizing all buds into distinct developmental phases [emergence (longitudinal break in the host bark), post-emergence (swelling in the host root), bract, perigone, and anthesis], and 3) identifying the measurements and durations associated with the bud stages.

Rainfall and temperature data during the study period were obtained from the nearest weather station of the Philippine

Atmospheric, Geophysical, and Astronomical Services Administration (PAGASA) located at the Municipality of Science City of Muñoz, Province of Nueva Ecija. Kendall rank correlation test was performed using RStudio Version 1.1.463 (package “ggpubr”) to determine the relationship between mortality or flowering data to rainfall and temperature, with the significance level set at 95% confidence interval.

RESULTS

A total of 512 buds were monitored across the three sites during the two-year study. The growth and development of these buds were divided into two general stages – namely, flower development (emergence from *Tetrastigma* to anthesis) and fruit development (flower senescence to the formation of fruits and seeds) (Table 1).

Flower Development

This starts when a bud emerges from the *Tetrastigma* host – the bud at the emergence phase. At this stage, a crack

Table 1. Development stages of *R. consueloae* buds classified according to morphological characteristics.

Phases	Characteristics	Size range	Duration
Stage 1: flower development			
Emergence	<ul style="list-style-type: none"> Bark of the <i>Tetrastigma</i> root breaks longitudinally at first, followed by slight crack across the longitudinal break, exposing more bud surface Appears as an irregularity in the <i>Tetrastigma</i> bark 	0.90 x 0.92 cm to 1.42 x 1.47 cm (n = 1)	≈ 100 d
Post-emergence	<ul style="list-style-type: none"> Rounded swelling in the <i>Tetrastigma</i> roots Rhytidome (carapace-like structure with patterns similar to a soccer ball) fully encloses bud 	0.69 cm to 3.85 cm (n = 282)	166 ± 39.95 d (n = 34)
Bract	<ul style="list-style-type: none"> Rhytidome starts to crack due to increase in bud size Bracts visible as white structures that eventually darken from tip to base, changing into dark brown, or reddish-brown in color Formation of cupule Perigone lobes still covered by bracts 	1.94 cm to 6.71 cm (n = 280)	110 ± 20.34 d (n = 12)
Perigone	<ul style="list-style-type: none"> Abaxial surface of at least one perigone lobe visible No sign of possible lobe detachment At least half of each bract already brown in color 	4.01 cm to 9.80 cm (n = 117)	42 ± 11.43 d (n = 37)
Anthesis	<ul style="list-style-type: none"> Bracts disengage (detach from the perigone lobes distally but not entirely) Lobes slowly detach until all lobes are straight upward Disk and diaphragm visible 	8.11 ± 0.78 cm (n = 101)	15 ± 5.85 h (n = 4)
Stage 2: fruit development			
Fruit development or senescence	<ul style="list-style-type: none"> Perigone lobes wrinkle and turn black If fruit is formed, perigone lobes and diaphragm drop, retaining the disk and the column structure which eventually harden into a rounded and grooved structure (young fruit); otherwise, the whole flower decays 	Less than the bloom diameter	Dropping of lobes for fruit development ≈ 1.5–2 mo; total decay (no fruit development) ≈ 1–2 mo
Fruit maturation	<ul style="list-style-type: none"> Exocarp cracks revealing brown seeds and white pulp Coconut apple-like smell 	6.55 ± 0.39 cm (n = 4)	145 ± 10.80 d (from bloom) (n = 5)

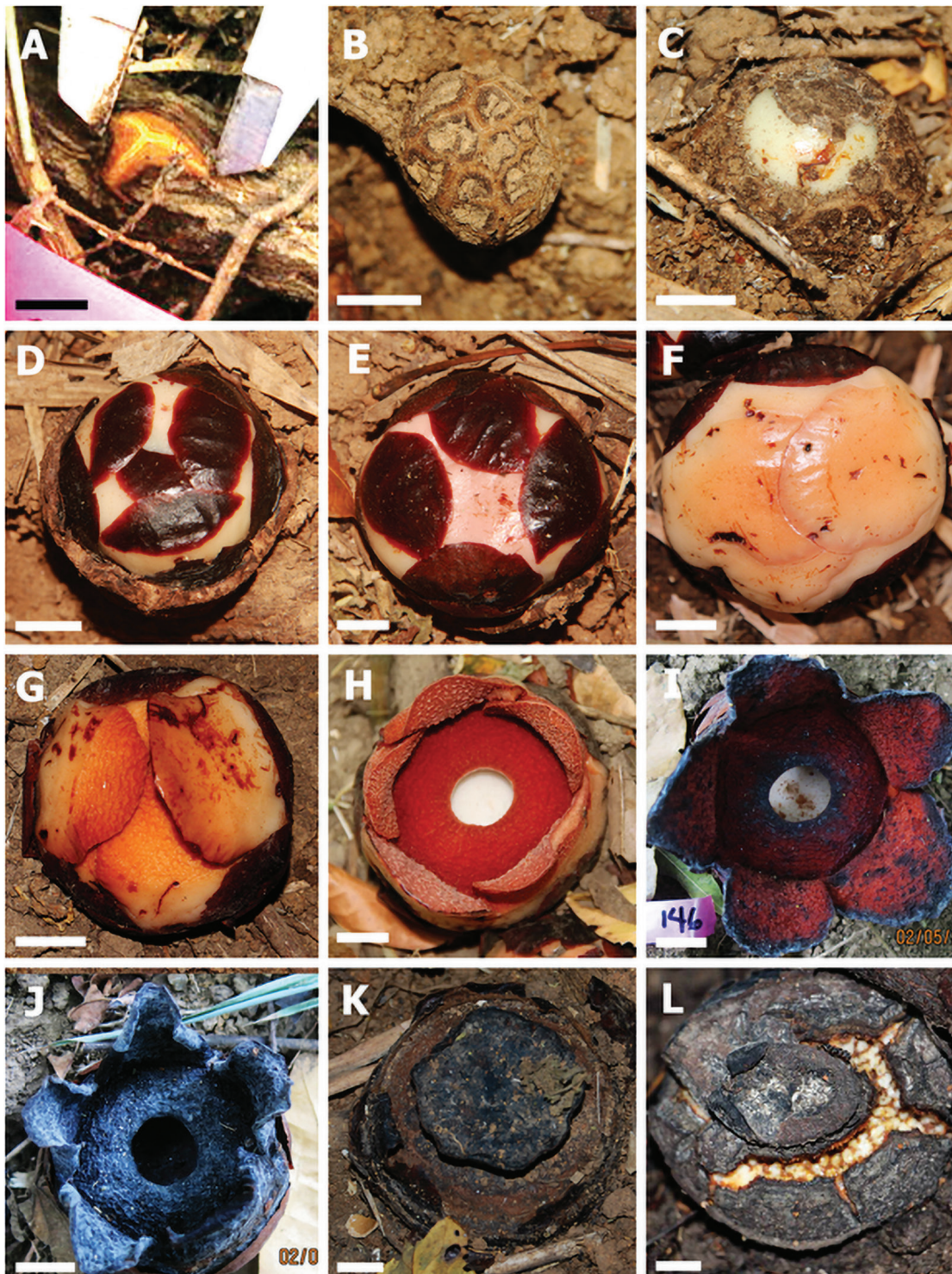


Figure 1. Development stages of *Rafflesia consueloae*: A) emergence from the *Tetrastigma*, B) post-emergence phase – rounded swelling where the true bud is still enclosed in *Tetrastigma* rhytidome, C) early bract phase – transition from post-emergence to bract phase, similar to the CBT stage of Susatya (2020), D) late bract phase – white bracts turned brown acropetally at the end of bract phase, E) early perigone phase – abaxial surface of lobes already seen, similar to the BPT stage of Susatya (2020), F) late perigone phase – substantial increase in size pushing bracts sideways, G) anthesis phase, H) fully-bloomed *R. consueloae*, I) start of flower senescence, J) decaying *R. consueloae* prior to young fruit development, K) developing young fruit, and L) matured *R. consueloae* fruit showing the white endocarp and brown seeds (scale bar: A = 0.5 cm; B–E, K–L = 1 cm; F–J = 2 cm). Photos by John Michael Galindon, Claire Ann G. Elmido, and Jaime M. Mangalindan.

or rupture from the root of the host could be seen (Figure 1A). Only one bud of this phase was clearly observed. The bud continued to grow slowly ~ 100 d after its discovery and eventually stopped growing for 300 d, after which it showed clear symptoms of decay.

Most observations and bud measurements started at the post-emergence phase or during the early development of buds (Figure 1B). During this phase, true buds were not yet visible but can be observed as rounded entities covered with *Tetrastigma* bark (rhytidome), with patterns resembling that of a soccer ball (bud diameter: 0.69–3.85 cm, $n = 282$). Since buds were mostly discovered during this phase, the calculation for the duration of days spent in this stage only included data from buds that were discovered at a diameter lower than 1.6 cm to avoid underestimation. After 166 ± 39.95 d ($n = 34$), the bud size increased which caused the *Tetrastigma* bark covering the bud to crack; this signals the start of the *bract* phase (bud diameter: 1.94–6.71 cm, $n = 280$) (Figure 1C). Whitish bracts were now exposed as a result of the cracking of the bark of the host. Within another 110 ± 20.34 d ($n = 12$), these white bracts darkened from tip to base, changed into light brown or reddish-brown, but still enveloped the perigone lobes (Figure 1D). This was followed by the perigone phase as soon as the abaxial surface of the perigone lobes became visible (bud diameter: 4.01–9.80 cm, $n = 117$). In this phase, bracts were gradually pushed sideward due to the significant increase in the size of the buds (Figures 1E–F). The perigone lobes matured and became pinkish to orange-red in color within 42 ± 11.43 d ($n = 37$).

The anthesis phase concluded the flower development stage. At the beginning of this phase, the uppermost perigone lobe started to detach from its attachment with other lobes (Figure 1G). One after the other, perigone lobes would slowly unfold until the diaphragm and disk became visible (Figure 1H). Unfolding of *R. consueloae* flower took 15 ± 5.85 h ($n = 4$) to complete. As soon as the *Tetrastigma* bark covering the bud started to crack and revealed the white bracts (bract phase), the number of days for a bud to reach anthesis was estimated to occur 166 ± 18.19 d after. Of the 512 studied individuals, only 101 (19.73%) buds reached anthesis. The highest bloom percentage was recorded in Site 2 (25.49%), followed by Site 1 (17.83%) and Site 3 (16.28%).

During the earlier stages of bud development from the post-emergence until the bract phase, a gradual increment in diameter growth was observed. This was followed by an immediate rapid growth increment during the perigone phase until the anthesis phase was reached. The average growth rate (every 4 d) for each phase was 1.79% during post-emergence ($n = 58$), 1.34% during the bract phase ($n = 12$), and 4.19% during the perigone phase ($n = 33$). In addition, based on the nine buds that had

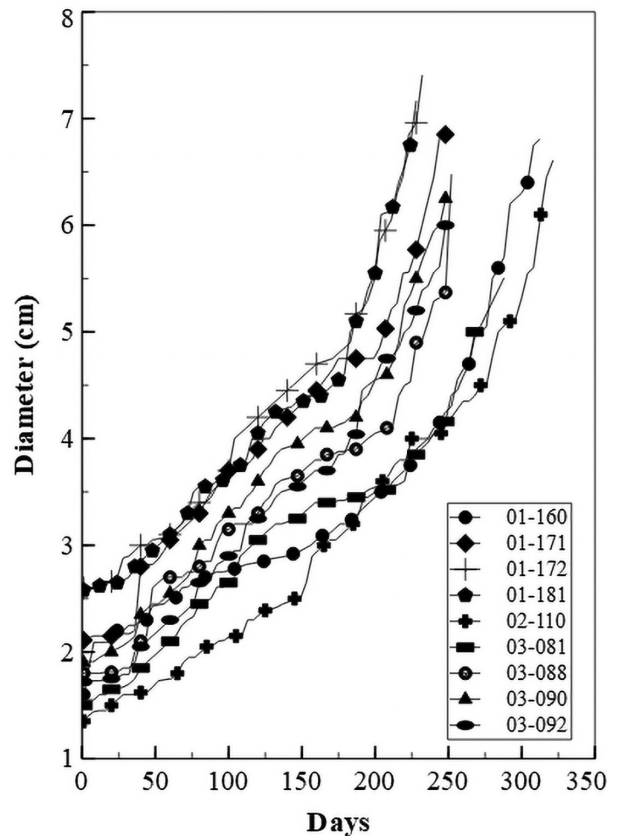


Figure 2. Growth curve of nine *R. consueloae* buds that completed flower development stage, monitored from post-emergence to anthesis phase. Legend shows bud code with the first two numbers indicating site (*i.e.* 01, 02, 03) and the last three numbers indicating bud number (*i.e.* 160, 171, 172).

records of the complete flower development (Figure 2), the overall growth rate of the diameter was 1.80% every 4 d. Furthermore, the study recorded bud mortality of 77.34% of the total population, at different stages before anthesis. The highest mortality occurred mostly during the bract phase (43.18%), followed by the post-emergence (36.11%), and least during the perigone phase (9.60%). Bud mortality during post-emergence appeared to be moderately correlated with minimum temperature ($r_t = 0.406$, $p < 0.01$) and weakly correlated with maximum temperature ($r_t = 0.372$, $p < 0.05$) and monthly average rainfall ($r_t = 0.297$, $p < 0.05$) (see Appendix II for statistical analysis). Such a relationship to environmental factors is not present in the bract and perigone phases.

Similar patterns in the frequency of flowering in 2014 and 2015 suggested a seasonality of the blooming of flowers. The peak of blooming happened between the months of December and April (Figure 3) when rainfall was low (distinct dry season from November–April). In addition, the temperature within these dry months was

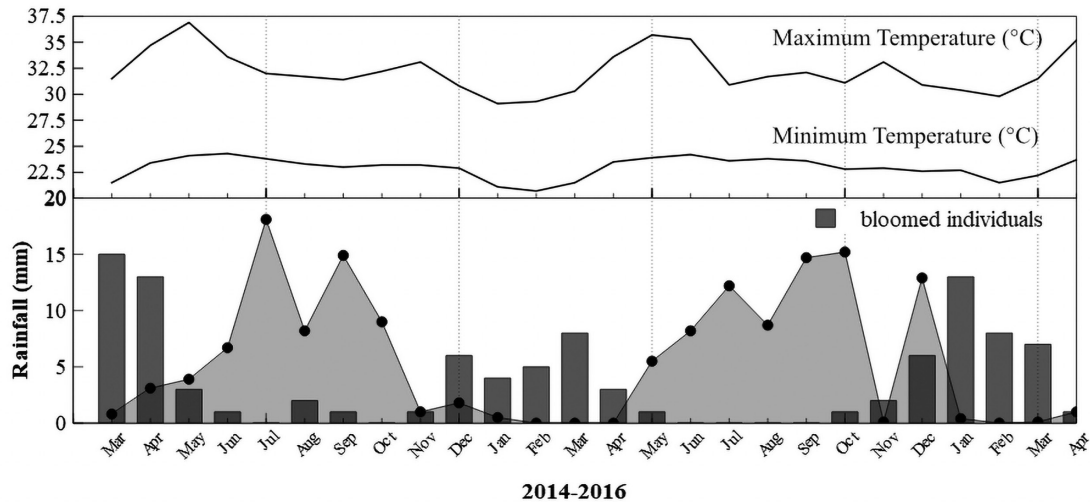


Figure 3. Rainfall and temperature data from the weather station of PAGASA located at Science City of Muñoz, Province of Nueva Ecija, for the period 2014–2016, in relation to the number of *Rafflesia consueloae* flowers recorded during the study period.

correspondingly low. Blooming of *R. consueloae* buds appeared to have a strong negative correlation with rainfall ($r_t = -0.505, p < 0.001$) and moderate negative correlation with average minimum temperature ($r_t = -0.487, p < 0.001$), suggesting that blooming events occur mostly during the driest and coldest periods. High rainfall data was recorded in December 2015 due to severe weather events such as Typhoon Nona (international name: Melor) and Tropical Depression Onyok that entered the Philippines; the latter traversed the central region of the country and brought torrential rains to the study sites.

Fruit Development

Senescence of flowers began 12–24 h after full bloom and within 5 d, the flower tissue turned completely dark (Figures 1I and J). The formation of a seed-bearing fruit took place after the flower completed senescence. At this stage, its lobes deteriorated with only the lower half of the flower remaining, which included the central column and the disk (Figure 1K). As it is uncertain whether a flower will develop into a fruit, all female flowers were assumed as entering the fruit development stage at once after senescence as long as there were no signs of decay and death of the fruiting body. From anthesis, six fruits matured with an average of 145 ± 11.03 d. Fruit maturation was characterized by cracking of the exocarp in designated grooves, revealing the white endocarp and minute, brown seeds (Figure 1L). In contrast to the faint rotten meat smell of the *R. consueloae* flower, the mature fruit smelled like young coconut meat. Matured fruit diameters are smaller than the flower’s bloom diameter. It is also notable that immature fruit diameter, observed after natural shedding of lobes, was smaller compared to diameter upon bloom. After shrinking a few centimeters, the diameter was

observed to increase until the fruit finally cracked.

Based on the two-year monitoring data collected throughout this study, we present a summary of what is currently known about the life history of *R. consueloae* (Figure 4), including the parts that remain unanswered.

DISCUSSION

Flower Development

The growth stages of *Rafflesia* prior to emerging from the host has been documented by Nikolov and co-authors (2014), and identifying the growth stages of *Rafflesia* after emergence from the host will contribute to the continuity of knowledge in *Rafflesia* life history. To date, there are very few studies documenting the growth and development of *Rafflesia*. Some studies on *Rafflesia* development assigned the stages according to bud diameter (Nais 2001; Mursidawati et al. 2014). Grouping according to size is the most convenient and least costly in terms of effort. Other authors such as Sofiyanti and co-authors (2007), Hidayati and co-authors (2000), and Susatya (2020) classified the developmental stages according to morphology and supplied corresponding diameter measurements for each stage. In this study, the buds of *R. consueloae* did not show definitive size ranges and, thus, did not allow specific grouping based on diameter only. This is due to distinct morphological changes in the buds which are independent of size. Hence, flower development was categorized based on the morphological characteristics of each bud to emphasize the transitional nature of the development from one stage to another from the emergence up to the anthesis phase (Table 1).

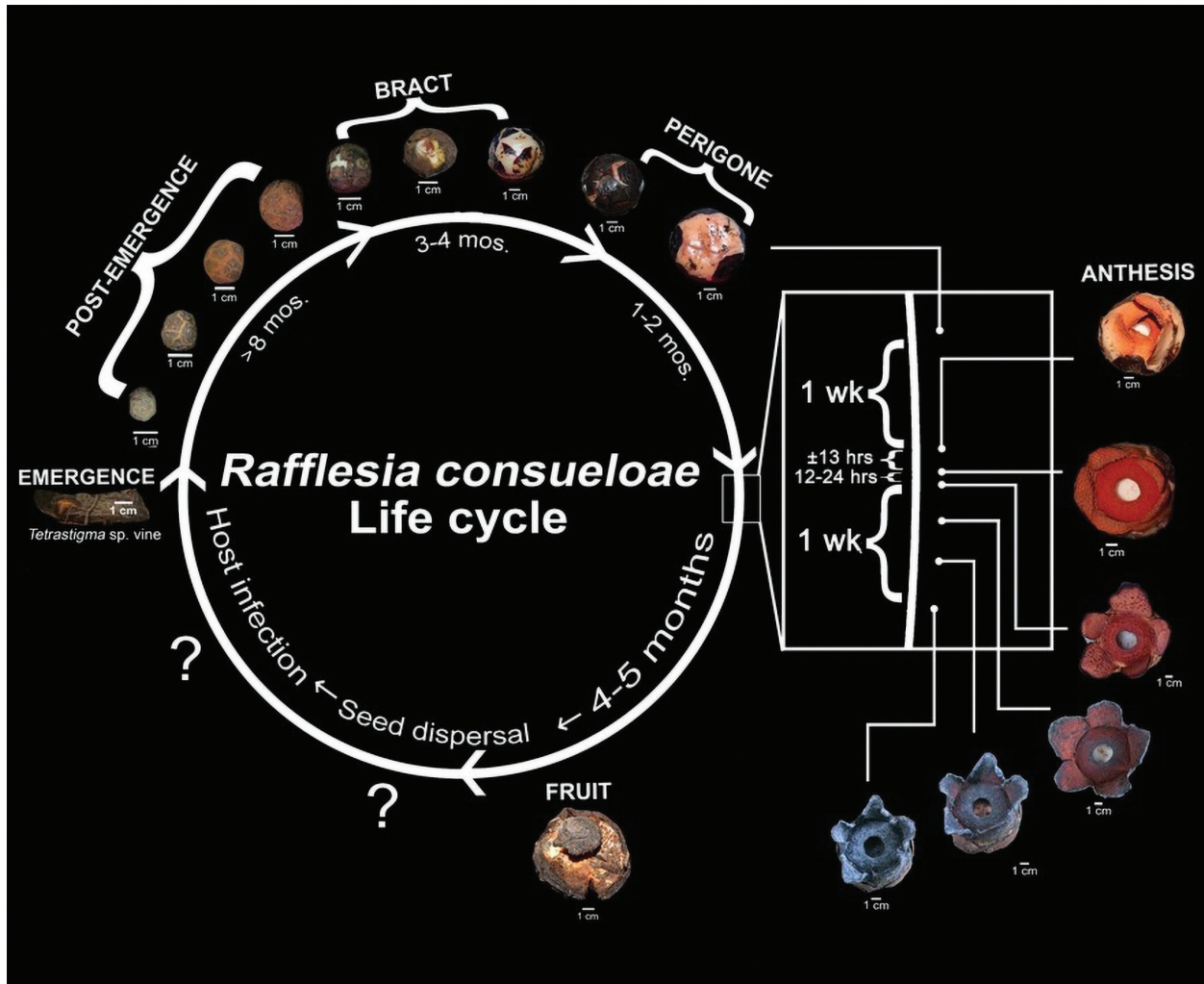


Figure 4. Life cycle of *Rafflesia consueloae*. The mechanism for seed dispersal and how the seeds infect the host plant remains a mystery. Layout by Josemaria Fuentes.

The emergence phase was observed only once in this study. This is mostly due to the difficulty in spotting the emergent bud firstly due to its size, and secondly due to its unrecognizable appearance as it is still not very distinct from the *Tetrastigma* bark, particularly as the root was usually covered with dust and dirt or was buried. In fact, this phase was only recorded once, during the discovery of Site 3 when the infected *Tetrastigma* was momentarily exposed and brushed in the process of searching for buds. The emergence phase has also not been reported in any other life-history studies in *Rafflesia* as most research works started the classification in what seems to be equivalent to the post-emergence phase. For example, the life history of *Rafflesia patma* has been studied by Hidayati and co-authors (2000) and they characterized the first of the four stages as a visible swelling under the *Tetrastigma* bark. Similarly, Mursidawati and co-authors (2014), although not stated in the text, showed a bud still

enclosed in a carapace-like structure in a photo of the T1 (first stage) of *R. patma* development. Susatya (2020) started the classification of the visible flower development stages of *Rafflesia* with the cupule stage, which is similar to the onset of the bract phase in *R. consueloae*. Our study is the first to mention and provide measurements as well as photographic evidence of an emergent *Rafflesia* bud (Figure 1A), which grew very slowly (from 0.9–1.47 cm in diameter) but eventually decayed.

Developmental stages assigned by Hidayati and co-authors (2000) on *R. patma* is the closest to the phases proposed in this study, except that the emergence phase was not mentioned. *R. patma*'s Stage 1 is similar to the post-emergence phase and these two stages surprisingly have almost similar ranges (0.69–3.85 cm for *R. consueloae* and 2–4 cm for *R. patma*) regardless of a big difference in average bloom diameter – 6.4–9.9 cm in *R. consueloae* (Galindon et al. 2016) and 40–52 cm in *R. patma* (Hidayati

et al. 2000). Stage 2 for *R. patma* was also similar with the bract phase. A noticeable difference however is that *R. consueloae* may enter this phase at a smaller size (1.75–3.9 cm) compared to *R. patma* (ca 4 cm). Sofiyanti and co-authors (2007) also mentioned a similar stage in *R. hasseltii* which was called the “cupula” stage. The 3rd stage reported by Hidayati and co-authors (2000) is also similar to the perigone phase. However, in *R. patma*, brown-colored bracts detach first to show the perigone. In *R. consueloae*, the lobes push the bracts sideward, which detach during the anthesis phase, similar to what was reported in *R. hasseltii* (Sofiyanti *et al.* 2007). Again, *R. consueloae* entered these stages in smaller diameter sizes compared to *R. patma*. Susatya (2020) observed morphological changes in *R. arnoldii* using vertical photographs of the buds and proposed six visible stages – including cupule, cupule-bract transition (CBT), bract, bract-perigone transition (BPT), perigone, and anthesis. These stages were all observed in *R. consueloae*; however, the cupule, CBT, and bract stages are compressed to the bract phase while the BPT and perigone stages are compressed to the perigone phase. This was done with the aim of establishing uniformity in determining the phases in *Rafflesia* development and using the “first appearance” of the bracts and perigone as a marker for entering the next phase. Moreover, the classification of the growth stages in Susatya (2020) was done using the side profile of the buds through vertical photographs while in *R. consueloae*, visual characterization was done from the top view, primarily because this is the most convenient angle in monitoring the small buds.

The growth rate of *R. consueloae* is exponential (Figure 2), similar to the growth curves exhibited by *R. keithii*, *R. pricei*, *R. tengku-adlinii* (Nais 1997, 2001), *R. patma* (Hidayati *et al.* 2000), the unidentified *Rafflesia* studied in Naha Jaley in Malaysia (Diway 2016), and *R. arnoldii* (Susatya 2020). The growth rate is fastest during the perigone phase and slowest during the bract phase, although the latter is not largely different from that of the post-emergence phase. The rapid growth in large buds of *Rafflesia* was also reported in *R. arnoldii* (Justesen 1922; Susatya 2020), *R. patma* (Hidayati *et al.* 2000), *R. keithii*, *R. pricei*, and *R. tengku-adlinii* (Nais 1997, 2001). Nikolov *et al.* (2014) reported bigger nuclei in *Rafflesia* cells relative to the surrounding host cells, which could indicate a massive genome that could result in a slower cell cycle brought about by genome rearrangements during mitosis. This could be another reason for the low growth rate in the earlier phases of *R. consueloae*. There were also some instances that the post-emergent buds of *R. consueloae* appeared to undergo a period of little or no growth, similar to that observed by Nais (2001). These dormant buds remained at the same size for some time but could not be considered dead since no signs

of decay were observed in the duration of the study. Monitoring of these buds presumed to be in the “lag stage” continued after the study period and was found to eventually decay with no recorded increase in size. This indicates that the “lag buds” might have already been dead for some time, but the decay of the outer carapace was not immediately evident.

Interestingly, the phases with low growth rates (post-emergence and bract) also have high mortality rates. The bract phase seems to be a crucial stage for bud survival, wherein beyond this phase the bud grew rapidly and usually proceeded to anthesis. High bud mortality has also been reported in other species of *Rafflesia* (Justesen 1922; Meijer 1958; Nais 1997; Hidayati *et al.* 2000; Yahya *et al.* 2010). Moreover, high mortality in smaller buds in *R. arnoldii* (cupule, CBT, and bract stage) and *R. bengkuenuensis* and low mortality in bigger buds were also observed by Susatya and co-authors (2017) and Susatya (2020).

Most *R. consueloae* buds decayed or aborted without any apparent reason. In other studies, insect larvae infestation on the perigone lobes of unopened buds was documented as a major cause of mortality (Nais 2001; Yahya *et al.* 2010), but this was not the case for *R. consueloae*. Although there are some buds that might have died due to herbivory, many buds that also had signs of herbivory were able to continue until anthesis. Animal predation was also a major cause of mortality in other *Rafflesia* species (Nais 1997; Hidayati *et al.* 2000) but was rarely observed in *R. consueloae*. Interestingly, *post-emergence* buds of *R. consueloae* seemed to be vulnerable during periods of higher minimum temperature, although the correlation was not strong, suggesting that other factors are driving bud death at this phase. Abraham and co-authors (2004) reported that bud mortality for *R. manillana sensu R. lagascae* (Pelser *et al.* 2013) occurred during the wet season between June and September. In *R. consueloae*, rainfall and minimum temperature appear to be factors that are possibly affecting death in the post-emergence buds but not on the bract and perigone phase buds.

A plausible explanation for the abortion of buds in the bract phase is nutrient limitation. *Rafflesia* species are completely dependent on their *Tetrastigma* hosts. However, the host has finite amounts of nutrients. In the same state, the *Tetrastigma* fitness during certain periods of the season could be varied from time to time, and when the host fitness is low but the buds are at the peak of their prime, the available nutrients inside the host plant could be more scarce than if the host is at prime. Growth was slow in the earlier phases (post-emergence and bract phases), probably due to a high number of buds that sequester the nutrients from the host (see Appendix III for the map of bud distribution). It appears as though the population undergoes survival of the fittest, and the fittest buds are

the only ones that could transition to the perigone phase. This idea makes sense, especially that the perigone phase shows the highest growth rate, and this is probably because a lot of its competitors for nutrients were already gone. Bud mortality is a form of natural population control, and death at the transition stage can amount to up to 70% of the population (Nais 1997).

Phenology

Plants respond to the changing seasons to initiate developmental programs precisely at particular times of the year (Janzen 1967), and flowering is best characterized by these seasonal responses. Selection on the timing of flowering is influenced by many different factors – including favorable temperatures, availability of water, light, and other resources. The optimal flowering time should often balance the need for a sufficiently long time for fruit and seed development against the risk of damage such as droughts (Rathcke and Lacey 1985). Flowering of *R. consueloae* appeared to occur seasonally (Figure 3). Several studies have shown how rainfall patterns and other climatic conditions greatly influence the seasonal patterns of growth and flowering for some species in tropical environments (Medway 1972; Reich and Borchert 1982; Sakai 2001). In this study, consistent timing of the flowering of *R. consueloae* coinciding with the coldest and driest months of the year is a strong indication that its flowering phenomenon could be triggered by climatic conditions and, probably, by other appropriate environmental cues.

The seasonality of other Philippine *Rafflesia* was also noted in other species. *Rafflesia baletei* had flowering peaks between December and May (Barcelona *et al.* 2009a, b). Meijer and Elliot (1990) reported *R. kerrii* flowering in Southern Thailand to be seasonal and also in the driest time of the year but in contrast to *R. consueloae*, it is in synchrony with the hottest period (January to March and May extended until June). *R. manillana sensu R. lagascae* (Pelser *et al.* 2013) was reported to have flowers throughout the year with a peak during the dry month of March (Abraham *et al.* 2004). *R. mira*, found in Mindanao, may also be considered seasonal since it was observed that flowers were recorded only between August and November (Barcelona *et al.* 2009a). On the other hand, *R. verrucosa* – also found in Mindanao – had peaks of flowering between October and December but continued intermittently throughout the whole year (Balet *et al.* 2010). *R. lobata* in Panay was also reported to have flowers the whole year, peaking only between February and June (Barcelona *et al.* 2009a). Knowledge on the timing of *Rafflesia* flowering will enable scientists and tourists to schedule their visits to coincide with the flowering season.

In several species, flower opening is independent of specific external regulation as it occurs at any time of the day, while some species showed otherwise. Blooming of *R. consueloae* generally occurred at night time, thus revealing nocturnal anthesis. This is correlated with a decrease in temperature and light intensity, and an increase in relative humidity. Experimental manipulation of flowering time can be done to further validate this hypothesis. Anthesis of *Rhizanthus zipellii* (Rafflesiaceae) was also observed to occur at night (Banzinger 1995), wherein buds were found flowering in the morning after still being closed in the previous afternoon. In *R. consueloae*, the first sign of lobe detachment was observed mostly at dusk or during early in the evening. By daylight, the lobes were upright. In several instances, buds were unable to complete their unfolding by daylight. These buds would then repose or undergo incomplete inactivity during daytime but resumed immediately at night time.

Flowers of *R. consueloae* have a very short life span. Pollinators such as blowflies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae) were attracted possibly due to the flower's smell (reminiscent of rotting meat), making *R. consueloae* another sapromyophilous species. The pollinators play a very critical role in the transfer of pollen from the anther (male flower) to the stigma (female flower) because of the short flower life span and the rarity of having a male flower and female flower at the same time. Nocturnal flowering of *R. consueloae* can also be explained as its strategy to immediately attract flies as soon as it is already in full display at daylight.

Fruit Development

Usually, after a week – once the whole flower dried up – fruit development commenced immediately. Normally, fruits of *Rafflesia* take 6–8 mo to mature (Bouman and Meijer 1994; Meijer 1997; Nais 1997; Hidayati *et al.* 2000). For the small-sized *R. consueloae*, it took only 4–5 mo. *R. manillana*, which was reassigned to *R. lagascae* by Pelser and co-authors (2013) was studied by Yahya and co-authors (2010), and revealed that *R. lagascae* flowers and fruits were produced from March–July (5-mo period), although no evidence of mature or ripe fruits was provided. Fruit maturation is characterized by cracking of the exocarp, thereby revealing the white endocarp and the brown seeds. All six fruits of *R. consueloae* monitored across the three sites matured in the month of July, although maturation was not only documented in July but in October as well in another site not included in this study. Other studies reported fruit discovery of *Rafflesia* during the months of August (Emmons *et al.* 1991) and November (Pelser *et al.* 2013).

The fruit of *R. consueloae* was a round/oval structure with black and hardened exocarp. The outer integument is woody and dry with distinct grooves and minute crevices. The fruit develops from a single inferior ovary. The *Rafflesia* fruit was considered a berry (Emmons *et al.* 1991) probably because of the fleshy endocarp and numerous seeds. However, it was observed that the *R. consueloae* fruit was dehiscent upon maturity. The case of the dehiscent berry was anomalous but had been reported in some cases, such as in *Ligustrum sempervirens* (Oleaceae) found in southwest China. In *L. sempervirens*, the fruits start off as a normal, fleshy, bluish-black berry, which gradually loses the fleshiness and become dry, eventually dehiscent (Lawrence and Green 1993). *R. consueloae* fruits, on the other hand, did not undergo drying of the endocarp. Instead, the fruit exposed the fleshy endocarp and the seeds upon dehiscence. Cracking of the exocarp occurred in the grooves and crevices noticeable during the immature fruit stage. The aroma of the mature fruit is reminiscent of freshly opened young coconut meat, which could probably be a mechanism to attract possible seed dispersers such as ants (Pelser *et al.* 2013) and small mammals (Emmons *et al.* 1991; Nais 1997; Hidayati *et al.* 2000). This would make *Rafflesia* another anomalous berry wherein the exocarp is hard (tougher than a pepo) and is dehiscent without drying of the endocarp.

Life History

Based on the results of the study, it is estimated that it would take roughly a year for the *R. consueloae* buds to develop until anthesis once a visible swelling in the root and stem of the host liana is observed. In the flower development stage, since the period from emergence to post-emergence was not measured, our results can only provide an accurate duration from the time of entering the bract phase until blooming, which took 4–6 mo. Cracking of the *Tetrastigma* bark upon entering the bract phase is a good starting point in counting the days until anthesis, as the transition is very obvious. A summary of the life cycle of *R. consueloae* showing a timeline of its major life-history events is presented in this study, with the knowledge on its seed dispersal and how new hosts are infected still remaining a mystery.

ACKNOWLEDGMENTS

Permission to collect samples of the *Rafflesia consueloae* for scientific research was covered by Gratuitous Permit Nos. III-2013-02 and III-2014-02 issued by the Department of Environment and Natural Resources – Region 03. We gratefully thank the First Gen Hydro Corporation (FGHPC) for providing resources and logistical support, the Protected Area Management Board that oversees the Pantabangan-

Carranglan Watershed and Forest Reserve, and the National Irrigation Administration – Pantabangan for access to the sites to undertake this study, specifically Federico R. Lopez, Francis Giles B. Puno, Ernesto B. Pantangco, Dennis P. Gonzales, Maria Christine T Mapanao, Janice O. Dugan, and Jose E. Jamito; Richard B. Tantoco and Agnes c. de Jesus of the Energy Development Corporation; Claire Ann G. Elmido, Jaime M. Mangalindan, Bobby Cabalic, Jose Maria Fuentes, DSRF and FGHPC staff, and local field guides for assistance and other logistic support.

DATA AVAILABILITY STATEMENT

The data used in this study will be archived at the Dryad Digital Repository.

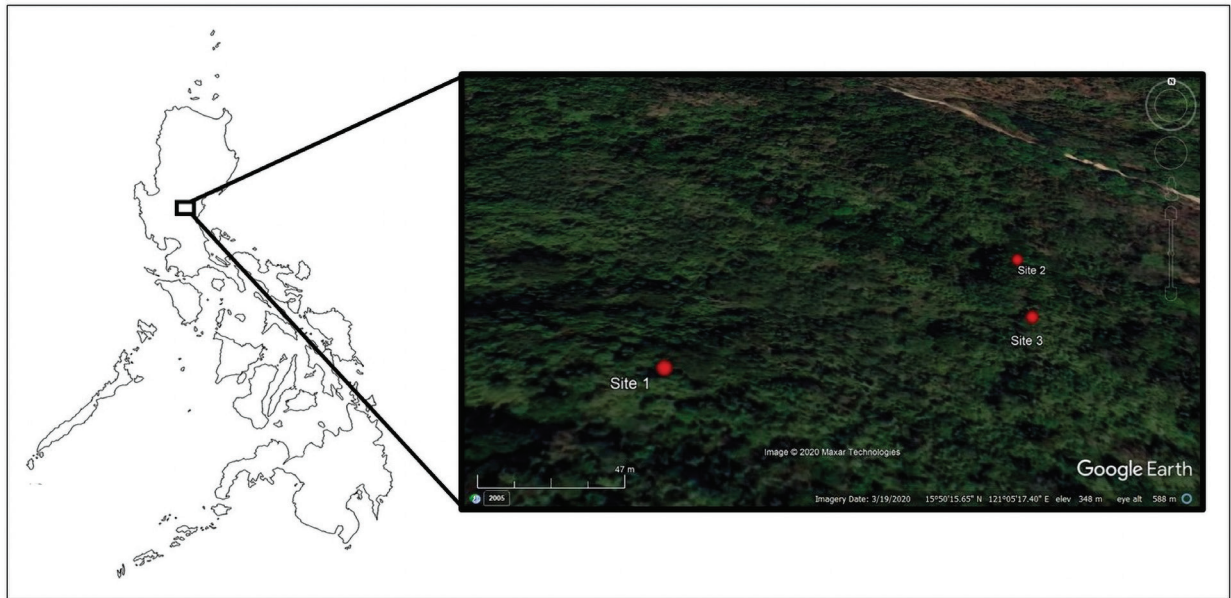
REFERENCES

- ABRAHAM ERG, BREVA RV, FERNANDO ES. 2004. Distribution and flowering of *Rafflesia manillana* Teschem. on Mt. Makiling, Luzon Island, Philippines. Proceedings of International Flora Malesiana Symposium, 20–24 Sep 2004, Los Banos, Laguna, Philippines. Retrieved from <http://agris.fao.org/agris-search/search.do?recordID=PH2005000383>
- BARKMAN TJ, KLOOSTER MR, GADDIS KD, FRANZONE B, CALHOUN S, MANICKAM S, VESSABUTR S, SASIRAT S, DAVIS CC. 2017. Reading between the vines: Hosts as islands for extreme holoparasitic plants. *American Journal of Botany* 104: 1–8. doi: 10.3732/ajb.1700117
- BALETE DS, PELSER PB, NICKRENT DL, BARCELONA JF. 2010. *Rafflesia verrucosa* (Rafflesiaceae), a new species of small-flowered *Rafflesia* from eastern Mindanao, Philippines. *Phytotaxa* 10: 49–57. doi: 10.11646/phytotaxa.10.1.8
- BANZINGER H. 1995. Ecological, morphological and taxonomic studies on Thailand's fifth species of Rafflesiaceae: *Rhizanthus zippelii* (Blume) Spach. *Natural History Bulletin of the Siam Society* 43: 337–365.
- BARCELONA JF, PELSER PB, BALETE DS, CO LL. 2009a. Taxonomy, ecology, and conservation status of Philippine *Rafflesia*. *Blumea* 54: 77–93. doi:10.3767/000651909x474122
- BARCELONA JF, CO LL, BALETE DS, BARTOLOME NA. 2009b. *Rafflesia aurantia* (Rafflesiaceae): a new species from northern Luzon, Philippines. *Gardens' Bulletin Singapore* 61: 17–27. Retrieved from <http://biostor.org/reference/146700>

- BOUMAN F, MEIJER W. 1994. Comparative structure of ovules and seeds in Rafflesiaceae. *Plant Systematics and Evolution* 193: 187–212. doi: 10.1007/BF00983550
- DIWAY BM. 2016. Reproductive ecology of the world's biggest flower – towards *in situ* and *ex situ* conservation. Annual Report of Pro Natura Foundation Japan 23: 165–176.
- EMMONS LH, NAIS J, BRIUN A. 1991. The fruit and consumers of *Rafflesia kethii* (Rafflesiaceae). *Biotropica* 23: 197–199. doi: 10.2307/2388307
- GALINDON JMM, ONG PS, FERNANDO ES. 2016. *Rafflesia consueloae* (Rafflesiaceae), the smallest among giants; a new species from Luzon island, Philippines. *Phytokeys* 61: 37–46. doi:10.3897/phytokeys.61.7295
- HIDAYATI SN, MEIJER W, BASKIN JM, WALCK JL. 2000. A contribution to the life history of the rare Indonesian holoparasite *Rafflesia patma* (Rafflesiaceae). *Biotropica* 32: 408–414. doi: 10.1111/j.1744-7429.2000.tb00487.x
- JANZEN DH. 1967. Synchronization of sexual reproduction of trees within dry season in Central America. *Evolution* 21: 620–637. doi: 10.2307/2406621
- JUSTESEN PT. 1922. Morphological and biological notes on *Rafflesia* flowers, observed in the highlands of Mid-Sumatra (Padangsche Bovenlanden). *Annales du Jardin Botanique de Buitenzorg* 32: 64–87.
- LAWRENCE TJ, GREEN PS. 1993. The anatomy of a dehiscent berry. *Kew Bulletin* 48: 53–57. Retrieved from <http://www.jstor.org/stable/4115747>
- MEDWAY L. 1972. Phenology of a tropical rain forest in Malaya. *Biological Journal of the Linnean Society* 4: 117–146. doi:10.1111/j.1095-8312.1972.tb00692.x
- MEIJER W. 1958. A contribution to the taxonomy and biology of *Rafflesia arnoldii* in West Sumatra. *Annales Bogoriensis* 3: 33–44.
- MEIJER W. 1997. Rafflesiaceae. *Flora Malesiana* (Ser. 1) 13: 1–42. Retrieved from <http://www.repository.naturalis.nl/document/570368>
- MEIJER W, ELLIOT S. 1990. Taxonomy, ecology and conservation of *Rafflesia kerrii* Meijer in southern Thailand. *Natural History Bulletin of the Siam Society* 38: 117–113. Retrieved from: http://www.siamese-heritage.org/nhbsspdf/vol031-040/NHBSS_038_2f_Meijer_TaxonomyEcologyAnd.pdf
- MURSIDAWATI S, WICAKSONO A, TEIXEIRA DA SILVA JA. 2019. Development of the endophytic parasite, *Rafflesia patma* Blume, among host plant (*Tetrastigma leucostaphylum* (Dennst.) Alston) vascular cambium tissue. *South African Journal of Botany* 123: 382–386.
- MURSIDAWATI S, IRAWATI, NGATARI. 2014. *Rafflesia patma* (Rafflesiaceae): Notes on its field study, cultivation, seed germination, and anatomy. *Buletin Kebun Raya* 17: 9–14. Retrieved from http://jurnal2.krbogor.lipi.go.id/index.php/buletin/article/view/17_1_2/96
- NAIS J. 1997. Distribution, reproductive ecology, and conservation of *Rafflesia* in Sabah. Proquest LLC, Ann Arbor, MI.
- NAIS J. 2001. *Rafflesia* of the World. Sabah Parks, Kota Kinabalu, Malaysia.
- NIKOLOV LA, TOMLINSON PB, MANICKAM S, ENDRESS PK, KRAMER EM, DAVIS CC. 2014. Holoparasitic Rafflesiaceae possess the most reduced endophytes and yet give rise to the world's largest flowers. *Annals of Botany* 114: 233–242.
- NIKOLOV LA, DAVIS CC. 2017. The big, the bad, and the beautiful: biology of the world's largest flowers. *Journal of Systematics and Evolution* 55: 516–524. doi: 10.1111/jse.12260
- PELSER P, NICKRENT DL, CALLADO JRC, BARCELONA JF. 2013. Mt Banahaw reveals: the resurrection and neotypification of the name *Rafflesia lagascae* (Rafflesiaceae) and clues to the dispersal of *Rafflesia* seeds. *Phytotaxa* 1311: 35–40. doi: 10.11646/phytotaxa.131.1.6
- RATHCKE B, LACEY EP. 1985. Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics* 16: 179–214. doi: 10.1146/annurev.es.16.110185.001143
- REICH PB, BORCHERT R. 1982. Phenology and ecophysiology of the tropical tree *Tabebuia neochrysantha* (Bignoniaceae). *Ecology* 63: 294–299. doi: 10.2307/1938945
- SAKAI S. 2001. Phenological diversity in tropical forests. *Population Ecology* 43: 77–86. doi:10.1007/PL00012018
- SOFIYANTI N, MAT-SALLEH K, PURWANTO D, SYAHPUTRA E. 2007. The note on morphology of *Rafflesia hasseltii* Surigar from Bukit Tiga Puluh National Park, Riau. *Biodiversitas* 9: 257–261. doi: 10.13057/biodiv/d080402
- SUSATYA A, PRANDEKA F, SAPRINURDIN, RAHMAN N. 2017. Population attributes of the very rare *Rafflesia bengkulensis* at Kaur Regency, Southern Bengkulu. *Buletin Kebun Raya* 20: 67–73.

- SUSATYA A. 2020. The growth of flower bud, life history, and population structure of *Rafflesia arnoldii* (Rafflesiaceae) in Bengkulu, Sumatra, Indonesia. Biodiversitas 21: 792-798
- WICAKSONO A, TEIXEIRA DA SILVA JA, MURSIDAWATI S. 2017. Dispersal of *Rafflesia patma* Blume endophyte in grafted host plant (*Tetrastigma leucostaphylum* (Dennst.) Alston). J Plant Develop. 24: 145–150.
- YAHYA A, HYUN JO, LEE JH, CHOI TB, SUN BY, LAPITAN PG. 2010. Distribution pattern, reproductive biology, cytotaxonomic study and conservation of *Rafflesia manillana* in Mt. Makiling, Laguna, Philippines. Journal of Tropical Forest Science 22: 118–126. Retrieved from <http://agris.upm.edu.my:8080/dspace/handle/0/10578>

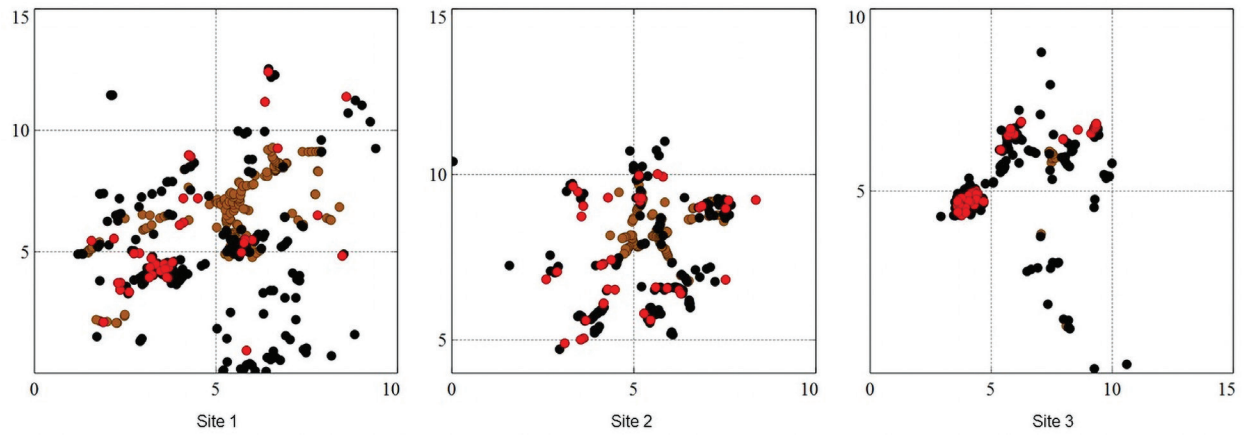
APPENDICES



Appendix I. Map of the study sites in Mt. Balukbok, Pantabangan, Nueva Ecija, Philippines.

Appendix II. Results for Kendall’s rank correlation test between mortality occurring in different phases and the environmental factors rainfall and temperature.

Average monthly mortality	Factor (monthly average values)	<i>z</i>	<i>p</i> -value	<i>tau</i>
Post-emergence	Rainfall (mm)	2.0329	0.0421	0.2965
	Maximum temperature (°C)	2.5650	0.0103	0.3724
	Minimum temperature (°C)	2.7898	0.0053	0.4061
Bract phase	Rainfall (mm)	0.9569	0.3386	0.1385
	Maximum temperature (°C)	-0.2222	0.8242	-0.0320
	Minimum temperature (°C)	-0.8893	0.3738	-0.1284
Perigone phase	Rainfall (mm)	-1.8217	0.0685	-0.2845
	Maximum temperature (°C)	1.2855	0.1986	0.1998
	Minimum temperature (°C)	-0.2912	0.7709	-0.0454
Anthesis phase	Rainfall (mm)	-3.4332	0.0006	-0.5054
	Maximum temperature (°C)	-1.6355	0.1019	-0.2396
	Minimum temperature (°C)	-3.3179	0.0009	-0.4874



Appendix III. X and Y plot of all buds from Sites 1–3. Color-coded dots represent the buds, where black represents monitored buds, red represents bloomed buds, and brown represents remnants. Monitored and bloomed buds were documented in this study while the remnants were counted prior to the start of the study. The 10 x 15 grid is in m.