FIELD NOTE

New locality and bud growth of the world biggest flower, Rafflesia tuan-mudae, in Naha Jaley, Sarawak, Malaysia

Bibian Diway1*, Yasuo Yasui2, Hideki Innan3 and Yayoi Takeuchi3, 4

1 Research Development and Innovation Division, Forest Department Sarawak, Km10 Jalan Datuk Amar Kalong Ningkan, 93200 Kuching, Sarawak, Malaysia
2 Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyou-ku, Kyoto 606–8502, Japan
3 School of Advanced Sciences, Graduate University for Advanced Studies, Kamiyamaguchi 1560–39, Hayama, Kanagawa 240–0193, Japan
4 Biodiversity Division, National Institute for Environmental Studies, 16–2 Onogawa, Tsukuba, Ibaraki 305–8506 Japan

* Corresponding author: bibianm@sarawak.gov.my

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ABSTRACT

A new Rafflesia population was found in Naha Jaley, Sarawak, in 2012. This study aimed to identify this Rafflesia species and investigate its bud growth. First, we described the flower characteristics and compared them with Rafflesia keithii and R. tuan-mudae, which are candidate species for the flower. Also, we investigated the phylogenetic position of this Rafflesia within Rafflesiaceae using DNA analysis. To estimate bud growth curve, we observed bud development from April 2013 to November 2013 in the field. Based on morphological comparisons and phylogenetic analysis, we confirmed the newly discovered population of the Rafflesia at Naha Jaley was R. tuan-mudae, which is the new locality of the species at the most Eastern side of the known distribution for this species. The results indicated that absolute growth rate was greater in larger buds. We also estimated that it took a year to bloom from the initial bud stage. The mortality in one of our sites was very high, with more than 80% of buds dead prior to flower opening. This flower would be vulnerable to extinction due to their extraordinary characteristics and anthropogenetic effects. Finally, we discussed how Rafflesia populations in Naha Jaley can be conserved both in-situ and ex-situ.

Key words: discovery, bud development, phylogenetic analysis, morphological comparison, Rafflesia keithii

INTRODUCTION

Rafflesia is an extraordinary plant in terms of its gigantic flower and holoparasitic relationship with its host plant, the Tetrastigma veins (Vitaceae). Rafflesia has no leaves, stem and roots and completely depends on the host for water, nutrients, and survival. Rafflesia comprises 37 species and is distributed only in tropical forests in the South East Asia region (Mat Salleh 1991, Nais 2001, Susatya et al. 2006, Adam et al. 2016, Susatya et al. 2017). In Borneo, a total of nine Rafflesia species are known (Adam et al. 2016), while only four species are known in Sarawak (Fig. 1a), namely Rafflesia tuan-mudae, R. precei, R. hasseltii, and R. keithii. First, R. tuan-mudae is found only in West Sarawak, including the Gunung Gading and Gunung Pueh areas in the Lundu District, Kampung (Kpg.) Timurang in Pedawan District, Kpg. Remun in Serian District, and Lanjak Entimau Wildlife Sanctuary (Nais 2001, Mat Salleh et al. 2006). It is also possibly found in Cagar Alam Gunung Raya Pasi near Singkawang, around Pontianak, and the upper Kayan River in Kalimantan. As bloomed flowers of R. tuan-mudae sometimes reach more than 50 cm diameter (Meijer 1997), this species is the main eco-tour target in Gunung Gading National Park and Kpg. Timurang. Other species are R. precei from Gunung Murud (Nais 2001, Beaman et al. 1988) and the recently discovered R. hasseltii from Tanjung Datu National Park (Ahmad Puad 2020). Lastly, R. keithii, which is mainly distributed in Sabah, was reported in Hose Mountains, by Nais (2001), albeit its locality description was vague, and no other records have since been reported.

All Rafflesia are totally protected plants in Sarawak under the Sarawak’s Wild Life Protection Ordinance (SWLPO 1998). However, both climatic and anthropogenic factors, such as flower collection, logging, and shifting cultivation, reduced the natural Rafflesia population (Nais and Wilcock 1998, Zuhud et al. 1999, Hikmat 2006, Suwartini et al. 2008, Mursidawati et al. 2014). For both in-situ and ex-situ conservation strategy of Rafflesia, fundamental knowledge of bud development and mortality causes are needed. However, due to its rareness, ecological information on Rafflesia, including distribution and growth
performance, are still scarce, and thus, conservation strategies for this species are difficult to implement.

Here, we report a new locality of *Rafflesia* in Naha Jaley, Belaga, Sarawak (Fig. 1, Fig. 2). In 2012, the local community of Naha Jaley discovered a *Rafflesia* population in a hill dipterocarp forest within the upper area of Bakun Hydroelectric Dam, Naha Jaley. Notably, this accidental discovery of *Rafflesia* stemmed from impoundment construction of Bakun Dam in 2010. Before this construction, the area was called Uma Balui and was occupied by more than 200 families of the Kayan ethnic group living in a village. Due to dam impoundment, many families were relocated to a new settlement area in the Asap River, Belaga. Some families instead remained and established a new village above the dam’s water level, naming the new location Naha Jaley. Before the dam impoundment, due to remoteness and distance, this new location was inaccessible. However, due to rising water levels from the dam, the area was accessible by boat, which led to a new discovery of several *Rafflesia* populations in the area. We hypothesized that this *Rafflesia* in Naha Jaley could be either *R. keithii* or *R. tuan-mudae*, which are morphologically similar; in fact, Naha Jaley is just 11 km from the Hose Mountains, which is a potential locality for *R. keithii* (Nais 2001) and 445 km from the *R. tuan-mudae* distribution area (Fig. 1a).

This study aimed to identify this *Rafflesia* species and investigate growth performance of buds within Naha Jaley populations. First, we described *Rafflesia* characteristics and compared these with *R. keithii* and *R. tuan-mudae*, the candidate *Rafflesia* species in Naha Jaley. Also, we investigated phylogenetic positions of this *Rafflesia* within Rafflesiaceae using DNA analysis. Next, we monitored bud development...
in the field and estimated bud growth curves. Former studies report animals, such as the long-tailed mountain rat *Niviventer rapit*, disturb *Rafflesia* buds, which is one of many potential mortality causes (Nais 1997). Therefore, we also conducted a camera-trap survey to identify bud predators. Finally, we discussed *in-situ* conservation, combined with local community development, as well as *ex-situ* conservation.

**MATERIALS AND METHODS**

**Study site**

Our field site was located in the upper Balui River, Naha Jaley, Belaga District, Sarawak, Malaysia (Fig. 1b). This area is covered by a hill mixed dipterocarp forest, where forests are mainly logged over and primary or pristine forests are confined on steep terrain where logging is prohibited by Sarawak’s law. The primary hill mixed dipterocarp forests were dominated by Dipterocarpaceae family members, such as *Shorea*, *Dipterocarpus*, and *Dryobalanops*, which are important commercial timber species. Also, we found dominant secondary forest species, such as *Macaranga* and *Mallotus*, especially in areas with large canopy gaps. Within village peripheries, we found patches of developed secondary forests following community driven shifting cultivation and agricultural farms. Pioneer tree species, such as *Macaranga*, *Glochidion*, and *Trema*, are most abundant in these secondary forests. The average annual rainfall was 4,556 mm during 1970–2014 in Belaga (N02°47’4.20”, altitude 762 m a.s.l.), which is located approximately 38 km from the study area.

In Naha Jaley, we found three *Rafflesia* population sites in a hill mixed dipterocarp forests (Fig. 1b). Site 1 (N2°23’22”, E113°58’50”, altitude 762 m a.s.l.) was located in a water catchment area of a logging camp, where the logging camp workers obtained water from a forest stream. Therefore, the forest was not disturbed and well conserved. Site 2 (N2°34’36.39”, E113°90’36.39”, altitude 232 m a.s.l.) was located at a Bukoh riverbank and was thus often affected by periodic flash floods. The topsoil was thin, mainly sandy, and skeletal soils. Bukoh river was often visited by the local community for recreational activities and fishing. Site 3 (N02°21’03.9”, E113°54’13.1”, altitude 252 m a.s.l.) was located near the Keboho river and adjacent to a paddy field. Site 3 lay in a valley along the seasonal stream. Physical soil examinations revealed that the topsoil was moist, dark in colour, and covered with thick humus.

Sites 2 and 3 were within the periphery of Naha Jaley villages.

**Field survey**

Our field survey in Site 1 was conducted in March 2012. We found a total of eight buds between stage 1 and 4 and collected three bud samples. In Site 2, we recorded 16 buds of stage 1 (less than 2.5 cm diameter) to stage 2 (range from 2.5–5 cm diameter) in November 2012 and also recorded their survival in November 2013. In Site 3, we monitored bud development and measured their diameters from April to October 2013 using calipers. Throughout the survey, we found nine buds in Site 3 range from stage 1 (2.5 cm diameter) to stage 3 (25 cm diameter). To describe the morphological characteristics of the flower buds, we collected five *Rafflesia* buds in Site 1, 2 and 3. Specifically, once the bud opened, we measured blooming flower diameter, diaphragm diameter, perigone lobe, anthers, and ramenta (Fig. 3). To identify predatory animals of *Rafflesia* buds, we installed a Bushnell camera trap to monitor animal movement at Site 3 from June to October, 2013.

**Growth analysis**

Using the monitoring data of bud development in Site 3 (N=6), we calculated absolute growth rate (AGR) for each observation according to Hunt (1990):

$$ AGR = \frac{(D_t - D_i)(t_f - t_i)}{t_f - t_i}, $$

where $D_t$ and $D_i$ are bud diameters measured at successive times $t_i$ and $t_f$. As we assumed that AGR would be bud size-dependent, we fitted Gompertz functions, which is one of the commonly used sigmoid models fitted to plant growth data (Paine et al. 2012). Here, the equation is described as follows:

$$ AGR = c + (d - c) \times \exp\{-\exp[b \times (D_t - e)]\}, $$

where b, c, d, and e are parameters. These four parameters were estimated using the *dmr* function in the *drc* package in R (R Core Team 2021). Using the obtained fitted model for AGR, we estimated bud growth curve and calculated the number of days to bloom from the initial bud (diameter = 0.1 cm).

**DNA extraction, sequencing, and phylogenetic analysis**

For DNA analysis to elucidate the *Rafflesia* species in Naha Jaley, we collected two *Rafflesia* samples: a bloomed
flower in Site 1 in Naha Jaley, collected in April 2012 (*Rafflesia* N. J.), and a bloomed *R. tuan-mudae*, collected at Kpg. Timurang (*R. tuan-mudae* K. T.) in April 2012 for reference. Total DNA was extracted from tissue samples using the cetyltrimethylammonium bromide (CTAB) method (Escaravage et al. 1998). Three DNA regions were selected for sequencing: that is, the atp6 and matR genes and nad1 B-C intron of the mitochondrial genome, which are commonly used in phylogenetic analysis in Rafflesiaceae (Barkman et al. 2008, Bendiksby et al. 2010, Pelser et al. 2019). Among the region, the nad1 B-C region acquired by Rafflesiaceae via horizontal transfer from Vitaceae (Davis and Wurdack 2004), this region would usefully resolve the relationships among Rafflesiaceae taxa (Pelser et al. 2019), as the horizontal gene transfer event occurred before the three Rafflesiaceae genera diverged (Barkman et al. 2008). The primer sets and polymerase chain reactions (PCR) conditions followed Barkman et al. (2008). After PCR products were purified with ExoSAP-IT (GE Healthcare), we performed cycle sequencing with BigDye Terminator v. 3.1 (Applied Biosystems, Foster City, California, USA) with the following cycles: 1 min at 96 °C; 25 cycles at 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 75 s. The sequenced samples were run in an ABI 3130 Genetic Analyzer. For phylogenetic analysis, obtained sequences were aligned using bioedit and edited sequences were deposited in DNA databank of Japan (DDBJ) (accession numbers: LC647810–LC647815). To evaluate the phylogenetic relationship among Rafflesiaceae, we also used sequences in Pelser et al. (2019). After aligning sequences in each region, we matched sequences according to the obtained region. After combining all three regions, we constructed a neighbor-joining (NJ) phylogenetic tree using MEGA X (Kumar et al. 2018) with 1,000 bootstrap replicates.

Fig. 3. Characteristics of *Rafflesia* in Naha Jaley. (a) Perigone lobes: five lobes, apex rounded, margin attire. (b) Central disk and processes: the number of processes was 35–44, apex covered with fine brittle. (c) White blots on the window. (d) Diaphragm aperture was about 12.5 cm. (e) Ramenta (also see the detail in Fig. 4). (f) The number of anthers of the male blooming flower was 40–41.
RESULTS

Morphological characteristic of the *Rafflesia* flower in Naha Jaley

To describe morphological characteristics, we observed flower buds at Site 1 and Site 3 and collected three bud samples, and a decomposed flower for anatomical observation (Table S1). We also collected an open flower from Site 2, two decomposed flowers and buds from Kpg. Timurang and Kpg. Remun in April and November 2012 for comparison with *R. tuan-mudae*, as described below.

**Perigone lobes** 5, reddish orange in colour with dense white wart, more or less of the same size, apex rounded, margin entire. **Diaphragm** pinkish orange, covered with dense white warts each surrounded by reddish brown margin. The warts were smaller than on the perigone lobe. Diaphragm aperture was 12.5 cm in diameter. **Window** exhibits concentric rings of roundish to elliptic white blots near the rim margin. **Processes** 36–44, unbranched, orange colour at the base and reddish brown toward the apex, apex conical or slightly laterally compressed, covered with fine bristles. Processes were arranged in three concentric circle.

**Ramenta** (Fig. 4): densely covered in a perigone tube and divided into lower, middle, and upper types. Lower type ramenta were solitary with apices unbranched, a slightly swollen head, and 2 mm long. Middle type ramenta were solitary with branched apices, but a frequently branched and swollen head, and 6 mm long. Upper ramenta were fascicle with apices branched and 10 mm long. **Mature male flower buds** (just before blooming, Fig. 5e): 25–30 cm in diameter. **Male flower** (Fig. 5h): 54 cm in diameter with 40–41 anthers. **Female flower**: not seen. **Host plant**: *Tetrastigma diepenhorstii* Miq.

The morphological characteristics of *Rafflesia* in Naha Jaley were summarized and compared with *R. tuan-mudae* and *R. keithii* (Table 1, Fig. 4), which are morphologically very similar. The key characteristic to identify *Rafflesia* species is the shape of the ramenta, as Meijer (1997) stated that different species exhibit different ramenta patterns. We found the ramenta shape of Naha Jaley’s *Rafflesia* was more similar to *R. tuan-mudae* (Fig. 4). We also observed a similar size of the blooming flower and diaphragm aperture to *R. tuan-mudae*, which also shared the same host plant species. Although the number of anthers was more similar to *R. keithii*, how the number of anthers varies within and

![Fig. 4. *Rafflesia* at Naha Jaley showing different types of ramenta. (a) Upper type, fascicle, apices branched, 10 mm long. (b) Middle type, solitary, apices some branched, but frequently branched and swollen head, 6 mm long. (c) Lower type, solitary, apices unbranched, some swollen head, 2 mm long. (d) Upper ramenta of *R. tuan-mudae*, fascicle, apices branched, 10 mm long.](image-url)
among species is unclear. Therefore, we concluded that the morphological characteristics were more similar to *R. tuan-mudae* than *R. keithii*.

### Phylogenetic position of *Rafflesia* in Naha Jaley

The sequences and other sequences of *Rafflesia* species were aligned and trimmed to defined start and end positions to yield fragments of the following sizes: 558 bp for atp6, 465 bp for matR, and 618 bp for nad1. As a result of the NJ tree (Fig. 6), we found a monophyly of Sundaic *Rafflesia* (75% bootstrap support). *Rafflesia N. J.* was a sister of *R. tuan-mudae K. T.* (99% bootstrap support), and the clade was nested with *R. tuan-mudae* (76% bootstrap support). Furthermore, the clade of *R. tuan-mudae* was nested with other Bornean *Rafflesia* species. Although *R. keithii* and *R. tuan-mudae* were morphologically similar, *R. keithii* was an outgroup of the *R. tuan-mudae* clade contrary to our expectation.

### Bud development and mortality

Based on field observations, we categorized six stages of bud development (Fig. 5). For stage 1, initial *Rafflesia* buds were observed as small and swollen (>2.5 cm diameter) on the inside bark of the host (*Tetrastigma diepenhorstii*). For stage 2, once the bark cracked, pinkish white cabbage-like buds emerged. In this stage, buds were protected by brown bracts. For stage 3, the brown bracts fell off, and buds proceeded to grow until the diameter reached >30 cm. For stage 4, buds matured and were ready to open. For stage 5, buds started opening. It took two days to fully bloom, and buds continued to open for 1–2 days. For stage 6, flowers started to immediately rot.

The observed AGR varied by 0.016–0.580 cm day\(^{-1}\) (Fig. 7a). The fitted Gompertz model exhibited significant parameters, where coefficients (± SD) of the model was \(b = -0.10 ± 0.04, t = 0.02; c = 0.03 ± 0.01, t = 0.08; d = 1.14 ± 0.43, t = 2.67; e = 19.70 ± 3.85, t = 5.12; p < 0.05\), which showed that AGR increased with bud size. The estimated length from initial bud to bloom was about 300–303 days.

| Table 1. Comparison of flower characteristics between *Rafflesia* at Naha Jaley, *R. tuan-mudae*, and *R. keithii*. |
|---------------------------------------------------------------|
| **Rafflesia in Naha Jaley** | **R. tuan-mudae** | **R. keithii** |
| Host plant | *Tetrastigma diepenhorstii* Miq. | *Tetrastigma rafflesiae* Planch. | *Tetrastigma leucostaphylum* (Dennst.) Ashton ex Mabb., very possibly also *T. diepenhorstii* Miq. |
| Diameter of open flower (cm) | 54 | (44-) 56 (-92) | 80–90 |
| Diameter of diaphragm aperture (cm) | 12.5 | 15–18 | NA |
| Diaphragm | The surface of diaphragm is covered with dense white warts each surrounded by reddish brown margin | NA | The surface of diaphragm is covered with dense white warts each surrounded by reddish brown margin rather dense numerous white warts, small ones interspersed with larger ones, the latter about 10–12 across the greatest breadth of the lobes |
| Perigone lobes | The numbers of white warts across the base of lobe are more than 8. | fewer blots across the perigone lobes, | |
| Ramenta structure and length branching | Upper type, is fascicle, apices branched, 10 mm long; Middle type, solitary, apices some branched but frequently branched and swollen head, 6 mm long; Lower type ramenta is solitary, apices unbranched, some swollen head, 2 mm long (Fig. 4a–c). | Upper Ramenta of *R. tuan-mudae*. fascicle, apices branched, 10 mm long. (Fig. 4d). | 5–6 mm long near the diaphragm and often fascicled (in bundles), only a few branched |
| Numbers of Anthers | 40–41 | 38 (Kmpg. Temurang, N = 1) | 40 |
| Reference | This study | Nais (2001) Meijer (1997) Zakaria et al. (2016) this study | Nais (2001) Meijer (1997) |
Rafflesia bud survival was observed at two sites, Sites 2 and 3. From a total of 12 buds monitored since November 2012 at Site 2, only two survived as of November 2013 (i.e., 83.3% of buds died). At Site 3, nine buds survived (100% survival rate was recorded) between April and November, 2013.

Camera traps showed no evidence of animals eating flower buds. Animals captured by the camera included a few protected animal roaming in the area such as a sun bear (*Helarctos malayanus*) and porcupine (*Hystrix sp.*); the sun bear and all porcupine species in Sarawak are protected under the Wild Life Protection Ordinance (WLPO 1998). We also found bearded pig (*Sus barbatus*), mouse-deer (*Tragulus javanicus*), greater mouse-deer (*Tragulus napu*), long-tailed macaque (*Macaca fascicularis*), and civet.

**DISCUSSION**

**New locality of Rafflesia tuan-mudae at Naha Jaley**

Our results of morphological comparison and phylogenetic analysis confirmed the newly discovered population of *Rafflesia* at Naha Jaley was *R. tuan-mudae*, which is the new eastern-most locality of this species within its known distribution. Nais (2001) reported occurrence and location of *R. keithii* within the Hose Mountain range adjacent to Naha Jaley. However, his flower descriptions were not clear which unable to fully identify species partly due to morphological confusion between *R. keithii* and *R. tuan-mudae*.

The *R. tuan-mudae* population in Naha Jaley remained undiscovered for long time because the area was too remote to be explored by local communities before the Bakun Dam impoundment. Recently, Ahmad Puad et al. (2020) reported the new locality of *R. hasseltii* from Tanjung Datu National Park in Sarawak, suggesting the distribution of *Rafflesia* in Sarawak may still be underestimated and more expeditions
may be needed to better understand species diversity of Rafflesia in Sarawak.

The entire Bakun area is one of the richest diversity hotspots in Sarawak. From wildlife monitoring and rescue operations conducted within the Bakun Dam reservoir area, a total of 86 animal species and 349 plant species have been recorded (Dagang et al. 2015). When the Bakun Hydroelectric Dam was impounded and reached its maximum level at 228 m a.s.l. in January 2012, a total of ~695 km$^2$ of forest area was submerged, causing massive losses to plant and animal habitats. Therefore, the Naha Jaley region is one of the remaining biodiversity hot spots in the Bakun area.

**Bud development and mortality of Rafflesia tuan-mudae at Naha Jaley**

We found size-dependent growth rate of Rafflesia in Naha Jaley; the estimated growth curve was exponential, with lower growth in initial and juvenile stages and rapid growth in mature stages. Exponential bud growth has been also reported in other Rafflesia species (Nais 1997, 2001, Hidayati et al. 2000, Susatya 2020, Tolod et al. 2021). Moreover, we estimated approximately one year from initial bud to bloom, which is consistent with a recent study by Susatya (2020) on the life history of R. arnoldii. The study showed that bud development took 1–1.5 years in the visible stage, while the whole life stage, including seed dormancy in the host bark, took 3.5–5 years. The study also reported that female flowers took longer to bloom than male flowers in R. arnoldii. Therefore, longer observation times are needed for R. tuan-mudae lifecycles to reveal the whole life cycle, including seed dispersal and incubation in the host stage when considering species characteristics and other environmental factors.

Mortality was inconsistent between Sites 2 and 3. This difference would be attributed to the physical environment where host plants and Rafflesia grow. Site 2 showed higher...
mortality rate near the riverbank with sandy and shallow topsoil, thus possibly poor nutrients, as well as frequent periodic flash floods. At Keboho river (Site 3), which exhibited lower mortality, soil was dark brown in colour and loose and thus rich in humus (B. Diway, personal observation). Furthermore, Site 2 is also frequently visited by the village locals for recreational activities whereas, Site 3 is privately owned land, so entry is restricted. Disturbance by locals can injure host plants. In a previous study on *R. precei*, Nais (1997) reported buds being eaten by long-tailed mountain rats, *Niviventer rapit*. Hidayati et al. (2000) also reported that the ~80% of flower buds die before opening, primarily due to consumption of buds by animals. However, in this study, we did not find any evidence of mammal predators in Naha Jaley. The cause of mortality also depends on life stage of buds, it might be higher mortality in early life stages of *Rafflesia* as Susatya (2020) reported high mortality in the small buds in *R. arnoldii* although our study could not examine the life stage effect on mortality due to the limitation of sample size. In this study, the observation period between sites 2 and 3 were different, we do not know the effect of the length of observation or season on mortality. As such we could not identify the cause of mortality in the study, further observation would be needed to clarify causes of mortality for planning conservation strategies for each area.

**Implications for conservation**

*Rafflesia* in Sarawak is a totally protected plant under the Wild Life Protection Ordinance Act 1998. The new discovery of *R. tuan-mudae* populations in Naha Jaley calls for urgent planning for habitat conservation, as the area was not a totally protected area and is also within the land inhabited by the Kayan community. Therefore, the population remains under pressure from surrounding human activities, particularly land clearing by local communities for agriculture. Besides most studies show *Rafflesia* had a high mortality rates before blooming, *Rafflesia* is suggested to be vulnerable to extinction due to four major reasons. First, the whole life of the plant depends on the host. Therefore, the host availability influences the survivorship of *Rafflesia*. While the host plant is relatively common throughout Southeast Asian forests, not all individuals can rear *Rafflesia*, which limits its occurrence. Second, mortality before blooming is quite high. Third, because of its bisexual flower, the success of pollination relies largely on simultaneous blooming of male and female flowers and pollinator efficiency (Beaman et al. 1988). In addition, inbreeding depression could be affecting reproductive success, although it is still unclear. Fourth, people collect the flower directly for medical use or as souvenirs in some areas (Ismail 1988). Habitat disruption is also a major concern in tropical areas. In fact, studies estimated that at

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**Fig. 7.** (a) Size-dependent changes in absolute growth rate. Line fitted with the Gompertz model. The dot indicates the observed data ($y$ is D1). Different colours indicate different individuals ($N = 6$). (b) Projected bud diameter along days is noted with a black line. At day 0, the diameter was 0.1 cm. When the diameter reached ca. 30 cm (red horizontal line), the flower started blooming. Dots indicate each observation. (a) First, we estimated the day of the latest record for each individual, and then, we back cast observation records.
least two species have already gone extinct (Meijer 1985). We are now making every effort to implement conservation action as soon as possible, which requires fundamental ecological knowledge of Rafflesia.

**In-situ conservation with local communities**

Some part of the newly discovered population in Naha Jaley was located within the locally protected forests for water catchment areas of Bakun Hydro reservoir. We recognize that these areas are important for securing drinking water for local people but also for providing habitat for *R. tuan-mudae* as an endangered species. For conservation management of these areas, one of the best protection measures is involving communities in the local conservation program. In fact, community-based conservation of wild species is a general worldwide trend, e.g., in Poring, Sabah, the Dusun community is highly aware of *Rafflesia* protection (Peters & Ting 2016). The Naha Jaley area not only contains *R. tuan-mudae* but also high biodiversity resources in animals and plants (Dagang et al. 2015). Using these natural resources, Naha Jaley can be promoted as an eco-tourism destination. Adding value to natural resources benefits domestic income as well as making people more conscious of *Rafflesia* and wildlife conservation. These community-based conservation programs have already been conducted in Sabah (Nais 2001) and showed promise. Finally, caution should be taken toward unguided tourists causing massive trampling and harming plants and their hosts.

**Importance of ex-situ conservation**

As is often the case with tropical regions, primary and secondary forests in Sarawak have been intensively logged and lands used for oil palm plantation and dam construction, as seen in the Bakun. Due to deforestation, biodiversity decreased in most Southeast Asian countries (Koh and Wilcove 2008, Sodhi et al. 2004). It is a tradition by local communities in Sarawak to clear land for shifting cultivation. Shifting cultivation and heavily degraded forest causes habitat loss for *Rafflesia*, which is very sensitive to environmental disturbance. To support an *in-situ* conservation program, the preservation of the species outside the habitat also plays an important role. For *Rafflesia*, it requires a special technique to cultivate the plant as it grows only in the host plant, Tetrastigma. Propagation of *Rafflesia* is also very challenging (Molina et al. 2017), but cultivation of *R. keithii* in Sabah (Nais 2001) and *R. rochussenii* in Indonesia already showed success, with recent studies demonstrating grafting as a useful propagation method for *Rafflesia* (Wicaksono et al. 2016).

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**Conflict of interest**

The authors have no conflicts of interest to declare.

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## APPENDIX

Table S1. Sample list for morphological description.

| Date       | ID | Place           | Sex   | Status     | No of anthers | Weight (kg) | Diameter of bud (cm) | Diameter of flower (cm) | No of process | Note          |
|------------|----|----------------|-------|------------|---------------|-------------|----------------------|------------------------|---------------|---------------|
| 2012. 3. 27| 1  | Site 1, Naha Jaley | female | bud        | –             | 0.8         | 12.3                 | –                      | –             |               |
| 2012. 3. 27| 2  | Site 1, Naha Jaley | female | bud        | –             | 0.2         | 8.9                  | –                      | –             |               |
| 2012. 3. 27| 3  | Site 1, Naha Jaley | male   | bud        | 40            | 4.5         | 28.4                 | –                      | –             |               |
| 2012. 11. 2| 4  | Site 3, Naha Jaley | male   | rotten flower | 40            | –           | –                    | –                      | –             |               |
| 2012. 11. 21| 5  | Site 2, Naha Jaley | male   | open flower | 41            | –           | 25.0*                | 54.0                   | 37            | Figure 2      |
| 2012. 4. 3 | 6  | Kpg. Temurang    | male   | bud        | 38            | –           | 8.0                  | –                      | –             | R. tuan-mudae |
| 2012. 11. 15| 7  | Kpg. Remun       | male   | rotten bud | 38            | –           | –                    | –                      | –             | R. tuan-mudae |

* just before blooming