

A REVIEW OF THE BIOLOGY OF *RAFFLESIA*: WHAT DO WE KNOW AND WHAT'S NEXT?

Review Biologi *Rafflesia*: Apa yang sudah kita ketahui dan bagaimana selanjutnya?

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Diterima/Received: 29 Desember 2015; Disetujui/Accepted: 8 Juni 2016

Abstrak

Telah dilakukan tinjauan literatur untuk meringkas informasi, terutama karya ilmiah yg baru diterbitkan, pada biologi *Rafflesia*. Sebagian besar publikasi terkini adalah pemberian nama species baru pada *Rafflesia*. Sejak tahun 2002, sepuluh spesies telah ditemukan di Filipina dibandingkan dengan tiga spesies di Indonesia. Karya terbaru filogenetik juga telah dieksplorasi (misalnya sejarah evolusi genus *Rafflesia* dan gigantisme, transfer horizontal gen dan hilangnya genom kloroplas) dan anatomi (misalnya endofit, pengembangan bunga); studi terbaru lainnya berfokus pada biokimia. Sayangnya, masih banyak informasi yang belum diketahui misalnya tentang siklus hidup, biologi dan hubungan ekologi pada *Rafflesia*. Kebanyakan informasi yang tersedia berasal dari hasil pengamatan. Misalnya penurunan populasi telah diketahui secara umum yang kadang kadang dikaitkan dengan kerusakan habitat atau gangguan alam tapi penyebab-penyebab yang lain tidak diketahui dengan pasti. Pertanyaan yang belum terjawab antara lain pada biologi reproduksi, struktur genetik populasi dan keragaman. Dengan adanya perubahan iklim secara global, kita amat membutuhkan studi populasi jangka panjang dalam kaitannya dengan parameter lingkungan untuk membantu konservasi *Rafflesia*.

Keywords: *Rafflesia*, Indonesia, Biologi, konservation, review

Abstract

A literature review was conducted to summarize information, particularly recently published, on the biology of *Rafflesia*. By far most of the recent papers on *Rafflesia* have named new species. Since 2002, ten species have been discovered in the Philippines compared to three species in Indonesia. Recent work also has explored phylogenetics (e.g. evolutionary history of the genus and of floral gigantism, horizontal transfer of genes and loss of the chloroplast genome) and anatomy (e.g. endophyte, flower development); other recent studies have focused on biochemistry. Unfortunately, we still know very little about the life cycle, biology and ecological relations of *Rafflesia*. Much of what we know comes from observations and not from experimental evidence. Population declines have been noted which are sometimes related to habitat destruction or natural

disturbances but other times the cause(s) are unknown. Questions remain unanswered on its reproductive biology and on its population genetic structure and diversity. With changing climates, we vitally need long-term population studies in relation to environmental parameters.

Keywords: *Rafflesia*, Indonesia, Biology, conservation, review

INTRODUCTION

Within the obligate holoparasitic plant family Rafflesiaceae, the genus *Rafflesia* attracts the most attention (Kupicha 1993; Meijer 1993; Nais 2001). This genus is restricted to tropical Southeast Asia – mostly Indonesia, Malaysia, and the Philippines. Its unusual growth habit – with no leaves, stems, or true roots and only a flower seen outside its host – produces the world's largest flower, up to 1 m wide in *R. arnoldii*. Moreover, a single genus of plant – the vine *Tetrastigma* – serves as its host.

Although the biology of *Rafflesia* has always fascinated scientists, there has been renewed interest in the genus over the past few years. New species have been discovered. Studies addressing issues such as the evolution of floral gigantism and endophyte anatomy have been published. All of this interest comes at a time when efforts for the conservation of *Rafflesia* are vitally important, with the pressing issues of climate change and tropical forest destruction.

The purpose of this paper is to review the biology of *Rafflesia*, paying special attention to recent studies. We briefly summarize the literature that has been published on taxonomy, phylogenetics, morphology and anatomy, biochemistry, ecology and conservation biology for species in the genus. To this end, we highlight aspects of the biology of *Rafflesia* that critically need to be addressed and make recommendations for future studies.

TAXONOMY

Meijer (1997) provided a comprehensive treatment of *Rafflesia* in Flora Malesiana. He listed 14 species: *R. arnoldii* R. Br. (*R. arnoldii* var. *arnoldii*), *R. atjehensis* Koord. [*R. arnoldii* var. *atjehensis*

(Koord.) Meijer], *R. cantleyi* Solms-Laubach, *R. gadutensis* Meijer, *R. hasseltii* Suringar, *R. keithii* Meijer, *R. kerrii* Meijer, *R. manillana* Teschemaker, *R. micropylora* Meijer, *R. patma* Blume, *R. pricei* Meijer, *R. rochussenii* Teijsm. & Binn., *R. schadenbergiana* Göpp., and *R. tengku-adlinii* Salleh & Latiff.

Rafflesia zollingeriana Koord. was considered a form of *R. patma* by Meijer (1997), even though Koorders described the species in 1919 as distinct. In the treatment, Meijer (1997) also included five “incompletely known species”: *R. borneensis* Koord., *R. ciliata* Koord., *R. titan* Jack, *R. tuan-mudae* Becc., and *R. witkampii* Koord. These five species were considered to be incomplete due to the lack of well-preserved specimens or a convincing type specimen, to a poor description, or to a description based on an immature bud. Nais (2001) re-instated *R. tuan-mudae*, *R. witkampii*, and *R. zollingeriana* in his account on *Rafflesia*.

Since Meijer's (1997) treatment, many new species have been described from Indonesia, Malaysia, and the Philippines. Only one species is known from Thailand and one from Brunei (Meijer 1997), with no additional species having been found in these countries. Three species have been discovered in Indonesia, all from Sumatra: *R. bengkulensis* Susatya, Arianto & Mat-Salleh (Susatya *et al.* 2005); *R. meijeri* Wiriadinata & Sari (Wiriadinata and Sari 2009); and *R. lawangensis* Mat-Saleh, Mahyuni & Susatya (Mat-Salleh *et al.* 2010). With Meijer (1997) and Nais (2001) assessments and the addition of these three new species, the total number of *Rafflesia* species in Indonesia is 14.

In Malaysia, Meijer (1997) recognized six species: *R. keithii*, *R. pricei*, and *R. tengku-adlinii* found in Sabah and Sarawak and *R. cantleyi*, *R. hasseltii*, and *R. kerrii* in Peninsular Malaysia. *Rafflesia hasseltii* was regarded as endemic to

Sumatra, Indonesia, until 1993 when it was discovered in Peninsular Malaysia (Wong and Latiff 1994). However, it would remain until 2002 for suitable specimens to be collected and studied. Upon further observation, Latiff and Wong (2003) distinguished *R. azlanii* Latiff & M. Wong with comparisons made to *R. cantleyi* and *R. hasseltii*. Due to flower similarity among these three species, they stated "...indeed Meijer in 2001 had hinted that the examples of the new species might be a natural hybrid between *R. cantleyi* and *R. hasseltii*." Sofiyanti *et al.* (2007) examined eleven *R. hasseltii* flowers from Indonesia and Malaysia and found that the flower diameter, ratio of aperture to diaphragm, and coverage and density of warts (blotches) were very diverse. The most recent discoveries of *Rafflesia* from Malaysia are *R. lima-lidah* Wong & Gan (Wong and Gan 2008); *R. su-meiae* Wong, Nais & Gan (Wong *et al.* 2009); *R. sharifah-hipsahiae* Adam, Mohammed, Aizat-Juhari & Wan (Adam *et al.* 2013); and *R. parvimaculata* Sofiyanti, Mat-Salleh, Khairil, Zuhailah, Mohd. Ros & Burslem (Sofiyanti *et al.* 2016), bringing the total of number of species in Malaysia to 12. However, *R. lima-lidah* has not been formally described.

Barcelona *et al.*'s (2002) discovery of *R. speciosa* Barcelona & Fernando from Panay Island was a historic event in the Philippines since the last *Rafflesia* identified from there was *R. schadenbergiana* in 1885 (Lays 2006) and until 2002 only two species were known (*R. manillana*, *R. schadenbergiana*) (Meijer 1997). The finding of *R. speciosa* was a turning point for *Rafflesia* discovery in the Philippines since several new species were recorded afterwards (Barcelona *et al.* 2009a). *Rafflesia manillana* was used to describe plants from Luzon and Samar by Meijer (1997), Nais (2001), and Fernando and Ong (2005). However, Madulid and Agoo (2007) re-examined these plants and concluded that the species in Samar was *R. manillana* while that in Luzon was new, *R. panchoana* Madulid, Buot & Agoo. Later, Pelsner *et al.* (2013) resurrected and neotypified *R. lagascae* Blanco for the Luzon population named as *R. panchoana*. *Rafflesia schadenbergiana* was rediscovered by Lays (2006),

with no collections made, and then by Barcelona *et al.* (2008a), with a collection of a newly opened flower. Fernando and Ong (2005) revealed *R. mira* Fernando & Ong (syn. *R. magnifica* Madulid, Tandang & Agoo) from Mindanao. In 2006, Barcelona *et al.* reported a new species from southern Luzon naming it *R. baletei* Barcelona & Cajano and Galang and Madulid found *R. lobata* Galang & Madulid from Panay Island. In 2007, two separate teams (Barcelona *et al.* 2007; Madulid *et al.* 2007) identified *R. philippensis* Blanco (syn. *R. banahawensis* Madulid, Villarriba & Agoo; *R. banahaw* Barcelona, Pelsner & Cajano) from Luzon. Other new species have been recognized from northern Luzon by Barcelona *et al.* (2008b, 2009b), *R. leonardi* Barcelona & Pelsner and *R. aurantia* Barcelona, Co & Balete, and from Mindanao, *R. verrucosa* Balete, Pelsner, Nickrent & Barcelona (Balete *et al.* 2010) and *R. mixta* Barcelona, Manting, Arbolonio, Caballero & Pelsner (Barcelona *et al.* 2014). Most notable, Galindon *et al.* (2016) described *R. consueloae*, the world's smallest *Rafflesia*, on Luzon Island. The total number of species in the Philippines is 13.

The total number of *Rafflesia* worldwide is about 36 taxa, with the highest number from Indonesia (14) and a nearly equal number from the Philippines (13) and Malaysia (12). The Philippines is leading the way in the most number of new species being described (11) since 2002. However, a small number of the 36 species are known from very poor specimens (e.g. only a bud), the taxonomic validity of a few species have been questioned, and some species have not been recorded for many years and might be extinct. Clearly, a current assessment of the known distribution and taxonomy of all species, particularly little known species, is urgently needed.

PHYLOGENETICS

Based on mitochondrial DNA, recent studies place Rafflesiaceae as a member of Malpighiales (Barkman *et al.* 2004, 2007; Wurdack and Davis 2009). Phylogenetic studies have revealed that floral gigantism in *Rafflesia* was relatively recent and was rapid with floral diameter increasing an average of 20

cm/million years (Davis *et al.* 2007; Barkman *et al.* 2008; Davis 2008); that genes in *Rafflesia* may possibly have been horizontally transferred from the host *Tetrastigma* (Davis and Wurdack 2004; Molina *et al.* 2014; Krause 2015); the potential loss of the chloroplast genome (Molina *et al.* 2014); that the Indonesian, Bornean, Peninsular Malaysian and Philippine species are monophyletic (Bendiksby *et al.* 2010); and that a dramatic increase in diversification may be explained by Mid-Miocene to Pliocene rainforest experiencing favorable conditions as well as natural selection promoting character displacement in flower size (Bendiksby *et al.* 2010).

MORPHOLOGY AND ANATOMY

Many studies have been carried out on the morphology and anatomy of *Rafflesia* (Justesen 1922; Olah 1960; Meijer 1958; Bouman and Meijer 1994; Bänziger 2004; Sofiyanti *et al.* 2007; Mursidawati 2012). Recent studies have illuminated fascinating aspects of the flower anatomy as well as the endophyte of *Rafflesia*. Using comparative studies of development and gene-expression patterns, Nikolov *et al.* (2013) showed that flowers of *Rafflesia* and *Sapria* are constructed very differently despite being morphologically very similar and are not homologous. Nikolov *et al.* (2014a) concluded that the endophyte develops from a proembryo and that the flowering shoot arises from the undifferentiated endophyte. The host does not react to the parasite except when the flower forms. In fact, they also suggested the possibility that the endophyte could be a commensal or mutually beneficial partner to its host. In addition, the surfaces of the floral apex and of the ovarial clefts are exposed secondarily during an extraordinary process of cell separation, which has not been documented in any other angiosperm (Nikolov *et al.* 2014b).

Bouman and Meijer (1994) suggested that flower diameter and size of ovules were positively related among species. In contrast, Sofiyanti and Yen (2012) found no pattern between these variables for *R. azlanii*, *R. cantleyi*, *R. hasseltii*, and *R. kerrii*. In

their study, *R. kerrii* which has the largest flower among the four species had a smaller ovule than *R. azlanii* and *R. cantleyi*. *Rafflesia* seeds are usually chestnut-shaped and brown when fresh and about 500-1500 µm in size (Mursidawati 2012; Sofiyanti and Yen 2012).

BIOCHEMISTRY

Only over the past eight years has the biochemistry and natural product chemistry of *Rafflesia* been explored. Plants of *R. kerrii* have been found to be rich in tannin (Kanchanapoom *et al.* 2007). Alkaloid (nicotine and caffeine) and phenolic (catechin, proanthocyanidin, phenolic acid) compounds have been detected in *R. hasseltii* (Sofiyanti *et al.* 2008; Zulkffle *et al.* 2014), and an antioxidant compound was detected from *R. kerrii* flower extract (Puttipan and Okonogi 2014). Extracts from *R. hasseltii* have been reported to be effective in accelerating wound healing (Abdulla *et al.* 2009). Furthermore, anti-microbial activity against *Candida albicans* was detected in an isolate of an endophytic fungus from *R. cantleyi* (Refaei *et al.* 2011).

ECOLOGY

Buds and flowering

High mortality rate is common in *Rafflesia* especially for buds that are less than 5 cm diameter and that grow on the ground (Meijer 1984; Hidayati *et al.* 2000; Galang 2007; Susatya 2007). The mortality can be due to natural disasters, human disturbances, high humidity/precipitation, insect infestations, trampling, predation, or resource limitation (Meijer 1985, 1997; Emmons *et al.* 1991; Zuhud *et al.* 1994; Hidayati *et al.* 2000; Nadia *et al.* 2012; Nikolov *et al.* 2014a). Justesen (1922) noted termites inside a dead bud of *R. arnoldii*; however, these buds may have already been dead before termites attacked them since he stated that termites would not attack healthy buds or immature fruits.

Most flowers of *Rafflesia* are found on the ground attached to the vine *Tetrastigma*; however,

sometimes they are observed on a vine up in a tree. For instance, Galang (2007) recorded five flowers of *R. lobata* at 5 m, two at 7 m, and one at 10 m above the ground. In general, there is no distinct flowering season for *Rafflesia*. For example, *R. arnoldii* and *R. patma* have been observed flowering at any time of the year (Hidayati and Susatya, pers. obs.; Mursidawati *et al.* 2014). In contrast, flowering appears to be seasonal, coinciding with the hottest and driest time of the year for *R. kerri* as open flowers have been reported from January to March (Meijer and Elliott 1990). The ratio of male:female flowers in populations is high: 22:8 (27% female flowers) in *R. keithii*, 89:2 (36% female flowers) in *R. pricei*, 7:2 (50% female flowers) in *R. tengku-adlinii* (Nais 2001), 6:1 (14%) in *R. lobata* (Galang 2007), and 15:1 (6%) in *R. manillana* (Yahya *et al.* 2010). Susatya (pers. comm.) observed seven locations of *R. arnoldii* for 3 years and found only two female flowers.

Pollination biology

Typically, more buds than flowers are observed in populations of *Rafflesia*, wherein flowering is also asynchronous (Justesen 1922; Meijer 1958; Olah 1960; Beaman *et al.* 1988; Bänziger 1991). Being a dioecious plant, *Rafflesia* needs pollinators for fertilization to occur. There is strong evidence that in *Rafflesia* both olfactory and visual cues are necessary to attract and hold pollinators long enough to carry or to transfer the pollen (Beaman *et al.* 1988). Moreover, Patiño *et al.* (2002) found that *R. tuan-mudae* is an endothermic flower which plays a role in the mimicry of the flower to attract blowflies. Flies were seen visiting both male and female flowers (Justesen 1922; Meijer 1958; Beaman *et al.* 1988; Bänziger 1991). In addition, Beaman *et al.* (1988) stated that the size and color of a *Rafflesia* flower that contrasts with the environment is an "...adaptation that increases the likelihood that the flower will be discovered by pollinators." Bänziger (1991) mentioned that pollination may occur between remote flowers that are blooming many days or weeks apart since fly-pollinators can travel a great distance in a few days. However, Nais (2001) found that pollen viability in *R.*

kerrii lasted only 72 hours. Nais (2001; see also Meijer 1997, Bänziger 2004) suggested that *Rafflesia* can produce seeds without fertilization, i.e. agamospermy, which is an issue that needs further study. *Lucilia* and *Chrysomya* were reported to be pollinators for *R. pricei* (Beaman *et al.* 1988), while *Chrysomya*, *Lucilia*, and *Sarcophaga* were found visiting *R. kerrii* (Bänziger 1991, 2004) and *R. patma* (Hidayati *et al.* 2000). Kahono *et al.* (2010) reported an additional five genera of flies visiting a female flower of *R. patma*.

Seed dispersal and host infection

The small seeds of *Rafflesia* are thought to have many possible dispersal agents like wild pigs, ground squirrels (Meijer 1958; Bouman and Meijer 1994; Zuhud *et al.* 1999; Nais 2001), ants, termites, pangolin (Justesen 1922; Kuijt 1969) and elephants (Kuijt 1969). Emmons *et al.* (1991) suggested that most likely it is small mammals that are the effective dispersal agents for *Rafflesia* seeds. They observed adult tree shrew (*Tupaia tana*) and adult plantain squirrel (*Callosciurus notatus*) feeding on a fruit of *R. keithii*. In contrast, Pelsner *et al.* (2013) found numerous ants (*Technomyrmex* sp., *Pheidologeton* sp.) inside the fruit wall of *R. philippensis*. They suggested that ants might be attracted to an elaiosome on the *Rafflesia* seed and carry seeds away from the fruit to their nest. Here, the seed germinates and infects the root of nearby *Tetrastigma*. Justesen (1922) believed that infection most likely took place on the underground part of the vine host. Likewise, Bänziger (1991) suspected that soil fauna, mainly arthropods and nematodes, serve as the main agents for new host infection.

Life cycle length

Meijer (1997) estimated that the length of the life cycle of *Rafflesia* is 3-4.5 years. More detailed studies have shown that the life cycle length (from seed to seed) for *R. patma* spans ca. 3-4 years (Hidayati *et al.* 2000) and for *R. arnoldii* is 3.5-5 years (Susatya 2007). The development of *R. rochussenii* from visible bud to ripe fruit was ca. 2.5 years (Zuhud *et al.* 1994). Due to this prolonged nature of bud

development and low incidence of flowering study of the life cycle of *Rafflesia* is difficult.

CONSERVATION BIOLOGY

Rarity

Due to its parasitic mode of life and its dependence on a limited number of species of the vine *Tetrastigma* (Chen *et al.* 2011; Zakaria *et al.* 2016), *Rafflesia* is rare and prone to extinction. Other aspects of its biology also leads to its rarity and potential for extirpation: high flower bud mortality, rare pollination events (flowers not opening simultaneously, populations having one sex or having a large sex ratio imbalance), unsuccessful pollination and seed dispersal, long flower development (3-4 years), and highly fragmented populations (Justesen 1922; Meijer 1958; Olah 1960; Hidayati *et al.* 2000; Hikmat 2006; Galang 2007; Yahya *et al.* 2010; Nadia *et al.* 2012).

Threats

Populations of some *Rafflesia*, along with the host, are declining in protected and unprotected forests due to natural disasters, flower collection, logging, and shifting cultivation (Nais and Wilcock 1998; Zuhud *et al.* 1999; Hikmat 2006; Suwartini *et al.* 2008; Mursidawati *et al.* 2014). The main threat for *Rafflesia* populations is loss of habitat due to logging or to collection of firewood and to conversion of forests into housing or into plantation monocultures of rubber, palm oil, and fruit gardens (Meijer 1985; Hikmat 1988; Ismail 1988; Meijer and Elliott 1990; Bänziger 1991; Nais and Wilcock 1998). In fact, populations of some recently discovered species (e.g. *R. lobata*, *R. manillana*, *R. philippensis*) have been found in severely degraded or fragmented forests (Barcelona *et al.* 2009a).

Direct human exploitation also is a contributing factor for *Rafflesia* rarity. Due to the conspicuous flowers that attract attention, people often cut them out of curiosity and in doing so also damage the host plants. People also collect buds or flowers because *Rafflesia* is believed to have medicinal properties and is used as a general tonic,

as a cure for fever or back ache, and for childbirth recuperation (Ismail 1988; Meijer and Elliott 1990; Hikmat 2006) or to increase women fertility (Zuhud *et al.* 1999). However, this last practice has not been used for a long time.

Population declines

Population declines have been noted for several species of *Rafflesia*, but the cause(s) of these declines are sometimes unknown. A site of *R. kerri* in Khao Sok Nature Reserve, Thailand visited in 1983 was not present in 1989 (Meijer and Elliott 1990). Three sites of *R. arnoldii* in Taba Penanjung, Bengkulu, Indonesia visited in 2003 did not contain plants in 2014 (Susatya, pers. comm.). Hikmat (2006) reported a rapid decline in populations of *R. zollingeriana* in East Java, Indonesia. In 1988, he recorded eight sites of this species along the coast of the Indian Ocean in Meru Betiri National Park. By 2003, only three sites contained plants: one site from the original eight and two new sites. Besides habitat destruction caused by a tsunami in 1994, Hikmat (2006) also noted harvesting of flower buds by local people. Suwartini *et al.* (2008) recorded 256 flower buds from five sites of *R. patma* in Pangandaran Nature Reserve, West Java, Indonesia in 1989 but only 96 from the same sites in 2007.

Ex situ conservation

Attempts to propagate *Rafflesia* for cultivation are certainly appealing, especially for conservation and for tourism attraction. However, there is little knowledge about how to inoculate host plants and germinate *Rafflesia* seeds. Attempts at germinating seeds in the laboratory or by placing them on living host plants and at tissue culture techniques have not been successful (Nais and Wilcock 1999; Mursidawati *et al.* 2014; Wicaksono *et al.* 2016). However, other attempts at inoculation have been successful (see Meijer *et al.* 1997; Vieldkamp 2007). *Rafflesia patma* and *R. rochussenii* growing on a host plant were successfully relocated to Bogor Botanical Garden in 1850, followed by *R. arnoldii* in 1856 (Meijer 1997; Vieldkamp 2007); recent relocations have not been successful for *R. patma* (Mursidawati *et al.* 2014). Recently, using grafting techniques *R. patma* flowered at the Bogor Botanical Garden (Mursidawati

et al. 2014; Wicaksono *et al.* 2016). Although pollination was not observed for the grafted plants at Bogor, manual pollination might be tried in the future (Bänziger 2004).

***In situ* conservation**

Habitat preservation is an essential part of the conservation of *Rafflesia*. However, many politicians and community leaders believe that conservation actions for *Rafflesia* are impediments to economic development (Ismail 1988). Collaboration, in mutual trust and respect, between local stakeholders, community leaders, agency heads, and scientists, as well as emphasis on the importance of conservation via social networking and mass media will greatly strengthen initiatives (Lestari *et al.* 2014). For example, an information network employing village heads and local people was set up in Sabah to report discoveries of new populations of *Rafflesia* to researchers at the University Kebangsaan Malaysia Sabah Campus. After a few years, at least eleven new sites of *Rafflesia* were found as compared to three sites known before the network. Many *Rafflesia* populations occur outside of protected areas or designated parks, and thus, eco-tourism is used in conservation efforts to relieve visitation pressure in protected areas. Ecotourism is a viable option that provides an economic return to landowners (e.g. entrance fees) and does not destroy habitat (e.g. for farming) (Nais and Wilcock 1998; Yulian 2002) as long as it fits within the cultural norms of community access to forest resources (Doolittle 2005). However, establishing ecotourism has to be carefully planned (e.g. from landowner hospitality and travel agency participation, to souvenir manufacturing) and monitored since sites can be over-used to the detriment of *Rafflesia* (Barcelona *et al.* 2009a; Lestari *et al.* 2014).

CONCLUSIONS

Progress has been made on the taxonomy, phylogenetics, morphology and anatomy, and biochemistry of *Rafflesia*; however, other aspects of

its biology remain poorly studied. Questions remain unanswered on its reproductive biology (such as agamospermy, seed dispersal and germination, and mode of host inoculation) and on its population genetic structure and diversity. Long-term monitoring of population dynamics as well as data on past and current distributions and status of populations are vitally needed. Preserving populations of *Rafflesia* and its host plant both outside (with eco-tourism) and inside of parks may become increasingly difficult with climate change. Yet, we know very little about the environmental conditions required for survival, growth, and reproduction of *Rafflesia* and its host. Efforts to involve local people in conservation strategies need to be continued as well as logistical and funding support for carefully planned ecotourism (e.g. construction of elevated walkways to reduce trampling). An education campaign (in schools and to the general public) on *Rafflesia* and its host would greatly help in conservation initiatives.

REFERENCES

- Abdulla, M.A., Ahmed, K.A., Ali, H.M., Noor, S.N., and S. Ismail. (2009). Wound healing activities of *Rafflesia hasseltii* extract in rats. *Journal of Clinical Biochemistry and Nutrition* 45: 304-308.
- Adam, H.J., Mohamed, R., Jauhari, M.A.A., Nik Ariff, N.N.F., and Wan, K.L. (2013). *Rafflesia sharifah-hapsahiae* (Rafflesiaceae), a new species from Peninsular Malaysia. *Turkish Journal of Botany* 37: 1038-1044.
- Balete, D.S., Pelsler, P.B., Nickrent, D.L., and Barcelona, J.F. (2010). *Rafflesia verrucosa* (Rafflesiaceae), a new species of small-flowered *Rafflesia* from eastern Mindanao, Philippines. *Phytotaxa* 10: 49-57.
- Bänziger, H. (1991). Stench and fragrance: unique pollination lure of Thailand's largest flower, *Rafflesia kerrii* Meijer. *Natural History Bulletin of Siam Society* 39: 19-52.

- Bänziger, H. (2004). Studies on hitherto unknown fruits and seeds of some Rafflesiaceae, and a method to manually pollinate their flowers for research and conservation. *Linzer Biologische Beitrage* 36: 1175-1198.
- Barcelona, J.F. and Fernando, E.S. (2002) A new species of *Rafflesia* (Rafflesiaceae) from Panay Island, Philippines. *Kew Bulletin* 57: 647-651.
- Barcelona, J.F., Cajano, M.A.O., and Hadsall, A.S. (2006). *Rafflesia baletei*, another new *Rafflesia* (Rafflesiaceae) from the Philippines. *Kew Bulletin* 61: 231-237.
- Barcelona, J.F., Pelsner, P.B., and Cajano, M.O. (2007). *Rafflesia banahaw* (Rafflesiaceae), a new species from Luzon, Philippines. *Blumea* 52: 345-350.
- Barcelona, J.F., Pelsner, P.B., Tagtag, A.M., Dahonog, R.G., and Lilangan, A.P. (2008a). The rediscovery of *Rafflesia schadenbergiana* (Rafflesiaceae). *Flora Malesiana Bulletin* 14: 162-165.
- Barcelona, J.F., Pelsner, P.B., Cabutaje, E.M. and Bartolome, N.A. (2008b). Another new species of *Rafflesia* (Rafflesiaceae) from Luzon, Philippines: *R. leonardi*. *Blumea* 53: 223-228.
- Barcelona, J.F., Pelsner, P.B., Balete, D.S., and Co, L.L. (2009a). Taxonomy, ecology and conservation status of Philippine *Rafflesia* (Rafflesiaceae). *Blumea* 54: 77-93.
- Barcelona, J.F., Co, L.L., Balete, D.S., and Bartolome, N.A. (2009b). *Rafflesia aurantia* (Rafflesiaceae): A new species from northern Luzon, Philippines. *Garden Bulletin of Singapore* 61: 17-28.
- Barcelona, J.F., Manting, M.M.E., Arbolonio, R.B., Caballero, R.B., and Pelsner, P.B. (2014). *Rafflesia mixta* (Rafflesiaceae), a new species from Surigao del Norte, Mindanao, Philippines. *Phytotaxa* 174: 272-278.
- Barkman, T.J., Lim, S.H., Mat Salleh, K., and Nais, J. (2004). Mitochondrial DNA sequences reveal the photosynthetic relatives of *Rafflesia*, the world's largest flower. *Proceedings of the National Academy of Sciences, USA* 101: 787-792.
- Barkman, T., McNeal, J.R., Lim, S.H., Coat, G., Croom, H.B., Young, N.D., and de Pamphilis, C.W. (2007). Mitochondrial DNA suggest at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evolutionary Biology* 7: 248.
- Barkman, T.J., Bendiskby, M., Lim, S.H., Mat Salleh, K., Nais, J., Madulid, D., and Schumacher, T. (2008). Accelerated rates of floral evolution at the upper size limit for flowers. *Current Biology* 18: 1508-1513.
- Beaman, R.S., Decker, P.J., and Beaman, J.H. (1988). Pollination of *Rafflesia* (Rafflesiaceae). *American Journal of Botany* 75: 1148-1162.
- Bendiksby, M., Schumacker, T., Gussarova, G., Nais, J., Mat Salleh, K., Sofiyanti, N., Madulid, D., Smith, S.A., and Barkman, T. (2010). Elucidating the evolutionary history of the Southeast Asian, holoparasitic, giant-flowered Rafflesiaceae: pliocene vicariance, morphological convergence and character displacement. *Molecular Phylogenetics and Evolution* 57: 620-633.
- Bouman, F. and Meijer, W. (1994). Comparative structure of ovules and seeds in Rafflesiaceae. *Plant Systematic and Evolution* 193: 187-212.
- Chen, P., Chen, L., and Wen, J. (2011). The first phylogenetic analysis of *Tetrastigma* (Miq.) Planch., the host of Rafflesiaceae. *Taxon* 60: 499-512.
- Davis, C.C. (2008). Floral evolution: dramatic size change was recent and rapid in the world's largest flowers. *Current Biology* 18: R1102-R1104.
- Davis, C.C., and Wurdack, K.J. (2004). Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from Malpighiales. *Science* 305: 676-678.
- Davis, C.C., Latvis, M., Nickrent, D.L., Wurdack, D.J., and Baum, D.A. (2007). Floral gigantism in Rafflesiaceae. *Science* 315: 1812.
- Doolittle, A.A. (2005). *Property and politics in Sabah, Malaysia: native struggles over land rights*. University of Washington Press, Seattle.

- Emmons, L.H., Nais, J., and Briun, A. (1991). The fruit and consumers of *Rafflesia keithii* (Rafflesiaceae). *Biotropica* 23: 197-199.
- Fernando, E.S. and Ong, P.S. (2005). The genus *Rafflesia* R.Br. (Rafflesiaceae) in the Philippines. *Asia Life Sciences* 14: 263-270.
- Galang, R. (2007). Population study of *Rafflesia lobata* in the northern section of the central Panay Island mountain ranges, Philippines. *Folia Malaysiana* 8: 87-98.
- Galindon J.M.M., Ong, P.S., and Fernando, E.S. (2016). *Rafflesia consueloae* (Rafflesiaceae), the smallest among giants; a new species from Luzon Island, Philippines. *PhytoKeys* 61: 37-46.
- Hidayati, S.N., Meijer, W., Baskin, J.M., and Walck, J.L. (2000). A contribution to the life history of the rare Indonesian holoparasite *Rafflesia patma* (Rafflesiaceae). *Biotropica* 32: 404-414.
- Hikmat, A. (2006). Kecenderungan populasi *Rafflesia zolingeriana* Kds. di Taman Nasional Meru Betiri, Jawa Timur (Population trend of *Rafflesia zolingeriana* Kds. in Meru Betiri National Park, East Java). *Media Konservasi* 11: 105-108.
- Ismail, G. (1988). Conservation of the giant *Rafflesia* in Sabah, Malaysia. *Trends in Ecology and Evolution* 3: 316-317.
- Justesen, P.T. (1922). Morphological and biological notes on *Rafflesia* flowers, observed in the highlands of mid-Sumatra. *Annales du Jardin Botanique de Buitenzorg* 32: 64-87.
- Kahono, S., Mursidawati, S., and Erniwati. (2010). Komunitas serangga pada bunga *Rafflesia patma* Blume (Rafflesiaceae) di luar habitat aslinya Kebun Raya Bogor Kota Bogor Provinsi Jawa Barat Indonesia. (Insects community on the flower of *Rafflesia patma* Blume (Rafflesiaceae) in its non-native habitat of Bogor Botanical Gardens, Bogor City, Province of West Java, Indonesia.) *Jurnal Biologi Indonesia* 6: 429-442.
- Kanchanapoom, T., Kamel, M.S., Picheansoonthon, C., Luecha, P., Kasai, R., and Yamasaki, K. (2007). Hydrolyzable tannins and phenylpropanoid from *Rafflesia kerrii* Meijer (Rafflesiaceae). *Journal of Natural Medicines* 61: 478-479.
- Krause, K. (2015). Grand-scale theft: kleptoplasty in parasitic plants? *Trends in Plant Science* 20: 196-198.
- Kuijt, J. (1969). *The Biology of Parasitic Flowering Plants*. University of California Press, Berkeley.
- Kupicha, K.K. (1993). Rafflesiaceae. Pages 177-178 In: V.H. Heywood (ed.). *Flowering Plants of the World*. Updated edition. Oxford University Press, New York.
- Latiff, A. and Wong, M. (2003). A new species of *Rafflesia* from Peninsular Malaysia. *Folia Malaysiana* 4: 135-146.
- Lays, P. (2006). Rediscovery of floral jewel in the Philippines archipelago: *Rafflesia schadenbergiana* Göppert, 1885 (Rafflesiaceae). *Lejeunia Revue de Botanique* 182: 1-6.
- Lestari, D., Hikamat, A., and Zuhud, E.A.M. (2014). Conservation strategy of *Rafflesia zollingeriana* Koord. in Meru Betiri National Park, East Java. *Jurnal Manajemen Hutan Tropika* 20: 9-16.
- Madulid, D.A., Tandang, D.N., and Agoos, E.M.G. (2005). *Rafflesia magnifica* (Rafflesiaceae), a new species from Mindanao, Philippines. *Acta Manilana* 53:1-6.
- Madulid, D.A., Tolentino, C.V.C, and Agoos, E.M.G. (2006). *Rafflesia banahawensis* (Rafflesiaceae), a new species from Luzon, Philippines. *Philippine Science* 43: 43-51.
- Madulid, D.A. and Agoos, E.M.G. (2007). On the identity of *Rafflesia manillana* Teschem. (Rafflesiaceae). *Philippine Scientist* 44: 57-70.
- Madulid, D.A., Buot Jr., I.E.B., and Agoos, E.M.G. (2007). *Rafflesia panchoana* (Rafflesiaceae), a new species from Luzon Island, Philippines. *Acta Manilana* 55: 43-37.

- Mat Salleh, K., Mahyuni, R., Susatya, A., and Veldkamp, J.F. (2010). *Rafflesia lawangensis* (Rafflesiaceae), a new species from Bukit Lawang, Gunung Leuser National Park, North Sumatra, Indonesia. *Reinwardtia* 13: 159-165.
- Meijer, W. (1958). A contribution to the taxonomy and biology of *Rafflesia arnoldii* in West Sumatra. *Annales Bogorienses* 3: 33-44.
- Meijer, W. (1984). New species of *Rafflesia* (Rafflesiaceae). *Blumea* 30: 209-215.
- Meijer, W. (1985). Saving the world's largest flower. *National Geographic Magazine* 168 (1): 136-139.
- Meijer, W. (1993). *Rafflesiaceae*. Pages 557-563. In: K. Kubitzki (ed.). *The Families and Genera of Vascular Plants*. Springer, Berlin.
- Meijer, W. (1997). *Rafflesiaceae*. *Flora Malesiana*, Series I, Vol. 13: 1-42.
- Meijer, W., and Elliott, S. (1990). Taxonomy, ecology and conservation of *Rafflesia kerrii* Meijer in southern Thailand. *Natural History Bulletin of Siam Society* 38: 117-133.
- Molina, J., Hazzouri, K.M., Nickrent, D.L., Geisler, M., Meyer, R.S., Pentony, M.M., Flowers, J.M., Pelser, P.B., Barcelona, J.F., Inovejas, S.A., Uy, I., Yuan, W., Wilkins, O., Claire-Iphanise, M., Locklear, S., Concepcion, G.P., and Purugganan, M.D. (2014). Possible loss of the chloroplast genome in the parasitic flowering plant *Rafflesia lagascae* (Rafflesiaceae). *Molecular Biology and Evolution* 31: 793-805.
- Mursidawati, S. (2012). Morfologi buah dan biji *Rafflesia patma* dan *R. arnoldii* (Fruit and seed morphology of *Rafflesia patma* and *R. arnoldii*). *Buletin Kebun Raya* 15: 20-29.
- Mursidawati, S., Irawati, and Ngatari. (2014). *Rafflesia patma* (Rafflesiaceae): catatan tentang studi lapangan, budidaya, perkecambahan biji dan anatominya. (*Rafflesia patma* (Rafflesiaceae): notes on its field study, cultivation, seed germination and anatomy.) *Buletin Kebun Raya* 17: 9-14.
- Nadia, W.A.Q., Siti-Munirah, M.Y., Zulhazman, H., and Razak, W. (2012). A preliminary study on the mortality rates of *Rafflesia kerrii* in the Lojing Highlands, Gua Musang, Kelantan, Malaysia. Proceedings of the Universiti Malaysia Terengganu 11th International Annual Symposium on Sustainability Science and Management, 9-11 July 2012, Terengganu, Malaysia. Pp. 456-459.
- Nais, J. (2001). *Rafflesia of the World*. Sabah Park in association with Natural History Publications (Borneo), Kota Kinabalu.
- Nais, J. and C.C. Wilcock. (1998). The *Rafflesia* Conservation Incentive Scheme in Sabah, Malaysian Borneo. *Sabah Parks Nature Journal* 1: 9-17.
- Nais, J. and Wilcock, C.C. (1999). The propagation of *Rafflesia* and its host plant: prospect for ex-situ conservation. *Sabah Park Nature Journal* 2: 111-120.
- Nikolov, L.A., Endress, P.K., Sugumaran, M., Sasirat, S., Vessbutr, S., and Kramer, E. (2013). Developmental origins of the world's largest flowers, Rafflesiaceae. *Proceedings of the National Academy of Sciences, USA* 110: 18578-18583.
- Nikolov, L.A., Tomlison, P.B., Manickam, S., Endress, P.K., Kramer, E.M., and Davis, C.C. (2014a). Holoparasitic Rafflesiaceae possess the most reduced endophytes and yet give rise to the world's largest flowers. *Annals of Botany* 114: 233-242.
- Nikolov, L.A., Staedler, Y.M., Manickam, S., Schonenberger, J., Endress, P.K., Kramer, E.M., and Davis, C.C. (2014b). Floral structure and development in Rafflesiaceae with emphasis on their exceptional gynoecea. *American Journal of Botany* 101: 225-243.
- Olah, L.V. (1960). Cytological and morphological investigations in *Rafflesia arnoldii* R. Br. *Bulletin of the Torrey Botanical Club* 87: 406-416.

- Pelser, P.B., Nickrent, D.L., Callado, J.R.C., and Barcelona, J.F. (2013). Mt. Banahaw reveals: the resurrection and neotypification of the name *Rafflesia lagascae* (Rafflesiaceae) and clues to the dispersal of *Rafflesia* seeds. *Phytotaxa* 131: 35-40.
- Patiño, S., Aalto, T., Edwards, A.A., and Grace, J. (2002). Is *Rafflesia* an endothermic flower? *New Phytologist* 154: 429-437.
- Puttipan, R. and Okonogi, S. (2014). Antioxidant activity of *Rafflesia kerrii* flower extract. *Drug Discovery and Therapeutics Journal* 8: 18-24.
- Refaei, J., Jones, E.B.G., Sakayaroj, J., and Santhanam, J. (2011). Endophytic fungi from *Raffleia cantleyi*: species diversity and antimicrobial activity. *Mycosphere* 2: 429-447.
- Sofiyanti, N., and Yen, C.C. (2012). Morphology of ovule, seed and pollen of *Rafflesia* R.Br. (Rafflesiaceae). *Bangladesh Journal of Plant Taxonomy* 19: 109-117.
- Sofiyanti, N., Mat-Salleh, K., Purwanto, D., and Syahputra, E. (2007). The note on morphology of *Rafflesia hasseltii* Suringar from Bukit Tiga Puluh National Park, Riau. *Biodiversitas* 9: 257-261.
- Sofiyanti, N., Wahibah, N.H., Purwanto, D., and Syahputra, E., and Mat-Salleh, K. (2008). Alkaloid and phenolic compound of *Rafflesia hasseltii* Suringar and its host *Tetrastigma leucostaphyllum* (Dennst.) Alston ex Mabb. in Bukit Tigapuluh National Park, Riau: a preliminary study. *Biodiversitas* 9: 17-20.
- Sofiyanti, N., Mat-Salleh, K., Mahmud, K., Mazlan, N., Hasein, M., and Burslem, D. (2016). *Rafflesia parvimaclata* (Rafflesiaceae), a new species of *Rafflesia* from Peninsular Malaysia. *Phytotaxa* 253: 207-213.
- Susatya, A. (2007). Taxonomy and ecology of *Rafflesia* in Bengkulu, Indonesia. PhD thesis, Universiti Kebangsaan, Malaysia.
- Susatya, A., Arianto, W., and Mat Salleh, K. (2005). *Rafflesia bengkuluensis* (Rafflesiaceae), a new species from south Sumatra, Indonesia. *Folia Malaysiana* 6: 139-152.
- Suwartini, R., Hikmat, A., and Zuhud, E.A.M. (2008). Kondisi vegetasi dan populasi *Rafflesia patma* Blume di Cagar Alam Leuweung Sancang. (Conditions of vegetation and population of *Rafflesia patma* Blume in Leuweng Sancang Nature Preserve.) *Media Konservasi* 13: 1-8.
- Vieldkamp, J.F. (2007). Some notes on the cultivation of *Rafflesia*. *Flora Malesiana Bulletin* 14: 50-53.
- Wicaksono, A., Mursidawati, S., Sukamto, L.A., and da Silva, J.A.T. (2016). *Rafflesia* spp.: propagation and conservation. *Planta* 244: 289-296.
- Wiradinata, H., and Sari, R. (2010). A new species of *Rafflesia* (Rafflesiaceae) from north Sumatra. *Reinwardtia* 13: 95-100.
- Wong, M., and Latiff, A. (1994). Rafflesias of Peninsular Malaysia. *Nature Malaysiana* 19: 84-88.
- Wong, M., and Gan, F. (2008). Lima-lidah, a potentially new species of *Rafflesia*. *Malayan Naturalist* 62: 40-42.
- Wong, M., Nais, J., and Gan, F. (2009). *Rafflesia sumeiae* (Rafflesiaceae). *Folia Malaysiana* 10: 89-98.
- Wurdack, K.J. and Davis, C.C. (2009). Malpighiales phylogenetics: gaining ground on one of the most recalcitrant clades in the Angiosperm tree of life. *American Journal of Botany* 96: 1551-1570.
- Yahya, A.F., Hyun, J.O., Lee, J.H., Sun, B.Y., and Lapitan, P.G. (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.
- Yulian, Hermawan, B., Okuda, N., and Fujime, Y. (2002). Studies on *Rafflesia* flower in Bengkulu, Indonesia. *Technical Bulletin of the Faculty of Agriculture, Kagawa University* 54: 35-40.

- Zuhud, E.A.M., Hikmat, A., and Nugroho, Y.A.F. (1994). Eksplorasi ekologi *R. rochussenii* T. et Binn. Untuk kegiatan konservasi dan penangkarnya di Gunung Salak. (Exploration on the ecological characteristics of *Rafflesia rochussenii* T. et Binn. for its conservation and cultivation in Gunung Salak.) *Media Konservasi* 14: 9-22.
- Zuhud, E.A.M., Hernidiah, N., and Hikmat, A. (1999). Pelestarian *Rafflesia hasseltii* Suringar di Taman Nasional Bukit Tigapuluh Riau-Jambi (Conservation of *Rafflesia hasseltii* Suringar in Bukit Tiga National Park, Riau-Jambi.) *Media Konservasi* 6: 23-26.
- Zakaria, W.N.F.W., Puad, A.S.A., Geri, C., Zainudin, R., and Latiff, A. (2016). *Tetrastigma diepenhorstii* (Miq.) Latiff (Vitaceae), a new host of *Rafflesia tuan-mudae* Becc. (Rafflesiaceae) in Borneo. *Journal of Botany* Article ID 3952323.
- Zulkffle, M.A., Osman, N.S., Yusoff, Z., Kamal, M.L., and Tajam, J. (2014). Identification of alkaloid compound and antioxidant activity of *Rafflesia cantleyi* and its host, *Tetrastigma tuberculatum*. *The Open Conference Proceeding Journal* 5: 18-20.