Journal of Tropical Forest Science 35(4): 489–495 (2023)
https://doi.org/10.26525/jtfs2023.35.4.489
ISSN: 0128-1283, eISSN: 2521-9847

RAFFLESIA AS A HAPAXANTHIC PLANT AND IMPLICATIONS FOR *RAFFLESIA* CONSERVATION AND MANAGEMENT

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Submitted March 2023; accepted May 2023

Rafflesia is famous for its enormous flowers attached to *Tetrastigma* vines. The theory to explain this phenomenon is that the *Rafflesia* flower is produced by a repeat-flowering entity that lives and grows as an endophytic parasite within its *Tetrastigma* host. Evidence is presented here to support the theory that *Rafflesia* is a hapaxanthic plant, flowering at the end of its life, with each plant terminating in a single flower; repeat flowering being the result of reinfection by seeds. This has existential implications for *Rafflesia*.

Keywords: Endophyte, parasite, totipotency, embryogenesis, ontogeny.

INTRODUCTION

Rafflesia was described by Robert Brown in 1821 as a flower living parasitically on a *Tetrastigma* host plant. It has been assumed that the flower is produced by an entity living within the host plant as an endophyte.

Nikolov et al. (2014) examined microscopic sections of infected *Tetrastigma* vines and found *Rafflesia* endophyte tissue in the form of cells with nuclei at least twice the diameter of the nuclei of host cells, embedded in the host tissues in the form of short uniseriate strands or in clusters that he referred to as protocorms. The amount of endophytic tissue was minute in relation to host tissue and there was no visible damage to host cells.

Mursidawati et al. (2019) examined the endophyte of *Rafflesia patma* on *Tetrastigma leucostaphylum*. The endophyte was in the form of small clusters of cells associated with the host cambium. They speculated that the endophyte is spread through the body of the vine through the activity of the cambium.

However, it is well-established that the cambium contributes only to radial growth of a stem or root through meristematic activity at the periphery of the stem or root, while linear extension is the result of meristematic activity at the apical meristems. There is no mechanism by which an endophytic body can spread or be spread along the length of a vine. There is nothing like a liquid stream that would allow a parasite to circulate in the body of its host and nothing resembling fungal mycelia that are thin enough to spread through the intercellular spaces between host cells.

A hapaxanthic plant is one that grows from seed and flowers once only, at the end of its life. As a hapaxanthic plant terminating in a single flower, each *Rafflesia* flower would be a whole plant produced by one seed, growing where it is lodged on the vine. Repeat flowering would be the result of reinfection by seeds.

Evidence from the location of *Rafflesia* flowers at or close to the ground

Rafflesia flowers are found only at ground level and up to a few meters above ground, rarely higher. This suggests that the flowers are the product of seeds that are dispersed by animals that frequent the *Tetrastigma* vine at or close to the ground, not the product of endophytes with the ability to spread internally to all parts of the host plant.

Evidence from the disappearance of *Rafflesia* from *Tetrastigma* hosts in natural sites

There used to be a popular *Rafflesia* colony in the Royal Belum State Park, Malaysia, that was carefully protected by the construction of a raised boardwalk to enable visitors to view the flowers without trampling on the *Tetrastigma* host plants. This construction was a project carried out in about 2017 at a site known as 'X-Ray' (Mazlan et al. 2019). It was expected that the plants of *Tetrastigma* would continue to produce *Rafflesia* flowers indefinitely. However, six years have elapsed since the construction of the boardwalk, and *Rafflesia* flowers have failed to reappear (Zubir Ismail, staff member of the Royal Belum State Park *pers. com.*, 2023).

In their review of *Rafflesia*, Hidayati and Walck (2016) listed several disappearances of *Rafflesia* from *Tetrastigma* host plants in their natural sites. One was a site of *R. kerrii* in Khao Sok Nature Reserve, Thailand, visited in 1983 but 'not present' in 1989 (Meijer & Elliott 1990). Three sites of *R. arnoldii* in Taba Penanjung, Bengkulu, Indonesia, visited by Susatya (*pers. comm.* with Hidayati) in 2003 did not contain plants in 2014. Hikmat (2006) recorded eight sites of *R. zollingeri* in 1988 in Meru Betiri National Park, East Java, but only one site had *Rafflesia* in 2003. Suwartini et al. (2008) recorded 256 flower buds of *R. patma* from five sites in Pangandaran Nature Reserve, West Java, in 1989 but found only 96 buds in the same sites in 2007. These disappearances suggest that there is no persistent repeat-flowering endophyte within the *Tetrastigma* host plants and that repeat flowering requires reinfection with seeds.

Evidence from attempts to establish *Rafflesia* through propagation of infected host plants

According to Meijer (1997), there were several locations near Bogor that had natural populations of *Rafflesia* but these were all wiped out partly by land clearance but also by repeated attempts to transfer *Rafflesia*-bearing *Tetrastigma* host plants to the Bogor Botanic Garden. Some transferred plants produced flowers in their new location but eventually, they stopped producing flowers. According to Meijer, the last reports of *Rafflesia* flowering in Bogor were in 1929. Meijer also mentioned the case of an infected host plant transported to Leiden and grown in the greenhouse in its Botanic Garden, that flowered in 1853. There was no report after that.

In an experiment reported by Linton et al. (2005), six pieces of infected vines 50–60 cm long and one cm thick were 'whip-grafted' to uninfected vines in the Tenom Orchid Centre in Sabah. Whip grafting is done by making two cuts on the recipient vine, into which the two ends of an infected piece are fitted. After eight years, *Rafflesia* buds appeared, but there has been no report after that.

The best explanation for temporary success followed by failure to repeat flowering is that flowering is due to the development of *Rafflesia* seeds already embedded in the transplanted plants and that without reinfection by seeds, the host plant cannot continue to produce *Rafflesia* flowers.

Evidence from ontogeny

The *Rafflesia* flower has five spreading perianth lobes and a dome-shaped corona (Figure 1 & 2) arising from a central stem. Before the flower opens, the flowering structure is in the form of a cabbage (Figure 3 & 4), with tightly overlapping corona, perianth lobes, and over 20 non-photosynthetic leaves. In plant morphological theory, a leaf is a lateral bifacial outgrowth of a stem, and a foliage leaf is one of many possible expressions of a leaf, to which various names have

Figure 1 A newly opened flower of *Rafflesia cantleyi* displaying its 5 perianth lobes (petals) and its dome-like corona with a circular window at its apex

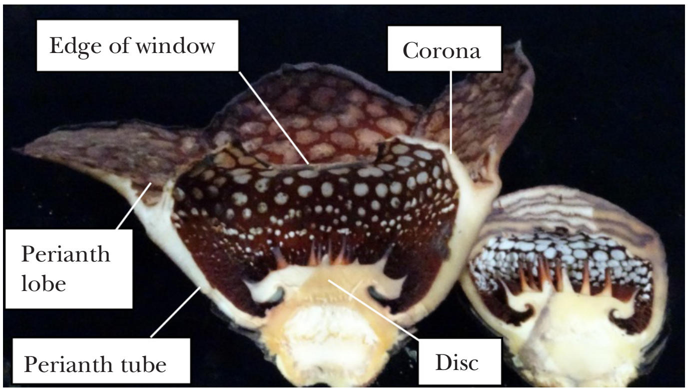

Figure 2 Newly opened flower (left) and shoot ball (right) in vertical section, the bracts having dried up and broken off

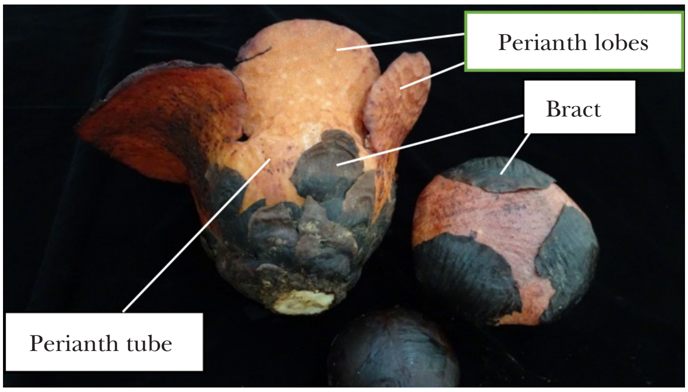

Figure 3 A shoot ball showing three bracts clasping the yet-to-open perianth (right) and a shoot ball with perianth lobes beginning to expand (left)

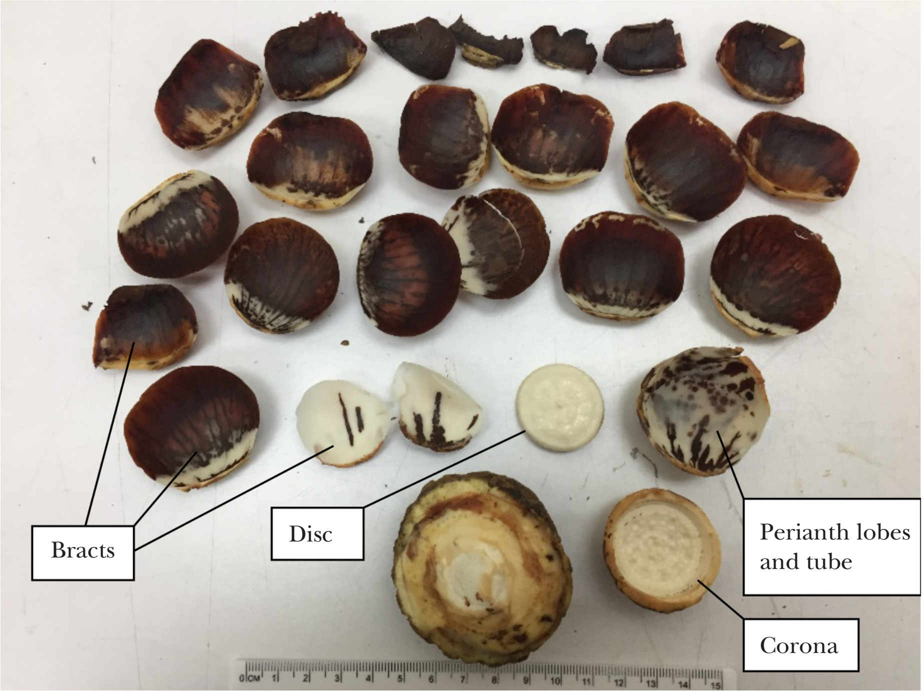

Figure 4 A 7 cm diameter bud dissected to separate its 23 bracts and perianth parts. Note the raised dots on the surface of the disc that will develop into finger-like processes and the impressions of those dots on the underside of the dome-shaped corona that has not yet expanded to reveal its window

been given, such as cotyledon, sepal, petal, tepal, stamen, carpel, scale leaf, sheath leaf, bract, or cataphyll depending on the form and function of the leaf and to some extent on the terminological preference of the author. The *Rafflesia* flower may be interpreted as a complete shoot system with a condensed stem bearing numerous bracts and terminating in a flower (Ng 2019).

The *Rafflesia* seed (Figure 5 & 6) is bilobed, up to 0.9 mm long, with an epidermis of a single layer of large thick-walled cells. Its distal lobe contains a small sac of undifferentiated thin-walled cells enclosed in a thin tough transparent membrane. The other lobe is the chalazal lobe, connected by a funicle to the placental tissues. The placental tissues are vertical anastomosing plates or columns packed into a large ovarian chamber (Fig. 7). I estimate at least 100,000 seeds per fruit. Other estimates are as high as 270,000 (Nais 2001).

The *Rafflesia* plant first becomes visible as a rounded bud 1.5–3.0 cm diameter protruding from its host vine. The bud is initially enclosed in bark tissue that is externally fissured into polygonal plates. In the vertical section (Figure 8), the basal part of the bud is in the form of a fleshy podium topped by a circular disc. The disc forms the apex of the *Rafflesia* flower and its upper surface bears small dots that will eventually become the finger-like processes

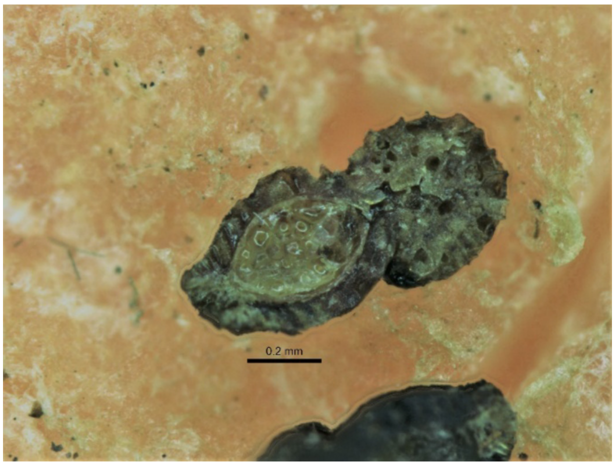

Figure 5 A seed in longitudinal section; the distal lobe containing a sac of thin-walled cells within a thin membrane

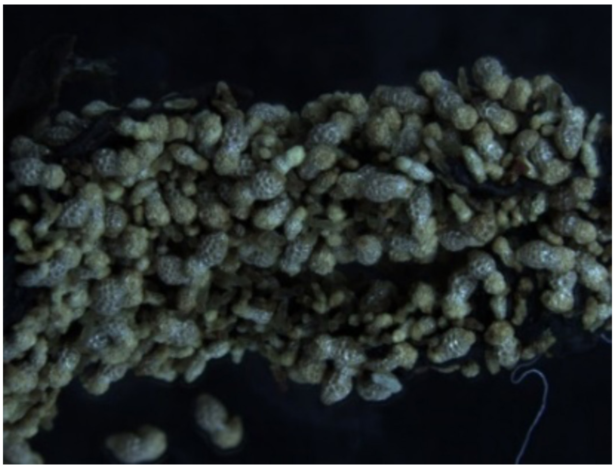

Figure 6 Seeds in surface view showing pattern of large epidermal cells

Figure 7 Vertical section of mature fruit showing seeds on the surface of vertical placental structures within a single large ovarian chamber

that are so characteristic of *Rafflesia* flowers. Around the disk are the overlapping layers of the embryonic corona, perianth lobes, and bracts. The underside of the edge of the disc provides the location for the stamens and the stigmatic band. The podium is the site of the embryonic ovarian chamber below the disc. The bud is therefore a complete plant in embryonic form. Subsequent development is a matter of differential enlargement and refinement of structures already predetermined.

The development of *Rafflesia* is best explained as an expression of hapaxanthy in which all its parts, including its flower, are formed during ontogenic development from seed. In contrast, the endophyte theory requires the somatic cells of an endophyte to do something theoretically impossible or highly unlikely, which is to resurrect defunct genes and restore genetic totipotency in order to produce embryonic plantlets.

Evidence from flowering data

As a general rule based on universal experience, the reproductive organ (flower/fruit) is the short-lived product of a longer-lived vegetative body. In practically all cases, the period from flower bud to fruit maturation is less than 12 months. However, in *Rafflesia*, the time span from bud to ripe fruit greatly exceeds one year. The recorded time spans are: *R. rochussenii* ca. 2.5 years (Zuhud et al. 1994). *R. patma*: 3–4 years (Hidayati et al. 2000), *R. arnoldii*: 3.5–5 years (Hidayati et al. 2016). These extraordinarily long time-spans do not support the theory that the *Rafflesia* flower is the ephemeral product of a long-lived endophyte. Instead, the 2.5–5 years' time span from bud to mature fruit is better interpreted as the life span of a female hapaxanthic plant.

The life-span of the male *Rafflesia* plant would end with flowering. This has been reported for several species, as follows:

R. azlanii: 40–50 days (Fatimah & Nor-Nafizah 2016).
R. arnoldii: 14 months (Olah 1960).
R. keithii: 13–17 months (Nais 2001).
R. pricei: 13–16 months (Nais 2001).
R. tengku-adlinii: 10–14 months in (Nais 2001).

All these measures are underestimates because we do not know how long it takes for the seed to reach the externally visible bud stage of about 2 cm diameter.

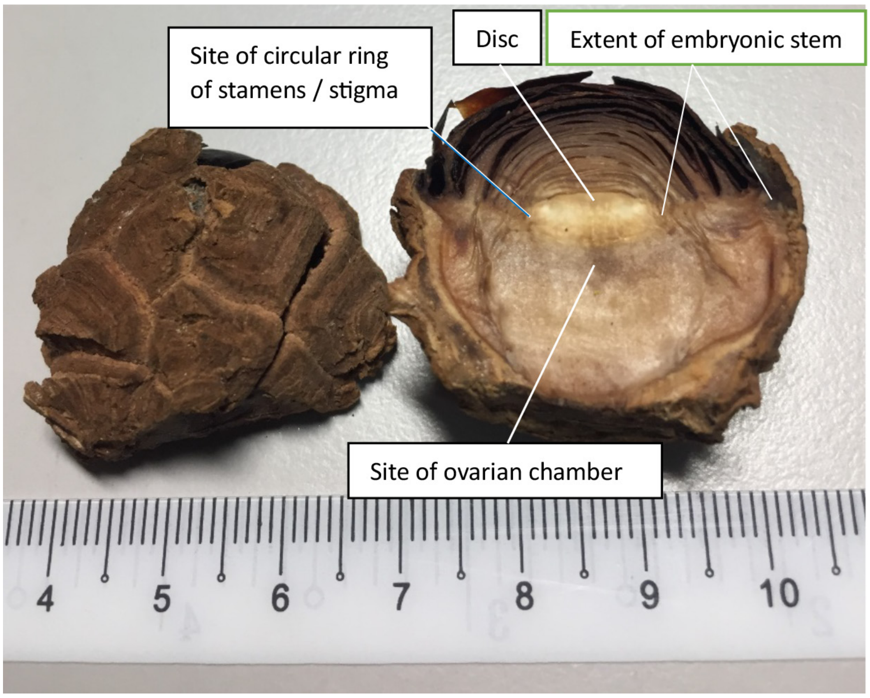

Figure 8 Vertical halves of a 3.5 cm diameter bud, showing the central axis and disc of the flower, topped by overlapping layers of embryonic perianth and bracts

Contradictory evidence from molecular biology

Barkman et al. (2017) analysed materials from 340 flowers representing three populations of *Rafflesia cantleyi*, two populations of *R. tuan-mudae,* and one population of the related *Sapria himalaya.* Most flowers were found to be genetically unique but they also found cases of flowers with the same genetic identity, spaced several metres apart. This is a matter that needs further investigation. Apomixis is a possibility.

Implications for conservation-management

The endophyte theory implies that *Rafflesia* is conserved as endophytes if the host plants are protected. This diverts attention from the role of seeds. If this theory proves to be false, the consequences for *Rafflesia* would be disastrous. In contrast, the hapaxanthic theory implies that every *Tetrastigma* site is an island with its population of *Rafflesia* sustained by a dynamic balance between the expiry of *Rafflesia* plants after flowering and the arrival of seeds. To ensure the conservation of *Rafflesia* we would need to improve our understanding of the sex ratio, the manner of seed dispersal, and the manner in which seeds germinate and establish themselves in the host.

Rafflesia flowers are unisexual and female flowers are usually outnumbered by males. Published figures are as follows:

R. keithii: 27% female (Nais 2001).
R. lobata: 14% female (Galang 2007).
R. manillana: 6% female (Yahya et al. 2010).
R. pricei: 36% female (Nais 2001).
R. tengku-adlinii: 50% female (Nais 2001).

Hidayati & Walck (2016) also had information that seven locations of *R. arnoldii* observed for three years produced only two female flowers. The sexual imbalance may affect conservation efforts.

The agents for seed dispersal need to be identified and protected. Shrews and squirrels have been observed feeding on fruits of *R. keithii* (Emmons et al. 1991). In my Institute, a captive tree shrew was fed with a ripe fruit of *R. cantleyi* after mixing the fruit placental pulp and its seeds with peanut butter. The animal ate the mixture but died the next day, suggesting that the seeds may be toxic to mammals. Since *Rafflesia* occurs in small patches even though *Tetrastigma* plants are common and widespread, the dispersal agent may be an animal with a limited home range, possibly an insect living in the bark of the host plant.

The minute size of *Rafflesia* seeds gives the impression that they have to germinate quickly or die but all attempts to induce germination artificially have not been successful. Nais & Wilcock (1999) tried to germinate seeds of *R. keithii* and *R. pricei* on filter paper and on filter paper soaked with tissue matter of *Tetrasperma leucostaphylum.* They terminated their experiment after four months. I tried to germinate *R. cantleyi* on moist tissue paper. I terminated mine earlier because the seeds deteriorated immediately after the epidermis had been cut. In my experience with other seeds (Ng 2014), cutting the seed coat does no harm and could promote germination, but *Rafflesia* is different. There are two different germination possibilities. One is for the seed to produce something that penetrates and spreads within the body of the host plant as an endophyte. The other is for the seed to develop directly into an embryonic hapaxanthic plant. This issue might be resolved by searching the bark of infected vines for evidence of germinating seeds.

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