Journal of Tropical Forest Science 37(2): 131–139 (2025)
https://doi.org/10.26525/jtfs2025.37.2.131
ISSN: 0128-1283, eISSN: 2521-9847

THE REPRODUCTIVE BIOLOGY OF RAFFLESIACEAE

Ng FSP

National Herbarium, Forest Research Institute Malaysia, 52105 Kepong, Malaysia

tropicalplantman@gmail.com

Submitted November 2024; accepted December 2024

Seeds in the family Rafflesiaceae do not germinate and produce seedlings. After their seeds get into their *Tetrastigma* hosts, their contents are released into the host tissues as strands or clusters of pro-embryonic cells. The clusters then develop into protocorms. A protocorm differentiates into an embryo by internal cleavage into the parts that become the various organs of the plant. Because all its organs are defined at the beginning of embryogenesis and no additions are made later, the plan of development in Rafflesiaceae is closed or determinate.

Keywords: closed versus open plan of development, embryogenesis, germination, parasitism, phanerogam, protocorm, *Tetrastigma*

INTRODUCTION

The family Rafflesiaceae, consisting of *Rafflesia, Rhizanthes,* and *Sapria,* is restricted to South-East and South Asia. The family includes the largest flowers in the world, measuring just over one meter in diameter. Its seeds are among the smallest, measuring about 0.9 × 0.5 mm. The seeds are produced in vast quantities, about 300,000 per fruit, but how they enter their host plants in nature is unknown. All species of Rafflesiaceae are completely parasitic. Of the thousands of genera of plants in its environment, only *Tetrastigma* is known to host Rafflesiaceae and only a small number of species of *Tetrastigma* are known to perform this role.

MATERIALS AND METHODS

This study is based on dissections of fresh specimens. For the finest dissections I use 'Gillette' razor blades, which are the sharpest and thinnest blades available. The chopping blocks are fresh pieces of carrot. One hand holds the specimen in place (with forceps or fingers) while the other hand holds the blade. I can slice a tiny seed into half by this method (Figure 1). The cut surfaces are clean, with the boundaries of tissues sharply defined. The carrot preserves the sharpness of the blade and provides the right amount of moisture to prevent small pieces from jumping and getting lost because of static electricity. The dissected specimens can be probed to determine the degree of bonding between adjacent tissues and the differences in their textures. I have used this method to document the fruits, seeds, and seedlings of over 600 species in 300 genera and 86 families of plants (Ng 2019).

Seeds and germination

The seed of Rafflesiaceae, as exemplified by *Rafflesia cantleyi,* is bilobed, measuring about 0.9 × 0.5 mm. One lobe is the swollen end of the funiculus that attaches the seed to its placenta. The other lobe contains a compact body of about 40 cells within a transparent protective membrane (Figure 1). This compact body is called the Brownian nucleus after Robert Brown (Ng 2019). All attempts to germinate *Rafflesia* seeds have failed and tissue culture methods and chemical treatments have had no effect (Nais 2001, Mursidawati et al. 2014, Molina et al. 2017). The cells inside the Brownian nucleus remain turgid and translucent even after months of storage under tropical room conditions, indicating that its protective membrane is very effective in preventing desiccation. However, we do not know how long the seeds retain viability because germination tests do not work on them. The failure of the seeds to germinate has been attributed to the absence of some key stimulant needed to trigger germination, but there is a morphogenetic reason. Germination involves the activation of apical meristems in an embryo to produce a seedling. In Rafflesiaceae the seeds do not contain an embryo and have no apical meristems.

This matter greatly troubled Robert Brown (1834) who wrote, "*I may here advert to one of the most difficult points in the economy of Rafflesiaceae, namely, by what means their minute embryos, which are at the same time of an extremely loose texture, are enabled to penetrate through the bark of the plants on which they vegetate, so as to account for such appearances as those exhibited in the nascent Rafflesia arnoldii ……in which I have been unable to trace any perceptible communication with the surface, and where the parasite seems rather to grow out of instead of into the stock.*" Brown observed embryos inside the host plant but could not explain how they got there.

Figure 1 *Rafflesia cantleyi* seed in longitudinal section, with the membrane of its Brownian nucleus indicated by an arrowhead

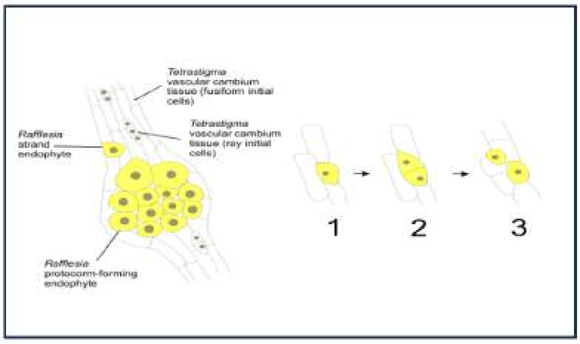

Figure 2 *Rafflesia patma* pro-embryonic cells detected in the cambium of *Tetrastigma leucostaphalum* (Mursidawati et al. 2019)

Under a microscope, *Tetrastigma* stems bearing *Rafflesia* buds have been found to contain short strands or uniseriate clusters of *Rafflesia* cells, identifiable by their large nuclei (Mursidawati & Wicaksono 2020). According to Wicaksono (*pers. comm.* 2024), the *Rafflesia* cells were found in the cambium and were observed on longitudinal sections of the *Tetrastigma* stem. They must be the contents of a Brownian nucleus released into the host plant by dissolution of the seed coat and the membrane of the Brownian nucleus.

Propagation of *Rafflesia* by inoculation with seeds has been carried out successfully at the Rafflesia Centre in Sabah for many years. Private growers nearby have also been propagating *Rafflesia* in their home gardens (Molina et al. 2017). However, *Rafflesia* buds only become visible two to eight years after inoculation by seeds. This indicates that pro-embryonic cells develop very slowly. Furthermore, they may move slowly through the stem of its host before settling down. This was indicated in an experiment in which a scion from a *Tetrastigma* stem infected with *Rafflesia* was grafted to a stock of a non-infected plant and *Rafflesia* buds appeared ten years later, at up to 205 cm from the point of grafting (Wicaksono 2017).

The protocorm

The protocorm is an ovoid body detectable externally as a small swelling on the surface of the stem of the *Tetrastigma* host (Ng 2024).

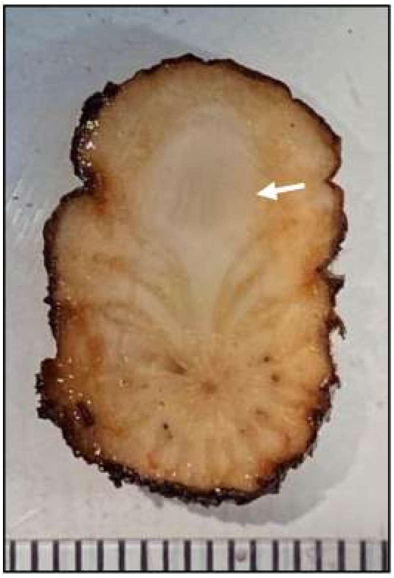

Figure 3 *Tetrastigma rafflesiae* freshly cut stem containing a protocorm (indicated by arrow-head) of *Rafflesia cantleyi*. Scale in mm

Figure 4 Same section as Figure 3 after allowing the tissues to oxidize overnight. The protocorm stands out as a translucent white body. Arrowheads indicate the line of cambial cells separating the inner woody core from the outer layer of bark

The position of the protocorm is precise. The stem of the host is divided by a line of cambium into an inner core of wood and outer layer of bark. The cells are organized in rays of vascular cells alternating with rays of parenchyma (Figures 3, 4 & 20). In the core, the vascular cells are xylem vessels whereas in the bark they are phloem vessels. The protocorm is embedded in the bark within a widened parenchyma ray, with its base in contact with the cambium. The protocorm is seamlessly united with the host and cannot be removed from the host without damage. The protocorm stands out visibly in Figure 4 because the section has been allowed to oxidise overnight and the *Tetrastigma* tissues darken while the protocorm remains translucent white.

Embryogenesis

The development plan of Rafflesiaceae is circular, with a central column functioning as an androgynophore, surrounded by a perianth and multiple bracts, all tightly packed upon a short stem-like podium. The perianth is divided into 5 lobes in *Rafflesia,* 10 in *Sapria*, and about 16 in *Rhizanthes*. The androgynophore bears the stamens and a stigmatic surface.

Figure 5 *Rafflesia cantleyi* male embryos at two stages of development. Arrowheads show (from top to bottom): layers of perianth lobes and bracts, the androgynophore, a stamen, the upper part of the podium which, in the female, would be the site of the ovary

Figure 6 *Rhizanthes infanticida* embryos in two stages of development Left: upper, middle and lower arrowheads show respectively the wall of the receptacle; the line of cambium in the host stem; the morphological centre of the host stem
Right: arrowheads show, from top to bottom: perianth lobes, androgynophore, a stamen, a bract, the upper part of the podium which, in a female, would be the site of the ovary

In *Rafflesia* the top of the androgynophore is expanded into a disc that overtops the stamens and stigmatic surface (Figure 5 & 10) but in *Rhizanthes* and *Sapria* there is no overhanging disc, and the stamens and stigmatic surface are on the sides of the androgynophore (Figures 6–9).

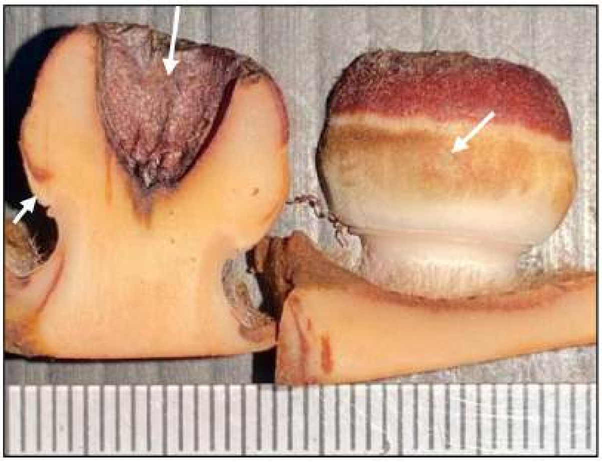

Figure 7 *Rhizanthes infanticida* young female
Left: androgynophore in longitudinal section, upper arrowhead indicates the space left by the emergence of the tips of the perianth lobes; lower arrowhead shows the flap of stigmatic tissue
Right: surface view showing the stigmatic surface (arrowhead) wrapped around the androgynophore

Figure 8 *Rhizanthes infanticida* male flower about to open. Arrowhead show (from top to bottom) perianth with its tips within the androgynophore; a stamen on the side of the androgynophore; abortive ovary

Figure 9 *Rhizanthes infanticida* male, showing stamens in a circle (indicated by arrowhead) around the androgynophore

As can be seen in Figures 5 & 6 the organs of the plant are defined by internal cleavage of the protocorm. Such a plan of development is said to be closed because no new organs are added later. The closed plan of development in Rafflesiaceae is most dramatically illustrated in *Rhizanthes* (Figure 6) in which the tips of the perianth lobes occupy the centre of the androgynophore. At anthesis, the perianth lobes straighten and their tips emerge, leaving a cavity in the centre of the androgynophore (Figure 7). The tips must have originated from the tissues in the centre of the androgynophore, not from outside and growing into a separately formed cavity.

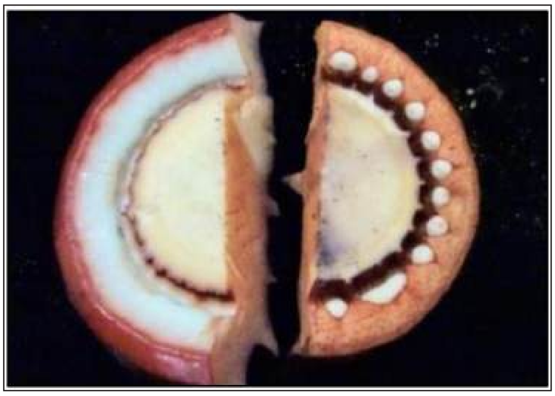

Figure 10 *Rafflesia cantleyi*
Left: underside of the rim of a female disc showing its stigmatic surface
Right: underside of the rim of male disc to showing its circle of stamens

As the embryo enlarges, it emerges through the bark of the host looking like a small cabbage. It consists of a flower bud in the centre covered by overlapping bracts. As the flower bud grows, the bracts take turns to grow and cover it. The outermost bracts cover the bud when it is smallest and the innermost ones cover the bud when it is largest (Figure 11). Eventually the flower bud emerges from the cover of the innermost bracts. It then opens to display its large perianth lobes (Figure 12). I think the *Rafflesia* 'flower' should be interpreted as a shoot system with a vegetative part of many bracts and a central solitary flower.

Figure 11 *Rafflesia cantleyi* cabbage dissected to show the bracts from outermost (smallest) to innermost, and the flower bud

Figure 12 *Rafflesia cantleyi*
Left: longitudinal section of female flower
Right: longitudinal section of male bud. Upper arrowhead shows the corona (also known as diaphragm); middle arrowhead shows the disc with processes on the upper surface; lowest arrowhead shows position of ovary

The ovary and fruit

In all three genera, the ovary is formed in the upper part of the podium (base of the androgynophore) by cleavage of previously solid protocorm tissue (Figures 13 & 14) into vertical plates of placentas that bear hundreds of thousands of ovules. In the male flowers the ovary is abortive.

Figure 13 *Rafflesia cantleyi* transverse section of an ovary with placentas formed by internal cleavage of previously solid protocorm tissue

Figure 14 Magnified vertical section of an ovary showing the placentas, bearing ovules

Figure 15 *Rafflesia cantleyi* fruits
Left: fruit, with remnants of the disc persisting on top
Right: older fruit with disk worn down

Rafflesia fruits are not formed from carpels and hence have no pericarp. In the absence of a pericarp, the fruit wall does not have the properties of pericarps such colour, scent, and resistance to decay. Brown (1821) included this passage from a letter by William Jack, '*The flower rots away not long after expansion and the seeds are mixed with the pulpy mass.*' Jack did not see any structure that he could recognize as a fruit. About five days after anthesis, the flower fades, collapses and begins to decay. However, in the female, the placental tissues remain alive for the ovules to mature into seeds but externally the fruit looks like dead wood (Figure 15).

The fruit is eventually broken, sometimes by small mammals that feed on its soft white placental tissues. Larvae of beetles and flies may also be found feeding on the placental tissues. After the *Rafflesia* tissues have rotted away only a woody receptacle remains. The receptacle is part of the xylem and phloem system of the host (Figure 16). In the case of *Rhizanthes* the perianth lobes dry up and the fruits look like dried flowers (Figure 17).

Figure 16 Receptacle formed by the *Tetrastigma* stem to accommodate *Rafflesia*. In this picture, the bark has been separated from the wood through the cambium and flipped over. The upper piece shows the xylem vessels and lower shows the corresponding phloem vessels

Figure 17 *Rhizanthes infanticida*. Top left shows a bud about to bloom. In the centre is an open flower placed face down. The rest are fruits in various stages of maturation

The significance of closed versus open plans of development

In a phanerogam, foliage leaves, bracts, sepals, petals, stamens, and carpels are formed in acropetal sequence by the activity of an apical meristem starting from a seedling. Since these organs originate in the same way, they are 'homologous' with each other. The principle of homology was the insight of Goethe (1790) and it represented such a big advance in morphological theory that Goethe is regarded as the founder of the science of morphology. A plan of development in which the body begins as a seedling and grows progressively by addition of organs is said to be open. An open plan allows a rooted plant to grow opportunistically to fill space in response to light and moisture, whereas a closed plan is characteristic of animals that can move from place to place in search of food.

In Rafflesiaceae, all the organs of the plant are defined by internal cleavage of a protocorm at the start of embryogenesis and no new parts are added later. Hence its plan of development is closed, making it fundamentally different from other plants. The stamens, stigma, ovary and seeds are all different in design from the corresponding organs in phanerogams (Ng 2019). Every feature when examined closely produces surprises.

The pollen grains are fluid, extruded through pores in the anthers of *Rafflesia* as bodies of viscous liquid within which the individual pollen grains are polygonal in shape due to mutual attraction but they slide freely around each other and form rounded bodies under surface tension. The individual grains lose their polygonal shape and become round when separated from each other in water. I have never seen other pollen grains behave in such a fluid manner. Immersion in water kills the pollen grains, reducing viability to 6% after 12 hours and zero after 24 hours. (Nais 2001). Pollen remaining in the anthers and free of water remain viable for 120 hours.

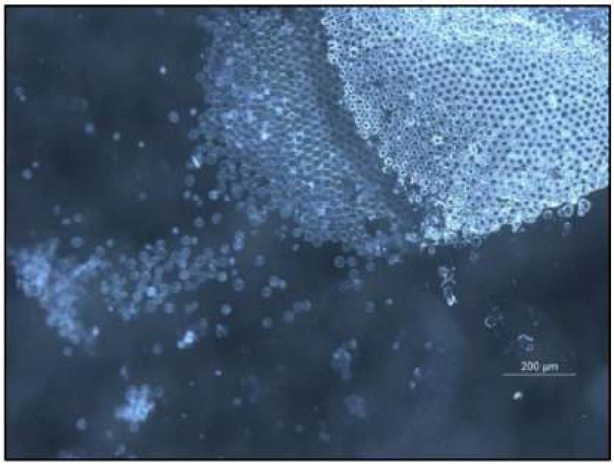

Figure 18 Pollen grains dissociating in water from a pollen blob. The outline of the grains changes from polygonal to round as they dissociate from each other

When Robert Brown described *Rafflesia arnoldii* in 1821, he classified it as a dicot because of its five-lobed perianth. Brown ran into difficulties when he tried to relate the structure of the ovary and stamens of *Rafflesia* to phanerogam and angiosperm models. He was baffled by the internal geography of the ovary. His comments in 1834 were *"The transverse section of the ovarium presenting an indefinite number of cavities irregular in form, having no apparent order, and over the whole of whose surfaces the ovula are inserted, is hardly reconcilable to the generally received type of the female organ…"* Brown also offered three interpretations to relate the stamens to other phanerogam stamens but was not convinced by any of them. In an amazing footnote to his second paper, in 1834, he wrote, *"In conclusion, therefore, it may perhaps be said that Rafflesia, in the structure both of ovarium and antherae, is not obviously reconcilable to any hypothesis hitherto proposed to account either for the origin or for a common type of the sexual organ of Phanerogamous plants."*

The botanical world ignored Brown's reservations and regarded *Rafflesia* as an angiosperm. Nikolov et al. (2014) claimed that *Rafflesia* has a shoot apex that "originates endogenously by formation of a secondary morphological surface" and that *Rafflesia* produces a seedling in which "the epicotyl dies soon after germination." In another paper, Nikolov and Davis (2017) included a page of diagrams (partially reproduced here as Figure 19) to illustrate how the organs in Rafflesiaceae are developed in sequence. My dissections (Figures 5, 6 and 7 show that all the organs in Rafflesiaceae are tightly packed from the start and can only have been formed *in situ* by internal cleavages in the protocorm. There is no trace of any shoot apex or seedling with an epicotyl. Anybody with a sharp blade and access to buds can see who is correct.

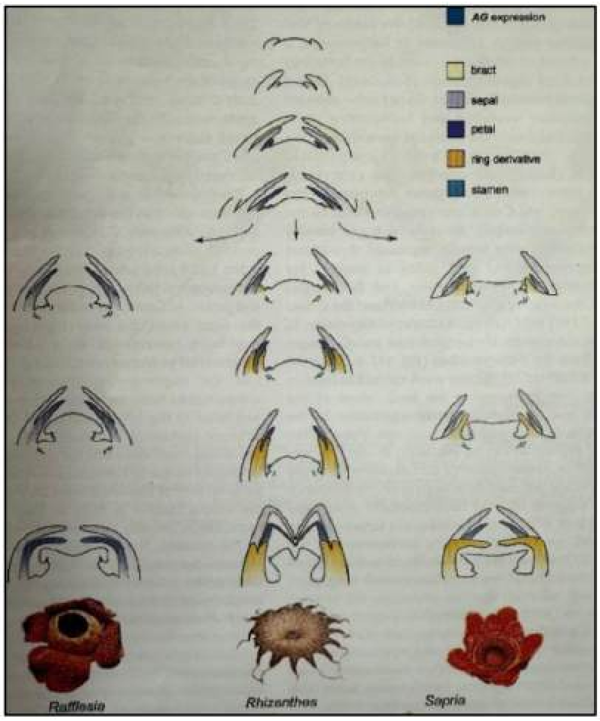

Figure 19 Part of a plate reproduced from Nikolov et al. (2014) to show the supposed sequence of development (from top to bottom) of the perianth and androgynophore in *Rafflesia, Rhizanthes* and *Sapria*

Does a mycelium-like endophytic vegetative body exist?

The theory that *Rafflesia* has a vegetative body living inside its host like a fungal mycelium was promoted by Kuijt who wrote in his book on parasitic plants (1969, p104). "*Evolution had stripped this parasite of irrelevant organs, leaving only an almost mycelial haustoria system and reproductive organs.*" On page 185, he further described the vegetative body as follows: "*The nature of the endophyte of Rafflesiaceae, constituting the entire vegetative body, defies description. The uniseriate filaments which form the youngest portion leave scarcely a tissue or an organ of the host unexplored. The endophyte has frequently been compared to a fungus mycelium, ramifying and anastomosing throughout the host.*" This was followed by Meijer's 1997 revision of Rafflesiaceae, which says that *Rafflesia* has an *endo*phytic body "*like a thallus inside the woody stems and root of the host plant*". There is an earlier study by WA Brown (1912) on *Rafflesia manillana* in which Brown reported the presence of *Rafflesia* cells as uniseriate strands of cells with large cell-nuclei permeating all the tissues of the host. However, the photographs by WA Brown are poor. What WA Brown saw were probably pro-embryonic *Rafflesia* cells.

If *Rafflesia* truly has an endophytic vegetative body ramifying all parts of the host stem, it should be easy to propagate *Rafflesia* by vegetative propagation of infected host stems and by now there should be gardens of cultivated *Rafflesia* all over the world. This has not happened despite numerous attempts documented in Meijer (1997) and Nais (2001). In the few instances of success, success has been short-lived and after the initial euphoria, there is no further news. There have also been failed attempts to establish *Rafflesia* in the Bogor Botanic Gardens by transferring *Rafflesia*-bearing *Tetrastigma* plants from their natural habitats to the Bogor Botanic Gardens. In addition, there are many cases of *Rafflesia* disappearing from where they used to occur (Siti Nur Hidayati & Walck 2016). There is a *Rafflesia* conservation site known as 'X-Ray' in the Royal Belum State Park in Perak, Malaysia, in which raised walkways were constructed in 2017 (Mazlan et al. (2019)). The *Rafflesia* flowers disappeared during the construction period and have not reappeared after seven years. There are cases of *Rafflesia* flowers reappearing months or years after their disappearance. Such reappearances seem to support the mycelium theory but there is a better explanation, that such plants contained dormant pro-embryonic cells. After these cells have been used up in flowering, there will be no more flowers.

The future for Rafflesiaceae

Every host plant will eventually die and any parasite it supports will die with it. Hence the survival of Rafflesiaceae depends on reinfection of new host plants with seeds. The mycelium theory appears to have no evolutionary significance but it has had the detrimental effect of distracting attention from the need to understand the dispersal of seeds and how they get into host plants. In Figure 20, a cluster of three seeds of *Rafflesia cantleyi* have been placed on a cross section of a stem of *Tetrastigma rafflesiae* to show how a 0.9 × 0.5 mm seed can easily fit into a split in the bark but this does not seem to happen in nature.

Figure 20 Three seeds of *Rafflesia cantleyi* (circled) placed on the phloem of a transverse section of *Tetrastigma rafflesiae;* arrowhead shows a split in the bark

Although *Tetrastigma* stems have been successfully inoculated with *Raffelsia* seeds the outcome of innoculation is unpredictable and inexplicable. Mursidawati & Wikaksono (2020) inoculated *Tetrastigma* with freshly harvested seeds of *Rafflesia patma* and reported that "Host plant inoculation in the natural habitat and at the Bogor Botanic Garden showed no sign of seed germination following 320 and 628 days past inoculation. The seeds were still fixed in their initial spots, looked fresh, and filled with viable tissue." Clearly, we are dealing with a phenomenon that needs a lot more study.

The lack of a seedling stage, the closed body plan with organs initiated by internal cleavage of a protocorm, and the unique structure and behaviour of the stamen, pollen, ovary, fruit, and seed, all indicate that Rafflesiaceae represents a branch of evolution (rafflesiosperms?) distinct from angiosperms and gymnosperms. Molecular taxonomists have placed Rafflesiaceae close to or within Euphorbiaceae but I have studied the fruits, seeds, and seedlings of 52 species in 27 genera Euphorbiaceae (Ng 2014) in detail and have found nothing to link *Rafflesia* with Euphorbiaceae.

Note: This paper is accessible free of charge from the FRIM/JTFS website or DOI link in which the images are in full colour and are enlargeable to show fine details.

ACKNOWLEDGEMENTS

This study was supported by Noorsiha Ayop and her team at the Forest Research Institute Malaysia that manages a *Rafflesia* conservation project in the Gerik District in the Malay Peninsula. Thanks also to the Librarian of the Singapore Botanic Gardens for copies of literature that I had difficulty accessing.

REFERENCES

Brown R. 1821. An account of a new genus of plants, named *Rafflesia*. *Transactions of the Linnean Society of London* 13: 1–42.

Brown R. 1834. Description of the female flower and fruit of *Rafflesia arnoldii* with remarks on its affinities, and an illustration of the structure of *Hydnora africana*. *Transactions of the Linnean Society of London* 19: 221–239.

Brown WA. 1912. The relationship of *Rafflesia manillana* to its host. *Philippines Journal of Science* 7: 209–236.

Goethe JWV. 1790. *Versuch die Metamorphose der Planzen zu erklaren. Available in English as Metamorphosis of Plants*. The MIT Press, Cambridge.

Kuijt J. 1969. *The Biology of Parasitic Flowering Plants*. University of California Press, Berkeley.

Mazlan MS et al. 2019. *Rafflesia Conservation and Management at Royal Belum State Park*. TNB Research Sdn. Bhd. Kuala Lumpur.

Meijer W. 1997. Rafflesiaceae. Flora Malesiana Ser.1. 13: 1–42.

Molina J et al. 2017. *Ex situ propagation of Philippine Rafflesia in the United States: Challenges and Prospects. Sibbaldia* No 15. Royal Botanic Garden Edinburgh, Edinburgh.

Mursidawati S, Irawati & Ngatari. 2014. *Rafflesia patma* (Rafflesiaceae): Notes on its field study, cultivation, seed germination and anatomy. *Bulletin Kebun Raya* 17: 9–14.

Mursidawati S, Wicaksono A & Teixeira da Silva JA. 2019. Development of the endophytic parasite *Rafflesia patma* Blume among host plant (*Tetrastigma leucostaphalum* (Dennst.,) Alston vascular cambium tissue. *South African Journal of Botany* 123: 382–386.

Mursidawati S & Wicaksono A. 2020. *Rafflesia patma*: conservation biology of a holoparasite. Indonesian Institute of Sciences (LIPI), Jakarta.

Nais J. 2001. *Rafflesia of the World*. Sabah Parks in association with Natural History Publications (Borneo), Kota Kinabalu.

Ng FSP. 2014. *Tropical forest fruits, seeds, seedlings and trees*. Forest Research Institute Malaysia, Kepong.

Ng FSP. 2019. Is *Rafflesia* an angiosperm? *Journal of Tropical Forest Science* 31: 286–297. https://doi.org/10.26525/jtfs2019.31.3.286

Ng FSP. 2024. *Rafflesia* realities and fantasies. *Journal of Tropical Forest Science* 36: 461–472. https://doi.org/10.26525/jtfs2024.36.4.461

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.

Nicolov LA et al. 2014. Floral structure and development in Rafflesiaceae with emphasis on their exceptional gynoecia. *American Journal of Botany* 101: 225–243.

Siti Nur Hidayati & Walck JL. 2016. A review of the biology of Rafflesia: What do we know and what's next. *Buletin Kebun Raya* 19: 67–78.

Wicaksono A, Teixeira da Silva JA & Mursidawati S. 2017. Dispersal of *Rafflesia patma* Blume endophyte in grafted host plant (*Tetrastigma leucostaphyllum* (Dennst.) Alston.). *Journal of Plant Development* 22: 143–150.