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Rafflesia harjatii (Rafflesiaceae) sp. nov., A New Species from East Kalimantan, Indonesia

(*Rafflesia harjatii* (Rafflesiaceae) sp. nov., Satu Spesies Baharu dari Kalimantan Timur, Indonesia)

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ABSTRACT

The vast and difficult to access region of Kalimantan is known to the home of eight species of *Rafflesia* including the arguable taxonomy status of three incomplete species. Flowering events of *Rafflesia* have been reported from East Kalimantan. However, their taxonomy statuses have not been verified. This information leads to the National Research and Innovation Agency (BRIN) to launch an initiative exploration investigating the taxonomy of *Rafflesia* in East Kalimantan. This exploration involved morphological comparisons and DNA sequencing analysis to determine the taxonomic status of the flowering *Rafflesia* found at ITCI-Kartika Utama Timber concession (ITCI-KU) in Sepaku, Penajam Paser Utara, East Kalimantan. The morphological comparisons between *Rafflesia* species from ITCI-KU and the eight *Rafflesia* recorded in Kalimantan showed the consistent differences with regard to the absence of windows, a single anulus, polymorphic processi, toadstool, and branched swollen apex of ramenta structures. It shares similarities with *R. tengku-adlinii* from Sabah, Malaysia, and *Rafflesia aurantia* from Luzon, Philippines, particularly regarding the absence of window structures, and the size of flowering. However, it differs from these two species in several diagnostic characters including the diaphragm openness, the structure and arrangement of ramenta, processus, column, groove, and annulus. The result of DNA analysis also confirmed that the *Rafflesia* from ITCI-KU differs from the all reported *Rafflesia* from Kalimantan. Thus, it has designated as *Rafflesia harjatii* sp. nov. An identification key to distinguish species of *Rafflesia* in Kalimantan is also provided.

Keywords: New taxon; Kalimantan; morphology; *Rafflesia*; ramenta

ABSTRAK

Wilayah Kalimantan yang luas dan sukar diakses diketahui menempatkan lapan spesies *Rafflesia*, termasuk tiga jenis tidak lengkap yang boleh dipertikaikan status taksonominya. Kejadian *Rafflesia* berbunga telah dilaporkan dari Kalimantan Timur, namun status taksonominya belum disahkan. Maklumat ini mendorong Agensi Penyelidikan dan Inovasi Negara (BRIN) melancarkan inisiatif penerokaan yang mengkaji taksonomi *Rafflesia* di Kalimantan Timur. Penerokaan ini melibatkan perbandingan morfologi dan analisis DNA untuk menentukan status taksonomi *Rafflesia* yang berbunga daripada konsesi Kayu ITCI-Kartika Utama (ITCI-KU) di Sepaku, Penajam Paser Utara, Kalimantan Timur. Perbandingan morfologi dengan lapan spesies Kalimantan yang direkodkan menunjukkan perbezaan yang tekal; termasuk ketiadaan tetingkap, anulus luaran tunggal, prosesi polimorfik dan toadstool bercampur dan ramenta apeks bengkak bercabang. Ia berkongsi persamaan dengan *Rafflesia tengku-adlinii* dari Sabah, Malaysia dan *Rafflesia aurantia* dari Luzon, Filipina terutamanya mengenai ketiadaan struktur tetingkap. Walau bagaimanapun, ia berbeza daripada kedua-dua spesies ini dalam beberapa aspek termasuk keterbukaan diafragma, struktur dan susunan ramenta, prosesi, ciri lajur dan anulus. Hasil analisis DNA menyokong hasil perbandingan morfologi. Oleh itu, *Rafflesia* berbunga yang diperhatikan di ITCI-KU mewakili species yang baru dikenal pasti dan ditetapkan sebagai *Rafflesia harjatii* sp. nov. Kunci pengenalan untuk membezakan spesies *Rafflesia* di Kalimantan juga disediakan.

Kata kunci: Kalimantan; morfologi; *Rafflesia;* ramenta; takson baharu

INTRODUCTION

The occurrence of *Rafflesia* on Kalimantan Island, also known as Indonesian Borneo, has been a subject of botanical inquiry for over a century (Erlinda, Iskandar & Widiastuti 2018; Koorders 1918; Lestari, Mahyuni & Iryadi 2020; Meijer 1997; Nais 2001; Sari et al. 2019). Recent research into the evolutionary relationships and geographical distribution of *Rafflesia* has indicated that this island may represent the ancestral habitat for various *Rafflesia* species (Pelser et al. 2019). Despite this, Kalimantan remains an underexplored region regarding the diversity and ecological aspects of *Rafflesia*, primarily due to its vast area and the difficulties associated with accessing *Rafflesia* habitats.

Botanists have engaged in extensive debates concerning the taxonomic classification of *Rafflesia* species found in Kalimantan. For example, *Rafflesia tuan-mudae* Becc., recognized as one of the largest flowers within the *Rafflesia* genus, is distributed across several locations, with its original discovery at Mt. Paoh in West Sarawak (Meijer 1997). Its range extends to Poteng Mountain in Singkawang, West Kalimantan (Erlinda, Iskandar & Widiastuti 2018), and Bukit Baka-Bukit Raya National Park in Central Kalimantan (Susatya 2011). Meijer (1997) classified *Rafflesia tuan-mudae* as a variant of *Rafflesia arnoldii* R. Br. based on similarities in the structures of diaphragm and ramenta. However, Nais (2001) and later Susatya et al. (2017) argued and recognized it as a distinct species, because of the differences in the window structures and wart patterns on the perigone lobes. The diaphragm's lower surface of *R. tuan-mudae* is exclusively covered by windows, whereas *R. arnoldii* exhibits both windows and ramenta in the same area (Susatya et al. 2017). Warts on *R. arnoldii* are characterized by two distinct sizes, with larger warts interspersed among smaller ones (Meijer 1997; Susatya 2007), while *R. tuan-mudae* features uniformly sized warts that are relatively spaced apart (Diway et al. 2022). Three additional species, namely *Rafflesia borneensis* Kds., *Rafflesia ciliata* Kds., and *Rafflesia witkampii* Kds., originating from Northeastern Kalimantan, were initially described by Koorders in 1918. However, Meijer (1997) did not recognize these three species as distinct entities, and categorized them instead as incomplete species due to the inadequacy of their herbarium specimens. A recent investigation conducted by Indonesia's National Research and Innovation Agency (BRIN) in collaboration with The University of Bengkulu showed that the original habitats of these three species have been transformed from forested areas to other land uses. Consequently, recollecting their herbarium specimens has become unfeasible, hindering the verification of their taxonomic status.

Among the eight named *Rafflesia* species in Kalimantan, four of them are recognized as distinct species including *Rafflesia hasseltii* Suringar, *Rafflesia keithii* Meijer, *Rafflesia pricei* Meijer, and *R. tengku-adlinii* Salleh & Latiff (Meijer 1997). *Rafflesia hasseltii* has been documented in Tanjung Datu, located at the border of Indonesia and Sarawak (Sofiyanti et al. 2007; Susatya 2011) as well as in Sambas, West Kalimantan (Sari et al. 2019). Its primary distribution extends from Kerinci-Seblat National Park and Bukit Tiga Puluh National Park in Riau (Sofiyanti et al. 2007) to Bengkulu in Sumatra, Indonesia (Meijer 1997). *Rafflesia keithii* has been identified in various sites, including the Croker Range, Ranau, Tenom, and Poring in Sabah, Malaysia. Its distribution appears to be restricted within Sabah, and there is no record of its presence beyond the Sabah region (Meijer 1984). *Rafflesia pricei* was first identified in the vicinity of the Mamut Copper Mine near Kinabalu, Sabah (Meijer 1997). Further investigations have shown two additional populations within Kayan Mentarang National Park in Northern Kalimantan Province, Indonesia (Jayasilan et al. 2004; Lestari, Mahyuni & Iryadi 2020). The occurrences of *R. hasseltii* in Tanjung Datu and Sambas, along with *R. pricei* in Kayan Mentarang National Park, respectively extend these species' geographical distributions to the east and south beyond their initial habitats. The most recently identified species from Borneo is *R. tengku-adlinii*, which was described in 1989. This species has a limited geographical distribution, and located on the eastern slopes of Mt Trusmadi and Gunung Lutong, at an elevation of 600 meters in Sabah, Malaysia (Mat-Salleh & Latiff 1989; Meijer 1997).

Recent efforts aimed at enhancing the understanding of *Rafflesia* taxonomy in Kalimantan have begun to yield encouraging outcomes. In 2022, a collaborative research initiative involving BRIN and the University of Bengkulu was launched to reassess the *Rafflesia* sites previously documented by Koorders (1918), Meijer (1997), and Atmoko (2014). The results of their investigation indicated that, although they could not locate any *Rafflesia* buds at the locations specified by Koorders and Meijer, they did observe flowering *Rafflesia* at Atmoko's site in November 2022. Atmoko (2014) had earlier reported the presence of *Rafflesia* within the forest concession area of ITCI-Kartika Utama (ITCI-KU) in Sepaku, East Kalimantan, but was unable to determine the species at that time, having only found several flower buds. Interestingly, nearly 34 years before Atmoko's observation, Smits (1980) documented flowering *Rafflesia* near a waterfall close to the sawmill and log yard at kilometer 41 of ITCI-KU.

The collaborative research also showed the presence of several flowering *Rafflesia* across six sites in proximity to the original locations identified by Smits. These specimens exhibited similarities to *R. tengku-adlinii* and *R. aurantia* Barcelona, Co & Balete. The geographical distribution of *Rafflesia tengku-adlinii* is known from the Trusmadi mountain range to Gunung Lutong, Sabah (Mat-Salleh & Latiff 1989). Meanwhile, *R. aurantia* has been documented in the Sierra Madre Mountain Range, located in Quirino Province in the Philippines (Barcelona et al. 2009). Both species are situated at considerable distances from the flowering *Rafflesia* observed at ITCI-KU in East Kalimantan. This significant geographical separation

raises speculations that the flowering *Rafflesia* at ITCI-KU may represent an undiscovered species. Therefore, we undertook comprehensive morphological and DNA assessments to clarify its taxonomic status.

MATERIALS AND METHODS

The study was conducted within the timber concession area of PT ITCI-KU located in Sepaku, Penajam Paser Utara, East Kalimantan. The research site was positioned adjacent to the logging road km 41, with an elevation ranging from 325 to 329 meters and a moderate slope of 10%-25%. The pH level and moisture content of the soil were measured using a portable soil testing device (DEMETRA PAT. 193478 E.M. System Tokyo, Japan) in a plot where *Rafflesia* was in bloom. A sample of soil pH was noted ranging between 5.4 and 7.0, while the soil humidity was recorded between 73% and 89%. This region was characterized by a secondary old-growth mixed-Dipterocarp forest. A herbarium specimen of male flowering *Rafflesia* was collected, examined, and subsequently deposited at Herbarium Bogoriense (BO). Comprehensive photographic documentations of this flowering *Rafflesia*'s structure were undertaken in the field, emphasizing the preservation of its natural and fresh morphological traits, including color and floral structures (Susatya et al. 2023). This documentation was essential due to the rapid changes in coloration and decay of the fresh flowering *Rafflesia* (Nais 2001). The morphological dimensions of *Rafflesia* were measured utilizing ImageJ software. A ruler was placed nearby by images, and used as a calibration reference for measuring the external structures of Rafflesia using ImageJ. The height of the disc rim and the processus, and the thickness of the toadstool ramenta were measured in the field to provide calibration references for the internal structures using ImageJ. The dimension values such as means, max-min, and standard deviations were reported where applicable.

A comparative morphological analysis between the observed species and other *Rafflesia* from Kalimantan was performed based on seven morphological characteristics, adhering to the methodologies established by Meijer (1997) and Susatya et al. (2017). The morphological characteristics examined included the presence of windows, the number of annuli, the structure and distribution of ramenta on the inner surface of the diaphragm and perigone tubes, the dimensions of the diaphragm, the patterns of warts on the diaphragm and perigone lobes, the number and structure of processus, and the number of anthers. The structure and distribution of ramenta were defined and described in accordance with the guidelines provided by Susatya et al. (2017).

To support the morphological comparison analysis and to establish the taxonomy status of the observed species, DNA analysis was conducted. Tissue samples from fresh perigone lobes and bracts of the flowering *Rafflesia* were collected in silica gel and deposited at BO. DNA was extracted using the Dneasy plant mini kit (Qiagen, Germantown, Maryland, USA) from these tissue samples. Two DNA regions, atp6 and matR were selected following the methods of Bendiksby et al. (2010) and Pelser et al. (2019) for phylogenetic analysis of *Rafflesia.* The atp6 and matR regions were PCR amplified by atp6 F and atp6 R, while matR F and matR R were amplified following Barkman et al. (2008). Phylogenetic analysis was performed for both atp6 (PP814626) and matR (PP814625). The DNA sequences were combined with the available *Rafflesia* sequence database at GenBank (Table 1) of National Institutes of Health (NIH) to establish the relationship between *Rafflesia* of ITCI-KU and the other *Rafflesia* species. Each locus was aligned independently using Muscle implemented in Mega 11 (Tamura, Stecher & Kumar 2021), and subsequently concatenated. Three outgroup species including *Sapria himalayana* Griff.*,* *Rhizanthes deceptor* Banziger*,* and *Rhizanthes infanticida* Banziger were used to construct the phylogenetic trees. The best nucleotide substitution model of GTR+I was determined by modeltest-ng v0.1.6. Maximum Likelihood (ML) phylogenetic reconstruction was performed with IQTree2 v2.1.4-beta (Minh et al. 2020) with 100,000 bootstraps. The generated tree was edited with TreeGraph 2 (Stöver & Müller 2010).

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DIAGNOSES

Rafflesia harjatii resembles to *R. tengku-adlinii* from Sabah, Malaysia, and *R. aurantia* from Luzon, Philippines, particularly with regard to the wart patterns, the coloration of the perigone lobes, and the absence of windows (Barcelona et al. 2009; Mat-Salleh & Latiff 1989). It can be distinguished from the other two species by several characteristics including the ratio of the diaphragm aperture to its diameter, the structure of the ramenta, and the quantity of annuli. This species exhibits the higher ratio of the diaphragm aperture to diameter compared to the other two species, and features slender and thick toadstool ramenta at the inner surface of its diaphragm, as well as a swollen apex and branched or lanate ramenta at the inner surface of its perigone tube. Furthermore, *R. harjatii* is characterized by a unique and single external annulus structure and possesses 34 polymorphic processi that are arranged in outer, mid, and inner concentric rings at its disc.

TYPE

INDONESIA, East Kalimantan, Penajam Paser Utara, Sepaku, East Kalimantan, Old-growth secondary mixed-Dipterocarp Forest, 0°58' 30.7" S, 116° 36' 03.3" E, 329 m altitude, 16 November 2022. Holotype: Ridha Mahyuni, Agus Susatya, Sudarmono, Dian Latifah, RASDL-ITCI 001 (BO). Male.

DESCRIPTION

Mature buds 8.4-10 cm. Male flower ca. 17-22 cm in diameter when fully opened. Perigone lobes 5.3-8.7 cm long, 7.6-9.0 cm wide. Warts light to dark orange, similar color to perigone lobes, irregular shapes, 0.3 cm to 0.5 cm wide. Perigone tube 5-6 cm high, light orange. Diaphragm pentangular to circular, 11.85-12.10 cm in diameter, 1.40-2.59 cm wide, the aperture 6.23-8.10 cm, the ratio of aperture diameter to the diaphragm 0.58-0.69, dark orange, darker than the color of perigone lobes, darker toward the diaphragm rims, numerous warts with a similar color to the diaphragm (Figure 1). Windows absent. Ramenta covering the inside surface just before the diaphragm rim to the bottom of the perigone tube, polymorphic structures: red maroon, slender and compound toadstools, 0.52-0.61 cm high, found across the inner surface of the diaphragm; red maroon slender toadstool, branched swollen apex, 1.1 cm high, swollen apex ramenta located across the inner surface of the perigone tube; swollen apex abundant at the lower part of the perigone tube (Figure 2). Disc 5.8-6.2 cm in diameter, milky white and gradually becoming dark red maroon toward its edge; disc rim 0.77 to 0.92 cm high. Processus 34, forming 3 concentric rings, outer, mid, and inner rings, respectively, consist of 18, 12, and 4 processi; varied in height, with outer, middle, and inner processi, respectively, 0.93-1.53 cm, 2.05 cm, and 1.82-2.45 cm high; polymorphic structures, rudimentary processus numerous, single or forming a group of two or three processi, arranged in a concentric ring, located close to disc rim; flattened with irregular truncate apex, flattened with spiky apex, cone, and spiky cone processi (Figures 1, 3). Central column 3.07-3.53 cm high; grooves running down from anthers at the disc to the base of the central column, canoe-shaped, 3.35-3.62 cm long, 0.41 cm at its widest, hairy. Anthers 20. Annulus interior absent, annulus exterior well developed (Figure 3).

ETYMOLOGY

The newly described species has been designated in honor of Mrs. Harjati Hashim Djojohadikusumo, recognizing her significant contributions to the field of nature conservation, particularly in relation to the forest habitat where this new species was found. For many years, Mrs. Harjati has played a pivotal role in leading various organizations, including the Yayasan Arsari Djojohadikusumo Nature Conservation and the Wadah Foundations, which promote a wide range of educational, social, and conservation initiatives.

DISTRIBUTION AND ECOLOGY

Distribution East Kalimantan, Penajam Paser Utara, Sepaku, 0° 58'30.7" S, 116° 36' 03.3" E, 325-329 m above sea level in an old-growth secondary mixed-Dipterocarp forest owned by ITCI-KU timber concession.

Habitat and ecology The new species was hosting on *Tetrastigma leucostaphyllum* (Dennst.) Alston ex Mabb. (Vitaceae) in an old-growth secondary mixed-Dipterocarp forest. The research site was previously logged over lowland Dipterocarp rain forests, and was part of the ITCI-KU timber concession. The dominant tree species included *Polyalthia tanuipes*, *Shorea laevis*, *Diplospora malaccensis*, *Neonaulea gigantea,* and *Xanthophyllum macrophyllum.* The new species was observed at 325 to 329 m above sea level with a moderate slope of 10%-25%, soil pH ranging from 5.4 to 7, and soil humidity at 89 %.

Flowering November-December.

TABLE 1. Genebank accession numbers of sequences used for the phylogenetic analysis[1]

Species	atp6	matR	Species	atp6	matR
Rafflesia harjatii [2]	PP814626	PP814625	*Rhizanthes deceptor*	EU882285	EU882323
Rafflesia patma	EU882270	EU882307	*Sapria himalayana*	EU882268	AY739006
Rafflesia gadutensis	EU882272	EU882309	*Rafflesia tuan-mudae*	LC647814	LC647813
Rafflesia pricei	EU882275	EU882312	*Rafflesia lobata*	MN055650	MN055670
Rafflesia speciosa	MN055647	EU882320	*Rafflesia schedenbergiana*	MN055652	MN055672
Rafflesia micropylora	EU882279	EU882316	*Rafflesia baletei*	MN055655	MN055675
Rafflesia kerrii	EU882278	EU882315	*Rafflesia consueloae*	MN055657	MN055677
Rafflesia arnoldii	EU882274	EU882311	*Rafflesia lagascae*	MN055663	MN055683
Rafflesia zollingeriana	EU882271	EU882308	*Rafflesia leonardi*	MN055659	MN055679
Rafflesia rochussenii	EU882269	EU882306	*Rafflesia manillana*	EU882282	EU882319
Rafflesia hasseltii	EU882273	EU882310	*Rafflesia mira*	MN055653	MN055673
Rafflesia tengku-adlinii	EU882280	EU882317	*Rafflesia mixta*	MN055651	MN055671
Rafflesia keithii	EU882276	AY453074	*Rafflesia philippensis*	MN055656	MN055676
Rhizanthes infanticida	EU882284	EU882322	*Rafflesia verrucosa*	MN055654	MN055674

[1]Source from National Institutes of Health (NIH); [2]Newly generated sequences (this study)

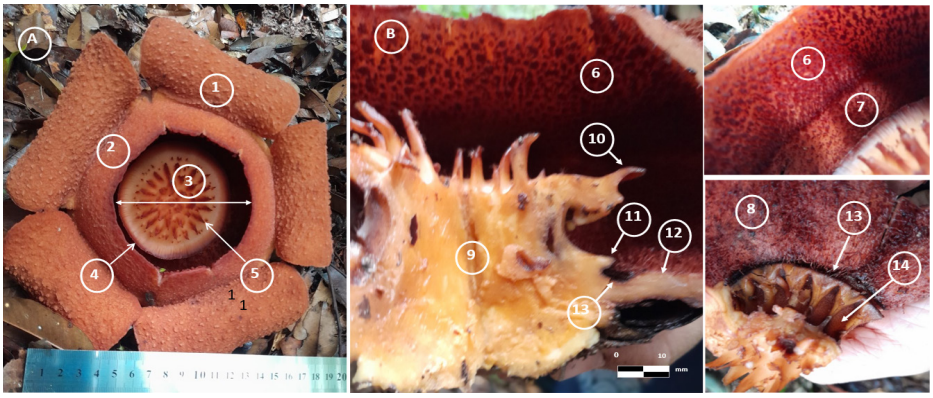

FIGURE 1. The flowering (A) and the inner view of *Rafflesia harjatii* (B). Perigone lobe (1), diaphragm (2), opening diaphragm (3), disc (4), and processus (5), the inner surface of the diaphragm without windows structure (6), the upper (7) and base (8) of the perigone tube, column (9), disc rim (10), the end of column (11), annulus exterior (12), gully (13), and groove (14). Specimen examined: holotype RSDL-ITCI 001. Photo: Agus Susatya

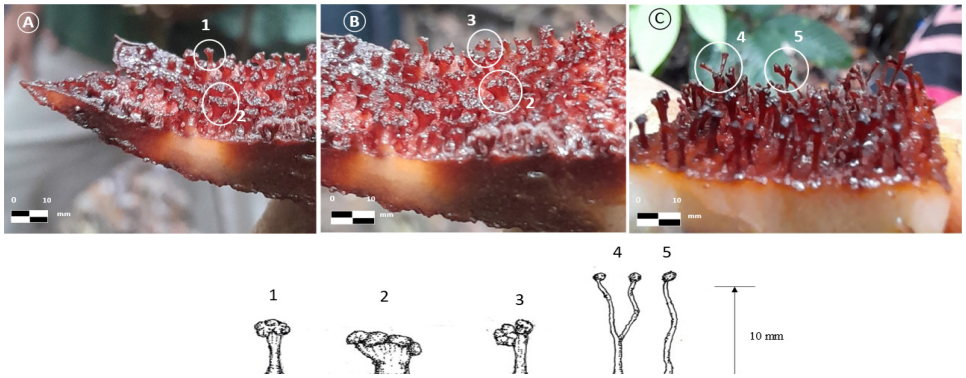

FIGURE 2. The ramenta structure and its distribution along the inner surface of upper (A) and lower diaphragm (B), and perigone tube (C). Slender toadstool (1, 3), thick toadstool (2), branched swollen apex ramenta (4), swollen apex ramenta (5). Specimen examined: holotype RSDL-ITCI 001. Photo and drawing provided by Agus Susatya

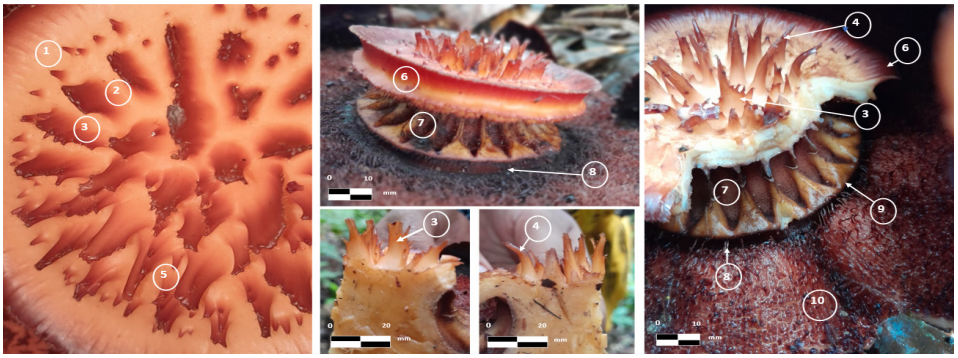

FIGURE 3. The detailed morphological structures of *R. harjatii*. Rudimentary (1), flattened processus with truncate apex (2), flattened processus with spiky apex (3), cone (4), spiky cone processus (5), disc rim (6), groove (7), gully (8), the end of column (9), and the lower part of the perigone tube (10). Specimen examined: holotype RSDL-ITCI 001. Photo: Agus Susatya

IUCN CONSERVATION ASSESMENT

The research location was established in a lowland rainforest that had previously undergone logging, but has since remained untouched by further logging activities. This area is categorized as an old-growth secondary forest. Following the identification of flowering occurrences of a newly discovered species, both the research site and its adjacent regions are being designated for conservation area. During our study, we identified six populations of this new species and estimated that there were no more than 50 individual flower buds present. Furthermore, our understanding of the species' geographical distribution is limited to this type locality. This newly described species is grouped as data deficient (DD) category, because of the available information is too limited to make reliable assessment of its conservation status (IUCN 2022).

RESULTS AND DISCUSSION

Species within the genus *Rafflesia* are typically characterized by ten distinct morphological traits, which include the dimensions of the flowers and buds, the wart patterns on both the perigone lobes and diaphragm, the diaphragm itself, the presence of windows on the inner surface of the diaphragm, the structure and distribution of ramenta on the inner surfaces of the diaphragm and perigone tube, the disc, the number and configuration of processus, the column, annulus, and anther (Beaman, Decker & Beaman 1988; Meijer 1997; Nais 2001; Susatya et al. 2017). These morphological traits serve as the basis for distinguishing the newly identified species, *R. harjatii,* from the other *Rafflesia* species especially found in Kalimantan.

Rafflesia harjatii can be distinguished from the four other distinct species found in Kalimantan, such as *R. hasseltii, R. keithii, R. tuan-mudae,* and *R. pricei*, based on several morphological features, including the size of flowering buds, the wart patterns on the perigone lobes and diaphragm, the presence of windows, the ramenta, and annulus structures. *Rafflesia harjatii* exhibits the smallest flowering *Rafflesia* among these species (Table 2). Its warts are numerous, small, and characterized by a dark orange color that matches the coloration of the perigone lobes and diaphragm. In contrast, the wart patterns of the other four species vary their sizes and patterns, and their colors from whitish to light orange against the darker orange perigone lobes. For instance, *Rafflesia keithii* features large warts interspersed with smaller ones, while *R. tuan-mudae* is characterized by uniformly spaced small warts on its perigone lobes (Diway et al. 2022; Susatya 2011). *Rafflesia hasseltii* has fewer but larger whitish to orange warts on its red maroon perigone lobes, and small dark orange warts at its whitish diaphragm (Nais 2001). Furthermore, *R. pricei* is noted for its large and occasionally confluent warts on both its perigone lobes and the diaphragm (Nais 2001). A significant morphological distinction between *R. harjatii* and the four other species lays in the presence of windows and the number of annuli. Specifically, *Rafflesia harjatii* has no windows on the inner surface of its diaphragm. Meanwhile, windows are present in the other four species. *Rafflesia harjatii* has only single annulus, while the other four species has two annuli (Table 2).

The ramenta structure of *R. harjatii* exhibits a variety of toadstool ramenta, a feature that is not present in the other three species, with the exception of *R. hasseltii.* Despite the similarities in the toadstool ramenta structures between *R. harjatii* and *R. hasseltii*, there is a notable distinction in the coloration of the apex of the toadstool. The ramenta of *Rafflesia harjatii* displays a consistent dark orange hue from the base to the apex, whereas *R. hasseltii* features a dark orange coloration with a contrasting white color at its apex. The importance of ramenta structure for distinguishing species within the genus *Rafflesia* is highlighted by Meijer (1997) and Susatya et al. (2017). Both emphasized that ramenta is a good diagnostic trait, attributing its consistent and invariant nature. Additionally, *R. harjatii* is characterized by a single exterior annulus, while the other four species possess both interior and exterior annuli (Table 2). The diagnostic significance of the annulus structure was first recognized by Koorders (1918) and later supported by Meijer (1997).

It is remarkably challenging to conduct a morphological comparison between *R. harjatii* and incomplete species such as *R. borneensis, R. witkampii,* and *R. ciliata*. The descriptions provided by Koorders (1918) for these three incomplete species were derived from immature buds preserved in alcohol, which were collected from the same site in Northeastern Kalimantan. These specimens exhibited fragmented and incomplete structures (Koorders 1918), which are inadequate for producing good and comprehensive descriptions (Meijer 1997). Analysing the morphological character data presented by Koorders (1918) (Table 3) shows that *R. harjatii* differs from these incomplete species with regard to the bud size, processus, ramenta, and the number of annuli. *Rafflesia harjatii* is characterized by smaller buds compared to *R. witkampii* and *R. ciliata*, while its size is comparable to that of *R. borneensis*. Additionally, *R. harjatii* displays a variety of ramenta types, ranging from swollen apex to toadstool forms, whereas the three incomplete species exhibit filiform ramenta with pointed and non-swollen apices. Furthermore, *R. harjatii* exhibitions polymorphic shapes of its processus including flattened, spiky, and conical forms, while the three incomplete species have only conical processus. Notably, *R. harjatii* features only an exterior annulus, while all the incomplete species were documented to hold both external and internal annuli (Table 3).

Among Kalimantan's *Rafflesia*, *R. harjatii* exhibits a notable similarity to *R. tengku-adlinii* in that both lack windows on the inner surface of their diaphragms. The absence of windows is a morphological trait shared by several *Rafflesia* species, including *Rafflesia patma* Blume (Meijer 1997), *Rafflesia zollingeriana* Koorders (Lestari & Mahyuni 2021; Meijer 1997; Susatya et al.

TABLE 2. Morphological traits of *R. harjatii, R. hasseltii, R. keithii, R. tuan-mudae,* and *R. pricei*

Trait	*R. harjatii*	*R. hasseltii*[1]	*R. keithii*[2]	*R. tuan-mudae*[2]	*R. pricei*[3]
a. Flower (cm)	17-22	35-70	80-90	44-92	25-37.5
b. Warts pattern	Small size, 0.3-0.5 cm, dark orange, similar colour to perigone lobes	Very large, size, 1-3 cm by 5-10 cm, 4-5 white wart across the base of the perigone lobe	Large circular warts interspersed by small wars, lighter orange color than dark orange perigone lobe, 10-12 warts	Large circular warts lighter orange color than dark orange perigone lobe, more than 10 warts	Large warts, confluence, 6-8 warts across the base of the perigone lobe, white to light orange color with dark orange perigone lobe
c. Diaphragm	Numerous warts with similar color to diaphragm	White to whitish orange colour, 1-2 dark orange warts across diaphragm	Lighter orange color than perigone lobes, dark orange warts forming 5 concentric rings	Light orange, numerous white warts with reddish brown margin	Much lighter orange color than perigone lobes, warts forming 4 rings, confluence and linear in the ring near the diaphragm perigone circular
d. Windows	Not present	3-4 concentric rings of white wart	5-6 concentric rings of white warts	6-8 concentric rings of circular to rectangular white warts near	4-5 concentric rings of with wart, without ramenta structure
e. Processus	32−34, polymorphic shapes	15-24, conical shapes	30-46, flattened processi	36-44, conical shapes	20-40, conical, and some flattened processi
f. Ramenta	Upper to middle tube: Red maroon slender and compound toadstools. Middle to lower tube: Branched swollen apex	Lower part of diaphragm: Compound toadstool. Upper perigone tube: Simple toadstool. Middle tube: Branched-toadstool, fascicle and swollen apex. Lower tube: Simple filiform	Middle to upper tube: Merged filiform. Lower to upper perigone tube: Filiform, 5-6 mm long, swollen apex ramenta	Upper tube: Fascicle, and branched apex and swollen apex (middle), swollen and unbranched ramenta, 6-10 mm long	Between diaphragm and perigone tube: Fence-like shape ramenta. Middle to upper perigone tube: Fascicle. Lower tube: simple swollen apex ramenta
g. Number of anthers	18─20	20	40	38-40	20
h. Annulus	Single annulus	2 distinct annuli	2 distinct annuli	2 distinct annuli	2 distinct annuli

[1]Meijer (1997); [2]Diway et al. (2022); [3]Nais (2001)

TABLE 3. The morphological characters of *R. hajatii* and three incomplete species of Kalimantan

Morphological characters	*R. harjatii*	*R. borneensis*[1]	*R. witkampii*[1]	*R. ciliata*[1]
Bud size (cm)	8.4-10 cm	7 cm	25 cm	17 cm
Warts	Numerous, small, 0.3-0.5 cm	Not described	Wart large, dense, rounded, 1.0-1.5 cm	Not described
Perigone tube (cm)	12 cm	13 cm	Not recorded	Not recorded
Diaphragm	1.40-2.59 cm, inside surface of diaphragm without wart	3 cm wide, fragmented specimens, 3inside surface without wart	Inside without wart/ without ramenta from base to near the upper edge	Inside scattered bristle hairs or glabrous without wart
Ramenta				
Size	0.5-1.2 cm	< 1 cm	Not recorded	0.5 cm
Structure	Red maroon slender and compound toadstools, branched swollen apex	Unbranched, pointed, and not swollen apex, scattered, small tubercle	Long and truncate filiform	Filiform, 0.5 cm high, simple, branched, acute apex
Disc diameter (cm).	5.8-6.2 cm diam	Fragmented, 8 cm diam	Not recorded	Not recorded
Disc rim	0.7-0.92 cm	Flattened rim	2 cm	Not recorded
Processus	32-34, polymorphic shape, flatted, spiky, conical shapes.	26, conical shape	Numerous processi, not recorded in number	Numerous styliform processi
Column	3.07 to 3.53 cm high	7 cm diameter, 3 cm high,	Not recorded	Not recorded
Anther	18-20	20 anthers	Not recorded	globular
Annulus	single	2 annuli, well-developed, winged annuli	2 annuli, interior annulus, winged shaped	Two annuli, wingless, internal annulus larger than external annulus

[1]Koorders (1918)

2023), *R. tengku-adlinii* (Mat-Salleh & Latiff 1989), *R. aurantia* (Barcelona et al. 2009), *Rafflesia consueloae* Galindon, Ong & Fernando (Galindon, Ong & Fernando 2016), *Rafflesia baletei* Barcelona & Cajano (Barcelona, Cajano & Hadsall 2006), *Rafflesia rochussenii* Teijsm. & Binn. (Jaarg 1851; Meijer 1997) and *Rafflesia meijerii* Wiriadinata & Sari (Sari & Wiriadinata 2010). *Rafflesia patma* (Meijer 1997), and *R. zollingeriana* (Susatya et al. 2023), both found on Java Island, are classified as mid-sized species, surpassing *R. harjatii* in size, with flowering buds measuring 30-50 cm in diameter for *R. patma* and 30-32 cm for *R. zollingeriana*, compared to 17-22 cm for *R. harjatii.* The primary distinguishing features among these three species lay in the wart patterns on the perigone lobes and the types of ramenta. Specifically, *R. patma* and *R. zollingeriana* are characterized by numerous light orange warts on dark orange or red maroon perigone lobes (Susatya et al. 2023), whereas *R. harjatii* displays a less distinct coloration between its warts and the background of its perigone lobes. Furthermore, the ramenta structures of these species differ significantly; *R. patma* and *R. zollingeriana* possess tuberculate ramenta, while *R. harjatii* features swollen apex, slender, and compound toadstool ramenta. Furthermore, *R. harjatii* can be differentiated from *R. rochussenii, R. meijerii,* and *R. consueloae* by the number of processi. It has 32-34 processi, in contrast to the near absence of processi in the other three species. When present, the processi in *R. rochussenii* are typically fewer than eight (Galindon, Ong & Fernando 2016; Meijer 1997; Sari & Wiriadinata 2010).

Rafflesia harjatii resembles *R. tengku-adlinii* and *R. aurantia* particularly in the dark orange color of the warts, perigone lobes, and diaphragm. Additionally, all three species lack the window structure on the inner surface of their diaphragms (Barcelona et al. 2009; Mat-Salleh & Latiff 1989). Nevertheless, *R. harjatii* can be distinguished from the other two species through several morphological features, including the degree openness of the diaphragm aperture, the number and arrangement of processus, and the structure of the ramenta. Although the diameters of the diaphragm of these three species are nearly identical, the ratio of the aperture to the diaphragm diameter of *R. harjatii* is the highest. This ratio shows that *R. harjatii* holds the widest aperture to its diaphragm diameter, and suggests that *R. harjatii* is the most pronounced openness among the three species (Table 4).

Rafflesia harjatii also exhibits the highest number of processi, ranging from 32 to 34, in contrast to *R. tengku-adlinii,* which has 18 to 25 polymorphic processi, and *R. aurantia* (Table 3). The dimensions of the processus in *R. harjatii* vary from 0.94 to 2.45 cm, surpassing those of *R. tengku-adlinii* and *R. aurantia*, which are limited to measurements of 0.5 to 1.0 cm. However, Mat-Salleh and Latiff (1989) did not provide specific measurements for the processus of *R. tengku-adlinii*. Barcelona et al. (2009) highlighted that the processi of *R. tengku-adlinii* are considerably smaller than those of *R. aurantia*. Both studies by Barcelona et al. (2009) and Mat-Salleh and Latiff (1989) identified the processi of *R. tengku-adlinii* and *R. aurantia* as exhibiting a flattened structure. A detailed analysis of *R. harjatii* shows a polymorphic processi, which includes not only various flattened forms but also other unique configurations. Specifically, *R. harjatii* is characterized by five distinct types of processus structures: rudimentary, flattened with a truncate apex, flattened with a spiky apex, cone, and spiky conical processi. The rudimentary processus can be found either alone or in small clusters of two or three, arranged in a discontinuous ring near the disk rim. The overall arrangement of the processus of *R. harjatii* forms three discontinuous rings, with the outer ring containing eight processi, the middle ring comprising twelve, and the central ring featuring six processi (Figure 3).

Ramenta serves as an important trait for distinguishing among *Rafflesia* species. Its consistent structure remains invariant across various climates and habitats (Meijer 1997). *Rafflesia harjatii* features both swollen apex ramenta, slender, and compound toadstool ramenta. According to the definitions provided by Meijer (1997) and Susatya et al. (2017), the mushroom-like formations of ramenta located on the inner surfaces of the diaphragm and perigone tube are identified as toadstool ramenta. In *R. harjatii*, both slender and compound toadstool ramenta are found on these two inner surfaces (Figure 2). In contrast, *R. tengku-adlinii* and *R. aurantia* lack these toadstool ramenta in the same locations, instead exhibiting lanate ramenta or swollen apex ramenta (Barcelona et al. 2009; Mat-Salleh & Latiff 1989). The lanate ramenta is exclusively located at the base of the perigone tube of *R. harjatii* (Figure 2). *Rafflesia tengku-adlinii* displays swollen apex or lanate ramenta extending to the rim of the diaphragm aperture (Mat-Salleh & Latiff 1989), whereas *R. aurantia* does not show similar lanate ramenta in the rim of the diaphragm (Barcelona et al. 2009). The arrangement of ramenta near the diaphragm aperture rim has been identified as a key distinguishing feature between *R. tengku-adlinii* and *R. aurantia* (Barcelona et al. 2009).

The other distinctive characteristics of *R. harjatii* include an annulus and canoe-like grooves that have not been observed in either *R. tengku-adlinii* (Mat-Salleh & Latiff 1989) or *R. aurantia* (Barcelona et al. 2009). In *Rafflesia harjatii,* a hairy, canoe-like grooves extend from the anther located in the disk to the base of the central column. Generally, this groove structure ends at the base of the central column, leading to a raised body that forms an interior annulus at the bottom of the perigone tube. However, *R. harjatii* presents a distinctive pattern where the canoe-like grooves end abruptly without creating an interior annulus, instead transitioning into a gully and an exterior annulus (Figure 1). This abrupt end of the groove and the lack of an interior annulus serve as additional distinguishing traits of this new species. The structure of the annulus has long been acknowledged as a crucial diagnostic feature for differentiating species within the genus of *Rafflesia*

(Koorders 1918; Meijer 1997). Koorders (1918) was the first to highlight the significance of the annulus structure in the taxonomy of *Rafflesia*, categorizing species based on the number of annuli present. Species with a single annulus are *R. zollingeriana, Rafflesia schanderbergiana,* and *Rafflesia manillana* (Koorders 1918). Initially, *Rafflesia* species characterized by two annuli included *R. arnoldii, R. patma, R. tuan-mudae, R. borneensis, R. witkampi,* and *R. ciliata* (Koorders 1918). Furthermore, Susatya (2007) identified an additional 15 species of *Rafflesia* that also possess two annuli.

Rafflesia harjatii also exhibits the largest number of processi, ranging from 32 to 34, in contrast to *R. tengku-adlinii,* which has 18 to 25, and *R. aurantia* (Table 3). The dimensions of the processus in *R. harjatii* vary from 0.94 to 2.45 cm, surpassing those of *R. tengku-adlinii* and *R. aurantia*, which are limited to the measurements of 0.5 to 1.0 cm. Mat-Salleh and Latiff (1989) did not provide specific measurements for the processus of *R. tengku-adlinii*. However, Barcelona et al. (2009) highlighted that the processi of *R. tengku-adlinii* are considerably smaller than those of *R. aurantia*. Both studies by Barcelona et al. (2009) and Mat-Salleh and Latiff (1989) identified the processi of *R. tengku-adlinii* and *R. aurantia* exhibiting a flattened structure. A detailed analysis of *R. harjatii* shows a polymorphic processi, which includes not only various flattened forms but also other unique configurations. *Rafflesia harjatii* is characterized by five distinct types of processus structures: rudimentary, flattened with a truncate apex, flattened with a spiky apex, cone, and spiky conical processi. The rudimentary processi can be found either alone or in small clusters of two or three, arranged in a discontinuous ring near the disk rim. The overall arrangement of the processus of *R. harjatii* forms three discontinuous rings, with the outer ring containing eight processi, the middle ring comprising twelve, and the central ring featuring four processi (Figure 3).

Ramenta serves as an important trait for distinguishing among *Rafflesia* species. Its consistent structure remains invariant across various climates and habitats (Meijer 1997). *Rafflesia harjatii* features both swollen apex ramenta, slender, and compound toadstool ramenta. According to the definitions provided by Meijer (1997) and Susatya et al. (2017), the mushroom-like formations of ramenta located on the inner surfaces of the diaphragm and perigone tube are identified as toadstool ramenta. In *Rafflesia harjatii*, both slender and compound toadstool ramenta are found on these two inner surfaces (Figure 2). In contrast, *R. tengku-adlinii* and *R. aurantia* lack these toadstool ramenta in the same locations, instead exhibiting lanate ramenta or swollen apex ramenta (Barcelona et al. 2009; Mat-Salleh & Latiff 1989). The lanate ramenta is exclusively located at the base of the perigone tube of *R. harjatii* (Figure 2). *Rafflesia tengku-adlinii* displays swollen apex or lanate ramenta extending to the rim of the diaphragm aperture (Mat-Salleh & Latiff 1989), whereas *R. aurantia* does not show similar lanate ramenta in that area (Barcelona et al. 2009). The arrangement of ramenta near the diaphragm aperture rim has been identified as a key distinguishing feature between *R. tengku-adlinii* and *R. aurantia* (Barcelona et al. 2009).

The other distinctive characteristics of *R. harjatii* include an annulus and canoe-like grooves that have not been observed in either *R. tengku-adlinii* (Mat-Salleh & Latiff 1989) or *R. aurantia* (Barcelona et al. 2009). In *R. harjatii,* a hairy, canoe-like grooves extend from the anther located in the disk to the base of the central column. Generally, this groove structure ends at the base of the central column, leading to a raised body that forms an interior annulus at the bottom of the perigone tube. However, *R. harjatii* presents a distinctive pattern where the canoe-like grooves end abruptly without creating an interior annulus, instead transitioning into a gully and an exterior annulus (Figure 1 & 3). This abrupt end of the groove and the lack of an interior annulus serve as additional distinguishing traits of this new species. The structure of the annulus has long been acknowledged as a crucial diagnostic feature for differentiating species within the genus of *Rafflesia* (Koorders 1918; Meijer 1997). Koorders (1918) was the first to highlight the significance of the annulus structure in the taxonomy of *Rafflesia*, categorizing species based on the number of annuli present. Species with a single annulus are *R. zollingeriana, R. schanderbergiana,* and *R. manillana*. Initially, *Rafflesia* species characterized by two annuli included *R. arnoldii, R. patma, R. tuan-mudae, R. borneensis, R. witkampi,* and *R. ciliata* (Koorders 1918). Furthermore, Susatya (2007) identified an additional 15 species of *Rafflesia* that also possess two annuli.

The sequencing analysis of the DNA for *R. harjatii* at the atp6 and matR regions shows an intriguing pattern in the phylogenetic relationship among species of *Rafflesia* from Kalimantan (Figure 4). The phylogenetic analysis clearly indicates that species of *Rafflesia* belong to two distinct clades; Sundaland and the Philippines groups. Despite of the unavailability of DNA sequence of *R. aurantia* at GenBank data base of NIH, we believe that this species most likely belongs to the Philippines group. This is because *Rafflesia* from the Philippines assembly into different clades according to their geographical origins due to limited dispersal capability and high endemism (Pelser et al. 2019). The Sundaland clade is further divided into Peninsular Malaysia-Kalimantan and Sumatera-Java subclades. *Rafflesia harjatii* is placed in the sub-clade of Peninsular Malaysia-Kalimantan, along with its five sister species: *R. keithii, R. pricei, R. kerrii* Meijer, *R. teuku-adlinii,* and *R. tuan-mudae*. Due to polytomy (Figure 4), *R. pricei, R. kerrii, R. teuku-adlini, R. tuan-mudae*, and the common ancestor of both *R. keithii* and *R. harjatii* have all evolved simultaneously from a common ancestor (Page & Holmes 1998). Interestingly, *R. harjatii* shares a common ancestor with *R. keithii,* not with *R. tuanku-adlinii.* The later has similarities to *R. harjatii* with regard to the flower size and the absence of windows. Both *R. harjatii and R. keithii* have distinctive morphological differences from

TABLE 4. Key characters of *R. harjatii* and *R. tengku-adlinii* and *R. aurantia*

Characters	*R. harjatii* (min-max; mean; stdev)	*R. tengku-adlinii*[1]	*R. aurantia*[2]
a. Mature Buds (cm)	8.4-10.0; 9.2; 5.8	13	8.5-9.5
b. Open flower (cm)	17.4-22.3; 20.2; 23.1	20-23.2	20
c. Perigone lobe			
1. Long (cm)	5.3-8.2; 6.7; 10.2	7-10	4.5-5.5
2. Width (cm)	7.6-9.0; 8.3; 4.9	12-16	6.3-7.7
d. Diaphragm			
1. Diameter (cm)	11.85-12.10; 11.6; 5.1	12.5	10
2. Width (cm)	1.40-2.59; 17.83; 2.84	5	Not recorded
3. Aperture (cm)	0.65-0.73; 0.68; 0.03	Not recorded	3.0-3.6
4. Aperture: diameter	0.58-0.69; 0.56; 0.05	0.42-0.44	0.36-0.37
e. Disc (cm)	5.8-6.2	7	5.5-6.0
f. Processus			
1. Number	32-34	18-25	Not recorded
2. Arrangement	Three concentric rings, outer, middle, and inner rings, respectively with 18,12, and 6 processi	Two concentric rings. Outer, and inner rings respectively with 17 and 8 processi	Not recorded
3. Structure	Polymorphic, rudimentary, flattened processi with truncate apex, flattened processi with spiky apex, cone, spiky cone	Not recorded	Polymorphic, variably branched, flattened processi
g. Ramenta			
1. Structure	red maroon slender toadstool, red marron compound toadstool, branched swollen apex, swollen apex ramenta	pustulate, sometimes clavate, branched with somewhat swollen apices at the opening, and thread-like form in the base of perigone	uniformly lanate, glabrous ramenta, slender, unbranched to furcate, entirely glabrous, tips swollen
2. Height (mm)	5-12	3.0-7.5	7-10
h. Number of anthers	18-20	20	12-14
j. Column (cm)	3.07-3.53 high, 3.41-5.75 cm wide	Not recorded	3
k. Annulus	single	Not recorded	Not recorded

[1]Mat-Salleh & Latiff 1989; [2]Barcelona et al. 2009

each other. *Rafflesia harjatii* is smaller with fewer processi and anthers than *R. keithii*. Furthermore, *Rafflesia harjatii* is characterized by the absence of windows and the presence of toadstool ramenta, while *R. keithii* has the windows at the lower surface of its diaphragm, but the absence of toadstool ramenta (Table 2). The fact that *R. harjatii* belonging to a different phylogenetic branch than *R. tuanku-adlinii* and the other four species from Kalimantan indicates the result of DNA analysis supports the morphological comparison analysis results (Figure 4).

Rafflesia keithii and *R. tengku-adlinii* is also from Sabah, the northern part of Borneo (Mat-Salleh & Latiff 1989; Meijer 1997), while *R. harjatii* is from the southeastern Borneo. These regions are separated by the Kalingkang-Kapuas Hulu mountains in the west and the Apau Kayan highland in the east. It is speculated that this geographical barrier has led *R. keithii, R. tengku-adlinii,* and *R. harjatii* to follow different speciation pathways.

IDENTIFICATION KEY FOR *RAFFLESIA* IN KALIMANTAN, INDONESIA

1. Windows absent .....……..……..…..2

Windows present .....……..……..…...…6

2.Swollenapexramentaabsent.....….…….....…….3

Swollenapexramentapresent...……...….....….5

3. Annuli wingless; mature bud 17 cm; warts large, round; pointed processi at disc; simple and branched filiform ramenta covering inside perigone tube, 5 mm long.... .....*R. ciliata*

Annuli winged ...……..…….....4

4. Thickened disc rim absent; ramenta simple and branched filiform at perigone tube, 5-7.5 mm long…… .....*R. witkampii*

Thickened disc rim present; ramenta tuberculate, < 1mm long.…..….....*R. borneensis*

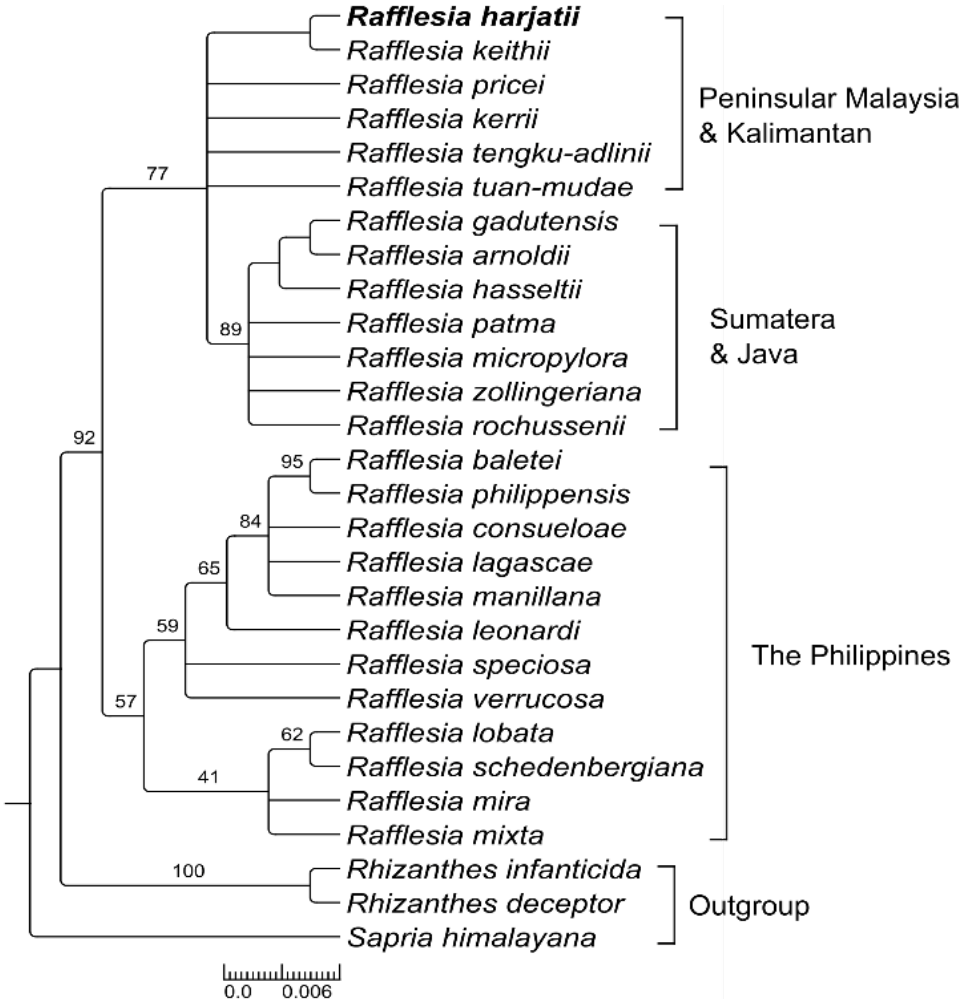

FIGURE 4. The maximum likelihood phylogenetic tree of *R. harjatii* based on DNA sequencing data of concatenated atp6 and matR regions. Numbers in each node is the bootstrap support (BS). Graph provided by Arief Priyadi

5. Toadstool ramenta absent; flower, 20-23.20 cm in diameter; diaphragm 5 cm wide; the ratio aperture to the diameter of the diaphragm 0.42-0.44; ramenta thread-like structure, 3-7.5 mm long; 18-15 processi; anther 20; annulus unknown……...…..... *R. tengku-adlinii.*

Toadstool ramenta present; flower,17-22 cm in diameter; diaphragm 1.4-2.6 cm wide, the ratio of aperture to the diameter of diaphragm 0.58-0.69; ramenta branched swollen apex; 32-34 polymorphic processi; anther 18-20, annulus one……..……...……..…..*R. harjatii*

6. Warts across perigone lobes more than …...……...…..7

Warts across perigone lobes less than ..…..…….....….8

7. Warts dimorphic, large warts interspersed with small warts; flowers 80-90 cm in diameter; windows partially covering the lower surface of the diaphragm; ramenta fascicled and branched, 5-6 mm long; processi 38-46. …….....……..…… *R keithii*

Warts monomorphic, equally distant from each other; flowers 44-92 cm in diameter; windows fully covering the lower surface of the diaphragm; swollen apex ramenta, 6-10 mm long; processi 36–44……. *R. tuan-mudae*

8. Confluent warts present at diaphragm; flower 25-37 cm in diameter; warts 6-8 across perigone lobes; diaphragm light orange with 4 concentric rings of warts; ramenta fascicled and branched, fence-like ramenta at the border between perigone tube and diaphragm present ……
…….....….*R. pricei*

Confluent warts absent at diaphragm; flowers 35-75 cm in diameter; warts 3-5 across perigone lobe; diaphragm whitish with 1-2 concentric rings of warts; ramenta toadstools, swollen apex, simple filiform, fence-like ramenta at the border between perigone tube and diaphragm absent ……
…….....….…..…. *R. hasseltii*

CONCLUSION

Rafflesia harjatii shares morphological similarities with *R. tengku-adlinii* from Sabah, Malaysia, and *R. aurantia* from Luzon, Philippines, particularly in terms of wart patterns, the coloration of the perigone lobes, and the lack of windows. However, it can be differentiated from these two species based on specific morphological traits, including the ratio of the diaphragm aperture to its diameter, the ramenta structure, and the number of annuli present. *Rafflesia harjatii* exhibits the highest ratio of diaphragm aperture to diameter among the three species, possesses slender and thick toadstool ramenta on the inner surface of its diaphragm, and features a swollen apex along with branched or lanate ramenta on the inner surface of its perigone tube. Additionally, this new described species is distinguished by a singular external annulus structure, in contrast to the two annuli found in the other two species. Furthermore, *R. harjatii* is characterized by the presence of 32 to 34 polymorphic processi, which are organized into outer, mid, and inner concentric rings on its disc. The results of DNA sequence analysis support the morphological analysis showing that *R. harjatii* differs to and is genetically distant to the other *Rafflesi*a species from Kalimantan.

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