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Morphological Variation of *Rafflesia zollingeriana* Koord. and its Recent Distribution in East Java, Indonesia

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Abstract. Locals in Lumajang have reported the growth of Rafflesia in Tempursari and Pasirian. Because species identification is fundamental for ecological monitoring, this study aims to identify species and record its geographical distribution. The Rafflesia at two locations were identified as *R. zollingeriana* after observing the specimens at the site and comparing them to herbaria from THBB and PHPB. This signifies that the distribution of *R. zollingeriana* in East Java has been updated. *R. zollingeriana* was formerly thought to only exist in conservation areas like Meru Betiri National Park and Watangan Nature Reserve, but this research demonstrates that it also exists outside of these protected/conservation areas. Only hills and steep cliffs are home to *R. zollingeriana* in Lumajang. This extremely limited and tough environment is assumed as a result of the conversion of forests into agricultural plantation, which only remained these sites for *R. zollingeriana* to grow. In order to prevent *R. zollingeriana* extinction in these areas, conservation strategies are required. This study offers conservation strategy recommendations, both in situ and ex situ. These recommendations expected could be used to establish a long-term conservation strategy for East Java's Rafflesia.

Key words: distribution, East Java, Indonesia, morphology, *Rafflesia zollingeriana*.

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INTRODUCTION

Species of Rafflesia is a unique biological entity because it has no leaf, no trunk, and true root system. The only observable organs are flower buds (knobs) and flowering Rafflesia. It is holoparasitic plant to one genus Tetrastigma (Barcelona et al., 2011). Its existence is not recognized until the knob grows and blooms successfully (Lestari et al., 2014a). As a result, Susatya (2020) categorizes the Rafflesia life cycle as having two stages: invisible and visible ones.

All species of Rafflesia are endangered due to their biological (Hidayati & Walck, 2016) and ecological characteristics (Lestari et al., 2014b; Susatya et al., 2017). Anthropogenic activities such as knobs exploitation (Fatmawati et al., 2018), illegal logging, the conversion of forest into large-scale agricultural uses, urbanization, and mass ecotourism put an additional threat to their extinctions (Fauzan et al., 2021; Wicaksono et al., (2016). Despite they are all endangered species, their biological and ecological attributes are unfortunately rarely studied. The comprehensive study of Rafflesia is difficult due to the lack of a large population size to observe routinely, the remoteness of Rafflesia sites, the irregularity of their flowering buds, and research fund (Tolod et al., 2021; Lestari & Rianto, 2017). Therefore, any new information of their biology is extremely valuable.

Basic data of Rafflesia is required as a reference point for attempts to conserve it, both in terms of the numbers and the preservation of its natural environment (Adnan el al. 2021), including its distribution. However, the distribution of three species of *Rafflesia, Rafflesia patma* Blume, *Rafflesia rochussenii* Teijsm. & Binn. and *Rafflesia zollingeriana* Koord., on the island of Java is rarely updated. The latest information about *R. patma* distribution was in 2017 (Triana et al 2017), *R. rochussenii* was in 2018 (Supartono & Herlina 2018) and *R. zollingeriana* was in 2018. *R. patma* reported not only distributed in Nusakambangan, Pangandaran, Leweung Sancang and Bojonglarang but also can

be found in Ciletuh, Sukabumi. *R. rochussenii* was not only distributed in Gunung Gede Pangrango National Park, but also can be founded in Kuningan, and *R. zollingeriana* is still exist near to Watangan Natural Reserve (Lestari & Mahyuni 2021).

Several people reported on social media about the flowering Rafflesia in Tempursari Lumajang, East Java in the middle of 2017. Three years later, there was another report regarding the blooming of Rafflesia on Mount Lincing, Pasirian, Lumajang. These reports must be verified, and the species must be recognized, as this is necessary before ecological monitoring can begin. Therefore, the objective of this study was to identify and morphologically describe the reported Rafflesia and record their geographical distribution. This study also offers in-situ and ex-situ conservation recommendations that could be used to establish a long-term conservation strategy for East Java's Rafflesia.

METHODS

Study Area

Two Rafflesia sites were visited to be observed their flowering Rafflesia. The first site was located at 450 m above sea level (asl) of the Argosari protected forest of Perhutani. The closest district to the site was Tempursari, Lumajang, East Java. The second site was located at 365 m a.s.l. of Lincing Mt, Bades, Pasirian Lumajang. Both locations were covered by secondary forests.

Plant Identification

The species was identified by observing the flowering Rafflesia in the site. The flower parts that observed were the perigone lobes, diaphragm, ramenta, disc, processi, annuli, and ovary. Those parts were measured with digital calliper and documented with a mirrorless camera and the dino-lite 3113 Series. The herbarium specimen was also collected in the first site, to get detailed examination in Tabanan Botanicus Baliensis Herbarium (THBB). All the results of the field observation and the examination on the herbarium were used to describe the reported species. The descriptions of all reported Rafflesia were morphologically compared to the collections of the specimens of *R. zollingeriana* at Tabanan Hortus Botanicus Baliensis (THBB) and Pasuruan Hortus Botanicus Purwodadiensis (PHPB) herbaria.

Rafflesia Geographical Distribution

Habitat coordinates were documented with GPS Garmin eTreck 10. These coordinates processed with ArcGIS 10.2 to update the previous *R. zollingeriana* distribution map in East Java.

Data Analysis

The data analysed descriptively. The data obtained is presented in figures, descriptions and tables.

RESULTS AND DISCUSSION

The two reported populations from Argosari forest respectively consisted of 4 individuals and 20 individuals, only one showed a blooming flower. The others were either decay or small diameter buds. Furthermore, one population found at Lincing Mt, consisting of a blooming flower, 5

Figure 1. Both Rafflesia lives on cliffs with a slope of more than 70 degrees. (a. Rafflesia in Argosari protected forest, b. Rafflesia in Lincing Mt (Rafflesia knobs signed with an arrow).

rotten flower and 1 bud with less than 11 cm in diameter.

The reported populations were located on relatively steep cliffs. Similar topography was also found in *R. arnoldi* and *R. rochussenii* in Kuningan (Supartono & Herlina 2018). In Bengkulu, the habitat of *R. arnoldi* close to human settlements, was usually in a steep terrain (Pariyanto & Hartati, 2021). It appears that all relatively flat terrains of a forest were converted to agriculture plantation, only steep cliffs remained and serving as Rafflesia's habitat. This is in line with Safniyeti et al. (2017) that the intensity of habitat exploitation activity has an impact on plant distribution.

Description

Mature female flower 30 – 33cm. ***Perigone lobes*** 5, size 8 – 9 cm with rounded apex. Perigone dominate by orange colour with regular pattern of white cream blotches. ***Diaphragm*** dominate by irregular white cream warts, with 13 – 21 cm in diameter. ***Opening* of diaphragm** 8 – 15 cm in diameter. ***Ramenta*** covered the perigone tube, from the lower surface of the diaphragm into bottom of the tube, tuberculate ramenta. ***Disc*** 7 - 9 cm in diameter, rim disc 0.4 – 1 cm high; rim disk orange, surface of the disc white cream. ***Processes*** orange, spiky flat cone, arranged in three rings. Processes range from 0.2 – 1.9 cm high, with some smooth spine at the top. ***Annulus*** covered with dense hairy*;* annulus exterior well developed, thick 4 mm; annulus interior weakly developed, 1 mm thick. ***Female flower*** with ovary in column, 2 cm from the cupula (flower base). **Anthers** unknown.

Rafflesia rochussenii, R. patma, and *R. zollingeriana* distributed in Java. One of these is believed to be Rafflesia of Lumajang. The presence of processii at the disc indicated that the reported Rafflesia did not belong to *R. rochussenii* (Figure 2) because *R. rochussenii* is distinguished from the other two species by its absence of processuss and the flat rim of the disc. There are only two options remaining: *R patma* or *R. zollingeriana*. Susatya et al., (2017) said that *R. patma* and *R. zollingeriana* have similar tuberculate ramenta structure. In term of distinctiveness of the species, Meijer (1997) considered *R. zollingeriana* as the variation of *R. patma.* He also mentioned that the only slightly different structure was the absence interior annulus or the very less developed interior on *R. zollingeriana*. However, Zuhud et al. (1998) and Susatya (2011) considered *R. zollingeriana* as a distinct species, not as a variation of *R. patma.* The

Figure 2. Rafflesia in Lumajang have some processi over the disk. (a) Rafflesia in Argosari grows right under the rock, so the flower is not perfectly round because the blooming process is obstructed by the stone; (b) Rafflesia in Lincing Mt.

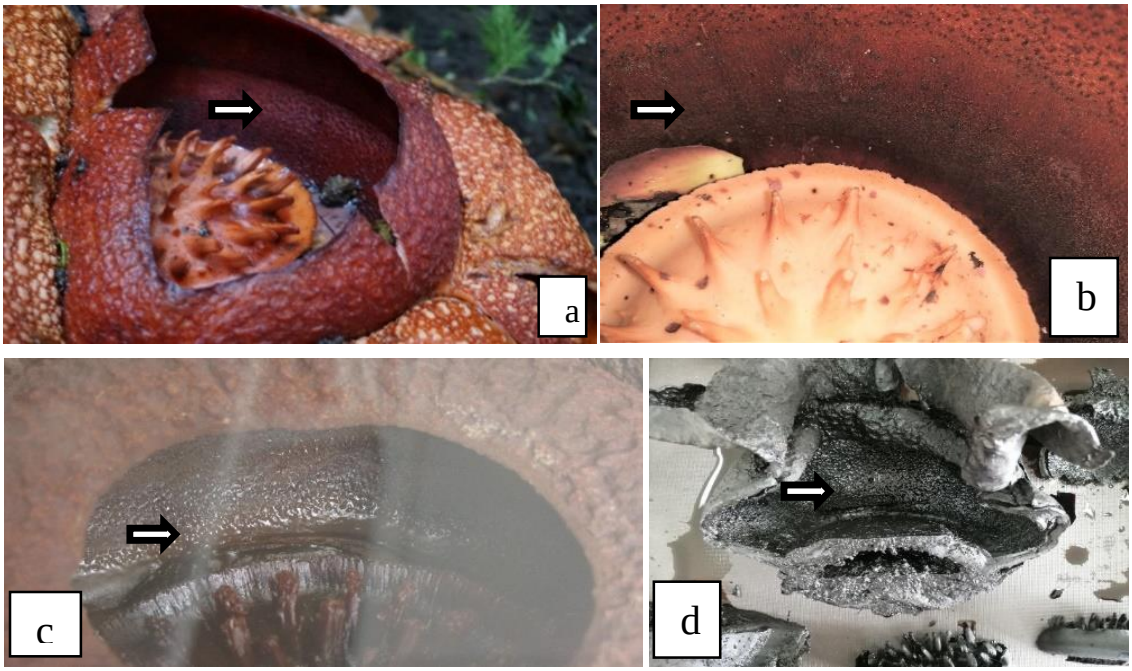

Figure 3. Ramenta covered the perigone tube, from lower diaphragma into bottom of the tube (a) Rafflesia in Argosari Protected Forest; (b) Rafflesia in Lincing Mt; (c) Wet specimen of *R. zollingeriana* in PHPB; (d) Wet specimen of *R. zollingeriana* in THBB.

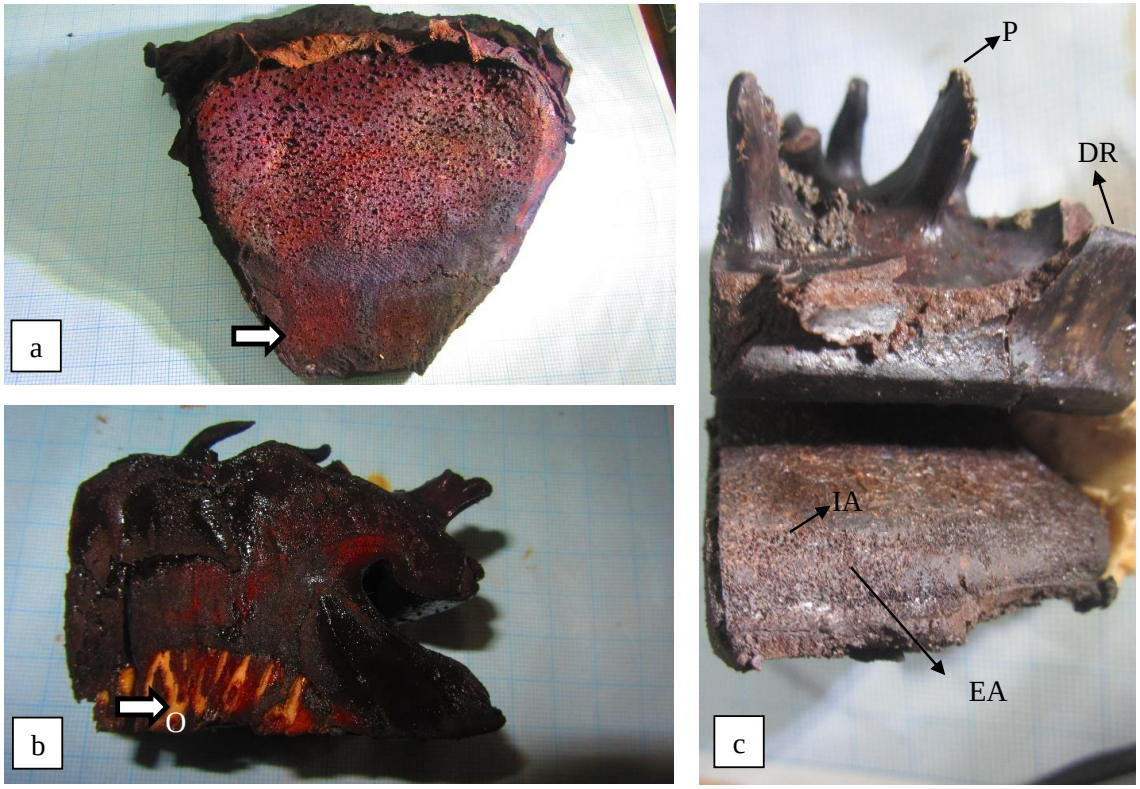

Figure 4. Rafflesia specimens from the Argosari Protected Forest (a) A perigone lobe revealed the ramenta from the lower diaphragma into the tube's bottom. Ramenta in the lower diaphragma is shallowly lobed tuberculate ramenta. Tuberculate ramenta in the middle of the perigone tube is longer than that of tuberculate ramenta in the bottom of the tube; (b) The presence of an ovary (O) at the base of the flower indicates that it is a female flower; (c). Processus was spiky flat cone, 1-6 mm long, disk rim 7 mm, exterior annulus (EA) well developed, 4mm width and interior annulus (IA) weakly developed, 1mm width.

Table 1. Morphological comparisons of *R. zollingeriana* of THBB, Argosari and Lincing Mt. Lumajang, East Java

Characteristics	***R. zollingeriana* wet specimen in THBB**	***R. zollingeriana* in Argosari**	***R. zollingeriana* in Lincing Mt**
Flower size	25x 27 cm	28x33 cm	26x30 cm
Number and size of perigone	5 10x12 cm	5 12x10 cm	5 10x11 cm
Diameter of the diaphragm	18x21 cm	15x21 cm	13x14 cm
Diameter of the opening of the diaphragm	17x 15 cm	15x8 cm	8 x 9 cm
Diameter of disc	9 cm	9 cm	8 cm
Number and length of processus	45 Size: 5-15 mm	37 Size: 2-19 mm	39 Size: 2-12 mm
White cream blotches on the perigone lobes and diaphragm	The diaphragm is dominated by irregularly white cream blotches, white cream blotches in perigone forming regular pattern	The diaphragm is dominated by irregularly white cream blotches, white cream blotches in perigone forming regular pattern	The diaphragm is dominated by irregularly white cream blotches, white cream blotches in perigone forming regular pattern
Colour on diapraghm and perigone lobes	white cream color dominated the diaphragm, orange color dominated the perigone lobes	white cream color dominated the diaphragm, orange color dominated the perigone lobes	white cream color dominated the diaphragm, orange color dominated the perigone lobes
Structure and length of ramenta and position of their occurrence	Tuberculate ramenta scattered in the tube, from lower of the diaphragm into bottom of the tube. Lower diaphragm covered by lobed tuberculate ramenta, the lower tube covered by simple tuberculate ramenta	Tuberculate ramenta scattered in the tube, from lower of the diaphragm into bottom of the tube. Lower diaphragm covered by lobed tuberculate ramenta, the lower tube covered by simple tuberculate ramenta	Tuberculate ramenta scattered in the tube, from lower of the diaphragm into bottom of the tube. Lower diaphragm covered by lobed tuberculate ramenta, the lower tube covered by simple tuberculate ramenta
Number of annulus	2, interior annulus less developed than exterior annulus	2, interior annulus less developed than exterior annulus	Not observed

presence or absence of the interior annulus can clearly be used and a strong character to differentiate both species. Later, Lestari & Mahyuni (2021) also reinstated *R. patma* and *R. zollingeriana* as a distinct species because of their differences in annulus, perigone and diaphragm pattern and distribution of their ramenta. Due to the ramenta that spread evenly in the inner surface of diaphragm into the inner of the tube, the perigone that dominated by orange color than white cream warts and the interior annulus that is less developed than exterior annulus, the reported Rafflesia are identified as *R. zollingeriana* Koord.

R. zollingeriana from Lumajang shares characters which similar to *R. zollingeriana* THBB, but showed some variations (Table 1). *R. zollingeriana* of Lincing Mt. appeared to have smaller diameter of diaphragm as well as the size of diaphragm openings. Both *R. zollingeriana* of Argosari and Lincing have fewer number and smaller size of processi than *R. zollengeriana* THBB. This variation is common in Rafflesia. *R. pricei* also has a variety of processi and diaphragm size (Lestari & Mahyuni, 2020).

Geographical Distribution Update

The presence of *R. zollingeriana* in Lumajang enriched and extended the geographical distribution of *R. pricei* in East Java, which has never been disclosed before. *R. zollingeriana* first described by Koorders from specimens that found in Watangan Puger, Jember, East Java (Koorders, 1918) and rediscovered in 5 km from Watangan Natural Reserve at 2018 (BKSDAE, 2018). *R. zollingeriana* can also be found in Meru Betiri National Park and its surrounding (Lestari et al., 2014b). Thus, *R. zollingeriana* in Lumajang is a new locality that potential to transform the approach to conservation schemes.

Conservation Strategy Recommendation

R. zollingeriana was formerly assumed to only exist in conservation areas like Meru Betiri National Park and Watangan Nature Reserve, but this research demonstrates that it also exists outside of these protected/conservation areas. *R. zollingeriana*'s habitat in Lumajang is in a local protected forest and community forest, close to residential areas. The majority of this forest has been converted into agricultural plantations, which produce corn, coffee, bananas, cloves, and other products. *R. zollingeriana* only exists on the slopes and cliffs. This habitat is scarce and vulnerable to landslides. The extinction of *R. zollingeriana* is likely to have been caused by this. To prevent *R. zollingeriana* extinction in these areas, conservation strategies are required, either the in-situ and ex-situ conservation strategy.

The in-situ conservation strategy proposed is building communities conservation awareness and developing ecotourism. According to Hidayati & Walck (2016), ecotourism is a viable option that

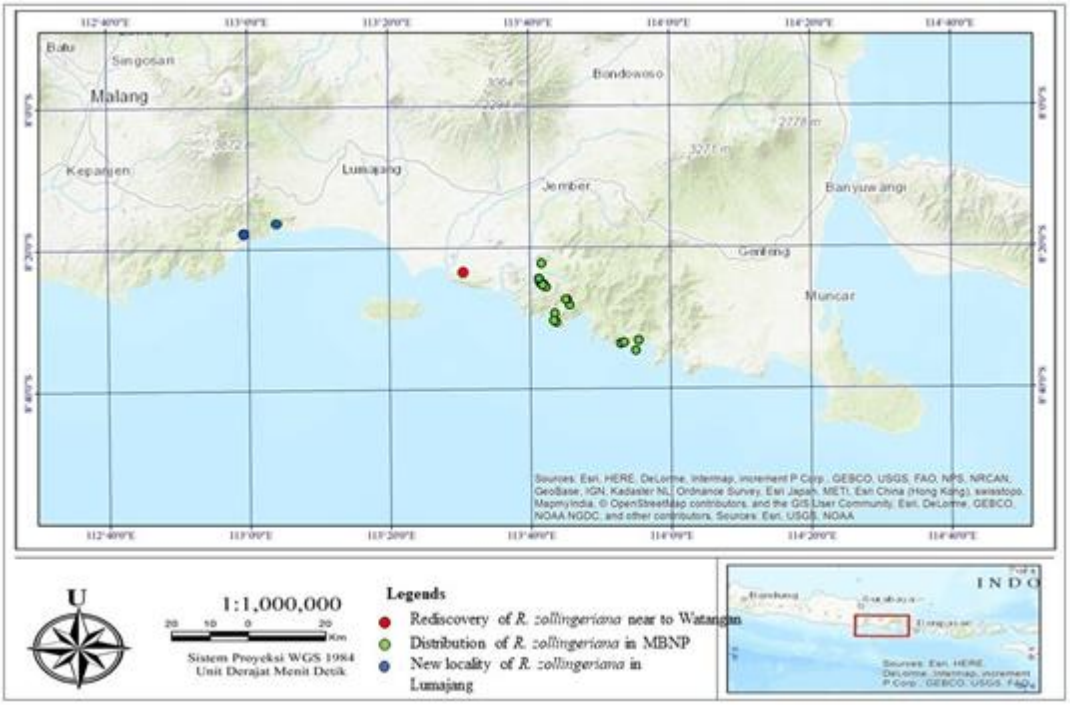

Figure 5. Updated distribution of *R. zollingeriana* in East Java.

provides an economic return to local people while not destroying habitat. According to the Minister of Environment and Forestry, the protection of Rafflesia is not only law enforcement but also public education (Mursidawati et al., 2015). Communities must be informed that the survival of the *R. zollingeriana* is living near them. They should not destroy or kill their host plants to keep the population alive because sustained Rafflesia is their pride and an important aspect of ecotourism (Lestari & Rianto, 2019). However, because the habitat in Lumajang is mostly on steep cliffs and is difficult to access, establishing ecotourism must be carefully planned.

Ex-situ conservation of *R. zollingeriana* is strongly suggested due to the severe external threat in the habitat. The presence of Rafflesia in ex-situ conservation areas is expected to be a solution to the difficulty of accessing Rafflesia in-situ for research (Tolod et al., 2021; Lestari & Rianto, 2017) and the lack of a large enough population to be observed regularly (Fauzan et al., 2021). Ex-situ conservation of Rafflesia is also expected to help with the provision of knowledge and data about the species, which is still scarce and shrouded in mystery. Studies of *R. patma* increased significantly after the plant was conserved ex-situ in the Bogor Botanical Gardens. Since then, about 15 study titles have been published that cover various aspects of *R. patma*.

Since 2006, Bogor Botanical Garden has been successful in conserving *R. patma* ex-situ using root grafting (Mursidawati et al. 2015). *R. zollingeriana* is hoped to be conserved in Purwodadi Botanical Garden as a result of this experience. Purwodadi Botanical Garden has been offered as an alternate ex-situ environment for *R. zollingeriana* since its traits are essentially equivalent to those of in situ habitats, namely lowland with higher temperatures and low humidity.

CONCLUSION

Rafflesia in Lumajang is identified as *R. zollingeriana*. These two habitats are new locality that updates distribution of *R. zollingeriana* in East Java. *R. zollingeriana*'s habitat in Lumajang is vulnerable to extinction and need to be conserved, either in-situ and ex-situ conservation. The in-situ conservation strategy proposed is building communities conservation awareness and developing ecotourism. The recommended ex situ conservation strategy is propagating *R. zollingeriana* to ex situ conservation areas, such Purwodadi Botanical Garden.

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