Final Evaluation Report

We ask all grant recipients to complete a project evaluation that helps us to gauge the success of your project. This must be sent in **MS Word and not PDF format**. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please DO NOT fill in and submit this form until the project has been completed.

Complete the form in English. Note that the information may be edited before posting on our website.

Please email this report to jane@rufford.org.

Your Details	
Full Name	Febrina Artauli Siahaan
Project Title	Ecological Investigation of Soil Microbiome in Rafflesia Zollingeriana Habitat: a Basis for Conservation and Preservation Action
Application ID	43116-1
Date of this Report	August 3, 2025

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Understand the composition, diversity, and potential functional roles of soil microbial communities associated with Rafflesia zollingeriana, and compare those found in infected and uninfected habitats assess their effects on the R.zollingeriana life cycle				Soil samples were collected in October 2024 from Papring Forest, Plot 68a, Sumbernanas Block, RPH Gombeng, Ketapang, area managed by Perhutani. Four sampling sites were systematically selected based on the presence of *Rafflesia zollingeriana* populations: three with *Rafflesia zollingeriana*-infected hosts (*Tetrastigma glabratum, T. rafflesiae, and T. dichotomum*), and one with uninfected host *T. glabratum* as a comparison site. Soil sampling was conducted carefully using gloves and a soil auger kit to avoid contamination. Samples were placed in sterile Falcon tubes, stored in a freezer box in the field, and promptly transferred to a refrigerator at the hotel, as the sampling process spanned several days. Environmental parameters were recorded at each site, and the chemical properties of the soil were analyzed. DNA extraction, PCR amplification, and Illumina NGS-based sequencing were performed, followed by microbiome analysis using QIIME2
To analyse interactions between R. zollingeriana, its host, and associated				We collected stems from three Rafflesia-infected host species (*Tetrastigma glabratum, T. rafflesiae,* and *T. dichotomum*), as

microbes				well as from one uninfected host species (*T. glabratum*). In addition, tissue samples of *R. zollingeriana* were also collected. All specimens were placed in sterile Falcon tubes, stored in a freezer box during field collection, and transferred to a refrigerator upon arrival at the hotel. DNA extraction, PCR amplification, and Illumina NGS-based sequencing were performed, followed by microbiome analysis using QIIME2. Microbial diversity analyses showed distinct community compositions and abundances among the stems of infected hosts, uninfected hosts, and the parasite. However, we are still working to identify the specific bacteria and fungi that play key roles in host–parasite interactions.
Educational campaign to increase the awareness through social media				All project activities were documented and disseminated through online platforms, primarily via social media. Additionally, we produced a short, animated video to raise public awareness, which was distributed through various digital media channels such as YouTube, Instagram, and community groups in facebook
Educational campaign to increasing awareness and understanding of *Rafflesia zollingeriana* conservation among local communities and relevant stakeholders.				We conducted an environmental education activity by inviting fellow conservation practitioner from Bengkulu to provide insights on Rafflesia ecotourism and strategies for establishing and sustaining community-based conservation initiatives. This activity included a presentation delivered to local communities, stakeholders; perhutani (which manage the area) and the

				Natural Resources Conservation Agency (BKSDA) under the Ministry of Forestry, which holds jurisdiction over the protection of endangered plant species.

2. Describe the three most important outcomes of your project.

a. Conducted ecological sampling, habitat characterization, population and microclimate data collection, in *Rafflesia zollingeriana* habitats as well as in sites with uninfected host plants

Four *R. zollingeriana* population sites were selected for soil and host stem tissue sampling, and one population site was selected for *R. zollingeriana* tissue sampling. The first site (RZ-01) was located on a riverbank slope, with host species were *Tetrastigma glabratum* (GB-01) and *T. rafflesiae* (Table 1 and Figure 1). Several individuals of *R. zollingeriana* were observed at different developmental stages, including buds (copule), bracts, and fruit (see Fig. 2). The second site (RZ-02) was relatively flat, characterized by dense bamboo leaf litter, with *T. rafflesiae* (RA-02) as the host. The third site, located at a higher elevation, contained several individuals of *R. zollingeriana* attached to *T. dichotomum* (TD-04). The fourth site was located a community garden approximately two kilometers from the nearest *R. zollingeriana* population. In this site we collected soil and stem samples from uninfected *T. glabratum*. In addition, perigone tissue from a blooming female flower (DP) was collected for microbiome analysis. This population consisted of eight female flowers: one in bloom and seven at the early fruit development stage.

Table 1. Sampling site Descriptions

Location code	Host	Elevation (m a.s.ll)	Slope (%)	Light intensity (lux)	Temperature (°C)	Relative Humidity (%)	Population *R. zollingeriana*
RZ-01	*T. glabratum* (GB-01), *T. Rafflesiae*	357	84	1009	29.1	68.5	3 copule , 4 bracts, 1 fruit, 5 post anthesis flower
RZ-02	*T. rafflesiae* (RA-02)	397	37	1213	31.2	73.4	1 copules, 1 bracts, 3 post anthesis flower
RZ-04	*T. dichotomum* (TD-04)	400	83	1026	32.4	67.6	2 copules, 1 post anthesis flower
NRZ-05	*T. glabratum* (NR-05)	327	0	3960	25	81.7	none
DP (perigone of *R. zollingeriana*)	*T. rafflesiae*	381	32	1870	32.1	68.6	1 female blooming flower, 7 post antheis flower, developing into fruits

Figure 1. Study Area of Rafflesia zollingeriana and uninfected host plant habitats.

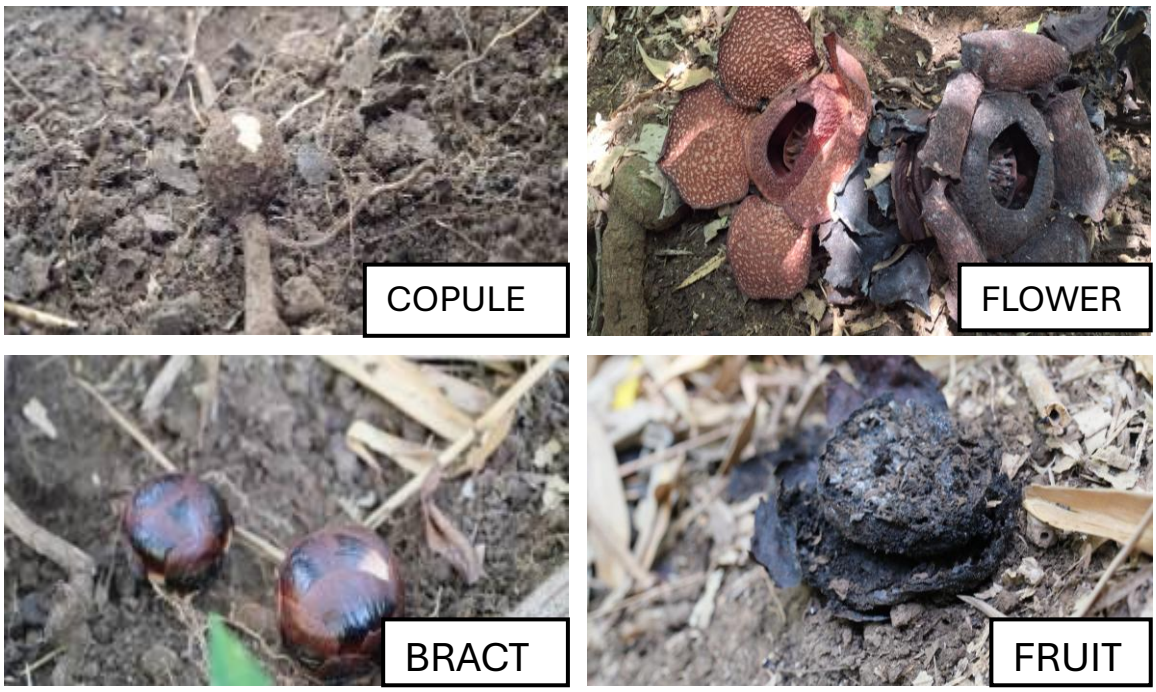


Figure 2. Developmental stages of *Rafflesia zollingeriana* - © febrina

Our soil analysis showed no significant differences between the soils in the infected and uninfected host plants. However, sulfur content was noticeably lower in the soil associated with uninfected hosts compared to the three other sites with infected hosts (Table 2).

Table 2. Soil chemical properties

Location code	Soil pH	Nitrogen [%]	Carbon [%]	Hydrogen	Sulfur [%]
RZ-01	87.55	0.27	2.35	2.013	0.101
RZ-02	84.93	0.41	4.25	1.904	0.067
RZ-04	86.90	0.28	2.71	1.560	0.062
NRZ-05	83.88	0.22	2.13	2.143	0.036

Figure 3. Sampling activities conducted in the habitat of *Rafflesia zollingeriana* - © Febrina

b. Composition, diversity, and potential functional roles of micobiomes in Infected and Uninfected Soils, infected and uninfected Hosts, and *R. zollingeriana*

We analyzed the microbiomes (bacteria and fungi) present in the soil of *Rafflesia zollingeriana* habitats and uninfected areas, as well as in infected and uninfected host plants tissue, and in *R. zollingeriana* itself. A total of 25 bacterial phyla and 14 fungal phyla were identified across soil, host, and *R. zollingeriana* samples. Figure 3a presents a comparison of the top 20 most abundant bacterial genera found in the host, parasite, and soil samples, including their presence in infected and uninfected tissues. We observed distinct differences in the abundance of several bacterial genera between infected and uninfected samples. The soil samples where Rafflesia population exists showed more diverse and even bacterial composition, compared to uninfected. Based on our data, we found the dominance of *Klebsiella* (63.1%) and *Pantoea* (20.3%) was observed in *R. zollingeriana* tissue, suggesting a potential specific bacterial association with parasitic tissue.

Our study also found that soil samples exhibited more even and diverse fungal distributions. In *R. zollingeriana* tissue, Penicillium and Cylindrocladiella (both Ascomycota) dominated the community, reaching 37.1% and 31.9% respectively, suggesting their strong association with parasitic tissues. Fusarium and Aspergillus were also present in moderate proportions (15.2% and 0.2%), while host samples revealed more variability with high levels of *Aspergillus*.

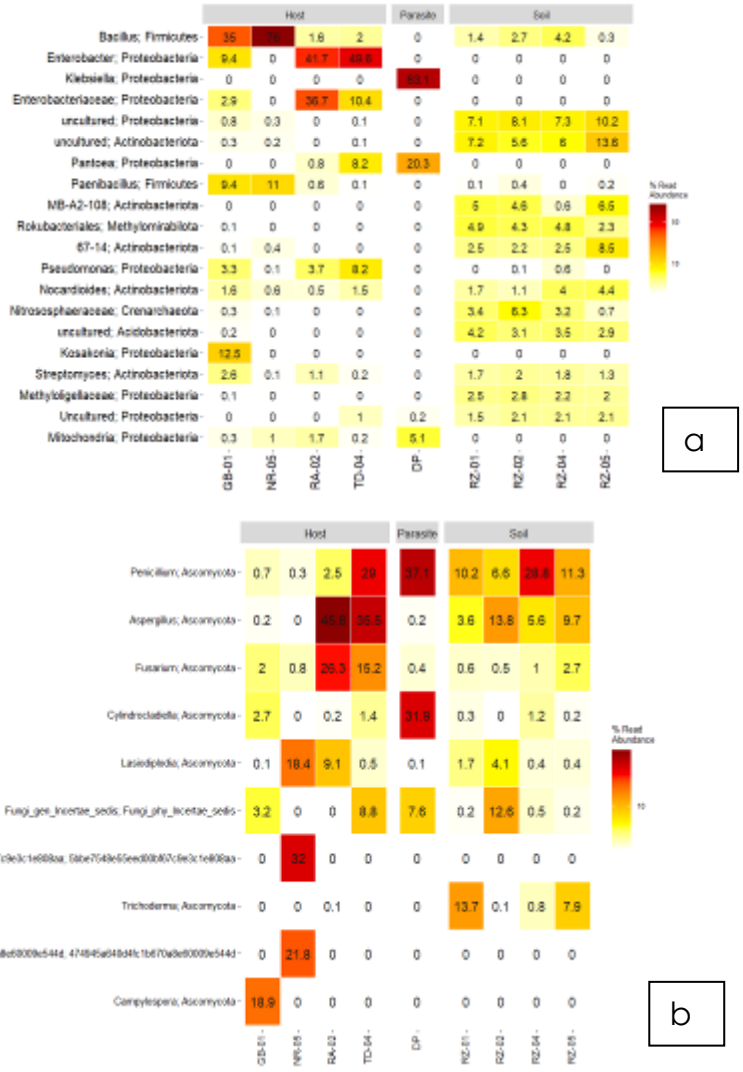


Figure 3. a Bacterial Community Composition in Host, Parasite, and Soil Samples , b. Fungal Community Composition in Host, Parasite, and Soil Samples of *Rafflesia zollingeriana*

c. Increasing Conservation Awareness and Stakeholder Engagement

Our educational campaign was conducted in two phases. In total, 54 participants attended the program. The age distribution was as follows: 17–25 years old – 8 participants; 26–35 years old – 14 participants; 36–40 years old – 9 participants; 41–45

years old – 13 participants; 46–50 years old – 6 participants; and above 50 years old – 4 participants. Among them, 81% were male and 19% were female.

In the first phase, we conducted presentations and discussions with key stakeholders, including Perhutani (the state forest management authority), BKSDA (Balai Konservasi Sumber Daya Alam – the national conservation agency under the Ministry of Forestry), local communities, and university students. This presentation attended by 37 people, with 16 people among them below 35 years old. This was part of an environmental education program focused on the biology *of R. zollingeriana*, the threats it faces, and practical actions that can be taken to protect this rare and iconic species. Many participants were unfamiliar with the biological nature of Rafflesia. They were surprised and intrigued to learn that Rafflesia is a holoparasitic plant with a unique life cycle and faces numerous threats to its survival.

Figure 4. Educational Campaign in Kampung Batara © Febrina

We also invited a conservation practitioner from Bengkulu: Septi Andriki, where community-based ecotourism has already been successfully implemented, to share his experiences and insights with the local participants. These sessions provided an overview of the need for collaborative, community-driven conservation efforts.

Figure 5. Septi Andriki, a Rafflesia conservation practitioner from Bengkulu, shared his experiences and gave a brief introduction on how to create Rafflesia-shaped souvenirs using simple materials © febrina

In the second phase, we organized a guided field visit to introduce *R. zollingeriana,* its habitat and host plants (*Tetrastigma* spp.) in Papring Forest, Plot 68a, Sumbernanas Block, RPH Gombeng, Ketapang. A total of 27 participants took part in this phase, the majority of whom were young people. Specifically, 18 participants were under the age of 35, while the rest were above 35 years old. We limited the number of people entering the forest due to the challenging terrain and to prevent damage to the *Rafflesia zollingeriana* population from trampling or other disturbances. In this field visit we primarily targeted young people living in the communities surrounding the forest area.

Participants were fortunate to witness three flowers in full bloom, offering and impactful firsthand experience. During the field trip, we practiced identifying the floral morphology of *Rafflesia zollingeriana*, showed the life cycle stages of *R. zollingeriana*, and explained how to distinguish between male and female flowers. We also showed representatives from Perhutani that over 30 populations *R. zollingeriana* flowers and 3 different host plants exist within their managed forest area, stated the urgent need for habitat protection and floral monitoring. We also educated local communities on the importance of not cutting the stems of host plants, to ensure the *Rafflesia zollingeriana* population attached to them remains undisturbed. To evaluate the impact of our campaign, we conducted pre- and post-campaign questionnaires to assess changes in participants' knowledge and awareness.

Figure 6. Field visit to introduce R. *zollingeriana*, its habitat host plants (*Tetrastigma* spp.) in natural habitat - © febrina

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.

Several unforeseen challenges arose during the project. One of the main difficulties was accessibility to the field sites. The target locations were rarely visited, resulting in overgrown vegetation and dense underbrush that made travel to the site was difficult. To address this, we adjusted our transportation arrangements by switching to a car with higher ground clearance on subsequent days to better navigate the terrain. Additionally, many of the sites were located on steep slopes, and during the rainy season, trails became muddy and unsafe. To mitigate this, we adjusted our field schedule based on weather forecasts and ensured that field teams were equipped with rain gear, in case of sudden rainfall. The guidance and support of our local guide, who were familiar with the landscape and trail condition helping us conduct fieldwork safely and efficiently.

Another significant challenge involved the preservation of biological samples due to the absence of cold storage facilities in the field. We overcame this by using portable freezer boxes with ice packs to maintain sample condition during transport. Additionally, we decided to stay at a nearby hotel and upgraded one of our room accommodations with the refrigeration facilities, allowing us to store specimens temporarily before transferring them to the laboratory.
One of the most challenging aspects of the project was conducting the educational campaign. Inviting residents to attend and actively participate in sessions about *Rafflesia zollingeriana* proved difficult, as many local community members were initially unfamiliar with the topic and hesitant to engage. We addressed this challenge by approaching and speaking directly with the village leader of Kampung Batara to personally communicate our intentions. This face-to-face engagement helped build trust and encouraged community members to attend and participate in the educational activities.

4. Describe the involvement of local communities and how they have benefitted from the project.

a. Increased awareness and knowledge of *Rafflesia zollingeriana*
Through our educational sessions and field visits, local residents especially who lived nearest forest gained a clearer understanding of *Rafflesia zollingeriana* as a holoparasitic plant with a unique life stage. Many participants had previously misunderstood Rafflesia as the fruit of the host plant, rather than as a parasitic flower that needs to be attached to the host. The program helped correct these misconceptions and fostered a deeper appreciation for this rare, endemic species and its conservation value.

b. Distribution of Educational Materials
To support long-term awareness and learning, we distributed booklets and shared a video animation that explained the life cycle, ecological function, and conservation status *of R. zollingeriana* in an accessible and engaging way.

c. Introduction to sustainable ecotourism opportunities
Local communities were introduced to the concept of Rafflesia-based ecotourism as a potential source of alternative income. By learning from successful models—such as the community-led ecotourism initiative presented by a practitioner from Bengkulu—participants began to view conservation not only as environmental protection but also as an opportunity for local economic empowerment.
In addition, participants were inspired to explore creative opportunities, such as make Rafflesia-shaped souvenir, developing batik patterns based on the Rafflesia flower and promoting *R. zollingeriana* as a unique cultural and ecological icon of their region. These ideas have the potential to increase local pride and drive community-based conservation.

5. Are there any plans to continue this work?

At this stage, we are still analyzing and comparing the diversity, functions, and roles of microbiomes found in the soil *of Rafflesia zollingeriana* habitats and uninfected areas, as well as in infected and uninfected host plants, and in *R. zollingeriana* itself. To understand the specific interactions and roles of dominant microbial taxa, more in-depth studies are required. In the next phase, we also plan to study the specific bacteria involved in the parasitic relationship between *R. zollingeriana* and its host, including how they influence the host-parasite interaction.

6. How do you plan to share the results of your work with others?

Our work related the educational campaign has already been published in online website of national news agency in Indonesia, and on social media platforms. We also plan to share our result on the microbiome and the edu-campaign's pre- and post-evaluation through conference presentations and scientific journal publications.

7. Looking ahead, what do you feel are the important next steps?

Long-Term Monitoring of *Rafflesia zollingeriana* Populations
Establishing a regular monitoring program is essential to track flowering events, population dynamics, and habitat conditions. This will help assess the effectiveness of current conservation actions and identify emerging threats over time.

Further Host-Parasite Interaction Research
Conduct in-depth studies to investigate the molecular and physiological mechanisms underlying the parasitic relationship between *R.zollingeriana* and its host, including studying the environmental, chemical, and biological cues that trigger its emergence and flowering, as well as identifying which bacteria drive the parasitic interaction and how they influence growth and survival of *R. zollingeriana.*

Building a Rafflesia Conservation Network
Conservation collaboration across regions is needed to protect Rafflesia species in all areas where they naturally grow. Strengthening partnerships among researchers, conservation agencies, local governments, and community groups can facilitate knowledge exchange, monitoring protocols, and promote joint conservation initiatives.

8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?
Yes, The Rufford Foundation logo was used in various materials produced throughout the project. Below are the details:

Flyers, Presentation Materials
The logo was included on flyers, and PowerPoint presentations used during field workshops, community engagement sessions, and stakeholder. These materials

helped introduce the project and its conservation message while crediting the support from The Rufford Foundation.

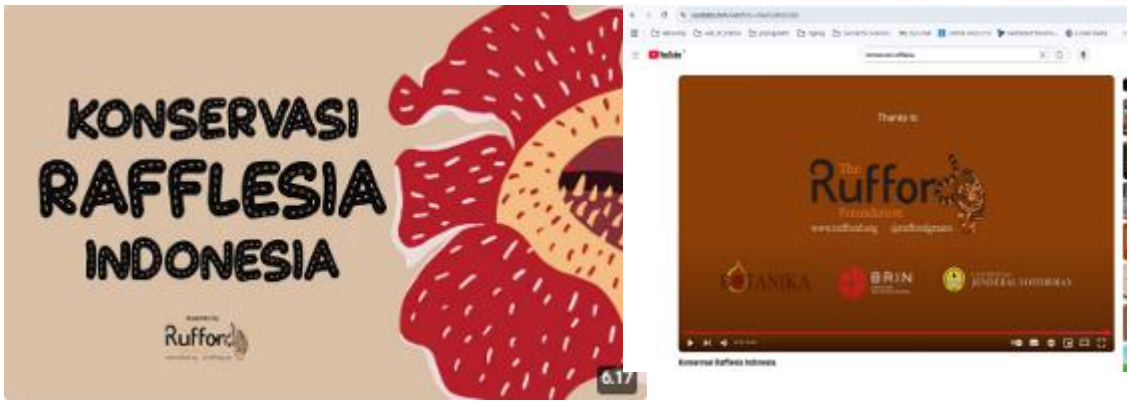
Figure 7. Flyers, Presentation Materials - © febrina

Booklet Publication
We designed and published an informative booklet about Rafflesia. The Rufford Foundation logo was placed on back covers. The booklet was distributed during public outreach events, field activities, and educational sessions with local communities and stakeholders.

Participant Gifts and Keyrings
As part of our outreach activities, we distributed small appreciation gifts to participants, including customized keyrings and souvenirs featuring The Rufford Foundation logo. These items were shared with local students, local communities, and stakeholders to raise awareness and appreciation for the project. We also provided shirts with the project name and The Rufford Foundation logo to local field guides who assisted in monitoring *R. zollingeriana* in the field.

Figure 8. *Participant Gifts and Keyrings (left),* shirts with the project name and The Rufford Foundation logo to local field guide bapak sapari (middle), and key rings (right) - © febrina

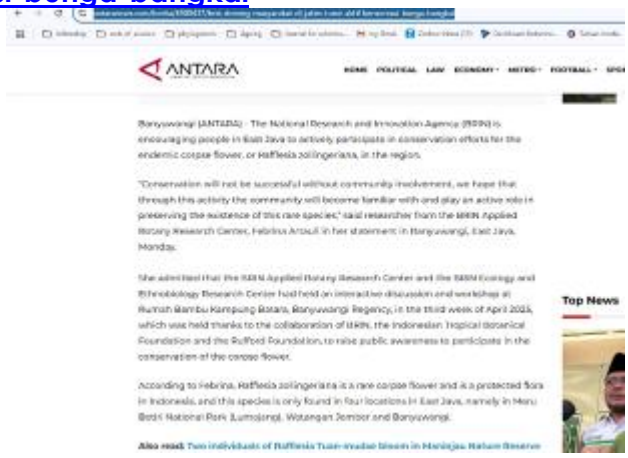
Social Media and Video Animation
We created and published a 6 minutes animated video to raise awareness about the conservation of Rafflesia. The video includes The Rufford Foundation logo in both the introduction and closing credits. It was shared across multiple social media platforms to reach a broader audience and engage the public in conservation efforts. We also shared the video with local stakeholders and community members to strengthen support.
Link youtube
https://www.youtube.com/watch?v=A0u1CMAO50A



In addition, our project activities were covered and published by Antara News Agency, a national news outlet in Indonesia.

Link to the news:

https://www.antaranews.com/berita/4800437/brin-dorong-masyarakat-di-jatim-turut-aktif-konservasi-bunga-bangkai



9. Provide a full list of all the members of your team and their role in the project.

Febrina Artauli Siahaan – Project Leader
Responsible for overall project coordination, including permit administration, data collection, data processing, data analysis, documentation, report writing, paper writing, and outreach activities.

Yuriza Eshananda – Lecturer and Researcher, Faculty of Biology, Jenderal Soedirman University
Contributed to data collection, data processing, data analysis, paper writing, and project documentation throughout the project.

Wilda Khafida – Lecturer and Researcher, Faculty of Biology, Jenderal Soedirman University
Involved in data collection, data processing, data analysis, paper writing and documentation throughout the project.

Dewi Lestari – Junior Researcher, Research Centre for Ecology and Ethnobiology, National Research and Innovation Agency (BRIN)
Participated in data collection, data processing, data analysis, documentation, paper writing and educational campaign.

10. Any other comments?

We would like to express our sincere gratitude to the community of Kampung Batara especially Bapak Widi, who warmly welcomed us and helped gather the local community for our educational campaign. We are also deeply thankful to our two local guides, Bapak Sapari and Bapak Jainoto, who accompanied and assisted us throughout the entire project.

Documentation

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Our team photos - © Wilda Khafida

Field work activities © Febrina, Dewi

Educational campaign © Febrina

After field work © Febrina

Rafflesia zollingeriana - © Febrina

Rafflesia zollingeriana - © Wilda

Rafflesia zollingeriana - © Febrina

ANNEX – Financial Report
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