Microbes of the Host-Holoparasite Holobiont: Implications for ex situ Conservation of *Rafflesia schadenbergiana*, the Philippines' Largest Corpse Flower

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ABSTRACT

Rafflesia schadenbergiana is the largest flower in the Philippines with a malodor that smells like rotting meat, hence the common name corpse flower. It is one of the most critically endangered plants in the world with less than 10 known individuals. This holoparasitic species lives entirely inside its host vine, *Tetrastigma,* making conservation especially difficult. Recent studies suggest that *Rafflesia,* its host, and associated microbes function together as a holobiont. This study characterized microbial communities associated with *R. schadenbergiana*-infected *Tetrastigma* roots using Oxford Nanopore 16S/ITS metabarcoding, with replicate samples independently sequenced using ZymoBIOMICS Illumina sequencing for comparison. Nanopore sequencing recovered diverse bacterial and fungal communities consistent with Illumina datasets. The characterized bacterial and fungal communities of the *Rafflesia-Tetrastigma* holobiont included taxa associated with nutrient cycling, nitrogen fixation, aromatic compound degradation, pathogen suppression, and stress tolerance. These findings support the hypothesis that microbial communities may contribute to host compatibility, parasite persistence, and future microbiome-informed conservation strategies for *R. schadenbergiana.*

INTRODUCTION

- *Rafflesia schadenbergiana* (Fig. 1) produces the largest flower in the Philippines with a malodor that smells like rotting meat, hence the common name corpse flower. It is among the world's most critically endangered parasitic plants with less than 10 known individuals, due to severe habitat loss and deforestation (Barcelona et al., 2008).
- *Rafflesia* is a holoparasitic plant that spends nearly its entire life cycle embedded within its host vine, *Tetrastigma* (Fig. 2). The parasite lacks leaves, stems, roots, and photosynthetic ability (Nais, 2001; Molina et al., 2014), and only emerges to flower.
- Successful parasitism is highly host-specific and depends on compatible *Tetrastigma* species and environmental conditions (Chen et al., 2011). Recent studies suggest that *Rafflesia*, its host, and associated microbial communities function together as a tripartite holobiont that may influence parasite survival and development (Molina et al., 2024).
- Plant holobiont microbial communities have been shown to contribute to nutrient cycling, stress tolerance, pathogen suppression, and host compatibility during parasitism (Fitzpatrick & Schneider, 2020).
- Metabolomic and microbiome studies have shown that infected *Tetrastigma* tissues possess distinct chemical and microbial profiles relative to uninfected hosts, suggesting microbial involvement in successful infection (Molina et al., 2025).
- This study characterized bacterial and fungal communities associated with *R. schadenbergiana* using Nanopore and Illumina metabarcoding to identify microbial taxa with potential applications in microbiome-assisted ex situ propagation and conservation.

Fig. 1. Blooming flower of *Rafflesia schadenbergiana* in the midst of a slash-and-burn forest patch, Bukidnon, Philippines.. Photo by Julie Barcelona ©

Fig. 2. Leaf of the *Tetrastigma* host plant (left). Infected *Tetrastigma* vine with *Rafflesia* buds (right)

MATERIALS & METHODS

- Two *Rafflesia schadenbergiana*–infected *Tetrastigma* host cuttings were collected from Bukidnon, Philippines under Philippine government and USDA permits.
- Small root cuttings were surface-sterilized and used for microbial DNA extraction using a modified Qiagen DNeasy Plant Mini Kit protocol. Separate PCR amplifications targeted bacterial 16S rRNA V3–V4 regions and fungal ITS2 regions using Oxford Nanopore–tailed primers.
- Amplicons were prepared using Oxford Nanopore ligation sequencing protocols (SQK-LSK114 with EXP-PBC001) and sequenced on a Flongle flow cell for 72 hours. Sequencing reads were basecalled, demultiplexed, filtered (300–500 bp), and aligned against SILVA and fungal ITS reference databases using BLASTn.
- Replicate samples were independently sequenced using ZymoBIOMICS Illumina 16S amplicon sequencing to validate Nanopore-derived bacterial community patterns.
- Ecological and functional traits of identified microbial taxa were evaluated through targeted literature review to infer potential roles in nutrient cycling, stress tolerance, host infection, and *Rafflesia* development within the *Rafflesia*–*Tetrastigma* holobiont.

RESULTS

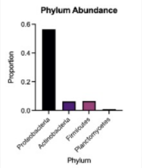

Figure 1. Phylum-level composition of bacterial communities associated with the *Rafflesia-Tetrastigma* holobiont. Proteobacteria exhibited the highest relative abundance compared to other detected phyla, including Actinobacteria, Firmicutes, and Planctomycetes. Bars represent the proportional abundance of each bacterial phylum within the total microbiome dataset.

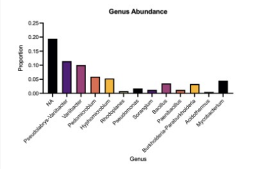

Figure 2. Relative abundance of bacterial genera identified in the *Rafflesia–Tetrastigma* microbiome. Unidentified taxa ("NA") showed the highest abundance, followed by *Pseudolabrys-Variibacter* and *Variibacter*. Other genera were detected at lower relative abundances. Bars represent proportions of the total bacterial community.

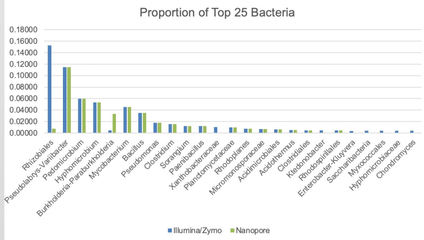

Figure 3. Relative abundance of the top 25 bacterial taxa detected by Illumina/Zymo and Nanopore sequencing. Both platforms show similar overall trends, with *Rhizobiales*, *Pseudolabrys-Variibacter*, and *Pedobacterium* among the most abundant taxa, though some differences in abundance are seen between methods.

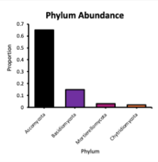

Figure 4. Bar chart showing the relative proportions of the most abundant fungal phyla in the samples. *Ascomycota* dominates the community (~65%), followed by *Basidiomycota* (~15%), *Mortierellomycota* (~3%), and *Chytridiomycota* (~2%).

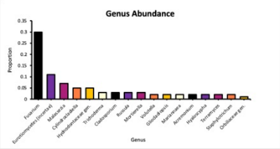

Figure 5. Relative abundance of the top fungal genera across the samples. *Fusarium* is the most abundant genus (~30%), followed by unclassified *Eurotiomycetes* (~11%), *Malassezia* (~7%), and several other genera present at lower proportions. The chart highlights the high diversity of fungal genera, with a long tail of low-abundance taxa.

DISCUSSION

- Nanopore metabarcoding generated ~533,700 filtered reads (~213.5 Mb), and Nanopore and Illumina datasets recovered broadly consistent dominant microbial taxa.
- The *Rafflesia-Tetrastigma* system functions as a tripartite holobiont, consisting of the parasitic plant, its host vine, and their associated microbial communities. Shared bacterial taxa between infected *Tetrastigma* tissues and *Rafflesia* suggest that microbial partners may contribute to host infection, nutrient transfer, stress tolerance, and parasite development throughout *Rafflesia*'s largely endophytic life cycle (Molina et al. 2024, 2025).
- Proteobacteria dominated the bacterial community, particularly Rhizobiales-associated genera linked to carbon cycling, nitrogen metabolism, and degradation of aromatic compounds (Compant et al., 2010).
- Plant growth-promoting bacteria including *Paenibacillus, Burkholderia-Paraburkholderia,* and *Pseudomonas* may enhance nutrient availability and host vigor during parasitic development (Grady et al., 2016). Stress-tolerant taxa such as *Bacillus, Clostridium,* and *Sorangium* may contribute antimicrobial activity, extracellular enzyme production, and microbial defense within infected tissues (Hashem et al., 2019).
- Fungal communities were dominated by taxa including *Trichoderma, Mortierella, Cladosporium,* and *Russula,* which are associated with nutrient cycling and plant-microbe interactions (Ozimek & Hanaka, 2021). *Trichoderma* species are recognized for antagonistic mycoparasitism and enhancement of plant nutrient uptake, suggesting possible roles in stabilizing infected host tissues (Contreras-Cornejo et al., 2016).
- Preserving microbial consortia associated with infected *Tetrastigma* tissues may improve ex situ propagation and conservation success for *Rafflesia schadenbergiana.* Now is the time because once extinct, we can no longer revive the corpse flower.

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