Science and Technology Group Annual Report FY2024

Petra Svetlikova Science and Technology Associate

1 Introduction

My research focuses on heterotrophic plants, i.e., plants that acquire at least part of their essential resources from other organisms. Heterotrophic plants include parasitic plants and mycoheterotrophic plants. Parasitic plants steal resources from other plants, while mycoheterotrophic plants steal resources from mycorrhizal fungi. I study their diversity, biology, ecophysiology, and genomics. I combine traditional ecophysiological methods with genomics to study physiology and evolution of parasitic and mycoheterotrophic plants. I mainly focus on Japanese, including Okinawan, species of various trophic strategies from partially (green) to fully heterotrophic (non-green) species.

2 Activities and Findings

I have recently focused mainly on these **three research projects** dedicated to the evolution and phylogenetics, genomics, transcriptomics, and metabolomics of selected Okinawan parasitic plants.

Evolution of full parasitic plants from Balanophoraceae

In collaboration with Dr. Huei-Jiun Su, Dr. Filip Husnik, and Dr. Kenji Suetsugu, I finalized our manuscript on the phylogenomics of parasitic plants from the family Balanophoraceae and the preprint is already available on bioRxiv (https://doi.org/10.1101/2025.03.29.646081).

In the manuscript, we sampled seven species of *Balanophora* (**Figure 1**) from 12 populations across Japan and Taiwan and analysed their transcriptomes and plastid-targeted nuclear-encoded proteins. By



Figure 1. Sampled *Balanophora* populations from Taiwan and Japan. (A) *B. japonica* (left and center: Kyushu, Japan; right: Taiwan), (B) *B. mutinoides* (Taiwan), (C) *B. tobiracola* (from left: Okinawa, Japan; Taiwan), (D) *B. subcupularis* (Kyushu, Japan), (E) *B. fungosa* ssp. *fungosa* (from left: Okinawa, Japan; Taiwan), (F) *B. yakushimensis* (from left: Kyushu, Japan; Taiwan), (G) *B. nipponica* (Honshu, Japan).

combining many diverse plastid-encoded and nuclear markers, we almost fully uncovered the relationships within the genus. Moreover, our phylogenies imply that the origins of obligate asexuality in Balanophora are correlated with island colonization. Another key focus of the study was on the non-photosynthetic plastids of Balanophora. Our findings suggest plastid genome reduction in Balanophora mainly occurred before the origin of the clade. Using both newly generated and publicly available transcriptomics data, we show that even though Balanophora plastids are among the most reduced organelle genomes, they are still predicted as highly metabolically active in most photosynthesisunrelated pathways usually present in chloroplasts. These results complement research on non-photosynthetic plastids in other parasitic eukaryotes, such as apicomplexans.

<u>Mitochondrial genomes of Balanophora species</u> <u>and horizontal gene transfer (HGT) between host</u> and parasite

In collaboration with Dr. Michelle Leger and Dr. Filip Husnik, I supervised Amy Morrell, a rotation student at OIST, examining the mitochondrial genomes and the presence of HGT from host species.

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In this project, we aim to analyze the genes presented in the mitochondrial genomes of various *Balanophora* plants and verify their origin. We both use diverse software to annotate *Balanophora* mitochondrial genes as well as compare our results with already reported mitochondrial genomes from mitogenomes of closely related species.

Metabolomics of a selected Okinawan parasitic plant

The last research project aimed at the metabolomics of a fascinating Okinawan holoparasitic plant, *Mitrastemon yamamotoi* (Figure 2), and it was the second part of Amy Morrell's rotation research project

in the Husnik Unit at OIST. In collaboration with Javier Tejeda (PhD student at Husnik Unit, OIST) and Dr. Filip Husnik, we sampled *Mitrastemon* from Yanbaru National Park in Okinawa and analyzed its metabolites.

3 Collaborations

Filip Husnik, OIST, Okinawa, Japan Michelle Leger, OIST, Okinawa, Japan Javier Tejeda, OIST, Okinawa, Japan Kenji Suetsugu, Kobe University, Kobe, Japan Su Huei-Jiun, University of Taipei, Taiwan

4 Mentoring

Amy H. Morrell, PhD rotation student, OIST, Okinawa, Japan (in collaboration with Dr. Michelle Leger and Dr. Filip Husnik)

Figure 2. Mitrastemon yamamotoi, a holoparasitic plant from tropical and subtropical forests of Southeast and East Asia.

5 Publications and other output

Preprint: Petra Svetlikova, Huei-Jiun Su, Kenji Suetsugu, Filip Husnik. 2025. Phylogenomics clarifies *Balanophora* evolution, metabolic retention of reduced plastids, and the origins of obligate

asexuality. bioRxiv. https://doi.org/10.1101/2025.03.29.646081

Conferences: Petra Svetlikova, Huei-Jiun Su, Kenji Suetsugu, Filip Husnik. The evolution of

Balanophoraceae: holoparasitic plants with extremely reduced plastid genomes. $17^{\rm th}$ World Congress on Parasitic Plants 2024, 3-7 June, Nara, Japan. Oral presentation.