Science and Technology Group
Annual Report FY2025

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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. Both life strategies have originated multiple times independently in the evolution of angiosperms. I study the diversity, biology, ecophysiology, and genomics of these unusual plants. I focus primarily on Okinawan plants, comprising the full spectrum of trophic strategies from partially (green) to fully heterotrophic (non-green) species.

2 Activities and Findings

Evolution of fully parasitic plants from Balanophoraceae

Our research on Balanophoraceae was recently published in New Phytologist, and the photo of *B. fungosa* from Okinawa was selected for the issue cover.

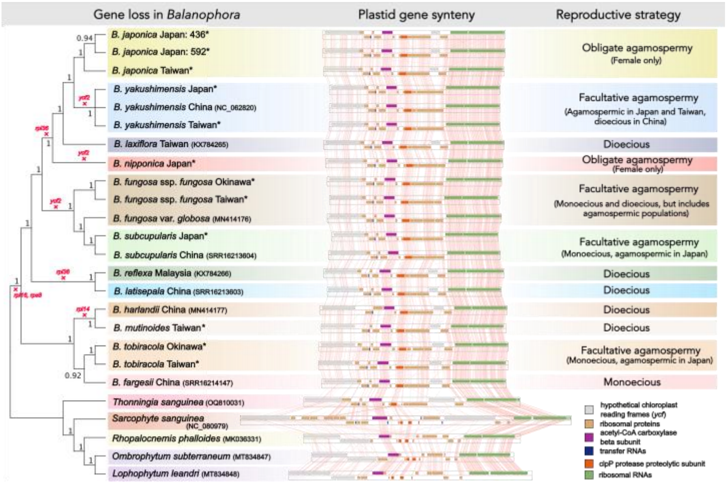

Figure 1. tBlastX alignment of whole plastid genomes of Balanophoraceae ordered according to their phylogeny and annotated with *Balanophora* reproductive strategies. The cladogram was inferred from the Bayesian phylogenetic tree based on 15 protein-coding plastid genes (LG + G model; 10 000 000 generations), and posterior probabilities are displayed next to nodes. Independent losses of specific plastid genes are highlighted in red. GenBank accession numbers for sequences obtained from NCBI are indicated in parentheses. All species studied in this study are highlighted by asterisks. It is plausible that other species in the genus may also possess facultative agamospermy. However, this has not yet been empirically tested in species other than *Balanophora fungosa* ssp. *fungosa*, *Balanophora tobiracola*, and *Balanophora subcupularis*.

In the manuscript, we sampled seven species of *Balanophora* from 12 populations across Japan and Taiwan and analysed their transcriptomes and plastid-targeted nuclear-encoded proteins. By 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 (Figure 1). 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 photosynthesis-unrelated pathways usually present in chloroplasts. These results complement research on non-photosynthetic plastids in other parasitic eukaryotes, such as apicomplexans.

Evolution of fully mycoheterotrophic plants from Thismiaceae

In collaboration with Dr. Filip Husnik and Dr. Kenji Suetsugu, we studied the phylogenomics of Thismiaceae. We sampled two species endemic to Japan: *Relictithismia kimotsukiensis* from Kyushu and *Oxygyne shinzatoi* from Okinawa (Figure 2). We assembled their plastid genomes and inferred the phylogeny of the family from various gene datasets.

Both plastomes are 30 kb in size; therefore, the largest plastomes of the family. The GC content of the *Relictithimia* and *Oxygyne* plastomes is 32.6 and 36.8%, which corresponds to the plastomes of their relatives. The plastome of *Relictithismia* includes 15 protein-coding ribosomal genes (11 unique),

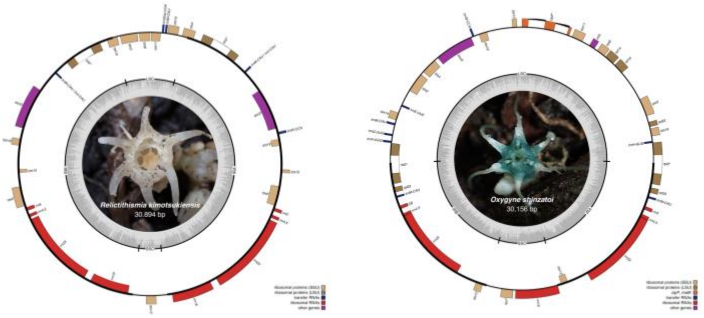

Figure 2. Circular maps of the *Relictithismia kimotsukiensis* (left) and *Oxygyne shinzatoi* (right) plastomes. Genes are color-coded according to functional categories. Genes inside the circle are transcribed clockwise, and those outside are transcribed counterclockwise.

2 other protein-coding genes (two copies of *accD*), 8 rRNA genes, and 5 tRNA genes **(Figure 2 left)**. The plastome of *Oxygyne* includes 19 protein-coding ribosomal genes (16 unique), 3 other protein-coding genes (*accD*, *clpP*, and *infA*), 7 rRNA genes, and 8 tRNA genes **(Figure 2 right)**. Both newly assembled plastomes include inverted repeat regions (IR) lost from *Haplothismia* and *T. panamensis*. This region covers the majority of the *Relictithismia* plastome. In spite of the relatively large size of the *Relictithismia* plastome, it has lost several genes present in other Thismiaceae plastomes.

We inferred phylogenetic trees from the nuclear 18S rRNA gene, concatenated alignments of two ribosomal RNA genes (16S and 23S rRNA), and 20 protein-coding genes. The phylogenies revealed *Relictithismia* as sister to all *Thismia* or to Old World *Thismia* species, which agrees with species morphology sharing traits with both *Haplothismia* and *Thismia. Oxygyne* is a sister to *Relictithismia* and all *Thismia* species. Currently, we are preparing an additional phylogeny based on mitochondrial genes, which we annotated for both studied species.

Mitochondrial genomes of *Balanophora* species and the ultrastructure of *Balanophora* cells under TEM

I supervised Sofiia Porkhun, an intern at OIST, from July to the end of November. We annotated the genes present in the mitochondrial genomes of various *Balanophora* plants and compared the results to already reported mitochondrial genomes of closely related species. In addition, we prepared the samples from *B. fungosa* inflorescence, stem, scale leaf, and tuber for transmission electron microscopy (TEM) and observed the final sections under TEM.

3 Collaborations

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

4 Mentoring

Sofiia Porkhun, intern at OIST, July–November 2025, Okinawa, Japan (in collaboration with Dr. Michelle Leger and Dr. Filip Husnik)

5 Publications and other output

Svetlikova P., Su H.-J., Suetsugu K. and Husnik F. (2026). Phylogenomics clarifies *Balanophora* evolution, metabolic retention in reduced plastids, and the origins of obligate agamospermy. New Phytol, 249: 2531-2546. https://doi.org/10.1111/nph.70761