

Science and Technology Group Annual Report FY2022

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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. 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

After returning to work from a parental leave last October, I have focused on the genomics and transcriptomics of heterotrophic plants. My main research aimed at the evolution of Japanese parasitic plants from the Balanophoraceae family. In addition, I studied the genomics of additional Okinawan parasitic and mycoheterotrophic plants.



Fig. 1: Studied parasitic plants from the *Balanophora* genus. Top left: *B. nipponica*; top and center right: *B. japonica*; center left: *B. tobiracola*; center: *B. yakushimensis*; bottom left: *B. fungosa* subsp. *fungosa*; and bottom right: *B. subcupularis*.

genomes of the same genus. The plastid genomes are highly reduced in size (14-16 kbp) and gene content (19-21 genes), highly AT-biased (>87%), and compact (**Tab. 1; Fig. 2**). Moreover, the studied plants utilize the same novel genetic code reported by Su et al. (2019), i. e., the code with the TAG reassignment from stop codon to tryptophan.

I also revealed the phylogeny of Balanophoraceae based on DNA and RNA-seq data and started to investigate the biogeography of studied plants, for example focusing on the origin of Japanese endemic species.

Evolution of full parasitic plants from Balanophoraceae

Currently, I am working on the preparation of manuscript dedicated to the evolution of Balanophoraceae. In collaboration with Dr. Filip Husnik and Dr. Kenji Suetsugu, I studied six *Balanophora* species from Japan. These plants are full heterotrophs parasitizing roots of their host trees and acquiring all essential resources from their vascular bundles. They stay most of the year inside hosts and appear aboveground only for reproduction during a few months every year.

We were able to sample all Japanese *Balanophora* species, including two species presented in Okinawa (*B. fungosa* and *B. tobiracola*; **Fig. 1**). I extracted their overall DNA and RNA and sent the samples for sequencing at OIST. Then I assembled and annotated plastid genomes of all six Japanese *Balanophora* and compared them to several already published plastid genomes of closely related species.

The studied *Balanophora* plastid genomes showed features similar to already reported plastid

Tab. 1: Plastid genome features of full parasitic plants from Balanophoraceae. *results of our study

species	plastid genome features				
	size (bp)	GC content (%)	protein-coding genes	rRNA genes	tRNA genes
<i>Balanophora harlandii</i>	16,056	12.9	16	3	1
<i>Balanophora fungosa</i> *	14,968	12.5	16	3	1
<i>Balanophora fungosa globosa</i>	15,130	12.6	16	3	1
<i>Balanophora japonica 436</i> *	15,624	12.1	16	3	1
<i>Balanophora japonica 592</i> *	15,646	12.0	16	3	1
<i>Balanophora nipponica</i> *	15,189	13.0	16	3	1
<i>Balanophora laxiflora</i>	15,505	12.2	16	3	1
<i>Balanophora reflexa</i>	15,507	11.6	16	3	1
<i>Balanophora subcupularis</i> *	14,259	12.9	16	3	1
<i>Balanophora tobiracola</i> *	16,255	12.3	17	3	1
<i>Balanophora yakushimensis</i> *	14,631	12.6	15	3	1
<i>Lophophytum leandri</i>	20,892	11.6	17	2	0
<i>Ombrophytum subterraneum</i>	17,313	14.0	18	2	0
<i>Rhopalocnemis phalloides</i>	18,622	13.2	19	2	1

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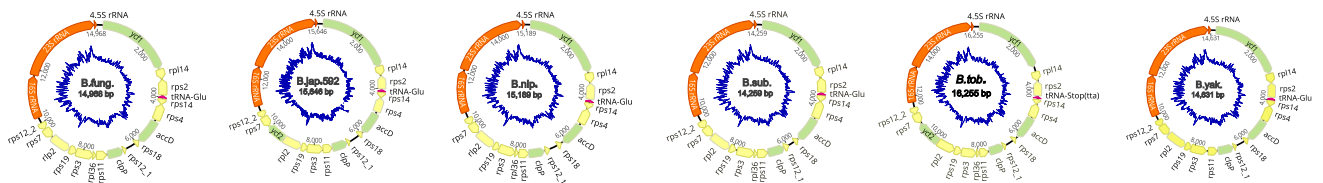


Fig. 2: Plastid genomes of six studied *Balanophora* plant species from Japan. From left: *B. fungosa* subsp. *fungosa*; *B. japonica*; *B. nipponica*; *B. subcupularis*; *B. tobiracola*; and *B. yakushimensis*. Plastid genome sizes are displayed under species names. Yellow: ribosomal protein-coding genes; green: genes of unknown or varying functions; orange: rRNA genes; and pink: tRNA genes. Blue represents GC content within the plastid genomes.

Genomics and transcriptomics of additional Okinawan heterotrophic species

I visited multiple sites around Okinawa for plants sampling, including Yanbaru, Ogimi, and Yomitan-son. Several years, I have been looking for a highly endangered mycoheterotrophic plant, *Oxygyne shinzatoi* (Fig. 3). This species is an endemic Okinawan species with intriguing biology that is disappearing from forest habitats due to lodging. This year, I successfully sampled this species (after receiving a research permit) and started to work on its genomics.

I optimized protocols for DNA and RNA extractions from newly sampled parasitic and mycoheterotrophic Okinawan plants and submitted the samples for sequencing. Recently, I have received the sequencing data and started to analyze them. I have successfully assembled plastid genomes of two Okinawan mycoheterotrophs, including the plastid genome of the above-mentioned *Oxygyne shinzatoi*.



Fig. 3: Okinawan endemic mycoheterotrophic plant, *Oxygyne shinzatoi*.

3 Collaborations

Filip Husnik, OIST, Okinawa, Japan
Kenji Suetsugu, Kobe University, Kobe, Japan

4 Publications and other output

Author list, *Title*, Journal or other reference, volume information (year)

- I updated an information booklet focused on Okinawan parasitic and mycoheterotrophic species and distributed these booklets among staff of the Environmental Research Support Section at OIST and OIST community members. This way people who are interested in heterotrophic plants and their protection can contribute to my research by e.g. finding new locations of heterotrophic plants on Okinawa.