

# On the way to heterotrophy: Ecophysiology and genomics of Okinawan parasitic and mycoheterotrophic plants

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## What is plant parasitism and mycoheterotrophy?

Not all plants rely only on own photosynthesis for nutrition. More than 24,000 plant species acquire at least a part of their essential resources by exploiting other organisms. Parasitic plants exploit other plant species (their hosts) and mycoheterotrophic plants exploit fungi. Both parasitism and mycoheterotrophy have originated multiple times independently in the evolution of land plants and comprise a whole spectrum of trophic forms from partially to fully heterotrophic plants (Fig 1). While partial heterotrophs (underlined in Fig 1) acquire a part of their essential resources still via own photosynthesis, full heterotrophs are non-green and acquire all resources through parasitism.

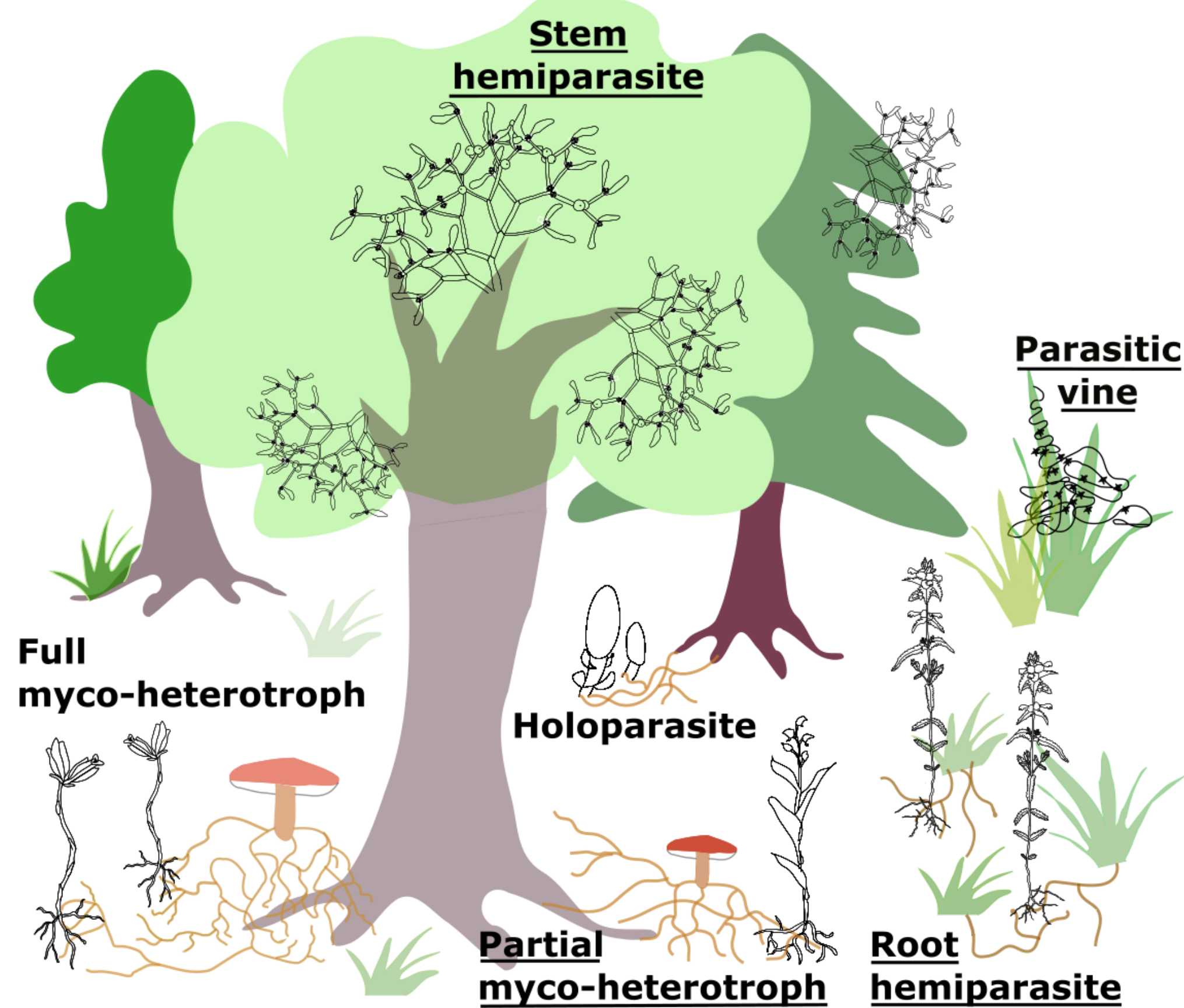


Fig 1: The most common functional types of parasitic and mycoheterotrophic plants. Underlined are species that photosynthesize. Hemiparasites (or partial parasites) connect either to roots or stems of their host species.

## Unique features of parasites and mycoheterotrophs

As a result of their close interaction with host plants and fungi, parasitic and mycoheterotrophic plants have unique physiology and genomes.

### Physiology

- unique mechanisms for maintaining a resource flow from host plants/fungi
- own photosynthesis often reduced compared to full autotrophs, but still important source of organic carbon in some species (e.g. root hemiparasites)

### Genomes

- frequent gene losses from plastid genomes
- most AT-biased plastid genomes to date
- bidirectional mRNA transfers between hosts and parasites
- horizontal gene transfers from diverse donors to their nuclear and mitochondrial genomes

## Aims

We combine traditional ecophysiological methods with genomics to study how is the ecophysiology of heterotrophic plants related to their genomes. We focus on Japanese, including Okinawan, species of various trophic strategies from partial to full heterotrophs. Although dozens of plastid genomes of parasites and mycoheterotrophs have been already sequenced, the genomes have been rarely studied simultaneously with the plant ecophysiology.

First question we would like to answer is: **How does the ecophysiology of parasitic and mycoheterotrophic plants relate to their plastid ultrastructure and genomes?**

## Parasitic and mycoheterotrophic plants in Okinawa

Several stem hemiparasites (or mistletoes), parasitic vines, and holoparasites can be found in Okinawa (Fig 2). In comparison to parasitic plants, mycoheterotrophs are much more diverse in Okinawa, including many orchid species and at least one full mycoheterotroph (*Sciaphila*; Fig 2 top). Many of these species are, however, rare and their environments need to be protected.



Fig 2: Selected parasitic and mycoheterotrophic plants from Okinawa. From top left to right: *Korthalsella japonica* (stem hemiparasite); *Sciaphila yakushimensis* var. *okinawensis* (full mycoheterotroph); *Balanophora tobiracola* and *B. fungosa* (holoparasites); *Cuscuta* sp. (parasitic vine); *Mitrastemon yamamotoi* (holoparasite); *Scurrula yadoriki* (stem hemiparasite); and *Cassytha filiformis* var. *filiformis* (parasitic vine).

## Preliminary results: Plastid genomes of 4 parasitic plants

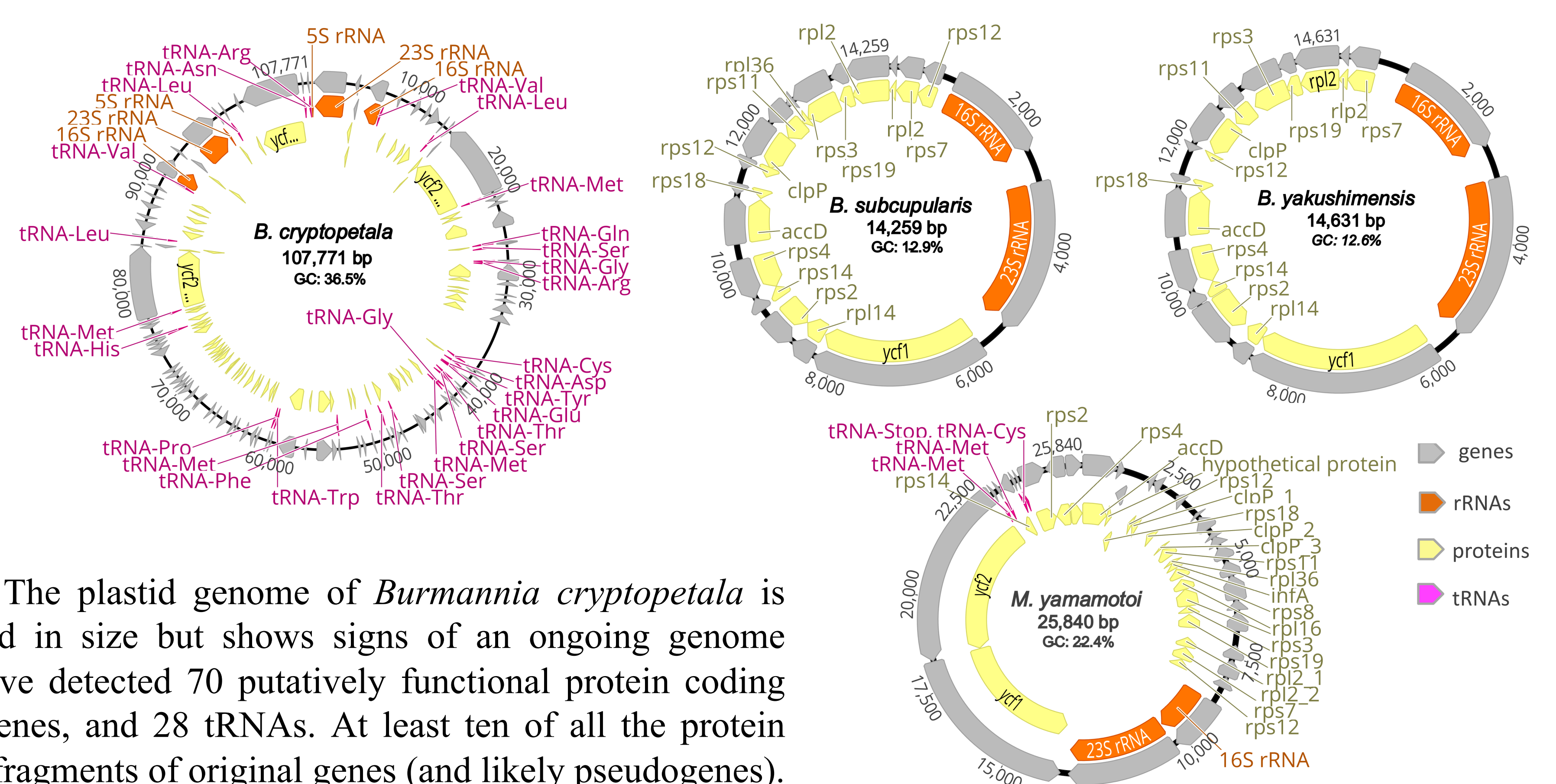


Fig 4: Plastid genomes of *Burmannia cryptopetala*, *Balanophora subcupularis*, *B. yakushimensis*, and *Mitrastemon yamamotoi*.

The plastid genome of *Burmannia cryptopetala* is not much reduced in size but shows signs of an ongoing genome shrinkage. We have detected 70 putatively functional protein coding genes, 6 rRNA genes, and 28 tRNAs. At least ten of all the protein coding genes are fragments of original genes (and likely pseudogenes).

The plastid genomes of *Balanophora subcupularis* and *B. yakushimensis* are highly reduced in size (Fig 4) and with two other known *Balanophora* plastomes, they have the most AT-rich genomes to date (> 87%). We have found 18 and 16 putatively functional genes in the plastid genomes, including 2 rRNA genes, 13 and 11 genes coding ribosomal proteins, and 3 protein-coding genes of varying or unknown function (*yef1*, *accD*, *clpP*). Our *Balanophora* species appear to utilize the same novel genetic code reported for their two relatives, i.e. the code with the TAG reassignment from stop codon to tryptophan (Su et al. 2019, PNAS).

The plastid genome of *Mitrastemon yamamotoi* is also highly reduced in size and gene content (26 genes), but not that extremely as the *Balanophora* species. Its genome is very similar to that described for its relative *M. kanehirai* from Taiwan (NCBI: MF372930; unpublished).

## Methods

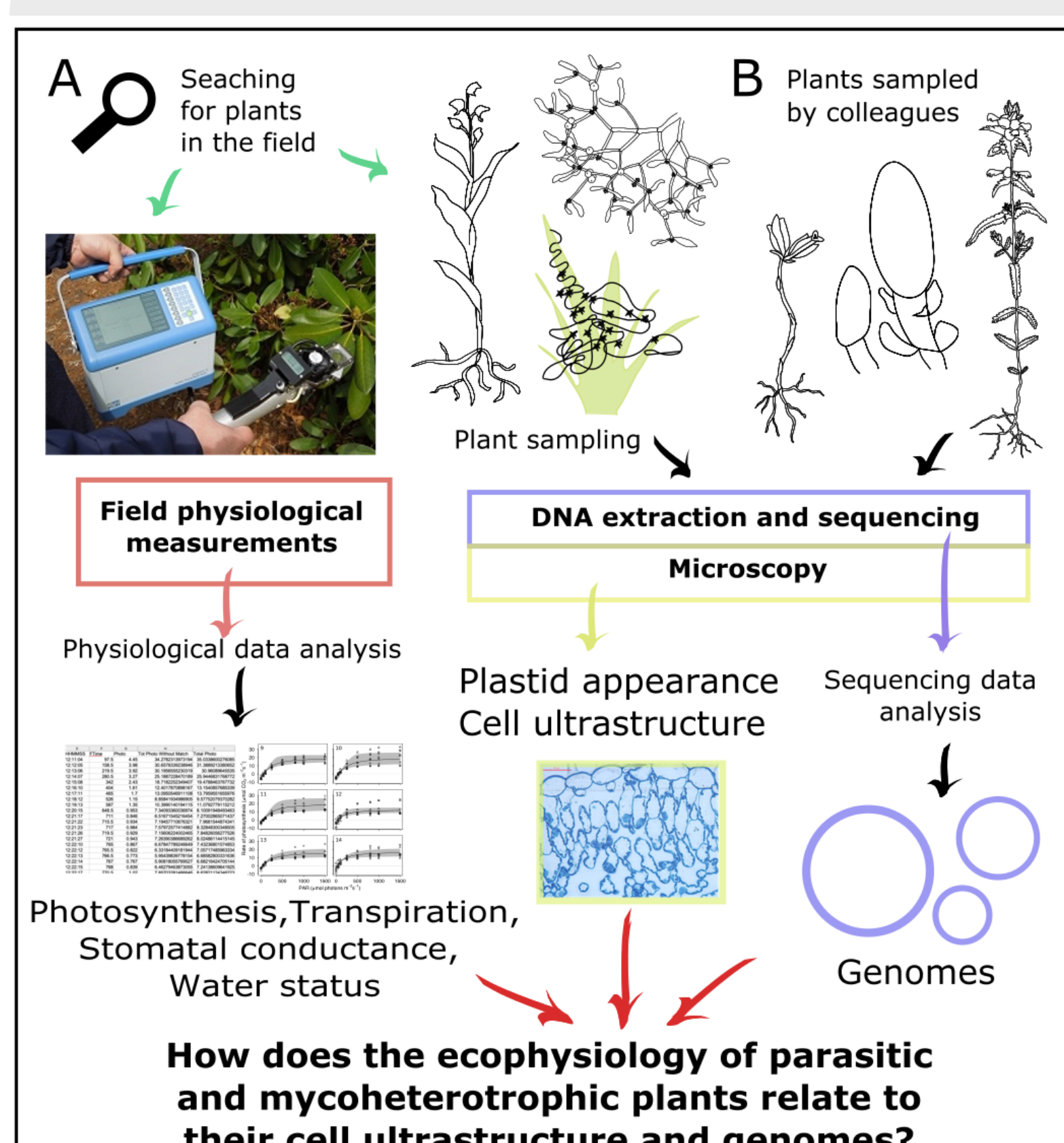


Fig 3: The workflow of the current project.

## Future prospects

- assemble plastid genomes of additional heterotrophic plants
- carry out field ecophysiological measurements on selected species:
  - *Cassytha filiformis* and its effects on host species
  - *Korthalsella japonica* and its ecophysiology
- sample species and prepare specimens for examining their cell ultrastructure and plastid appearance by electron microscopy