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Terrestrial Biodiversity and Ecosystems

Phylogenomics of *Phalaenopsis* and Conservation Genomics of *Phalaenopsis equestris* and *Phalaenopsis formosana* of Taiwan

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Doctoral Dissertation Defense Presentation

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Abstract

The moth orchid (*Phalaenopsis* Blume), commonly known as "butterfly or moth orchids," belongs to the Orchidaceae, a species-rich, widespread, and commercially important plant family. Moth orchid comprises over 80 species, including a few recorded varieties, subspecies, and natural hybrids. Like other flowering plants, advanced genomic studies are needed to clarify specific taxonomic issues. Despite their economic value over the years, almost all moth species face population decline due to deforestation and over-collection in the wild. In Taiwan, two native species, *Phalaenopsis equestris*, and *Phalaenopsis formosana,* are on the verge of extinction. *P. equestris* survives only in fragmented forests on Little Lanyu Island but is becoming more threatened due to human and natural pressures. At the same time, *P. formosana* faces a more critical situation as there are no recent sightings of it in the known type distribution.

This Ph.D. dissertation primarily aims to strengthen Dr. Cecilia Koo Conservation Center's (KBCC) conservation plan for saving the two botanical Taiwanese moth orchid heritage species from extinction. We aim to provide new insights into the taxonomic classification and evolutionary divergence within the genus *Phalaenopsis.* We used the Double digest restriction-site associated DNA sequencing (ddRAD-seq), and genome skimming on available *Phalaenopsis* nursery stocks from KBCC sourced from different nurseries in Taiwan.

In the first chapter, we generated the phylogenetic trees to stabilize the taxonomic classification of *Phalaenopsis.* These phylogenies were also compared to historical groupings, and diagnosable characters were imposed onto the plastome tree to infer character evolution. The resulting trees supported the present classification and aligned with previous Sanger-based methods, but now with a higher bootstrap support.

Our study estimated that the genus originated in the early Oligocene and diversified during the Miocene and Pliocene periods. The Plastome time tree provided the time estimates for traits like pollinia evolution and life habits.

In the second chapter, we used 42 *Phalaenopsis* species nursery stocks (32 *P. equestris* and one wild type from Little Lanyu Island). Our ddRAD-seq tree revealed four distinct genetic groups within *P. equestris*. One cluster contained the identified seven individuals genetically related to the one wild type, which exhibited sufficient levels of genomic heterozygosity. The four genetic groups within *P. equestris* also show distinct genetic structures and new insights into the morphological differences from the observed dissected flowers, especially among the individuals from Visayas and Mindanao. This discovery of wild individuals in human-cared nurseries marks a pivotal achievement and will provide a solid foundation for KBCC's cross-breeding plan.

In the third chapter, we utilized 96 moth orchid nursery stocks, including 56 individuals of *P. formosana* with three known wild genotypes and nine presumed wild nursery stocks. In our ddRAD-seq phylogeny, the 56 nursery stocks of *P. formosana* were split into four well-supported (100% BS) genetic clusters. In one cluster, all the 12 recorded wild-type (presumed and known wild type) and three additional individuals genetically allied to them were merged into this cluster. This result indicates they are the most genetically linked as wild individuals of *P. formosana*, among other samples. Moreover, sufficient genetic heterozygosity was revealed in these wild-type stocks, which could be used in breeding programs at KBCC future reintroduction programs in Taiwan. Significantly, our study has provided the first genomic evidence supporting *P. formosana* as an evolutionarily distinct lineage.

Keywords: ddRADseq, connectivity, nursery collections, population genomics, threatened species