**ABSTRACT**

Diplazium molokaiense is an endangered fern species endemic to Hawaii. It is an extremely rare species with only estimated 61 individuals left in the wild. Taxonomic classification of this species is an important aspect of its conservation. Using the following morphological characters: sori size and shape, scale size and shape, and frond size, D. molokaiense was preliminarily placed into the Diplazium genus. Six regions (rbcL, matK, atpA, apg1, trnL-F, and rps4-trnS) of the chloroplast genome were amplified and sequenced using Sanger sequencing. Sequences for outgroup taxa and 20 Diplazium species were downloaded from GenBank and used to infer a phylogeny. Our data provide evidence for the phylodetic placement of D. molokaiense and can aid in its conservation.

**INTRODUCTION**

Diplazium molokaiense (Fig. 1) is an extremely rare fern species endemic to Hawaii. The species is listed as critically endangered on the IUCN Red List, indicating that D. molokaiense is at the highest risk of extinction (Wood 2006). A 2006 survey conducted by co-author HO, estimated only 61 individuals remain on the Hawaiian Islands. Diplazium molokaiense has not been placed on the tree of life, meaning that its taxonomic position has not been determined and very little is known about its relationship with other fern species. Understanding the taxonomic classification of this species will be beneficial to conservation efforts and, ultimately, to the survival of the species.

**METHODS**

**Morphological:** Because of the critical status of the species, we were not allowed to remove fern tissues from the field for study in the lab; therefore, we visited the ferns in the field and took multiple photographs for further study. Additionally, we studied images of herbarium specimens and written descriptions by Wood (2006). Particular attention was paid to scale shape and color as well as sori shape. D. molokaiense has toothless, lanceolate scales that are brown/grey and concolorous (Fig. 2). The leaves are imparipinnate (Fig. 1), and the sori are elongated and flattened (Fig. 3).

**Sequences:** Molecular: We obtained dried frond material from a specimen in Maui. DNA was extracted using IBI Scientific Genomic (Dubuque, IA) DNA Mini Kit. The six regions – four plastid genes (atpH, matK, rps4, and rbcL) and two atp genes (atpH, matK, and rbcL) and two intergenic spacers (rps4-trnS and trnL-F) were amplified with PCR and purified using Exo-SAP. The resulting DNA was sent to the Genomics Core Facility at the University of Nebraska Medical Center for sequencing with Genewiz. The sequences were uploaded to GenBank. Sequences for 18 additional Diplazium species and two Athyrium (outgroup) species were downloaded from GenBank (Houser et al. 2016; Wei et al. 2013) and uploaded to GenBank as well. Athyrium is a closely related genus and a suitable outgroup. The sequences were aligned and concatenated using MAFFT (Katoh et al. 2013) and used to estimate a maximum likelihood phylogeny (Fig. 4) using RAxML (Stamatakis, 2014).

**Taxonomic Classification:** The high conservation status of the species requires urgent attention. A robust phylogenetic study is necessary to set taxonomic boundaries and aid in conservation efforts. Molecular phylogenetic studies have shown that Diplazium species are monophyletic with Athyrium species (Wei et al. 2013) and Athyrium molokaiense is sister to Diplazium heterocarpum, but with only weak support.

**RESULTS**

Combining results from two lines of evidence – morphological and molecular – our data place Diplazium molokaiense in Main Clade II (Fig. 4, Wei et al. 2013). The placement is based on sori shape, scale shape/color, geographic distribution, and six chloroplast markers. D. molokaiense is sister to Diplazium heterocarpum, but with only weak support.

**CONCLUSIONS & FUTURE DIRECTIONS**

- **Morphological and molecular characters place Diplazium molokaiense in Main Clade II and is sister to Diplazium heterocarpum.**
- **D. molokaiense has never been placed in the tree of life before, and new information can aid in the conservation and propagation of the species.**
- **Sequences of additional DNA regions are needed to boost support for some clades in the phylogeny.**
- **Diplazium esculentum, Diplazium sandwichianum, and Diplazium arnottii are fern species also found in Hawaii. DNA from frond tissue from these species are being extracted and sequenced. A more robust Diplazium phylogeny will further conservation and knowledge of Hawaiian Diplazium species.**

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