Genomic and Metagenomic Exploration of Microbial Endophytes and a new Potential *Phytophthora* Species in the Monkey Puzzle Tree *Araucaria araucana* in Chile[1]

Sebastián Márquez,[2] Francisca Venegas,[2] Gabriela Jiménez,[2] Guus Teunisse,[2] Freddy Boehmwald,[2] Pablo Zamora,[2] Álvaro Castro,[2] Jaime Alarcón,[3] and Eduardo Castro-Nallar[3]

Abstract

Araucaria araucana (Araucaria or monkey puzzle tree; Class = Pinopsida; Family = Araucariaceae) is an endangered conifer with a fragmented and relict distribution in southern Chile and Argentina. Araucaria is the type species of a genus of relatively old conifers with an ancient distribution that covered the continent of Gondwana, and that now comprises 19 extant species distributed in Oceania and South America.

Araucaria has been historically threatened by logging (banned in 1990), wildfires, overgrazing, invasive trees, and extensive human harvesting of Araucaria seeds. More recently, the Chilean forest authority reported extensive damage spread throughout its geographic distribution in Chile and Argentina, which is characterized by browning of branches and needles following a "bottom-up" pattern and radiating from the trunk to the tip of the branches. While 90% of *A. araucana* population is affected there is only a 2% mortality rate in Chile, according to the Corporación Nacional Forestal; Chilean National Forestry Corporation, CONAF. The disease was dubbed DFA as "foliar damage of the Araucaria tree" for its acronym in Spanish. While there are several hypotheses regarding the cause of DFA including approximately a 10-year drought in the region, the widespread nature of the disease which covers all of its geographic distribution at various intensities, plus gardens, nurseries, and public squares, suggests the influence of a pathogen, opportunistic or otherwise.

Here, we use amplicon sequencing targeting the 16S rRNA and ITS taxonomic marker genes to reveal the structure and composition of Araucaria's microbial communities throughout its geographic distribution (n > 600). Community analyses suggest that Araucaria's microbial communities are structured primarily within tree by tissue, and secondarily by sampling site, i.e., Andes or Nahuelbuta mountain ranges and north/south gradient.

---

[1] A version of the paper was presented at the Seventh Sudden Oak Death Science and Management Symposium, June 25-27, 2019, San Francisco, California.

[2] UC Davis, Chile Life Sciences Innovation Center, Santiago, Chile.

[3] Universidad Andrés Bello, Center for Bioinformatics and Integrative Biology (CBIB), Santiago, Chile. Corresponding author: A. Castro, acastro@ucdavischile.org.

To get a better view of Araucaria's microbial communities, we complement these analyses with shotgun metagenomic sequencing, which revealed that up to 20% of the endophytic communities are dominated by a *Phytophthora* lineage closely related to subclade 8c species that include *P. ramorum*, *P. lateralis*, *P. hibernalis*, and *P. foliorum*. We extracted contigs from this new lineage and were able to reconstruct a preliminary phylogeny. Ongoing efforts include culturing and isolation of this *Phytophthora* member, as well as PCR screening over 300 Araucaria samples (healthy and infected) from its entire geographic distribution. We discuss our results and future experiments in the light of testing whether this lineage of *Phytophthora* is the causal agent of DFA.

Acknowledgements

Funded by CONAF (Corporación Nacional Forestal; Chilean National Forestry Corporation), executed by UC Davis Chile Life Sciences Innovation Center, and supported by Universidad Andrés Bello, Center for Bioinformatics and Integrative Biology (CBIB) and Fundación Mar Adentro.