

Peer-Reviewed Scientific Article

Title: A metabarcoding protocol targeting two DNA regions to analyze root-associated fungal communities in ferns and lycophytes.

Simplified Title (non-expert audience): A protocol to identify fungi in fern and lycophyte roots.

Audience: Researchers, technicians, and specialists in plant biology, ecology, and mycology

Reference: Applications in Plant Sciences (2023)

This article describes the development and optimization of a protocol to identify fungi in the roots of ferns and lycophytes. Two step-by-step molecular procedures were validated, from DNA extraction to fungal classification and statistical analyses. This piece demonstrates scientific expertise, structured methodology, peer-reviewed writing experience, and the capacity to summarize technical data in compliance with scientific standards.

Received: 31 August 2022 | Accepted: 4 April 2023
DOI: 10.1002/aps3.11523

APPLICATION ARTICLE

Applications
in Plant Sciences

A metabarcoding protocol targeting two DNA regions to analyze root-associated fungal communities in ferns and lycophytes

Thais Guillen-Otero¹ | Soon-Jae Lee² | Cheng-Wei Chen³ | Peter Szoevenyi¹ | Michael Kessler¹

¹Department of Systematic and Evolutionary Botany, University of Zurich, Zurich, Switzerland
²Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland
³Biodiversity Program, Taiwan International Graduate Program, Academia Sinica and National Taiwan Normal University, Taipei 115, Taiwan

Correspondence
Thais Guillen-Otero, Department of Systematic and Evolutionary Botany, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland.
Email: shunomey@gmail.com

This article is part of the special issue "Emerging Methods in Botanical DNA/RNA Extraction."

Abstract
Premise: Detailed studies of the fungi associated with lycophytes and ferns provide crucial insights into the early evolution of land plants. However, most investigations to date have assessed fern–fungus interactions based only on visual root inspection. In the present research, we establish and evaluate a metabarcoding protocol to analyze the fungal communities associated with fern and lycophyte roots.
Methods: We used two primer pairs focused on the ITS rRNA region to screen the general fungal communities, and the 18S rRNA to target Glomeromycota fungi (i.e., arbuscular mycorrhizal fungi). To test these approaches, we collected and processed roots from 12 phylogenetically distant fern and lycophyte species.
Results: We found marked compositional differences between the ITS and 18S data sets. While the ITS data set demonstrated the dominance of orders Glomerales (phylum Glomeromycota), Pleosporales, and Helotiales (both in phylum Ascomycota), the 18S data set revealed the greatest diversity of Glomeromycota. Non-metric multidimensional scaling (NMDS) ordination suggested an important geographical effect in sample similarities.
Discussion: The ITS-based approach is a reliable and effective method to analyze the fungal communities associated with fern and lycophyte roots. The 18S approach is more appropriate for studies focused on the detailed screening of arbuscular mycorrhizal fungi.

KEYWORDS
amplicons, DNA sequencing, ferns, ITS, metabarcoding, mycorrhizal fungi, lycophytes, 18S rRNA

Resumen
Premisa: El estudio de los hongos asociados a licofitas y helechos proporciona información crucial sobre la evolución temprana de las plantas terrestres. Sin embargo, hasta el momento, la mayoría de las investigaciones ha evaluado las interacciones helecho–hongo basándose solamente en la observación directa de las raíces. En la presente investigación, establecemos y evaluamos un protocolo de metabarcoding enfocado en dos regiones de ADN para analizar las comunidades fúngicas asociadas a las raíces de helechos y licofitas.
Métodos: Utilizamos dos pares de primer orientados hacia la región ITS ARNr, para la detección de las comunidades fúngicas generales, y la región 18S ARNr, para captar hongos pertenecientes al phylum Glomeromycota (i.e., hongos micorrícicos arbusculares). Para evaluar estos procedimientos, nosotros recolectamos y procesamos raíces de 12 especies de helechos y licofitas distantes desde el punto de vista filogenético.

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