Combing molecular and morphological studies to explore the fungal diversity of Costa Rican Oak Forests

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Background

Fungi are the second most species-rich organism group after insects. However, they are a very much understudied and underexplored group with more than 90% of them still unknown to science. In this project, we explored the fungal diversity in a neotropical *Quercus* forest in Costa Rica using molecular studies as the first step. Is the fungal diversity in a Neotropical *Quercus* Forest in Costa Rica similar/same as other *Quercus*/Oak forests in America?

I hypothesize the fungal diversity in Costa Rica Quercus Forest will be different from other Oak forests

Figure 1. *Lactarius* specimen from Quercus forest in Costa Rica. Collection number MHCR 2

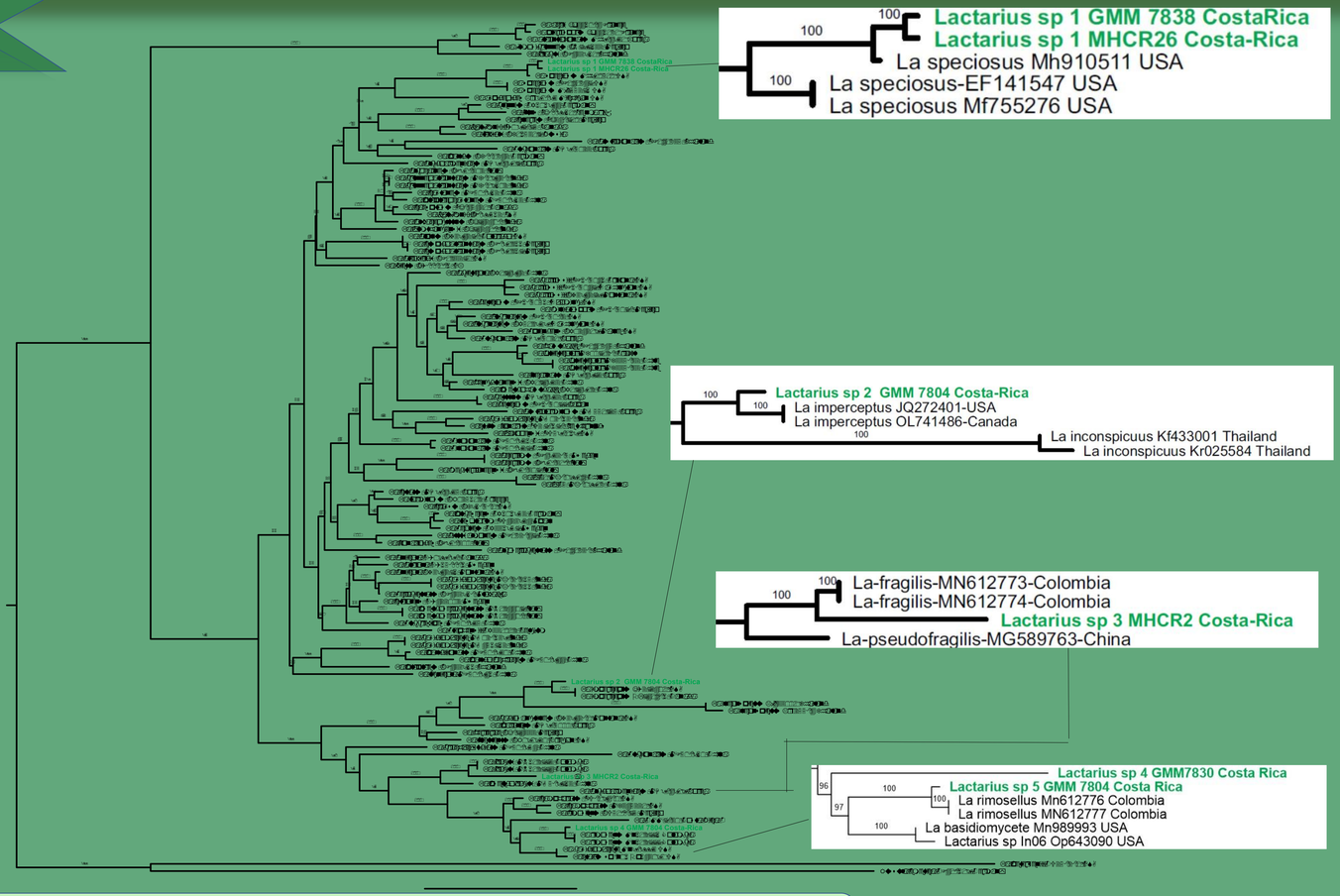

Figure 2. Phylogenetic relationships within *Lactarius* samples from Costa Rica inferred from ITS sequences.
Bootstrap values are indicated on the tree branches. Costa Rican samples are highlighted in green.

Results & Discussion

The overall fungal diversity after completing DNA sequencing consisted of *Lactarius*, *Amanita*, *Russula*, and 22 other genera. Our preliminary results from the phylogenetic tree show that there are 5 potentially new species of *Lactarius*, highlighted in green. The *Lactarius* species collected in Costa Rica haven't been previously found in other *Quercus* forests. Furthermore, they seem to be phylogenetically close with American and Colombian sequences. Moving further in this study, morphological studies would have to be done to propose the new *Lactarius* species found in this project.

Figure 3. *Lactarius* specimen from Quercus forest in Costa Rica. Collection number MHCR 26

Methods

DNA extraction, PCR and sequencing

DNA was extracted from dried specimens harvested from the San Gerardo de Dota. *Quercus* (Oak) forest in Costa Rica using Alkaline Extraction Process. The nuclear rDNA ITS region was amplified using primers ITS1F-ITS4, PCR products were purified and then sequenced with a 3730xl DNA Analyzer (Thermo Fischer) at the Pritzker Lab of Molecular Systematics and Evolution (Field Museum). The obtained sequences were assembled and edited using BioEdit.

Phylogenetic analyses

From all our sequences we selected *Lactarius* species to perform phylogenetic analysis to understand the phylogenetic relationships and identify potential new species, as it was the most abundant genus found. The obtained sequences were assembled and edited using Sequencher. Relevant sequences were downloaded from GenBank and included in the analyses. Separate data sets for the two markers were aligned using MAAFT. and maximum likelihood (ML) was performed using IQ-TREE

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Works Cited

Montoya, Leticia & Bandala, Victor & Haug, Ingeborg & Stubbe, Dirk. (2011). A new species of Lactarius (subgenus gerardii) from two relict Fagus grandifolia var. mexicana populations in Mexican Montane Cloud forests. Mycologia. 104. 175-81. 10.3852/11-051.

Nguyen I. T., Schmidt H. A., Haeseler A. & Minh B. Q . 2014. — IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood Phylogenies. Molecular Ecology and Evolution

Hall T. 1999. — BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95-98.

Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013 Apr;30(4):772-80. doi: 10.1093/molbev/mst010.