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RESEARCH NOTE

Xylariales: First results of mycological exploration in the Sangay and Llanganates National Park, Ecuador [version 1; referees: awaiting peer review]

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Abstract

In the present study fungi ~~collections~~ were sampled in the Sangay (SP) and Llanganates (LP) National Parks, from which sequences of the ~~regions of the~~ internal transcribable spacer (ITS1-5.8S-ITS2) of the ribosomal DNA were obtained (RDNA).
The taxonomic identification of fungi of the order Xylariales was achieved with the bioinformatic tools, to further study the phylogenetic relationships among the collected individuals and thus contribute with base information on their biological diversity, necessary to design and implement measures for the conservation of fungi.
All records belong to the genus Xylaria, of these eight belong to PL and two to SP. A record was not identified at the species level, suggesting that it could be a new species. A phylogenetic tree of Maximum Likelihood was built.

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Introduction

Sangay (SP) and Llanganates (LP) National Parks in Ecuador are considered as high priority conservation units in the Tropical Andes, due to their high biodiversity and high endemism[1,2]. However, their mycological diversity is still unknown. This study aims to contribute to the conservation of fungi, showing the results of their diversity, based on molecular taxonomy, by analyzing the ITS (internal transcribed spacer) regions. ITS is the accepted 'barcode' for fungi[3]. For this, the DNA sequence of specimens of exploratory fungal collections were analyzed within the aforementioned parks. Here we present results exclusively for the Xylariales order.

Methods

~~Obtaining the sequences~~ and molecular identification

Sample collection was carried out during the months of January and February 2015. The fruiting bodies collected were deposited in the QCAM Fungarium (Catholic University Mycology Collection, Quito). Table 1 displays the collection codes, as stored at the QCAM. The ITS1-5.8S-ITS2 region was amplified by PCR with primers ~~(provided by Invitrogen Co., Carlsbad, CA, USA)~~ ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3')[4] and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')[5]. The amplified fragments were sent for sequencing to Macrogen Inc. (Seoul, South Korea). The ~~obtained sequences were edited~~ in Geneious R8 (Biomatter Ltd. 2005–2012) ~~by selecting the "*de novo* assemble" tool and then trimming the ends. The consensus sequence was manually edited,~~ and submitted to GenBank. Sequence data were analyzed by comparison with sequences available in GenBank. An assignment to the lower taxonomic level was made by direct homology of the consensus sequences with the search results in BLASTn (NCBI) optimized for highly similar sequences (megablast), alignments that presented 100

Phylogenetic analysis

Sequence data were aligned with Geneious R8 and later manually adjusted with Mesquite version 3.04[6]. Public sequences are available in GenBank that corresponded to specimens that gave the greatest homology in BLASTn with the sequences of the collected specimens were included in the analyses. Phylogenetic trees were constructed in Geneious R8 using the PhyML[7] plugin for Maximum Likelihood (ML) with a Custom (010230) substitution model determined by jModelTest 2.1.4.[8,9], according to Corrected Akaike Information Criterion (AICc)[10,11]. A bootstrap of 1000 replica~~s~~ was used.

Results

All the specimens collected were of the genus *Xylaria.* The eight specimens from Llanganates National Park were identified as *X. enterogena, X. fissilis, X. schweinitzii, X. telfairii* and three unidentified species. For the two samples from Sangay National Park, one was *X. telfairi* and the other was an unidentified species. Differences in the number of samples found at each park could be due to the sampling effort that was different in each park, however, this is a sample of the high biodiversity in LP and SP. The unidentified species were different in each park. The analysis shows that there are no shared species of *Xylaria* ~~at~~ the two parks (Table 1), this is important for conservation decisions. The phylogenetic relationships recovered from the analysis of the ITS sequences (Figure 1) show~~s~~ two major groups. The first major group, composed by clades A and B, is well supported (bootstrap > 95) includes specimens from LP and PS. Clade A includes all *X. entogena* specimens and is well supported (bootstrap > 95). Clade B includes all *X. telfairii* specimens and *Xylarya* sp.1 specimens (bootstrap < 50), it could be supposed that *Xylarya* sp.1 belongs to the *X. telfairii* species, but because the high difference among the

Table 1. Fungi of the order Xylariales collected in the Sangay and Llanganates National Parks in Ecuador.

QCAM Fungarium Code	GenBank Accession Number	Identification	Sampling location	
			National Park	Altitude
QCAM4663	MG768840	*Xylaria enterogena*	Llangantes	1370
QCAM4551	MG768839	*Xylaria enterogena*	Llangantes	1387
QCAM4537	MG768834	*Xylaria fissilis*	Llangantes	1377
QCAM4540	MG768836	*Xylaria schweinitzii*	Llangantes	1377
QCAM4232A	MG768832Â	*Xylaria telfairii*	Sangay	2885
QCAM4550	MG768838	*Xylaria* sp. 1	Llangantes	1387
QCAM4666	MG768841	*Xylaria* sp. 1	Llangantes	1379
QCAM4306A	MG768833	*Xylaria* sp. 2	Sangay	2885
QCAM4545	MG768837	*Xylaria* sp. 3	Llangantes	1373
QCAM4539	MG768835	*Xylaria* sp. 4	Llangantes	1377

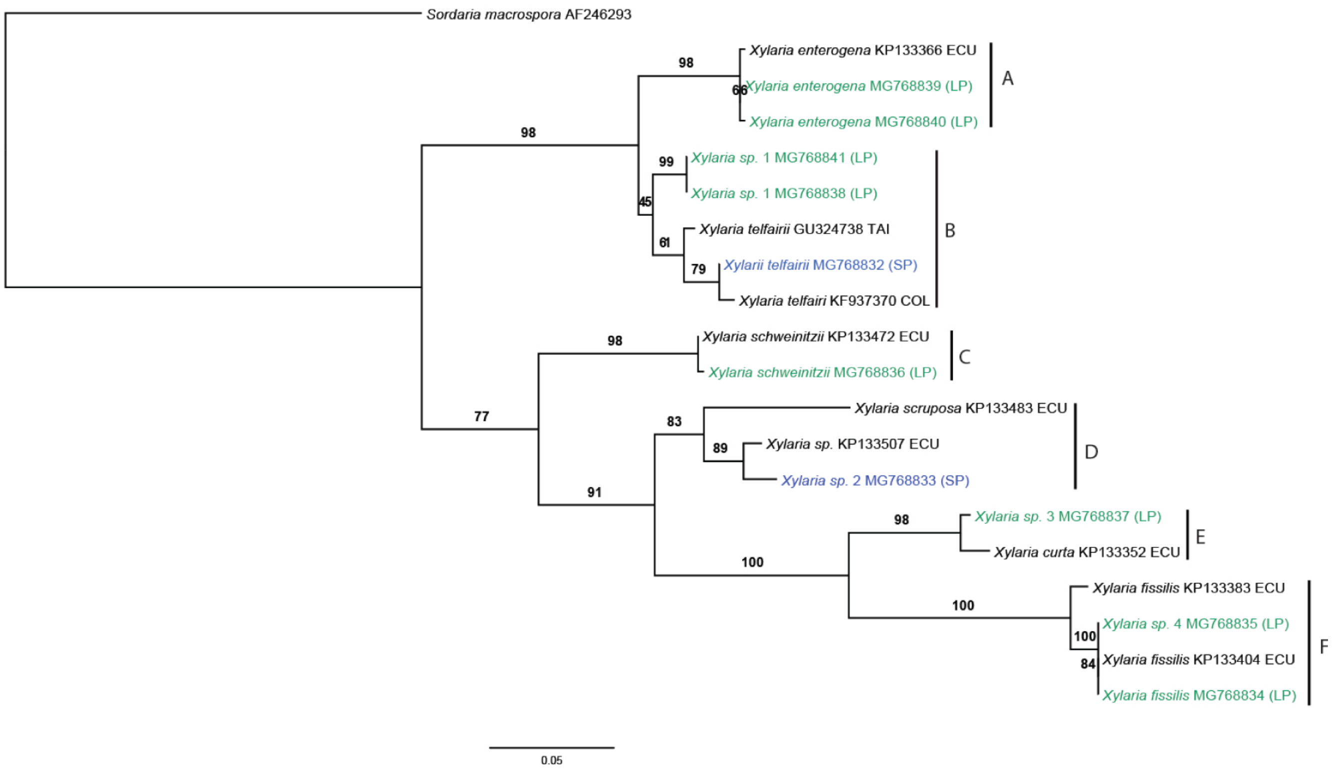

Figure 1. Maximum likelihood phylogenetic tree of specimens of the Xylariales order obtained in Sangay and Llangantes National Parks, based on sequences of the ITS (internal transcribed spacer) region.

sequences it is considered a different species. In the second major group (bootstrap > 75), clade C is sister to clades D, E and F; this major group includes specimens from LP and SP~~, too.~~ Clade C includes all *X. schweinitzii* specimens (bootstrap > 95). Clade D (bootstrap > 80) includes *Xylaria* sp. 2, the closest sequence to *Xylaria* sp. 2 from SP was a previously reported collection also from Ecuador[12] in a cloud forest in the province of Imbabura, that was also identified only at the genus level. Clade~~s~~ E (bootstrap > 95) shows Xylaria sp. 3, the closest sequence to this individual~~s~~ belongs to the same previously reported collection[12], identified only at the genus level. Clade F (bootstrap = 100) includes *Xylaria fissilis* sequences from LP and one from 12; clade F also includes *Xylaria* sp. 4 an unidentified specimen.

These unidentified specimens might represent new species. Additional loci and more detailed morphological analyses are needed to determine this. The genus *Xylaria* is probably the largest in the family Xylariaceae, with 35 estimated genera[13], but the real number remains unknown[14]. Studies ~~in relation~~ to the biological diversity of this order in the National Parks of Ecuador are scarce, more systematic field studies would surely reveal a greater diversity of families, genera and species within the Xylariales in SP and LP, as well as other regions and protected areas of Ecuador, especially if we take into account the cosmopolitan distribution of *Xylaria*[13]. In fact, new fungal species in SP, belonging to the Agaricales, have recently been described[15].

Conclusions

The results obtained allow us to establish a baseline for the biological diversity of the Xylariales in SP and LP, an important step to the conservation of fungi. This is the main contribution of this study. We found four species of *Xylaria*: *X. enterogena*, *X. telfairii*, *X. schweinitzii*, *and X. fissilis*, and four potential new species; the species found in LP are different from those found in SP. However, there is much more to discover. A huge and complex task is pending. To advance our understanding of this Kingdom we must start by deciphering the diversity of fungi present in these sites.

Data availability

The sequencing data are available on the NCBI Genbank webpage:

Xylaria enterogena: https://www.ncbi.nlm.nih.gov/nuccore/MG768840

https://www.ncbi.nlm.nih.gov/nuccore/MG768839

Xylaria fissilis: https://www.ncbi.nlm.nih.gov/nuccore/MG768834

Xylaria schweinitzii: https://www.ncbi.nlm.nih.gov/nuccore/MG768836

Xylaria telfairii: https://www.ncbi.nlm.nih.gov/nuccore/MG768832

Xylaria sp. 1: https://www.ncbi.nlm.nih.gov/nuccore/MG768838

https://www.ncbi.nlm.nih.gov/nuccore/MG768841

Xylaria sp. 2: https://www.ncbi.nlm.nih.gov/nuccore/MG768833

Xylaria sp. 3: https://www.ncbi.nlm.nih.gov/nuccore/MG768837

Xylaria sp. 4: https://www.ncbi.nlm.nih.gov/nuccore/MG768835

Competing interests

No competing interests were disclosed.

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