RESEARCH NOTE

Xylariales: First results of mycological exploration in the Sangay and Llanganates National Park, Ecuador [version 1; peer review: 2 approved with reservations]

María-Fernanda Guevara ^{1,2}, Paula Salazar³, Bence Mátyás ⁴,
María-Eugenia Ordoñez⁵

¹Biotechnology of Natural Resources, Universidad Politécnica Salesiana, Quito, 170525, Ecuador

²Environmental Research Group, Secondary Metabolites and Animal Biotechnology NUNKUY-WAKAN, Universidad Politécnica Salesiana, Quito, 170525, Ecuador

³Carrera de Biología de los Recursos Naturales, Universidad Politécnica Salesiana, Quito, 170525, Ecuador

⁴Grupo de Investigación Mentoría y Gestión del Cambio, Universidad Politécnica Salesiana, Cuenca, 010102, Ecuador

⁵School of Biological Sciences, Pontifical Catholic University of Ecuador, Quito, 170143, Ecuador

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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.

Keywords

Diversity, ITS, Llanganates, National Park, Sangay, Xylarial

This article is included in the **Ecology and Global Change** gateway.

Open Peer Review

Approval Status

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version 2 (revision) 17 May 2018	 view	 view	 view
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version 1 23 Feb 2018	 view	 view	

1. **Francisco J. Flores** , Universidad de las Fuerzas Armadas-ESPE, Sangolquí, Ecuador
2. **D. Jean Lodge**, USDA Forest Service, Luquillo, USA
University of Georgia, Athens, USA
3. **Juan Enrique Tacoronte Morales** ,
University of Georgia, Athens, USA

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Bence Mátyás (bmatyas@ups.edu.ec)

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Competing interests: No competing interests were disclosed.

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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 fungi³. 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 3.04⁶. 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 PhyML⁷ 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 replicas 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. telfairii* 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**) shows 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. enterogena* specimens and is well supported (bootstrap > 95). Clade B includes all *X. telfairii* specimens and *Xylaria* sp.1 specimens (bootstrap < 50), it could be supposed that *Xylaria* 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	<i>Xylaria enterogena</i>	Llangantes	1370
QCAM4551	MG768839	<i>Xylaria enterogena</i>	Llangantes	1387
QCAM4537	MG768834	<i>Xylaria fissilis</i>	Llangantes	1377
QCAM4540	MG768836	<i>Xylaria schweinitzii</i>	Llangantes	1377
QCAM4232A	MG768832	<i>Xylaria telfairii</i>	Sangay	2885
QCAM4550	MG768838	<i>Xylaria</i> sp. 1	Llangantes	1387
QCAM4666	MG768841	<i>Xylaria</i> sp. 1	Llangantes	1379
QCAM4306A	MG768833	<i>Xylaria</i> sp. 2	Sangay	2885
QCAM4545	MG768837	<i>Xylaria</i> sp. 3	Llangantes	1373
QCAM4539	MG768835	<i>Xylaria</i> 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 Ecuador¹² in a cloud forest in the province of Imbabura, that was also identified only at the genus level. Clades E (bootstrap > 95) shows *Xylaria* sp. 3, the closest sequence to this individuals belongs to the same previously reported collection¹², 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 genera¹³, but the real number remains unknown¹⁴. 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 described¹⁵.

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.

Grant information

The Secretaria de Educación Superior, Ciencia, Tecnología e Innovación del Ecuador (SENESCYT), Arca de Noé Initiative and the QCAM Fungarium supported the project.

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Version 1

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D. Jean Lodge

¹ Department of Agriculture, USDA Forest Service, Luquillo, PR, USA

² Department of Plant Biology, University of Georgia, Athens, GA, USA

Introduction. Because the aim is to compare these two parks in Ecuador, it is important that the elevation ranges of each are included, as well as the distance between the two parks.

Methods, p. 3, last line seems to be an incomplete sentence. It ends with 'BLASTn' (NCBI) optimized for highly similar sequences (megablast), alignments that presented 100' It is probably meant to say 'overlapped 100%.'

Results: Text for Clade E does not match the phylogram. The text says that sp. 3 was related to another collection identified only at the genus level. However, the phylogram shows a well-supported clade (98% BS) comprised of sp. 3 and *X. curta* KP133352 ECU. I would expect *X. curta* to be reasonably common there. There is either an error in identification of KP133352 ECU or the text in results needs to be changed, and the abstract and first line of results and conclusions also need to be changed to include *X. curta*.

That also means that the unidentified species need to be renumbered in the text, table, and phylogram, with sp. 4 becoming sp. 3.

There are likely 3 undescribed species included in this study. The journal allows for photographs, and photos should be added to the manuscript. Even if photos were not taken of the fresh specimens, photos of the dried specimens can be used, and are very helpful. If photos of the asci, especially the ascus plug stained with iodine/Melzer's reagent, and photos of the ascospores showing the germ slits, that would be very helpful also for future work. The ascospore length and width ranges, the shape and extent of the germ slit, and the shape and size of the ascus plug would be helpful additions.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 01 March 2018

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Francisco J. Flores

Departamento de Ciencias de la vida y la Agricultura, Universidad de las Fuerzas Armadas-ESPE, Sangolquí, Ecuador

The article "Xylariales: First results of mycological exploration in the Sangay and Llanganates National Park, Ecuador " provides internal transcribed spacer sequences from *Xylaria* isolates collected from Ecuadorian national parks. Information about fungal diversity in Ecuador is still scarce, which makes this manuscript relevant. Nevertheless, I have several concerns, mainly:

- Phylogenetic analysis using only the ITS sequence is very superficial, and, most likely, it won't show an accurate representation of the real evolutionary relationships between isolates.
- There is no mention about the morphological characteristics of isolates. Is the morphology congruent with the genotype?

The manuscript needs major revision to improve clarity.

Several suggestions are made in the file linked below.

https://f1000researchdata.s3.amazonaws.com/linked/196501.Bence_Matyas_rev.pdf

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phylogenetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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