RESEARCH NOTE

REVISED Xylariales: First results of mycological exploration in the Sangay and Llanganates National Parks, Ecuador [version 2; referees: 2 approved with reservations]

María-Fernanda Guevara [1,2], Bence Mátyás [3,4], María-Eugenia Ordoñez[5]

[1]Biotechnology of Natural Resources, Universidad Politécnica Salesiana, Quito, 170525, Ecuador
[2]Environmental Research Group, Secondary Metabolites and Animal Biotechnology NUNKUY-WAKAN, Universidad Politécnica Salesiana, Quito, 170525, Ecuador
[3]Grupo de Investigación Mentoria y Gestión del Cambio, Universidad Politécnica Salesiana, Cuenca, 010102, Ecuador
[4]Grupo de Investigación Ambiental para el Desarrollo Sustentable- GIADES, Universidad Politécnica Salesiana, Quito, Ecuador
[5]School of Biological Sciences, Pontifical Catholic University of Ecuador, Quito, 170143, Ecuador

v2 **First published:** 23 Feb 2018, **7**:222 (doi: 10.12688/f1000research.13623.1)
Latest published: 17 May 2018, **7**:222 (doi: 10.12688/f1000research.13623.2)

Abstract

Fungal samples were collected in the Sangay (SP) and Llanganates (LP) National Parks in Ecuador. Sequences of the internal transcribed spacer regions (ITS1-5.8S-ITS2) of the ribosomal DNA ~~of the samples~~ were analyzed.Taxonomic identification of fungi of the order Xylariales was done through phylogenetic analysis using a Maximun Likelihood method. All analyzed collections presented here belong to the genus Xylaria, ~~of these eight belong to PL and two to SP~~. Four samples were not identified at the species level, suggesting it could be a new species. This data contributes with base information on the biodiversity of the Parks, necessary to design and implement measures for the conservation of fungi in Ecuador.

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

Open Peer Review

Referee Status: ? ?

	Invited Referees 1	2
REVISED version 2 published 17 May 2018		
version 1 published 23 Feb 2018	? report	? report

1 **Francisco J. Flores**, Universidad de las Fuerzas Armadas–ESPE, Ecuador

2 **D. Jean Lodge**, USDA Forest Service, USA
University of Georgia, USA

Discuss this article

Comments (0)

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

Author roles: Guevara MF: Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation; **Mátyás B**: Project Administration, Writing – Review & Editing; **Ordoñez ME**: Conceptualization, Investigation, Methodology, Project Administration, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

How to cite this article: Guevara MF, Mátyás B and Ordoñez ME. **Xylariales: First results of mycological exploration in the Sangay and Llanganates National Parks, Ecuador [version 2; referees: 2 approved with reservations]** *F1000Research* 2018, **7**:222 (doi: 10.12688/f1000research.13623.2)

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.

First published: 23 Feb 2018, **7**:222 (doi: 10.12688/f1000research.13623.1)

REVISED **Amendments from Version 1**

According to the referee's suggestions we have made the following changes in the manuscript:

- The abstract, introduction and methods were re-written for ease of comprehension.
- The results are explained objectively
- The table and figure legends are also modified for ease of comprehension of the content.
- In addition, we rephrased 2 sentences in Conclusions.
- We have removed one of the authors from the second version of the article as an error was made when she was included in the moment of submitting the first version of the article. We were working on several articles at the same time and because of confusion, her name was included in this article. Paula Salazar is a valuable person for the Universidad Politécnica Salesiana and she has collaborated in several projects, but unfortunately she did not collaborate in this research done in association with Pontificia Universidad Católica.

See referee reports

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 as primary fungal barcode marker for fungi[3,4]. 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, other fungal orders were also collected but are not shown here.

Methods

Sequencing 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 ITS1-5.8SITS2 region was amplified by PCR with primers ITS1F (5'-CTTGGTCATTT AGAGGAAGTAA-3')[5] and ITS4 (5'-TCCTCCGCTTATTGATA TGC-3')[6]. The amplified fragments were sent for sequencing to Macrogen Inc. (Seoul, South Korea). The forward and reverse sequences obtained for each isolate were assembled in Geneious R8 (Biomatter Ltd. 2005–2012), 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% coverage and at least 99% identity with a sequence

Table 1. Fungi of the order Xylariales collected in the Sangay and Llanganates National Parks in Ecuador. *SNPs: Single nucleotide polymorphisms found between the sample analyzed and the closest hit on the BLASTn search.

QCAM Fungarium Code	GenBank Accession Number	Identification	Sampling location	
			National Park	Altitude (m)
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

previously reported in GenBank were considered. The results were compared with the previously made morphological identification at the QCAM Fungarium, to check the taxonomic designation.

Phylogenetic analysis

Sequence data were aligned with Geneious R8 and later manually adjusted with Mesquite version 3.04[7]. Public sequences available in GenBank that corresponded to specimens that gave the greatest homology in BLASTn with the sequences of the collected specimens were included. Phylogenetic trees were constructed in Geneious R8 using the PhyML[8] plugin for Maximum Likelihood (ML) with a custom substitution model (010230), determined by jModelTest 2.1.4.[9,10], according to Corrected Akaike Information Criterion (AICc)[11,12]. ~~A bootstrap of 1000 replica was used.~~

Results

All the specimens analyzed 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, and not necessarily to the richness of the Xylareales in the Parks. The unidentified species were different in each park. The analysis shows that there are no shared species of Xylaria at the two sampled sites (Table 1), this is important for conservation decision making. 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, it includes specimens from LP and PS. Clade A includes all X. entogena specimens and Clade B includes all X. telfairii specimens, and Xylarya sp.1 specimens. It is possible that Xylarya sp.1 might belong to the X. telfairii group, but due to the differences among the sequences it is likely a different species. In the second major group, clade C is sister to clades D, E and F. This major group includes specimens from both LP and SP. Clade C includes all X. schweinitzii specimens. Clade D includes Xylaria sp. 2. The closest sequence to Xylaria sp. 2 from SP was a previously reported collection also from Ecuador[13] in a cloud forest in the province of Imbabura, that was also identified only at the genus level. Clade E shows Xylaria sp. 3, the closest sequence to this individual belongs to the same previously reported study[13], identified only at the genus level. Clade F includes Xylaria fissilis sequences from LP and one from 13. Clade F also includes Xylaria sp. 4, an unidentified specimen. The number of nucleotide differences (SNPs) between the sample and the closest hit on the BLASTn search suggests that these specimens may belong to new species (Table 1). 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[14], but the real number remains unknown[15]. Studies related to the biological diversity of this order in the

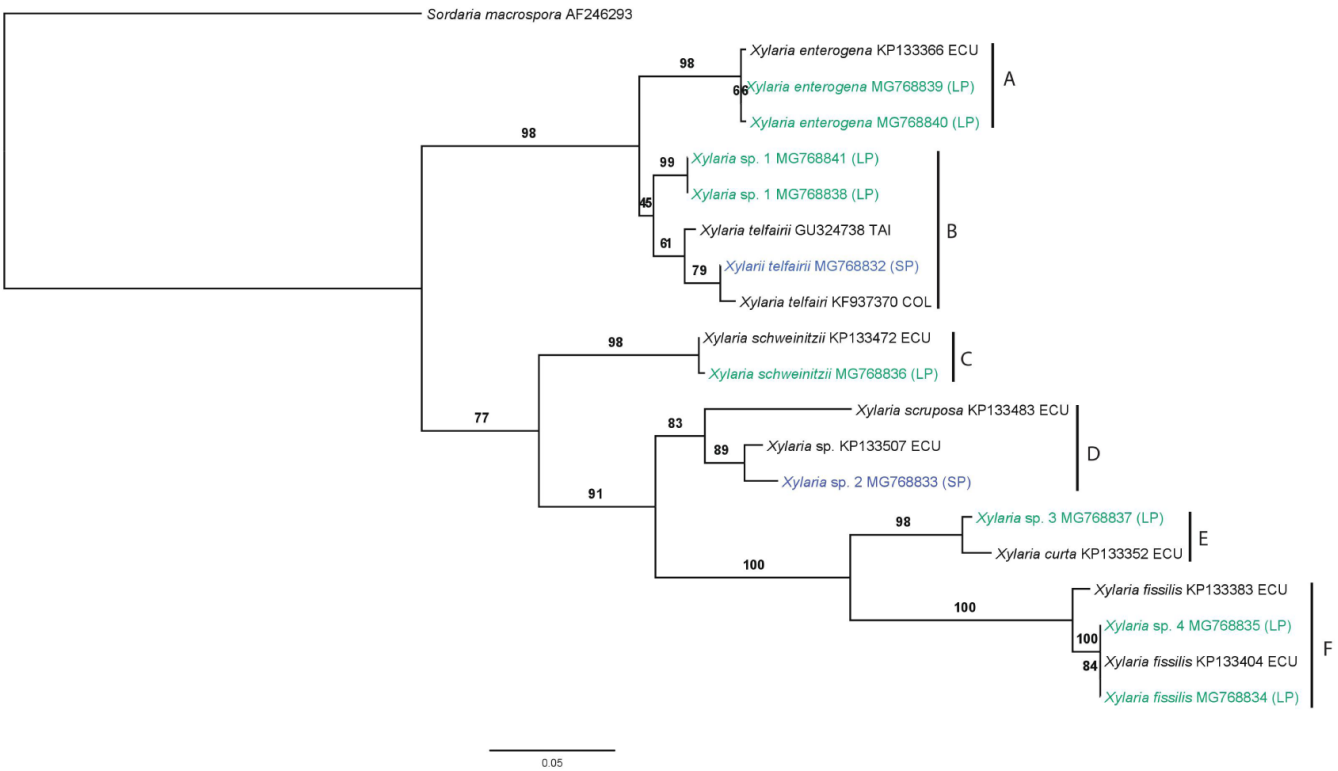

Figure 1. Maximum likelihood phylogenetic tree of specimens of the Xylariales order obtained in Sangay and Llangantes National Parks, based on sequences of the ITS (internal transcribed spacer) region.

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[16].

These entire 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[15]. 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 PS, belonging to the Agaricales, have recently been described[16].

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 based on ITS sequences ~~divergence~~; 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 the Kingdom Fungi we must start by deciphering the diversity of species 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 from "Pontificia Universidad Católica del Ecuador" supported the project.

Acknowledgements

We are grateful to Marcel A. Caminer, Charles W. Barnes and Cristina E. Toapanta for their contribution to the development of the present research. This research has been carried out according to the MAE-DNB-ARRGG-CM-2014-002 "Contrato Marco de Acceso a Recursos Genéticos".

References

1. Bajaña F, Rivas J, Sánchez D, *et al.*: **Informe de la evaluación inicial del Parque Nacional Sangay como Sitio de Patrimonio Natural de la Humanidad.** *Quito-Ecuador: Ministerio del Ambiente, Fundación Natura, EcoCiencia y UICN-Sur.* 2002.
2. Ministerio del Ambiente del Ecuador: **Plan de Manejo Parque Nacional Llanganates**. Quito-Ecuador, 2013.
Reference Source
3. Schoch CL, Seifert KA, Huhndorf S, *et al.*: **Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*.** *Proc Natl Acad Sci U S A.* 2012; **109**(16): 6241–6.
PubMed Abstract | Publisher Full Text | Free Full Text
4. Schoch CL, Robbertse B, Robert V, *et al.*: **Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi.** *Database (Oxford).* 2014; **2014**; pii: bau061.
PubMed Abstract | Publisher Full Text | Free Full Text
5. Gardes M, Bruns TD: **ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts.** *Mol Ecol.* 1993; **2**(2): 113–118.
PubMed Abstract | Publisher Full Text
6. White TJ, Bruns T, Taylor J: **Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics.** *Mycologia.* 2013; **64**(1).
Reference Source
7. Maddison WP, Maddison DR: **Mesquite: a modular system for evolutionary analysis.** Version 3.04. 2015.
Reference Source
8. Guindon S, Dufayard JF, Lefort V, *et al.*: **New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0.** *Syst Biol.* 2010; **59**(3): 307–321.
PubMed Abstract | Publisher Full Text
9. Darriba D, Taboada GL, Doallo R, *et al.*: **jModelTest 2: more models, new heuristics and parallel computing.** *Nat Methods.* 2012; **9**(8): 772.
PubMed Abstract | Publisher Full Text | Free Full Text
10. Guindon S, Gascuel O: **A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood.** *Syst Biol.* 2003; **52**(5): 696–704.
PubMed Abstract | Publisher Full Text
11. Akaike H: **Information theory and an extension of the maximum likelihood principle.** In: Petrov BN, Csaki F. (Eds.), *2nd International Symposium on Information Theory.* Budapest: Akademiai Kiado, Budapest, Hungary, 1973; 267–281.
Reference Source
12. Posada D: **jModelTest: phylogenetic model averaging.** *Mol Biol Evol.* 2008; **25**(7): 1253–1256.
PubMed Abstract | Publisher Full Text
13. Thomas DC, Vandegrift R, Ludden A, *et al.*: **Spatial Ecology of the Fungal Genus *Xylaria* in a Tropical Cloud Forest.** *Biotropica.* 2016; **48**(3): 381–393.
Publisher Full Text
14. Lee JS, Ko KS, Jung HS: **Phylogenetic analysis of *Xylaria* based on nuclear ribosomal ITS1-5. 8S-ITS2 sequences.** *FEMS Microbiol Lett.* 2000; **187**(1): 89–93.
PubMed Abstract | Publisher Full Text
15. Hsieh HM, Lin CR, Fang MJ, *et al.*: **Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily.** *Mol Phylogenet Evol.* 2010; **54**(3): 957–969.
PubMed Abstract | Publisher Full Text
16. Crous PW, Wingfield MJ, Burgess TI, *et al.*: **Fungal Planet description sheets: 558–624.** *Persoonia.* 2017; **38**(1): 240–384.
PubMed Abstract | Publisher Full Text | Free Full Text

Open Peer Review

Current Referee Status: ? ?

Version 1

Referee Report 19 April 2018

doi:10.5256/f1000research.14800.r32603

? **D. Jean Lodge** [1,2]

[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 resutls 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 have read this submission. I 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.

Referee Report 01 March 2018

doi:10.5256/f1000research.14800.r31232

? **Francisco J. Flores**
Carrer of Engineering Biotechnology, 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.

Referee Expertise: Phylogenetics

I have read this submission. I 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.