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Keywords: Broussonetia papyrifera, decaying wood, fungal endophytes, Peroneutypa scoparia, invasive tree.

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Abstract: Fungal endophytes comprise a highly diverse group of particular interest for their relevant implications to the ecosystems they inhabit. The objective of this study was to infer the phylogenetic affinity between strains of Peroneutypa scoparia exhibiting different lifestyles to elucidate possible shifts in ecological roles. Specimens and living cultures used in the present study were obtained from decaying wood and from live stem tissues of the invasive host species Broussonetia papyrifera. The similarity between the fungal strains was studied through molecular analyses. The results showed a close phylogenetic link and high genetic similarity between endophytic and saprotrophic strains. The main findings suggest that P. scoparia has primary access to the substrate as an endophyte and then, this organism may change its use of the available resources presenting a saprotrophic growth. These results provide valuable information about the roles that diatrypaceous fungi play as endophytes or as decaying wood inhabitants and contributes to evaluate the ecological significance of this group.

Suggested Reviewers:

Opposed Reviewers:

Response to Reviewers: List of responses to the comments: Please find below the changes we have incorporated into the manuscript.

-References: Please check your references and format them as demonstrated:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51-59.
It was checked. Several changes to improve the format were made

-Journal names should be abbreviated according to the list of title word abbreviations:
http://www.issn.org/2-22661-LTWA-online.php.
It was checked.

Reviewer #2:
-Please go again carefully through the whole text and correct it where appropriate (e.g., line 94: some word is perhaps missing?
Yes. The reviewer is right. We added “in” in the sentence (line 94). Others minor additions, marked in the text, were also included.

-lines 264-266: repetition of what has already been said in Materials and Methods (lines 103-105)
We have deleted the paragraph. We began the Discussion with a new introductory text “Fungal endophytes exhibit a broad range of lifestyles. The present study provides insight as to whether diatrypaceous endophytic fungi manifest themselves as saprotrophs when the host dies.”

-lines 267-276: mostly repetition of what has already been said in Results (lines 201-217). In fact, the real discussion starts at the line 280. Overall, the Discussion chapter is quite short and brings only few new ideas (supported by the results obtained in this study and compared with relevant literature).
Yes the reviewer is right. We prefer to leave this paragraph because we consider that in this specific case, it serves as a brief introduction and gives the reader the context in which the discussion is based. Anyway, if the editor considers appropriate reviewer's suggestion, we can eliminate paragraph of the manuscript.

**Cover Letter**

Buenos Aires, 11/07/2014

Dear Editor in Chief Dr. Hermann Heilmeier,

We are returning to you the revised version of the manuscript Ms. Ref. No.: FLORA-D-14-00072. "**Plant-fungal association in trees: insights into changes in ecological strategies of *Peroneutypa scoparia* (Diatrypaceae)** by Andrés de Errasti, M. Victoria Novas and Cecilia C. Carmarán[*].

We thank you and the reviewer for the new suggestions.

In the present submission we included: the revised files (Cover letter and MS) and those without changes (Highlights, Graphical abstract, Fig1, Fig 2 and Fig 3). In the new manuscript we remarked the changes (in yellow those of the first revision and in green the new corrections) to make them easier to view. The references format and the abbreviations have been checked and some modifications have been done (not marked in the text).

We hope you find this new version of the manuscript suitable to be published in the Journal.

Thank you very much in advance, looking forward to hearing from you.

Cecilia Carmaran
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UNIVERSIDAD DE BUENOS AIRES

Buenos Aires, 11th July 2014

Dear Dr. Bohumil Mandak

Editor Flora

Morphology, Distribution, Functional Ecology of Plants

We are returning to you the revised version of the manuscript Ms. Ref. No.: FLORA-D-14-00072R1. "**Plant-fungal association in trees: insights into changes in ecological strategies of *Peroneutypa scoparia* (Diatrypaceae)** by Andrés de Errasti, M. Victoria Novas and Cecilia C. Carmarán*.

We thank you and the reviewers for the suggestions that have really improved our manuscript. We have included most of your suggestions in the manuscript.

Below there is a point by point description of the reviewer comments.

**List of responses to the comments:** Please find below the changes we have incorporated into the manuscript.

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Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51-59.

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**Anyway, if the editor considers appropriate reviewer's suggestion, we can eliminate paragraph of the manuscript.**

*Manuscript



# "Plant-fungal association in trees: insights into changes in ecological strategies of *Peroneutypa scoparia* (Diatrypaceae)."

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## ABSTRACT

Fungal endophytes comprise a highly diverse group of particular interest for their relevant implications to the ecosystems they inhabit. The objective of this study was to infer the phylogenetic affinity between strains of *Peroneutypa scoparia* exhibiting different lifestyles to elucidate possible shifts in ecological roles. Specimens and living cultures used in the present study were obtained from decaying wood and from live stem tissues of the invasive host species *Broussonetia papyrifera*. The similarity between the fungal strains was studied through molecular analyses. The results showed a close phylogenetic link and high genetic similarity between endophytic and saprotrophic strains. The main findings suggest that *P. scoparia* has primary access to the substrate as an endophyte and then, this organism may change its use of the available resources presenting a saprotrophic growth. These results provide valuable information about the roles that diatrypaceous fungi play as endophytes or as decaying wood inhabitants and contributes to evaluate the ecological significance of this group.



**Key words:** *Broussonetia papyrifera*, decaying wood, fungal endophytes, *Peroneutypa scoparia*, invasive tree.


## INTRODUCTION

Fungal endophytes, i.e. fungi that live within plant tissues without causing symptoms, are found in all plant lineages. (Arnold, 2008; Petrini, 1991; Rodriguez et al., 2009). This diverse group of fungi can modulate the ecology of plant communities, conferring resistance to abiotic and biotic stresses (Rodriguez et al., 2009).

The ecological roles of endophytes are just starting to be elucidated. So far, it is known that endophytes may be neutral, parasitic or mutualistic inhabitants of their hosts (Arnold, 2008; Hyde and Soytong, 2008, Partida-Martínez and Heil, 2011). The high diversity of endophytes harbored by a single host probably includes species with the ability to either play play just one of these roles or change roles over time or under certain conditions (Arnold, 2008). Previous studies based on molecular and enzymatic approaches have suggested that, fungal organisms that occur as endophytes, can switch their nutritional mode (from saprotrophic to parasitic or vice versa) or/and lifestyles (soil, fungi, and decaying and living plants) (Chaverri & Samuels 2013; Delaye et al., 2013; Duong et al., 2008; Hyde et al., 2007; Oses et al., 2008; Promputtha et al., 2007; Promputtha et al., 2010; Parfitt, 2010).

Many studies have assessed the connection between parasitic and saprotrophic fungi in wood; however, few of them performed an experimental design to establish a direct relationship between these nutritional modes or habitat preferences (Álvarez-Loayza, 2011; Chaverri and Samuels, 2013).

The diversity of members of the family *Diatrypaceae* (Xylariales, Ascomycota) have been extensively studied in wood of different hosts (Rappaz, 1987, Vasilyeva and Stephenson, 2004, among others). Species of this family have been mostly described as saprotrophic on dead wood and only few species have been characterized as pathogens (Trouillas and Gubber, 2010). Previous reports indicate that members of this group are able to grow in living trees as endophytes; *Libertella* sp on *Picea excelsa* (Carroll et al., 1977), *Cryptosphaeria populina* (Chapela, 1989) and *Cryptosphaeria lignyota* on *Populuts tremuloides* Michx (Hutchinson, 1999) and more recently *Peroneutypa scoparia* was recorded from *Garcinia* species

(Phongpaichi et al., 2006).

In Argentina several works have been carried out in order to characterize the diversity of saprotrophic species of *Diatrypaceae* (Carmarán and Romero, 1992; Carmarán, 2002; Carmarán et al., 2006; Pildain et al., 2005). In a previous work, we isolated endophytic strains of *P. scoparia* (anamorphic state, *Libertella* sp.) from living branches of *Broussonetia papyrifera* (L.) L'Her. ex Vent, considered an invasive tree (de Errasti et al., 2010), turning this tree species into an interesting model to study the relationship between strains that occur as endophytes and as fallen wood-inhabitants of the same species of fungi.

The aims of the present study were to determine the occurrence of an endophytic stage of saprotrophic fungi within Diatrypaceae, by performing an ecological survey at the field, and to evaluate the relationship between the obtained strains through phylogenetic analyses based on DNA sequence comparison of the ITS region and the β-tubulin gene.

## MATERIALS AND METHODS

### Study area and plant material

The study area is located in Dr. Carlos Spegazzini Reserve (a natural Mycological Reserve within an urban area in Lomas de Zamora district (34° 47' S; 56° 56' W), Buenos Aires province, Argentina. This area is characterized by a slightly acid soil, with pH values ranging from 5.4 to 5.8. The maximum and minimum average five-year span temperatures are 22.2°C and 10.7°C, respectively. The average rainfall is 938 mm and 73% of relatively humidity. The land occupies approximately 60 hectares and the plant community is characterized by a naturalized forest (De Magistris, 1996).

*Broussonetia papyrifera* (L.) L'herit (Moraceae), the host tree from which we have previously isolated diatrypaceous fungi as woody-tissue endophytes (de Errasti et al., 2010), was chosen to carry out the present study. In Argentina, *B. papyrifera* is an exotic invasive tree (INBIAR 2013) in protected areas due to its high colonization rate.

*Isolates from living trees*

The diatrypaceous strains used in the present study were those previously obtained from woody tissue of living trees of *B. papyrifera* (de Errasti et al., 2010). They are deposited in the culture collection as *BAFCcult* 3321, 3322, 3390 and 3391 (Culture collection of the Facultad de Ciencias Exactas y Naturales, University of Buenos Aires).

*Experimental design*

To evaluate the occurrence of an endophytic stage of saprotrophic strains in the same host, we carried out the following analyses on the same individual trees from which we have previously isolated diatrypaceous fungi as endophytic strains. A total of five trees of *B. papyrifera* were included in this study and three healthy branches were cut off from each tree (2-6 cm in diameter), resulting in a sampling of 15 branches (modified from Hendry et al., 2002). From each branch, two 10 cm-long sections (herein after referred to as "units") were cut, kept in sterile polyethylene bags and processed in the laboratory within 48 h after collection (according to the section *Survey of newly saprotrophic infections of diatrypaceous fungi*). The remaining parts of the branches (remaining branches) were labeled and left on the ground to perform further analyses (Fig. 1).

*Survey of preceding saprotrophic infections of diatrypaceous fungi*

To examine infections established previous to the beginning of this study, fallen branches with typical diatrypaceous stromata were collected from the sampling area. The sampling was performed at the beginning of the study and six months later. At the laboratory, isolates were obtained from the hamathecium.

*Survey of newly saprotrophic infections of diatrypaceous fungi*

To study the development of new saprotrophic infections two procedures were carried out. First, the cut units, kept in sterile polyethylene bags, were sterilized by autoclave and then within 48 hs, they were returned to the sampling site (herein after referred to as "sterilized units") to the same location point of the "remaining branches". From remaining branches, units 10 cm-long were cut (herein after referred to as "field units") and left on the ground near to sterilized units. Then, the sterilized units and the field units were checked to detect the emergence of diatrypaceous fungi (teleomorphic or anamorphic stromata) six months and one year after the sampling. When specific fungal structures were noticed, isolates from the hamathecium were performed.

The experiment began in April 2007 (autumn), when the healthy branches were cut off from the trees, and continued until the same month the following year. Therefore the two types of units (sterilized *vs* field) were subjected to the same environmental conditions.

*Evaluation of epiphytic and endophytic fungi of bark*

To discard potential colonization of the branches by epiphytic fungi, portions of bark from each individual tree were analyzed: a) by pressing the external surface of the

bark on a sterile plate with malt extract agar (MEA 2%) b) by plating small pieces of bark, previously surface-sterilized, on the same medium. The presence of diatrypaceous fungi was assessed for the following 15 days.

*Fungal identification*

Two replicates of each strain, obtained from the different procedures, were grown on MEA and Potato dextrose agar (PDA) for at least ten days. Pure cultures were examined periodically for sporulation. The morphological identification of the strains was carried out only for members of the family *Diatrypaceae*. Identified species were preserved in the *BAFCcult* (Holmgren et al., 1990).

*DNA extraction, amplification and sequencing*

The diatrypaceous strains obtained from the different procedures (BAFCcult 3320, BAFCcult 3323, BAFCcult 3324, BAFCcult 3325, BAFCcult 3326, BAFCcult 3327, BAFCcult 3328) and two diatrypaceous fungi obtained in our previous work (BAFC 3321, BAFC 3322) (de Errasti et al., 2010) were grown on MEA 0.1 % (w/v) and incubated at 25°C for 21 days in light/darkness. DNA was extracted from the cells using the UltraCleanTM Microbial DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, USA) according to the manufacturer's instructions. The ITS region of the strains was amplified using the universal primers ITS1 and ITS4 (White *et al.*, 1990), whereas a fragment of the β-tubulin gene was amplified using the primers Bt2b and T2 (Glass & Donaldson, 1995; O'Donnell and Cigelnik, 1997). In some cases, best amplification results were achieved by adding 6% bovine serum albumin (BSA, Promega Corp.) to the PCR reaction mix. PCR products were purified using a QIAGEN Gel Extraction kit (QIAGEN Inc.). Both strands of each fragment

were sequenced by Macrogen Service Center. *Xylaria berteri* and *X. curta* were chosen as outgroups.

*Molecular and phylogenetic analyses*

A total of 34 sequences from ITS and 31 from β-tubulin genes, including 10 from isolated strains from the Dr. Carlos Spegazzini reserve (de Errasti et al., 2010), were used in the molecular analyses (Table 1). Environmental samples of NCBI BLAST were tested to identify sequences with high similarity. No significant results were obtained. The sequences obtained in this study were pair-wise compared to estimate percentage of similarity.

A dynamic homology analysis was performed using the program POY 4 (Varón et al., 2010). The commands build (10) and swap (100) were used (cost 5 was assigned for gap opening). To determine the support for each clade, a Bremer analysis with 2000 replications was performed.

For static homology analysis, BioEdit sequence alignment editor, version 7.0.5.3 (Hall, 1999) was used in the sequence manipulation. The parsimony method was used for the phylogenetic analysis. The alignments are deposited in TreeBASE (Submission ID 11053). Phylogenetic analysis of sequence data was performed using NONA version 2.0 (Goloboff, 1997) with all characters equally weighted and gaps scored as missing data. The analysis was performed with 700 replications, cost 5 for gap opening and 2 for gap extension. Overall, 3.5% of the data matrix cells were scored as gaps. To determine the support for each clade, a bootstrap analysis with 2000 replications was performed.

*Germination and viability assays*

To test whether conidia have a functional role in fungal spread, strains *BAFCcult* 3321 (endophytic) and 3325 (saprotrophic) were used to test conidial viability and germination rate. Conidia produced on MEA after 10 days were harvested in sterile water. For the viability assays, conidia were stained with propidium iodide (50 mgr/ml) for 10 minutes and counted using a New Bauer's device with fluorescent microscopy. One hundred conidia were observed for each isolate. For the germination assay, conidia were spread on a plate containing MEA. The germination percentage was evaluated between 8 and 48 h afterwards. A total of 12 fields from two different plates were observed for each strain. The conidium was considered as germinated when the length of the germination tube reached more than 50 % of its total length.

## RESULTS

*Saprotrophic strains of diatrypaceous fungi*

Eight saprotrophic strains (four from *B. papyrifera*, two from *L. lucidum* and two from an unknown host) were obtained from the different procedures (Table 2).

No evidence of diatrypaceous infections was found in the units that were sterilized before being left in the sampling area. However, two strains of *Peroneutypa scoparia* were obtained from the "field units" of *B. papyrifera* (*BAFCcult* 3325 and 3326).

Another two specimens identified as *Eutypella leprosa* and *Peroneutypa. scoparia* (*BAFCcult* 3320) were collected from old fallen branches of *B. papyrifera*, so they were considered as saprotrophic infections previous to the beginning of this study.

The survey to detect preceding saprotrophic diatrypaceous fungi in fallen branches of

213 other host species allowed us to identify a strain of *P. scoparia* (*BAFCcult* 3324) and
214 the anamorphic state (*Libertella* sp. *BAFCcult* 3323) on fallen branches of *Ligustrum*
215 *lucidum*. We also detected two strains of *P. scoparia* (*BAFCcult* 3327 and 3328) in
216 fallen branches of an unknown host that were incorporated to the analysis.
217 Neither epiphytic nor bark endophytic diatrypaceous fungi were recovered using
218 the procedures explained in the Materials and methods.

219

220 *Phylogenetic analysis*

221 ITS region

222 The phylogenetic analysis using static homologies (NONA) (157 informative
223 characters) yielded one most-parsimonious tree of length (L) =363, consistency index
224 (Ci) =63, and retention index (Ri) =87. The most parsimonious tree is shown in Figure
225 2. When using dynamic homologies, we obtained one most-parsimonious tree of L
226 = 1225 (Fig. 2). Bremer values are shown above the branches. The *Diatrypaceae*
227 species included in the analysis are strongly supported as monophyletic (100%
228 bootstrap value) using *Xylaria curta* as the outgroup taxon. Both analyses showed an
229 evident cluster of *BAFCcult* 3321 and 3322 (endophytic isolates) with *BAFCcult*
230 3325 and 3326 (saprotrophic isolates), together with *P. scoparia.*

231 β-tubulin gene

232 The phylogenetic analysis using static homologies (NONA) (500 informative
233 characters) yielded two most-parsimonious trees of L= 1293, Ci= 67, and Ri= 85.
234 Figure 3 shows one of the most parsimonious trees. When using dynamic
235 homologies, we found one most-parsimonious tree of L= 3629. Figure 2 shows a
236 comparison between the trees obtained with NONA (A) and POY (B). Bremer

values are shown above the branches. The *Diatrypaceae* species included in the analysis are strongly supported as monophyletic (100% bootstrap value) using *Xylaria berteri* as the outgroup taxon. The results using the β-tubulin gene showed a topology similar to that of the ITS trees. An inner clade with a high support value (100% bootstrap value) clustered *BAFCcult* 3321, 3322 (endophytic isolates), 3325 and 3326 (saprophytic isolates), in a similar way to that observed in the ITS analyses, although including isolates *BAFCcult* 3320 and 3328 (without results for the ITS gene). Slight differences can be observed in the analyses using dynamics homology approach.

*Sequence similarity*

The ITS genotype groups based on 98 % ITS sequence similarity (pairwise comparisons, Table 3) showed a high congruence with the phylogenetic relationship observed between the saprotrophic and endophytic isolates from *B. papyrifera* (clade A) inferred by static and dynamic homologies. This relationship, with the addition of the saprotrophic isolates (*BAFCcult* 3320 and *BAFCcult* 3328), can be also inferred by the β-tubulin genotype groups based on 90 % ITS sequence similarity (Table 4).

*Germination and viability*

The germination rates obtained (0.9% for *BAFCcult* 3321 and 0.0% for *BAFCcult* 3325) were not significantly different ($\chi^2$ = 1.04; FD=1; p=0.3085). The viability rates were 47.5 ± 9.7% and 51.7 ± 8.3% for *BAFCcult* 3321 and *BAFCcult* 3325 respectively. The difference observed was not significant (Kruskal-Wallis: H=3.75; P= 0.0528).

## DISCUSSION

Fungal endophytes exhibit a broad range of lifestyles. The present study provides insight as to whether diatrypaceous endophytic fungi manifest themselves as saprotrophs when the host dies. Here eight strains of diatrypaceous fungi were obtained from the different procedures performed to study the saprotrophic state. Specimens of *Eutypella leprosa* and *P. scoparia* were obtained from fallen branches of *L. lucidum* and from an unknown host within the study area, both during the survey and six months later, thus offering evidence of previous infections in the area. We did not observe the presence of diatrypaceous infection in sterilized branches after one year. Additionally, stromata of *P. scoparia* (*BAFCcult* 3325, 3326) were observed on field units of *B. papyrifera*. These were the same branches from which we had isolated diatrypaceous endophytes in a previous work (de Errasti et al., 2010). These results support the assumption that the inoculum was available, but was eliminated through the sterilization procedure, and that the observed stromata had developed from the endophytic infections detected in our previous work (de Errasti et al., 2010).

These results led us to hypothesize that the life cycle of some diatrypaceous species can include an endophytic stage that may lead them to become saprotrophs at host senescence being then responsible for the colonization after the branch fell, as suggested for other groups of fungi (Chaverri and Gazis, 2011; Hyde and Soytong, 2008; Oses et al., 2008; Pildain et al., 2005).

The phylogenetic association between endophytic and saprotrophic strains, agrees with the idea that they may have a common origin, supporting our previous hypothesis from the field trials. Sequence analyses of the studied fungi suggest

287 that the isolated strains from *B. papyrifera* belong to *P. scoparia*, supporting the
288 taxonomic position of the members of this clade.

289 In addition, in concordance with that found by Jacobs et al. (1988) and Glawe and
290 Rogers (1984), we recorded a low germination rate and high viability. Therefore the
291 most plausible explanation is that conidia represent a relict state and ascospores
292 represent the only source of inoculum in this species.

293 *Peroneutypa scoparia* is a cosmopolitan species with low host specificity (Rappaz,
294 1997). Our findings suggest that this species has primary access to the substrate as an
295 endophyte, therefore external factors (not analyzed in the present study) as light,
296 temperature and gaseous regimes, among others, could determine a change in its use of
297 the available resources, then presenting a saprotrophic growth. *P. scoparia* is a species
298 with great ability to decay wood, as reported in a previous study, in which we detected
299 strains of *P. scoparia* showing uniform degradation ability, typical of white rot fungi
300 (Pildain *et al.*, 2005). Saprotrophic fungi with an endophytic stage could have
301 advantages over those that are strict saprotrophs (Muller et al., 2001; Parfitt et al.,
302 2010), so when the tree or some parts of it dies (fallen) these fungi should be the first
303 colonizers of the decaying wood material.

304 Studies on the associations between invasive trees and fungi have focused on how
305 this interaction may promote invasion (invasion meltdown) (Nuñez et al., 2009). We
306 consider that some attention should be focused on the modifications involving
307 communities of saprophytic fungi. Our findings suggest that some invasive trees,
308 capable of carrying a high diversity of endophytes, as *B. papyrifera* (de Errasti et al.,
309 2010), could promote the establishment of some fungi, leading to some changes in the
310 relative compositions of saprophytic communities in invaded natural ecosystems, thus
311 displacing saprotrophic native species incapable of using the advantages of the

endophytic stage.

**Acknowledgements**

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430 Table 1 **List of taxa used in the phylogenetic analysis**

| Taxon | GenBank β-tubulin | GenBank ITS |
|---|---|---|
| *Diatrype bullata* (Hoffm.) Fr. | DQ007002 | |
| *Diatrype flavovirens* (Pers.) Fr. | DQ006959 | AJ302430 |
| *Diatrype stigma* (Hoffm.: Fr.) Fr. | DQ007003 | |
| *Diatrypella sp.* | DQ007001 | |
| *Diatrypella sp.* | AY684217 | |
| *Eutypa armeniaca* Hansf. & M.V. Carter | | DQ006948 |
| *Eutypa lata* (Pers.) Tul. & C. Tul. | AY684215 | DQ006932 |
| *Eutypa lata* (Pers.) Tul. & C. Tul. | AY684213 | AY684228 |
| *Eutypa lata* (Pers.) Tul. & C. Tul. | AY684214 | AY684232 |
| *Eutypa lata* (Pers.) Tul. & C. Tul. | DQ006989 | AY684233 |
| *Eutypa lejoplaca* (Fr.:Fr.) Fuckel | AY684197 | AY684221 |
| *Eutypa lejoplaca* (Fr.:Fr.) Fuckel | AY684196 | AY684238 |
| *Eutypa leptoplaca* (Mont.) Rappaz *BAFC 51667* | FJ267699 | |
| *Eutypa leptoplaca* (Mont.) Rappaz | AY684211 | AJ302453 |
| *Eutypa leptoplaca* (Mont.) Rappaz | AY684210 | AY684235 |
| *Eutypa leptoplaca* (Mont.) Rappaz | DQ006963 | AY684236 |
| *Eutypa leptoplaca* (Mont.) Rappaz | DQ006961 | DQ006924 |
| *Eutypa maura* (Fr.:Fr.) Fuckel | DQ006967 | AY684224 |
| *Eutypa maura* (Fr.:Fr.) Fuckel | AY684198 | DQ006926 |
| *Eutypa petrakii* Rappaz | DQ006971 | |
| *Eutypa sparsa* Romell | AY684201 | AY684220 |
| *Eutypa sparsa* Romell | | AY684219 |
| *Eutypa tetragona* (Dubby) Sacc. | AY684202 | DQ006923 |
| *Eutypa tetragona* (Dubby) Sacc | | AY684223 |
| *Eytupella alsophila* (Mont.) Berk. | | AY302467 |
| *Eytupella kochiana* Rehm | | AJ302462 |
| *Eutypella leprosa* (Pers.) Berl. | | AJ302463 |
| *Eutypella scoparia* Schwein. | | EU436689 |
| *Eutypella scoparia* Schwein. | | EU436688 |

| | | |
|---|---|---|
| *Eutypella scoparia* Schwein. | | AJ30246 |
| *Eutypella scoparia* Schwein. | | AF378624 |
| *Eutypella scoparia* Schwein. | GQ294029 | GQ29362 |
| *Eutypella vitis* (Schwein.) Ellis & Everh. | DQ006999 | |
| *Libertella* sp. *BAFCcult* 3321 | EU728698 | EU72451 |
| *Libertella* sp. *BAFCcult* 3322 | EU728699 | EU72452 |
| *Libertella* sp. *BAFCcult* 3323 | EU864425 | EU72473 |
| *Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero *BAFCcult* 3320 | EU728697 | |
| *Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero *BAFCcult* 3324 | | EU72454 |
| *Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero *BAFCcult* 3325 | EU864426 | EU72455 |
| *Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero *BAFCcult* 3326 | EU864427 | EU72456 |
| *Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero *BAFCcult* 3327 | EU864428 | EU72457 |
| *Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero *BAFCcult* 3328 | EU864429 | |
| *Xylaria berteri* (Mont.) Cooke | AY951763 | |
| *Xylaria curta* Fr. | | DQ32244 |

449 **Table 2** Diatrypaceous fungi analyzed

| **Isolates as** | **Type of wood tissue or procedure applied** | ***Broussonetia papyrifera*** | ***Additional strains*** |
|---|---|---|---|
| Endophyte | Inner bark | Ns | Ns |
| | Wood | *Libertella* sp. *BAFCcult* 3321<br>*Libertella* sp. *BAFCcult* 3322<br>*Libertella* sp. *BAFCcult* 3390<br>*Libertella* sp. *BAFCcult* 3391 | Ns |
| Saprotroph | Established infections | *E. leprosa*<br>*P. scoparia BAFCcult* 3320 | *P. scoparia BAFCcult* 3328[a]<br>*P. scoparia BAFCcult* 3327[a] |
| | Sterilized units | Ns | Np |
| | Field units | *P. scoparia BAFCcult* 3325<br>*P. scoparia BAFCcult* 3326 | *Libertella sp. BAFCcult* 3323[b]<br>*P. scoparia BAFCcult* 3324[b] |
| | Superficial bark | Ns | Np |

450

451 (b) = obtained from *Ligustrum lucidum*. (a) obtained from unknown host

452 Ns= No additional diatrypaceous strains were obtained

453 Np= No performed

454

455 **Table 3** Similarity between sequences using ITS markers (pairwise comparison)

| | BAFC 3321 | BAFC 3322 | BAFC 3323 | BAFC 3324 | BAFC 3325 | BAFC 3326 | BAFC 3327 |
|---|---|---|---|---|---|---|---|
| BAFC 3321 | | | | | | | |
| BAFC 3322 | 0.985 | | | | | | |
| BAFC 3323 | 0.747 | 0.749 | | | | | |
| BAFC 3324 | 0.804 | 0.809 | 0.755 | | | | |
| BAFC 3325 | **0.989** | **0.98** | 0.749 | 0.801 | | | |
| BAFC 3326 | **0.989** | **0.98** | 0.749 | 0.801 | 1 | | |
| BAFC 3327 | 0.802 | 0.807 | 0.746 | 0.89 | 0.799 | 0.799 | |
| *P. scoparia** | 0.897 | 0.895 | 0.683 | 0.739 | 0.902 | 0.902 | 0.73 |

456 BAFC: Culture Collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires,

457 Argentina. In bold are comparisons between sequences from strains isolated from fallen branches.

458 (BAFC3326, 3325) and as endophytes (BAFC 3321, 3322). The sequences marked as *P. scoparia**

459 refer to *Eutypella scoparia* accession number.

460

**Table 4** Similarity between sequences using β-tubulin marker (pairwise comparison)

| | BAFC 3320 | BAFC 3321 | BAFC 3322 | BAFC 3323 | BAFC 3324 | BAFC 3325 | BAFC 3326 | BAFC 3327 |
|---|---|---|---|---|---|---|---|---|
| BAFC 3320 | | | | | | | | |
| BAFC 3321 | **0.895** | | | | | | | |
| BAFC 3322 | **0.995** | 0.899 | | | | | | |
| BAFC 3323 | 0.508 | 0.504 | 0.507 | | | | | |
| BAFC 3324 | 0.432 | 0.433 | 0.432 | 0.415 | | | | |
| BAFC 3325 | 0.87 | **0.951** | **0.87** | 0.512 | 0.432 | | | |
| BAFC 3326 | 0.883 | **0.952** | **0.884** | 0.505 | 0.429 | 0.949 | | |
| BAFC 3327 | 0.589 | 0.594 | 0.59 | 0.556 | 0.476 | 0.59 | 0.596 | |
| BAFC 3328 | 0.95 | **0.899** | **1** | 0.507 | 0.432 | 0.874 | 0.884 | 0.524 |

BAFC: Culture Collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. In bold are comparisons between sequences from strains isolated as saprotrophic (BAFC 3320, 3325, 3326, 3328) and endophytic (BAFC 3321, 3322)

**Fig. 1** Diagram of procedures to obtain strains from fallen and living branches (as endophytes).

**Fig. 2** ITS cladograms based on maximum parsimony. **a** Static homologies analysis (NONA 2.0). BioEdit sequence alignment editor, version 7.0.5.3 (Hall, 1999), was used in the sequence manipulation. All characters equally weighted and gaps scored as missing data. The analysis was performed with 700 replications. Cost 5 for gap openings and 2 for gap extensions were assigned. A bootstrap analysis was performed with 2000 replications (values indicated above branches). **b** Dynamic homology analysis (POY4). The commands build (10) and swap (100) were used (cost 15 for gap opening was assigned). To determine the support for each clade, a Bremer analysis was performed with 2000 replications (values indicated above branches). Rectangles indicate isolated strains from Argentina. Eu.=*Eutypella* E.=*Eutypa* D.=*Diatrype* P.=*Peroneutypa*.

**Fig. 3** β-tubulin cladograms based on maximum parsimony. **a** Static homology analysis (NONA 2.0). BioEdit sequence alignment editor, version 7.0.5.3 (Hall, 1999), was used in the sequence manipulation. All characters equally weighted and gaps scored as missing data. The analysis was performed with 700 replications. Cost 5 for gap openings and 2 for gap extensions were assigned. A bootstrap analysis was performed with 2000 replications (values indicated above branches). **b** Dynamic homology analysis (POY4). The commands build (10) and swap (100) were used (cost 15 for gap opening was assigned). To determine the support for each clade, a Bremer analysis was performed with 2000 replications (values indicated above branches). Rectangles indicate isolated strains from Argentina. Eu.=*Eutypella* E.=*Eutypa* D.=*Diatrype* P.=*Peroneutypa*.

**Plant-fungal association in trees: insights into changes in habitat preferences of diatrypaceous fungi**.

Andrés de Errasti M. Victoria Novas Cecilia C. Carmarán

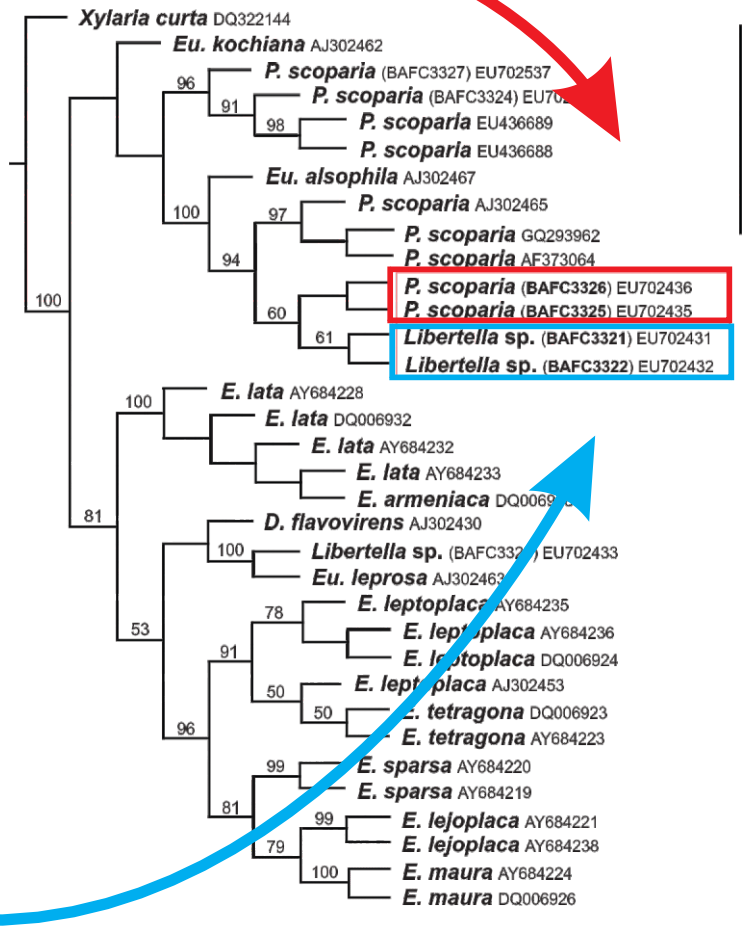
*Highlights*

- The relationship between fungal strains with different lifestyles was studied.
- Analyses shown a strong association between strains isolated as endophytes and as saprotroph
- *Peroneutypa scoparia* has access to the substrate as wood endophyte.
- Some diatrypaceous fungi are capable to change their life strategy from endophytes to saprobes

Graphical Abstract (for review)

Strains isolated from living tree

Strains isolated from fallen branches



Figure

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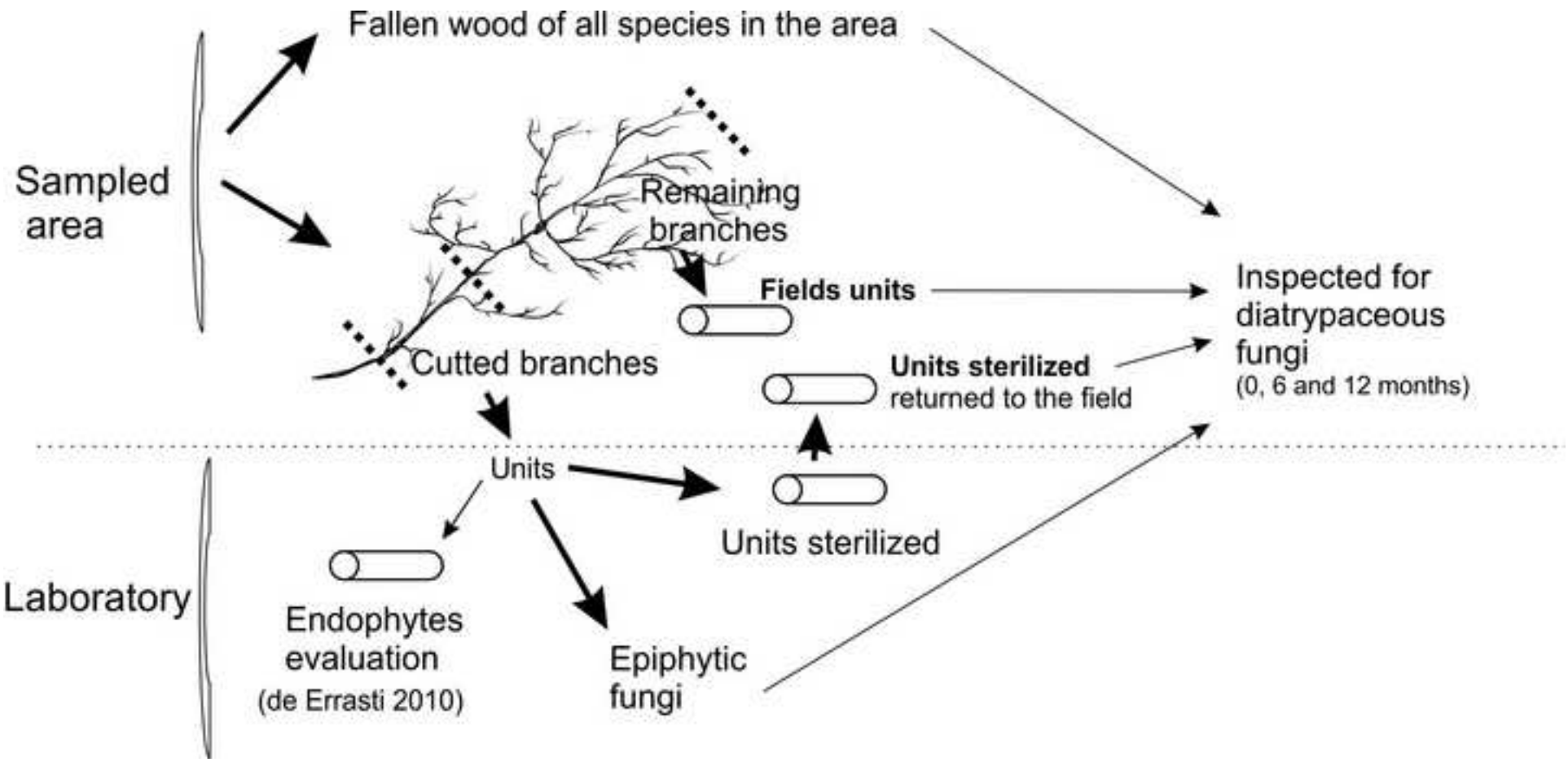

**Figure 2**

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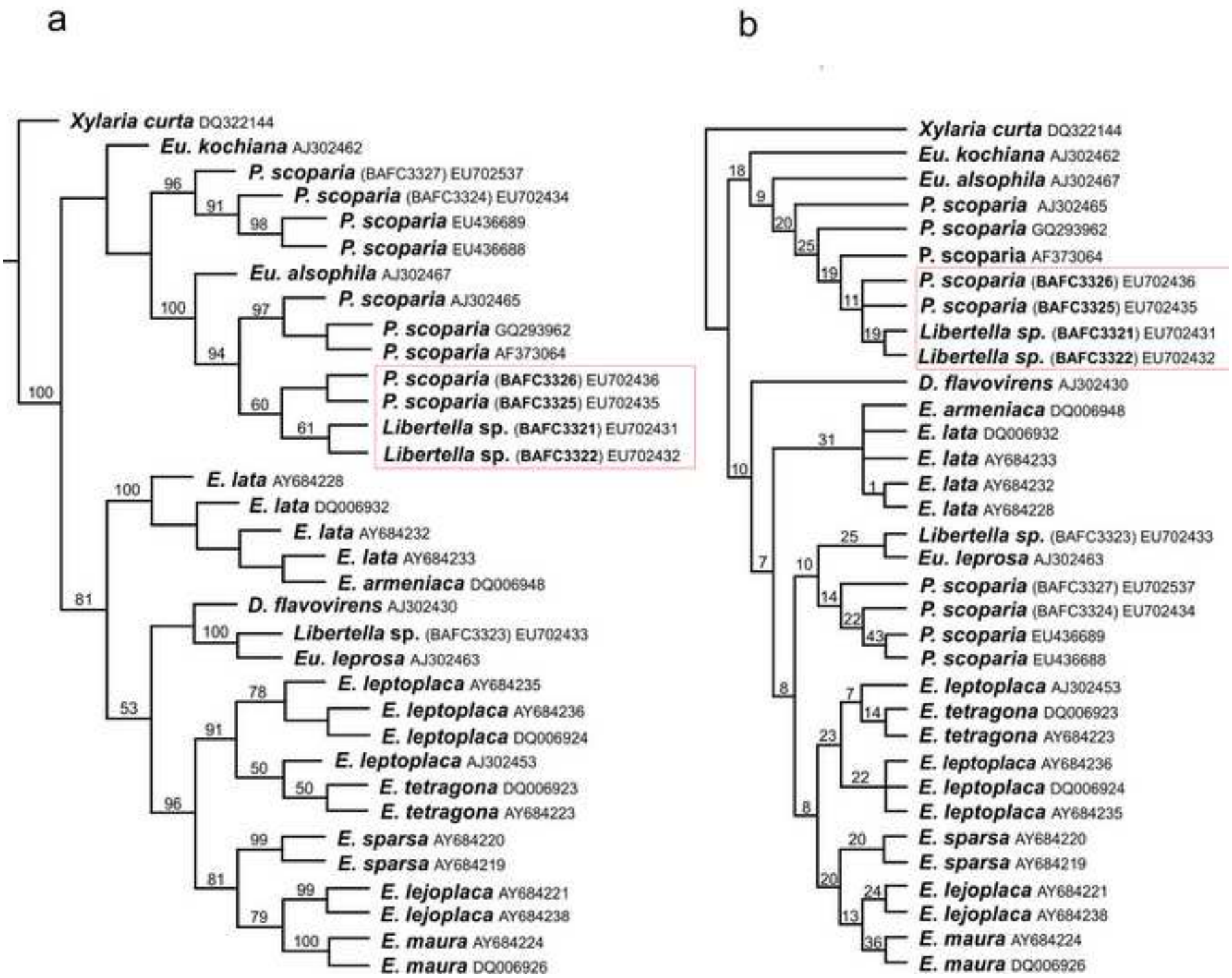

**Figure 3**

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