Botryosphaeriaceae from *Eucalyptus* and Native Myrtaceae in Uruguay*

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Summary

Species of the Botryosphaeriaceae are important pathogens causing cankers and die-back on many woody plants. In Uruguay, *Neofusicoccum eucalyptorum* (=*Botryosphaeria eucalyptorum*), *N. ribis* (=*B. ribis*) and *B. dothidea* have previously been associated with stem cankers on plantation grown *Eucalyptus globulus*. These fungi also exist as endophytes in healthy *Eucalyptus* leaves, twigs and stems, typically causing disease after the onset of stress. There is good evidence to suggest that species of the Botryosphaeriaceae, other than those previously reported, could cause cankers on *Eucalyptus* spp. and native Myrtaceae trees in Uruguay. In this study, we identified the Botryosphaeriaceae present on *Eucalyptus* spp. and on native Myrtaceae trees, and considered the genetic diversity between isolates found on both groups of hosts. Symptomatic and asymptomatic material was collected countrywide from *Eucalyptus* spp. and native Myrtaceae. Monosporic cultures were identified based on conidial morphology and comparisons of DNA sequences for the ITS region of the rDNA operon. Results revealed that isolates of the *N. parvum-N. ribis* complex, and *B. dothidea* were present on both *Eucalyptus* spp. and native Myrtaceae. In contrast, *N. eucalyptorum* was found only on *Eucalyptus* spp. and *Diplodia seriata*-related (=*B. obtusa*) isolates were obtained only from native trees. This study expands the knowledge of the occurrence of Botryosphaeriaceae on native and introduced Myrtaceae in Uruguay.

Key words: *Botryosphaeria* canker; *Eucalyptus*; Myrtaceae

Resumen

Botryosphaeriaceae aisladas de *Eucalyptus* y Mirtáceas nativas en Uruguay

Varias species residentes en la familia Botryosphaeriaceae son importantes patógenos causantes de cancros y «die-back» en numerosas plantas leñosas. En Uruguay, cancros del fuste observados en plantaciones de *Eucalyptus globulus* han estado asociados a *Neofusicoccum eucalyptorum* (=*Botryosphaeria eucalyptorum*), *N. ribis* (=*B. ribis*) y *B. dothidea*. Estos hongos también habitan como endófitos en hojas, ramas y fustes asintomáticos de *Eucalyptus* volviéndose patógenos luego de la ocurrencia de algún estrés. Existen evidencias que sugieren que otras especies de Botryosphaeriaceae, además de las antes mencionadas podrían causar cancros en *Eucalyptus* spp. y mirtáceas nativas en Uruguay. En este estudio, identificamos las especies Botryosphaeriaceae presentes en *Eucalyptus* spp. y en

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mirtáceas nativas, y analizamos la diversidad genética entre los aislamientos aislados de ambos grupos de hospederos. Muestras de material vegetal de *Eucalyptus* spp. y mirtáceas nativas con y sin síntomas fueron colectadas desde diversos puntos del país. Los cultivos fúngicos monospóricos obtenidos de dichas muestras, fueron identificados en base a la morfología de conídios y a técnicas moleculares mediante comparación de secuencias de ADN correspondientes a la región ITS del ADN ribosomal. Los resultados muestran que aislamientos identificados dentro del complejo *N. parvum-N. ribis,* y otros identificados como *B. dothidea* fueron aislados de ambos hospederos, *Eucalyptus* spp. y mirtáceas nativas. Por el contrario, *N. eucalyptorum* fue aislado únicamente de *Eucalyptus* spp., mientras que aislamientos de una especie relacionada a *Diplodia seriata* (=*B. obtusa*) fueron obtenidos únicamente de árboles nativos. Este estudio permite expandir los conocimientos acerca de las especies Botryosphaeriaceae presentes en mirtáceas nativas e introducidas en Uruguay.

Palabras clave: *Botryosphaeria* canker; *Eucalyptus*; Myrtaceae

Introduction

Eucalyptus is one of the most important hardwood crops in the world (Turnbull, 2000), and it is currently the major forest tree in Uruguay (MGAP, 2005). In the last 10 years, the area planted to *Eucalyptus* in Uruguay has tripled. This increase is associated with planting primarily two species, *Eucalyptus globulus* ssp. *globulus* Labill (hereafter *E. globulus*) and *Eucalyptus grandis* Hill ex Maid. that together make up over 480.000 ha (MGAP, 2005). Large numbers of these trees are being generated by vegetative propagation and stands of selected clones are commonly found.

The use of clonal material with similar genetic characteristics over large areas of the country increases the risk of disease outbreaks. In Uruguay, very little work has been done on *Eucalyptus* pathogens and almost nothing is known about the epidemiology and population structure of the most important pathogens occurring on these trees. Correct species identification and characterization of these pathogens is a prerequisite for effective breeding programs focused on obtaining durable genetic resistance to diseases.

Eucalyptus spp. are exotic in Uruguay and pathogens affecting these trees could also be introduced. Native trees could also represent an important source of *Eucalyptus* pathogens, as is being found elsewhere in the world (Wingfield, 2003). Frequently, species belonging to the Myrtaceae have been shown to be potential hosts of pathogens that can infect *Eucalyptus* spp. (Coutinho *et al.,* 1998; Seixas *et al.*, 2004; Wingfield *et al.*, 2001; Wingfield, 2003).

Severe diseases caused by fungi belonging to the Botryosphaeriaceae have been reported on *Eucalyptus* spp. worldwide. Stem cankers and die-back of *Eucalyptus* spp. have been associated with *Botryosphaeria dothidea* (Moug. : F.) Ces. and De Not. (Barnard *et al.*, 1987; Old and Davison, 2000; Smith *et al.*, 1994; Yuan and Mohammed, 1999), although these reports probably refer to a number of different species of Botryosphaeriaceae. However, very little is known about Botryosphaeriaceae infecting exotic *Eucalyptus* or native Myrtaceae trees in Uruguay. Endophytic *Botryosphaeria dothidea*, *Neofusicoccum eucalyptorum* (Crous, Smith ter and Wingf.) Crous, Slippers and Phillips and *N. ribis* (Slippers, Crous and Wingf.) Crous, Slippers & Phillips have been found in some *Eucalyptus* spp. (Alonso, 2004; Bettucci and Alonso, 1997; Simeto *et al.*, 2005), while *Myrceugenia glaucescens* (Camb.) Legr. and Kaus. is the only native Myrtaceae host where a species of Botryosphaeriaceae, *B. dothidea* has been found (Bettucci *et al.,* 2004). Further investigation on endophytic populations of native Myrtaceae and species of *Eucalyptus* is very important since it is well known that certain endophytic fungi become pathogenic in stressed trees (Old *et al.*, 1990; Pusey, 1989; Wene and Schoeneweiss, 1980). Thus, the aim of this work was to increase the knowledge of species of Botryosphaeriaceae occurring on *Eucalyptus* as well as native Myrtaceae in Uruguay.

Materials and Methods

Fungal isolates

With the aim of isolating and identifying fungi present on native Myrtaceae and exotic *Eucalyptus* species, during 2005 and 2006, *Eucalyptus* plantations and natural forest growing in close (<1 km) proximity to *Eucalyptus* were scouted throughout Uruguay. Surveys included the provinces of Cerro Largo, Durazno, Florida, Lavalleja, Maldonado, Paysandú, Río Negro, Rivera, Tacuarembó, Treinta y Tres and Rocha. A total of 21 Myrtaceae species native to Uruguay and 10 species of *Eucalyptus* were examined (Table 1). Symptomatic and asymptomatic material was collected. Endophytic

microorganisms were isolated from asymptomatic fresh material. Leaf, petiole and twig sections were sequentially surface-sterilized in 70 % ethyl alcohol for 1 min, immersed in 0.4 % sodium hypochlorite for 2 min, then rinsed twice in sterile distilled water and blotted dry on sterile filter paper. Surface sterilized plant tissue was placed on 2 % malt extract agar (MEA) (2 % malt extract, 1.5 % agar; Oxoid, Basingstoke, England). Plates were incubated at room temperature (~20º C) for one week. Colonies resembling Botryosphaeriaceae were selected for this study, and maintained in 2 % MEA at 8º C. To verify the efficacy of the surface sterilization and to assure the growth of only endophytic microorganisms, imprints of sample surfaces were made on MEA plates and observed for one week to confirm that fungi did not grow.

To stimulate isolates to produce fruiting structures (pycnidia) and conidia, they were grown on 1.5 % water agar (WA) (Sigma Chemicals, St. Louis, MO) with sterilized pine needles placed onto the medium surface. Plates were incubated at 22º C under continuous black light until pycnidia were observed on the pine needles (approx. 3 weeks after plating). Monosporic cultures were generated by plating a spore suspension taken from two pycnidia, suspended in 300 ìl of sterile water on WA. Germinating conidia were lifted from the agar plates and transferred to fresh 2 % MEA.

Table 1. List of species of native Myrtaceae and exotic *Eucalyptus*, sampled in this study.

Myrtaceae species native to Uruguay	***Eucalyptus* species**
Acca sellowiana	*E. camaldulensis*
Agariota eucalyptides	*E. cinerea*
Blepharocalyx salicifolius	*E. dunnii*
Calyptranthes concinna	*E. ficifolia*
Eugenia involucrata	*E. globulus*
E. mansonii	*E. grandis*
E. repanda	*E. maidenii*
E. uniflora	*E. robusta*
E. uruguayensis	*E. tereticornis*
Gomidesia palustris	*E. viminalis*
Hexachlamis edulis	
Myrceugenia euosma	
Myrce. glaucescens	
Myrcianthes cisplatensis	
Myrci. pungens	
Myrciaria tenella	
Myrrhinium atropurpureum var. octandrum	
Psidium luridum	
P. incanum	
P. pubifolium	

Morphology

For morphological characterization, pycnidia and conidia produced on pine needles were mounted on microscope slides, examined under a standard light microscope Nikon Eclipse E600 and photographed with a Nikon Digital Camera DXM1200F (Nikon Inc., Melville, NY). A total of 53 isolates with structures resembling the Botryosphaeriaceae were obtained from different hosts. Isolates were grouped by conidial morphology and only one specimen per group was further analyzed from each sampled tree, totalizing 29 isolates.

DNA extraction, PCR, sequencing and phylogenetic analysis

For DNA extraction, the 29 isolates listed in Table 2 were grown in 2 % malt extract agar (MEA) at room temperature for 10 days. Mycelium was scrapped directly from the colonies on the plates and transferred to Eppendorf tubes (1.5 ml) with 1-mm glass beads and extraction buffer (Qiagen Inc., Valencia, CA). These were vigorously shaken using a vortex mixer and placed in a water bath at 60º C for 1 hr. DNA extraction was performed using the Qiagen Plant DNeasy Mini Kit (Qiagen Inc., Valencia, CA) following manufacturer's instructions.

Primers ITS1 (5' TTC GTA GGT GAA CCT GCG G 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') (White *et al.*, 1990) were used to amplify the internal transcribed spacer region of the ribosomal DNA operon (ITS). Polymerase Chain Reactions (PCR) were performed in a 25-µl reaction mixture of 1.0 µl of 0.05% casein, 12.5 µl of Amplitaq Gold PCR Master-Mix (Applied Biosystems, Foster City, CA), 1.0 µl of 10 mM ITS1, 1.0 µl of 10 mM ITS4, 8.5 µl of ddH_2O and 1.0 µl of DNA template. PCR amplifications were performed in a MJ Research PTC 200 DNA Engine Thermal Cycler PCR (MJ Research, Reno, NV) with the following parameters: 5 min at 94º C; 1 min at 94º C; 1 min at 50º C; 1 min at 72º C; cycle to step 2, 35 times; 5 min at 72º C; hold at 10º C.

PCR products were visualized on agarose gels, purified and prepared for sequencing using ExoSAP-IT PCR clean-up kit (USB Corp., Cleveland, OH) following manufacturer's instructions. Sequencing reactions were performed using the same primers with the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) and an ABI Prism 377 automated DNA sequencer. Sequences were obtained in both directions and assembled using ChromasPro software version 1.33 (Technelysium Pty. Ltd., Eden Prairie, MN). Sequences obtained in this study were aligned with sequences of different species in the Botryosphaeriaceae available in GenBank (Table 2). Multiple sequence alignments were made by using Discovery Studio Gene v1.5 (Accelrys Inc., San Diego, CA).

Phylogenetic analysis was performed using PAUP Version 4.0b10a (Swofford, 2002). Neighbor-joining analysis used the uncorrected «p» substitution model. Gaps generated in the alignment process during the comparison were treated as missing data and all characters were treated as unordered and of equal weight. Ties were broken randomly when found. Maximum parsimony analysis was performed using the heuristic search option with simple taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Support for the nodes of the shortest trees was determined by analysis of 1,000 bootstrap replicates (Hillis and Bull, 1993). Tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated. The trees were rooted using the GenBank sequences of *Guignardia philoprina* Berk. and Curtis and *Teratosphaeria africana* (Crous and Wingf.) Crous and Braun.

Results

Fungal Isolates

A total of 29 isolates of Botryosphaeriaceae were obtained from different *Eucalyptus* spp. and native Myrtaceae trees (Table 2). Isolates UY37 and UY88 were obtained from dead tissue of *E. grandis* pruning residue, and isolates UY1050, UY1263, and UY1366 from stem canker lesions. The remaining isolates were obtained from asymptomatic plant material. All isolates produced conidiomata after three weeks of incubation on water agar with pine needles. Monosporic cultures were obtained from these structures.

Morphology and DNA sequence comparisons

Phylogenetic analysis showed that the 29 analyzed isolates reside in the Botryosphaeriaceae. The alignment contained 58 ingroup taxa and 2 outgroup taxa. Out of 503 total characters, 305 were constant, 74 variable characters were parsimony-uninformative and 104 were parsimony informative. Heuristic search analysis of the data resulted in one tree (TL = 342 steps; CI = 0.711; RI = 0.894; RC = 0.635; HI = 0.289) (Figure 1).

Table 2. List of isolates used in this study, including those for which sequences downloaded from GenBank+.

Culture ID#	Fungus species	Host species	GenBank accession #
UY9 *	*Botryosphaeria dothidea*	*Blepharocalyx salicifolius*	EU080907
UY16*	*Neofusicoccum parvum-N. ribis*	*Blepharocalyx salicifolius*	EU080908
UY37*	*N. parvum-N. ribis*	*Eucalyptus grandis*	EU080909
UY40*	*Neofusicoccum eucalyptorum*	*Eucalyptus grandis*	EU080910
UY48*	*B. dothidea*	*Eucalyptus grandis*	EU080911
UY52*	*N. parvum-N. ribis*	*Eucalyptus grandis*	EU080912
UY88*	*N. eucalyptorum*	*Eucalyptus grandis*	EU080913
UY107*	*Diplodia seriata* -related	*Myrcianthes cisplatensis*	EU080914
UY118*	*N. parvum-N. ribis*	*Eugenia uruguayensis*	EU080915
UY119*	*B. dothidea*	*Eugenia uruguayensis*	EU080916
UY231*	*N. parvum-N. ribis*	*Blepharocalyx salicifolius*	EU080917
UY339*	*B. dothidea*	*Myrceugenia glaucescens*	EU080918
UY394*	*N. eucalyptorum*	*Eucalyptus dunnii*	EU080919
UY543*	*N. parvum-N. ribis*	*Eugenia repanda*	EU080920
UY587*	*N. eucalyptorum*	*Eucalyptus tereticornis*	EU080921
UY671*	*D. seriata* -related	*Hexachlamys edulis*	EU080922
UY672*	*Dothiorella iberica*- related	*Hexachlamys edulis*	EU080923
UY693*	*D. seriata* -related	*Eugenia uniflora*	EU080924
UY719*	*B. dothidea*	*Myrrhinium atropurpureum var. octandrum*	EU080925
UY754*	*N. parvum-N. ribis*	*Eucalyptus ficifolia*	EU080926
UY788*	*D. seriata* -related	*Blepharocalyx salicifolius*	EU080927
UY1050*	*N. parvum-N. ribis*	*Eucalyptus globulus*	EU080928
UY1070*	*N. eucalyptorum*	*Eucalyptus maidenii*	EU080929

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UY1190*	*N. eucalyptorum*	*Eucalyptus globulus*	EU080930
UY1225*	*D. seriata* -related	*Acca sellowiana*	EU080931
UY1233*	*N. eucalyptorum*	*Eucalyptus viminalis*	EU080932
UY1263*	*D. seriata* -related	*Myrciaria tenella*	EU080933
UY1366*	*N. parvum-N. ribis*	*Blepharocalyx salicifolius*	EU080935
CMW10122	*Neofusicoccum parvum*	*Eucalyptus grandis*	AF283681
CMW10125	*N. eucalyptorum*	*Eucalyptus grandis*	AF283686
CMW7775	*D. seriata*	*Ribes sp.*	AY236954
CBS115041	*Do. iberica*	*Quercus ilex*	AY573202
CMW6235	*N. parvum*	*Tibouchina lepidota*	AY615136
CMW6804	*N. eucalyptorum*	*Eucalyptus dunnii*	AY615139
CMW6229	*Neofusicoccum eucalypticola*	*Eucalyptus grandis*	AY615142
CMW6217	*N. eucalypticola*	*Eucalyptus rosii*	AY615143
CMW14077	*Lasiodiplodia gonubiensis*	*Syzygium cordatum*	AY639595
CMW13434	*Pseudofusicoccum stromaticum*	*Eucalyptus* hybrid	AY693974
WAC12396	*Neofusicoccum ribis*	*Eucalyptus grandis x E. camaldulensis*	AY744369
WAC12398	*Dichomera eucalypti*	*Eucalyptus diversicolor*	AY744371
WAC12402	*D. eucalypti*	*Eucalyptus camaldulensis*	AY744373
WAC12399	*Neofusicoccum australe*	*Eucalyptus diversicolor*	AY744374
WAC12400	*N. australe*	*Eucalyptus marginata*	AY744375
WAC12403	*Dichomera versiformis*	*Eucalyptus camaldulensis*	AY744376
VPRI31988	*D. versiformis*	*Eucalyptus pauciflora*	AY744377
WAC12404	*B. dothidea*	*Eucalyptus calophylla*	AY744378
CMW15950	*N. parvum*	*Eucalyptus globulus*	DQ093193
CMW15948	*Neofusicoccum macroclavatum*	*Eucalyptus globulus*	DQ093197

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CMW15947	*N. macroclavatum*	*Eucalyptus saligna*	DQ093199
CMW14012	*N. ribis*	*Syzygium cordatum*	DQ316073
CMW14030	*N. parvum*	*Syzygium cordatum*	DQ316077
CMW13998	*Neofusicoccum magniferae*	*Syzygium cordatum*	DQ316081
CMW14009	*B. dothidea*	*Syzygium cordatum*	DQ316084
CMW14071	*Neofusicoccum luteum*	*Syzygium cordatum*	DQ316088
CMW14074	*N. australe*	*Syzygium cordatum*	DQ316089
CMW14116	*Lasiodiplodia theobromae*	*Syzygium cordatum*	DQ316092
CMW568	*D. seriata*	*Malus* sp.	DQ836726
CMW3025	*Teratosphaeria africana*	*Eucalyptus viminalis*	AF283690
CMW7063	*Guignardia philoprina*	*Taxus baccata*	AF312014

(+) Cultures marked with a (*) were sequenced in this study. Sequence data for other cultures were taken from GenBank and result from the studies of Barber *et al.,* 2005; Burgess *et al.*, 2005; Gure *et al.*, 2005; Mohali *et al*., 2007; Pavlic *et al.*, 2007; Slippers *et al.,* 2004b, 2007

Based on the analyzed DNA sequences, five different Botryosphaeriaceae species are represented in the 29 isolates analyzed. Thus, five isolates clustered with *B. dothidea*, eight isolates clustered with *N. eucalyptorum*, six isolates were closely related to *Diplodia seriata* De Not. (=*B. obtusa* (Schwein.) Shoemaker), and nine isolates clustered with the *N. parvum-N. ribis* complex. The remaining isolate is likely related to *Dothiorella iberica* Phillips, Luque and Alves (=*B. iberi*ca Phillips, Luque and Alves), but the bootstrap value was low.

Morphological comparisons confirmed the results of the DNA sequence comparisons. *Botryosphaeria dothidea* was found endophytically in *Eucalyptus grandis* and in four native Myrtaceae species namely *Blepharocalyx salicifolius* (Kunth) Berg, *Eugenia uruguayensis* Cambess, *Myrceugenia glaucescens* and *Myrrhinium atropurpureum* Schott var. *octandrum* Benth. Isolates identified as *N. parvum-N. ribis* complex were found in both *Eucalyptus* spp. and native Myrtaceae. These were obtained from asymptomatic plant tissue from *Blepharocalyx salicifolius*, *Eugenia uruguayensis* and *Eugenia repanda* Berg, while the isolate UY1366 was obtained from a stem canker on *Blepharocalyx salicifolius*. On *Eucalyptus*, however, they were isolated as endophyte (in *E. grandis* and *E. ficifolia* Muell.). They were also found sporulating on dead tissue (on *E. grandis* debris) and from stem cankers (on *E. globulus*).

Seven isolates emerging from this study were identified as *N. eucalyptorum*. Six of these were endophytes on different *Eucalyptus* species *(E. grandis, E. dunnii* Maiden, *E. tereticornis* Sm., *E. maidenii* Muell, *E. globulus and E. viminalis* Cunn. ex Schauer*),* and the remaining was found sporulating on dead *E. grandis* tissue. On the other hand, *Dothiorella seriata*-related isolates were obtained only from Myrtaceous trees but not found on *Eucalyptus* samples. Finally, a *Dothiorella iberica*-related species was found as endophyte in *Hexachlamys edulis* (Berg) Kausel and Legrand, a native Myrtaceous tree.

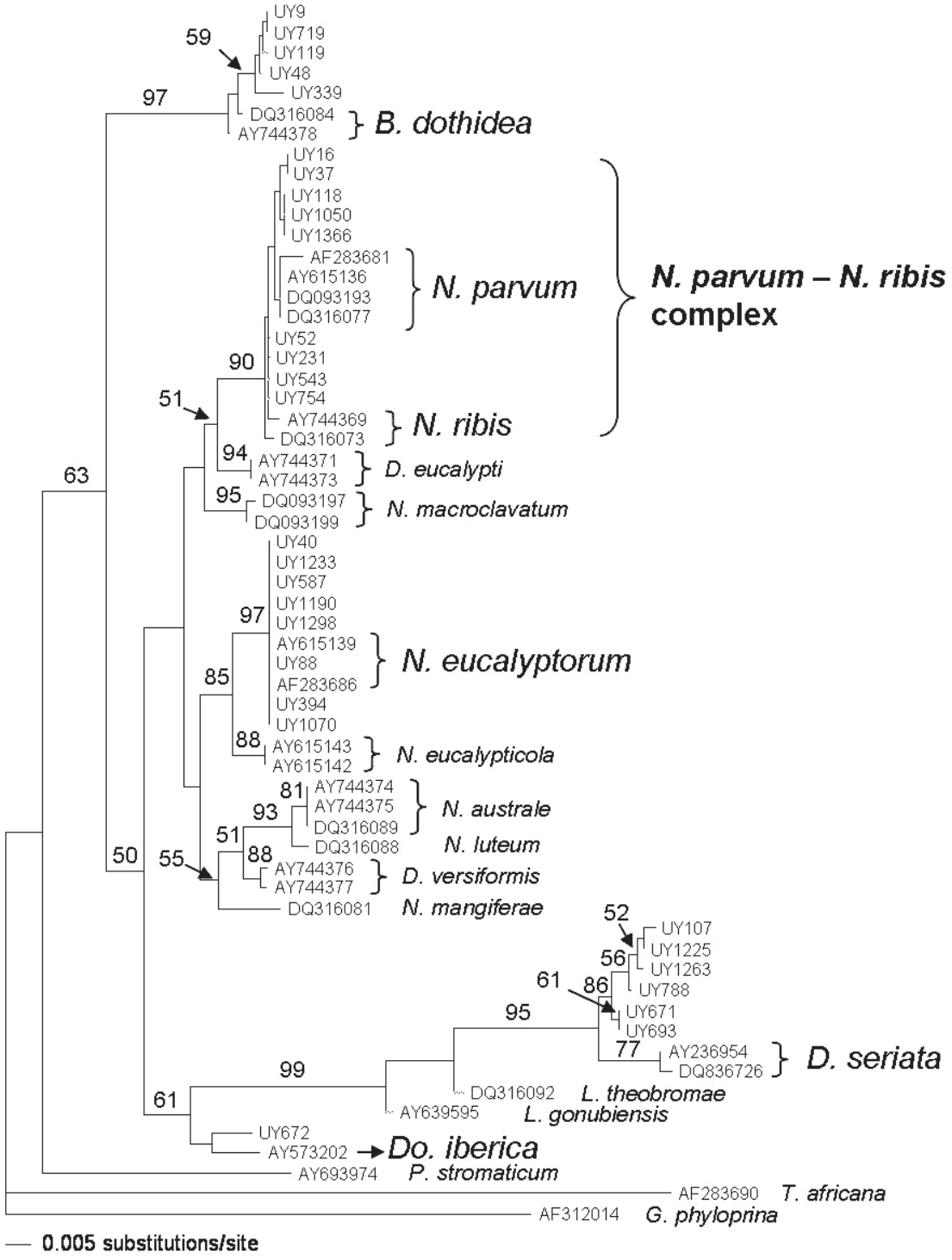

Figure 1. Phyogenetic relationship among the fungal isolates obtained in this study from native Myrtaceae trees and exotic *Eucalyptus* plantations in Uruguay and sequences of Botryosphaeriaceae downloaded from GenBank. The phylogenetic tree was constructed using neighbor-joining analysis, with the uncorrected «p» model on the ITS rDNA sequences. The tree was rooted with *Mycosphaerella africana* and *Guignardia phyloprina*. Bootstrap values greater than 50% from 1000 replications of the heuristic search are shown at the nodes.

Discussion

In this study, we found five species of Botryosphaeriaceae on *Eucalyptus* and native Myrtaceae in Uruguay. *Botryosphaeria dothidea* was previously reported as an endophyte infecting eucalypts (Bettucci and Alonso, 1997; Smith *et al.*, 1996) and also causing stem cankers on eucalypts in Uruguay (Balmelli *et al*., 2004) and other countries (Smith *et al.*, 1994). However, identification of species of Botryosphaeriaceae prior to the application of DNA sequence comparisons indicates that reference to *B. dothidea* probably implies a suite of different species. Thus, some of the isolates previously considered to be *B. dothidea* have subsequently been identified as *Neofusicoccum parvum* (Pennycook and Samuels) Crous, Slippers and Phillips and *N. ribis* (Slippers *et al*., 2004b).

With the recent taxonomic concept of Botryosphaeriaceae (Crous *et al*., 2006), *B. dothidea* has been infrequently isolated from *Eucalyptus* spp. and it has been suggested that this fungus may not be an important pathogen of these trees (Slippers *et al.*, 2004b; Pavlic *et al*., 2007). Nevertheless, little is known about the fungus and pathogenicity tests using different isolates should be carried out in order to more accurately evaluate its importance as a pathogen of *Eucalyptus*.

Bettucci *et al.* (2004) reported the presence of endophytic *B. dothidea* in *Myrceugenia glaucescens*, a Myrtaceous tree native to Uruguay. In this study, we confirmed this finding and also found endophytic infections of *B. dothidea* on *Blepharocalyx salicifolius*, *Eugenia uruguayensis*, and *Myrrhinium atropurpureum* var. *octandrum*. These results support the wide host range for the fungus reported by Michialides *et al*. (2001).These findings also emphasize the need to consider native Myrtaceae when sampling for a population structure study of *B. dothidea* in Uruguay.

Collection of isolates residing in the *Neofusicoccum parvum-N. ribis* complex, was not surprising as this fungus is known to be common on *Eucalyptus*. Slippers *et al.* (2004a) used a multiple gene genealogy to provide strong evidence that *N. parvum* and *N. ribis* represent different species. They also recommend caution when distinguishing between these two species based on morphology or sequence data for only a single DNA locus. Therefore, the isolates identified as belonging to *N. parvum-N. ribis* complex in this study must await more detailed comparisons using multiple gene approach.

Neofusicoccum parvum has been found on a wide range of hosts including certain Myrtaceae trees worldwide (Barber *et al.*, 2005; Burgess *et al.*, 2005; Gure *et al.,* 2005; Mohali *et al.*, 2007; Pavlic *et al.,* 2007; Slippers *et al*., 2004b). Slippers *et al.* (2004b) showed that *N. parvum* rather than other species of the Botryosphaeriaceae was associated with disease of *Eucalyptus* in South Africa. In addition, *N. parvum* was reported as an important die-back and stem canker pathogen of *Eucalyptus* in Ethiopia, Republic of Congo, and Uganda (Gezahgne *et al*., 2004; Smith *et al.*, 1994, Roux *et al.*, 2001). To the best of our knowledge, this species has not previously been found in Uruguay. However, Alonso (2004) reported the presence of *N. ribis* on *Eucalyptus globulus* based on the morphology and comparisons of sequence data for the ITS region of the rDNA operon. Due to the complexity of distinguishing between *N. parvum* and *N. ribis*, further analyses are required to confirm this report.

Neofusicoccum ribis has a wide host range and it has been found on certain *Eucalyptus* spp. (Barber *et al.*, 2005; Mohali *et al.*, 2007), Myrtaceae species (Pavlic *et al.*, 2007) and other non-Myrtaceous hosts (Denman *et al.*, 2003; Zhou *et al.*, 2001). This fungus was associated with the death of *E. radiata* in Australia (Shearer *et al.*, 1987), and even with some concerns raised about the species identification at that time, Pavlic *et al* (2007) concluded that *N. ribis* is the most pathogenic species of Botryosphaeriaceae on the *Eucalyptus* clones used in their study. These reports reinforce the need for correct identification as well as to assess the pathogenicity of Botryosphaeriaceae occurring on eucalypts.

Neofusicoccum eucalyptorum has previously been reported in Uruguay as an endophyte in *Eucalyptus globulus* or from bark lesions (Alonso, 2004; Bettucci, 2003). In the present study, this fungus was found on a large number of different *Eucalyptus* spp. and many of these are new host records for this fungus. It was not found on native Myrtaceae and it might represent a non–native pathogen accidentally introduced into Uruguay.

Smith *et al.* (2001) analyzed the pathogenicity of several isolates of *N. eucalyptorum,* and concluded that even when isolates of *N. eucalyptorum* were less virulent than those of *B. dothidea*, it was clear that *N. eucalyptorum* is pathogenic to eucalypts. Therefore, pathogenicity tests are needed to assess the importance of isolates obtained in this study. Likewise, it will be important to confirm that the fungus does not occur on native Myrtaceae, in which case it might also pose a threat to these trees in Uruguay .

Diplodia seriata-related *(='Botryosphaeria' obtusa*) isolates were found as endophytes on five different

native Myrtaceae. It was also isolated from a stem canker on *Myciaria tenella*. The phylogenetic tree shows that these isolates are related to *D. seriata* but they formed a defined group that suggests they could be a different species. Since spores produced by the fungus resembled those produced by *D. seriata*, multiple gene analysis is required to resolve the identity of this group of isolates. *Diplodia seriata* has been reported causing severe disease on many different hosts (Britton and Hendrix, 1989; Brown-Rytlewski and McManus, 2000; Phillips *et al.*, 2007; Pusey, 1993; Swart and Botes, 1995). However, it has not been found on *Eucalyptus* species elsewhere in the world, and was also not isolated from this host during this study. The presence of this fungus on native Myrtaceae might indicate a potential threat to *Eucalyptus* plantations. Pathogenicity tests are thus planned to gain a better understanding of its potential hazard to introduced *Eucalyptus* species.

A single isolate in this study was related to *Dothiorella iberica* (='*Botryosphaeria*' *iberica*). This fungus was found as an endophyte on a member of the Myrtaceae, *Hexachlamys edulis*. *Dothiorella iberica* has recently been described by Phillips *et al.* (2007) and the only known hosts are *Malus* and *Quercus* (Phillips *et al.*, 2005). Further investigation is needed to confirm the identity of the isolate and test its pathogenicity to *Eucalyptus* spp.

This study expands the knowledge of the occurrence of Botryosphaeriaceae on native and introduced Myrtaceae in Uruguay. It has not been experimentally shown whether organisms that produce diseases affecting these trees in Uruguay are native or have been introduced. Since Uruguay has a reasonably large number of native trees in the Myrtaceae family that are related to *Eucalyptus* it could be hypothesized that these native trees could be the hosts of some pathogens affecting *Eucalyptus* spp. Results of this study evidence *B. dothidea* and *N. parvum-N. ribis* complex occurring in both native and introduced trees. Future work will focus on understanding the relationships among these fungi and their respective host. Reciprocal pathogenicity tests and population biology studies should be undertaken to obtain a better understanding of this pathosystem and to better assist breeding programs aimed at elevating resistance to diseases.

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