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ORIGINAL ARTICLE

# Three new species of Ophiostomatales from *Nothofagus* in Patagonia

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**Abstract** The Ophiostomatales (Ascomycota) include mainly insect and mite-associated fungi, the majority of which are found on trees. Very little is known regarding the occurrence or diversity of these fungi in South America. The aim of this study was to consider their occurrence on native *Nothofagus* trees in the Patagonian Andes of Argentina. Isolates were collected in national parks and provincial reserves in Patagonia between 2009 and 2011. These were grouped based on morphology, and 22 representative isolates were included in phylogenetic analyses based on sequence data of multiple loci (LSU, ITS, beta-tubulin and translation elongation factor-1 alpha genes). The isolates could be assigned to ten different taxa, and included eight species of *Ophiostoma s. l.*, one species of *Leptographium*, and one species in the *Sporothrix lignivora* complex. Three of the species are described as new, including *Ophiostoma patagonicum*, *Leptographium gestamen*, and *Sporothrix cabralii. Ophiostoma quercus* and *O. noveae-zelandiae* are reported for the first time from Argentina, and we show that the latter species is distinct from *O. pluriannulatum*, in contrast to a previous suggestion that they represent the same taxon.

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## Introduction

The 'ophiostomatoid' fungi is a convenient term for a group of species that produce either ascospores or conidia, or both spore types, in sticky drops on elevated ascomatal necks or conidiophores (De Beer et al. 2013a). These traits are considered an adaptation for dispersal by arthropods, most commonly bark and ambrosia beetles (Coleoptera: Scolytinae) and sap-feeding beetles (Coleoptera: Nitidulidae). Morphological evidence suggested that this group is polyphyletic (De Hoog 1974; Weijman and De Hoog 1975), a fact confirmed later by biochemical (Jewell 1974; Spencer and Gorin 1971), physiological (Harrington 1981), and molecular data (Berbee and Taylor 1992; Hausner et al. 1992, 1993; Spatafora and Blackwell 1994). The two main 'ophiostomatoid' genera, *Ceratocystis* Ellis & Halst. and *Ophiostoma* Syd., that were taxonomically confused for many years are currently treated in different orders: the Ophiostomatales and Microascales, respectively (De Beer et al. 2013a, 2014).

The most recent and comprehensive taxonomic reassessment of the Ophiostomatales, the focus of the present study, included 266 species (De Beer and Wingfield 2013). The authors considered *Ceratocystiopsis* Upadhyay & Kendr., *Fragosphaeria* Shear, *Graphilbum* Upadhyay & Kendr., and *Raffaelea* Arx & Henneb. sensu stricto as monophyletic lineages. *Leptographium* Lagerb. & Melin sensu *lato* were shown to be polyphyletic, including at least ten species complexes. *Ophiostoma s. l.* comprises the *Ophiostoma s. str.* that includes the *O. ips*, *O. pluriannulatum*, and *O. ulmi* complexes, and several

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smaller lineages. The *Sporothrix schenckii* - *O. stenoceras* complex, which forms part of *Ophiostoma s. l.*, includes several asexual *Sporothrix* spp., as well as those with known sexual states at present still treated as species of *Ophiostoma*. The genus *Sporothrix* will be redefined to accommodate all of these species (De Beer and Wingfield 2013). However, one *Sporothrix* species, *S. lignivora* de Mey., Z.W. de Beer & M.J. Wingf., was shown to form a distinct lineage with some sequences of unidentified taxa from environmental studies. It is against the background of these eight major generic lineages that we explored the Ophiostomatales in the present study.

During the course of the past century, the Ophiostomatales have been well studied in North America and Europe (Jacobs and Wingfield 2001; Wingfield et al. 1993). In the last two decades, many novel species have been reported from Africa (De Meyer and De Beer 2008; Grobbelaar et al. 2010; Kamgan Nkuekam et al. 2008, 2012; Zhou et al. 2006), East Asia (Kirisits et al. 2013; Masuya et al. 2013; Zhou et al. 2013), and Australasia (De Beer et al. 2003; Harrington et al. 2001; Kamgan Nkuekam et al. 2011; Thwaites et al. 2013), bringing the total number of accepted species to 295 (De Beer et al. 2013b). Of these, the small number of ophiostomatoid fungi reported from South America might leave the impression that the continent does not harbor a large diversity of these fungi, although it may also indicate that South America has been poorly explored in this regard.

The Ophiostomatales of the temperate rainforest of Chile have been treated in a number of past studies, at a time when *Ceratocystis* and *Ophiostoma* were considered collectively. Butin and Aquilar (1984) described *O. valdivianum* (Butin) Rulamort and *O. nothofagi* (Butin) Rulamort on *Nothofagus* spp. and *O. araucariae* (Butin) de Hoog & R.J. Scheff. on *Araucaria araucana* (Molina) Koch (Butin 1968). They also reported *O. piceae* (Münch) Syd. and *O. piliferum* (Fr. : Fr.) Syd. on *Nothofagus* spp. (Butin and Aquilar 1984). The presence of *O. piceae* and *O. piliferum* was confirmed by Billings (1993). These reports were based exclusively on morphological criteria, with the only exception being *O. araucariae*, of which an isolate was included in a DNA-sequence-based analysis of the genus *Ophiostoma* (Zipfel et al. 2006). Subsequent studies in Chile focused on these fungi infecting species of *Pinus* and *Eucalyptus* spp. (Peredo and Alonso 1988; Zhou et al. 2004). There have been no prior studies on the Ophiostomatales from the *Nothofagus* deciduous forests (Veblen et al. 1996) of Argentina. The aim of this study was thus to characterize the morphology, phylogenetic affinities, and taxonomy of the Ophiostomatales found in the *Nothofagus* forests of Argentina, and to compare the emerging data with previous reports of these fungi from the geographically-linked native rainforests of Chile.

## Materials and methods

### Collection of isolates

Four national parks (Lanín, Lago Puelo, Los Alerces, and Nahuel Huapi) in the Chubut, Río Negro, and Neuquén provinces of Argentina were surveyed for ophiostomatoid fungi during the autumns of 2009, 2010, and 2011. Four additional sites in Chubut (Corcovado, Huemules, and Villarino) and Tierra del Fuego (Reserva "Corazón de la Isla") provinces were surveyed during the spring and summer months of the same period. Declining, dead, and fallen trees showing symptoms of infection by ophiostomatoid fungi, including wood staining and the production of typical fruiting bodies, were selected for sampling.

Samples collected from trees were taken to the laboratory in plastic bags to maintain a moist environment. When fruiting structures were present, isolates were obtained by lifting spore masses from the apices of ascomata or conidiophores and transferring these to 2 % (*w/v*) malt extract agar (MEA; 20 g Difco agar, 20 g Difco malt extract). When no fruiting bodies were observed, sapwood tissue was incubated in sealed moistened plastic bags for 5–25 days, until sporulation was evident, after which spore masses were transferred to isolation media. Pure cultures were obtained by transferring single hyphal tips to uninoculated plates. Isolates used in this study are maintained in the culture collection of the Centro de Investigación y Extensión Forestal Andino Patagónico, Argentina. Holotypes and duplicates of type cultures were deposited at the BAFC herbarium, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina, and at the culture collection of the Forestry and Agricultural Biotechnology Institute (CMW), University of Pretoria, South Africa, for taxonomic use only.

For comparative purposes, the ex-holotype isolate of *Ophiostoma novae-zelandiae* (Hutchison & Reid) Rulamort (UAMH 9559) was obtained from the University of Alberta Microfungus Collection and Herbarium, Canada.

### Morphology

Morphological traits were assessed for 7-, 14-, and 21-day-old cultures on MEA. Structures on host tissue were also characterized. Cultures were incubated at 25 °C in the dark. Colony

**Fig. 1** Phylogram obtained from ML analyses of the LSU region showing the overall placement in the Ophiostomatales of isolates obtained from Argentina. Sequences obtained in the present study are printed in bold type and colored boxes indicate groups that include Argentinian isolates. MP and ML bootstrap support values (1,000 replicates) above 75 % are indicated at the nodes as MP/ML. Posterior probabilities (above 90 %) obtained from BI are indicated by bold lines at the relevant branching points. * = bootstrap values lower than 75 %. T = ex-type isolates. Scale bar = total nucleotide difference between taxa


LSU
*/84
CIEFAP440 KT362222
O. quercus DQ294376
*/84
O. australiae EF408608
O. novo-ulmi DQ294375
O. ulmi DQ294374
93/97
CIEFAP463 KT3622243
CIEFAP431 KT362223 T
Ophiostoma patagonicum sp. nov.
A
O. ulmi complex
O. distortum DQ294369
75/95
CIEFAP450 KT362225
O. canum DQ294372
O. piceae AF234837
O. flexuosum DQ294370
B
O. piceae complex
O. karelicum EU443756
O. floccosum AF234836
O. ainoae DQ294368
96/97
Ophiostoma sp. EU984282
80/78
O. tingens AF282871
O. macrosporum AF282873
100/97
O. californicum AF137280
O. conicolum AF155689
O. longirostellatum AF155688
*/*
O. retusum L05783
O. multiannulatum DQ294366
CIEFAP447 KT362227
O. pluriannulatum DQ294365
CIEFAP423 KT362226
75/100
O. subannulatum DQ294364
O. carpenteri DQ294363
C
O. pluriannulatum complex
77/75
O. ips DQ294381
O. pulvinisporum DQ294380
O. montium DQ294379
H. ips AF282872
O. japonicum GU134184 T
O. piliferum DQ294377
Ophiostoma s. s.
Ophiostoma s.l.
O. nigricarpum DQ294356
CIEFAP464 KT362228
D
92/92
S. brasilensis JQ070141
S. schenckii DQ294352
O. phasma DQ316151
98/99
CIEFAP458 KT362230
CIEFAP456 KT362229 T
Sporothrix cabralii sp. nov.
E
*/75
S. stylites EF139115
S. pallida EF139121
S. humicola EF139114
O. palmiculminatum DQ316143
O. gemellus DQ821531 T
82/85
O. africanum DQ316147
O. protearum DQ316145
*/77
S. variecibatus DQ821537
S. inflata DQ294351
O. stenoceras DQ836904
O. lunatum DQ294355
O. fusiforme DQ294354
O. stenoceras - S. schenckii complex
S. lignivora EF139119 T
CIEFAP451 KT362231
S. lignivora complex
F
83/87
Cs. brevicomis EU913683 T
83/89
Cs. ranaculosa DQ294357
Cs. manitobensis DQ294358
87/94
Cs. pallidobrunnea EU913682
Cs. minima DQ294361
100/100
Cs. minuta EU913645
Cs. minuta-bicolor DQ294359
Cs. minuta EU913656 T
Ceratocystiopsis
100/99
F. reniformis AB189155
F. purpurea AF096191
Fragosphaeria
95/98
G. aenigmatica DQ294391
G. piceaperda DQ294392
G. laricis DQ294393
L. lundbergii DQ294388
G. huntii DQ294387
G. yunnanensis AY707207
G. aurea DQ294389
L. terebrantis EU296777 T
84/95
L. procerum EU296776 T
L. sinoprocerum EU296773 T
L. alethinum EU502800
G. wageneri DQ294396
82/92
G. francke-grosmanniae DQ294395
G. serpens JN940174 T
G. galeiformis DQ294383
86/85
G. leptographioides DQ294382
97/93
G. olivacea AJ538337
G. olivaceapini AJ538336
G. cucullata AJ538335
G. crassivaginata DQ294386
G. abieticola GU134177 T
L. pruni AB104900
G. grandifoliae DQ294399
*/86
CIEFAP457 KT362233
CIEFAP453 KT362232 T
Leptographium gestamen sp. nov.
100/100
L. abietinum EU177472
G. penicillata DQ294385
G. abiocarpa AJ538339
G
Leptographium s.l.
81/79
R. quercivora AB496454 T
100/100
R. montetyi EU984301
100/100
R. sulphurea EU177463 T
R. amasae EU984295
95/96
R. lauricola EU177440 T
97/98
Raffaelea sp. EU984281
R. brunnea EU177457
R. sulcati EU177462 T
R. santoroi EU984302
R. albimanens EU177452 T
R. subalba EU177443 T
R. tritirachium EU177464 T
R. subfusca EU177450 T
R. ambrosiae EU177453 T
100/98
R. gnathotrichi EU177460 T
R. scolytodis AM267270
R. ellipticospora EU177446 T
88/97
R. canadensis EU177458 T
97/99
Raffaelea sp. EU177466
Raffaelea sp. EU177465
Raffaelea s.s.
90/92
Gra. rectangulosporium AB235158 T
Gra. microcarpum GU134186
Graphilbum sp. GU221905
100/99
Podospora decipiens AY780073
Sordaria fimicola AY545728
Graphilbum
0.02

colors were described using the Munsell Color Charts (Munsell 1912). Conidiophores and ascomata were mounted on glass slides in distilled water or distilled water and phloxine for microscopic examination. For species descriptions, fifty measurements of each characteristic taxonomic structure were made. Averages (mean), standard deviation (sd), and minimum (min) and maximum (max) measurements are presented for each structure as (min) mean minus sd – mean plus sd (max).

### DNA extraction, PCR, and DNA sequencing

DNA was extracted from fungal mycelium (ca.100 mg) grown in DIFCO Malt Extract Agar (2 % malt extract, 2 % agar) incubated for 2 weeks in the dark at 25 °C. Total DNA was extracted using an Ultraclean® Microbial DNA extraction KIT (Mo Bio Laboratories, Carlsbad, CA), following the manufacturer's directions.

For DNA sequencing, five gene regions were chosen based on those used in recent literature for generic placement and species resolution in the different groups within the Ophiostomatales. Part of the 28S gene region of ribosomal DNA (LSU) was amplified with primers LROR and LR5 (Vilgalys 2013). The 18S partial sequence, internal transcribed spacer 1, 5.8S, internal transcribed spacer 2, and 28S partial region of ribosomal DNA (ITS) was amplified with primers ITS1-F and ITS4 (White et al. 1990). A portion of the *β-tubulin* gene (*BT*) was amplified with primers Bt2a and Bt2b (O'Donnell and Cigelnik 1997), while the ITS2-LSU region was amplified with primers ITS3 and LR5 (White et al. 1990), and the *eukaryotic translation elongation factor 1-α* (*EF*) with primers EF1F and EF2R (Jacobs et al. 2004).

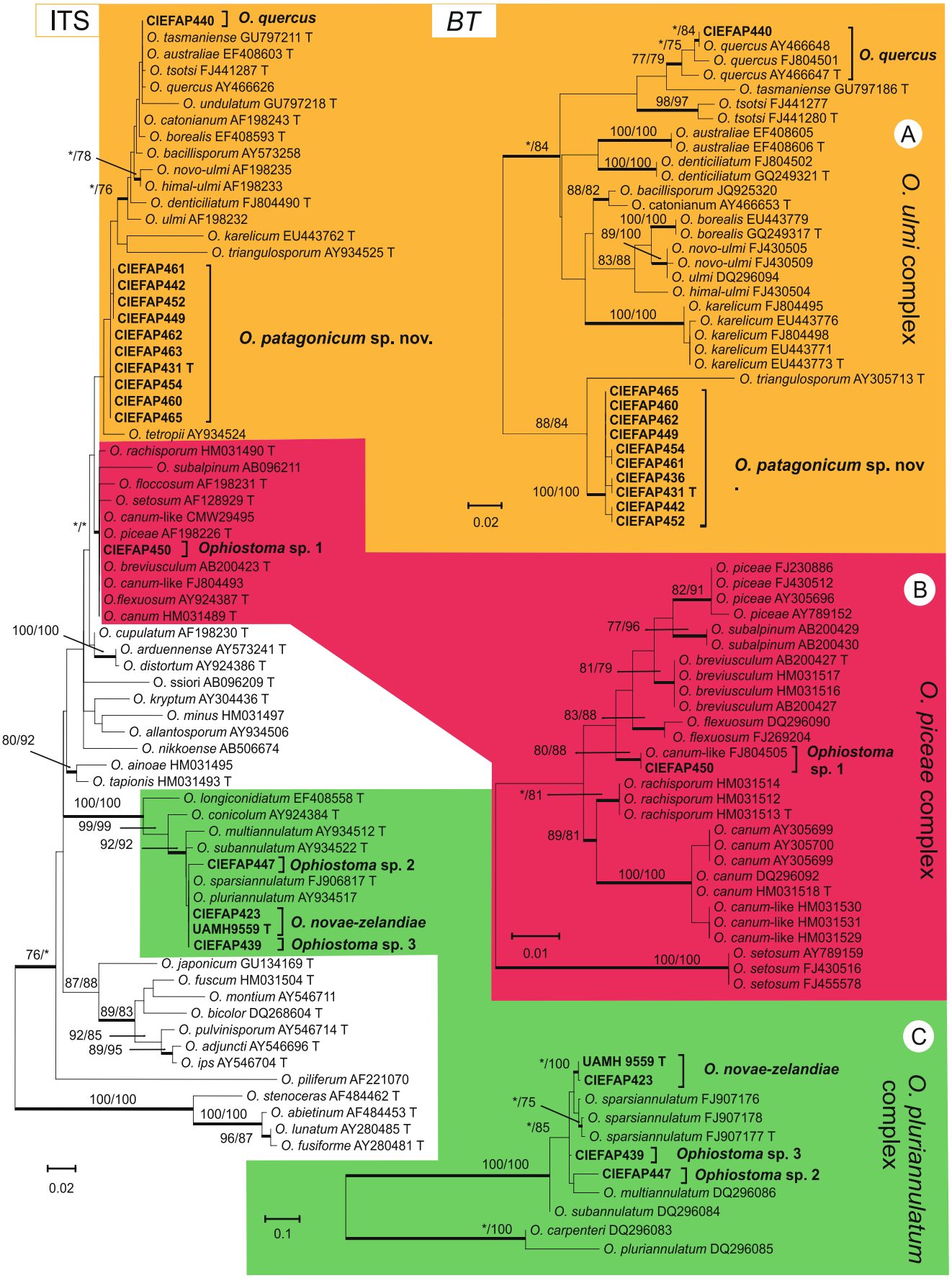
Reaction mixtures (25 mL total volume) consisted of 2.5 mL polymerase chain reaction (PCR) reaction buffer, 2.5 mM $MgCl_2$, 200 mM each of deoxynucleotide (dNTP), 0.2 mM each of primer, 1 U FastStart Taq DNA Polymerase (Roche Applied Science, Mannheim, Germany), and 2 mL diluted genomic DNA solution. Amplifications were performed in an Eppendorf MasterCyclerH gradient (Eppendorf, Hamburg, Germany) thermocycler with the following conditions: an initial denaturation step at 96 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C annealing for 30 s, 72 °C extension for 60 s, and a final extension step at 72 °C for 8 min. Amplification of the respective genes was confirmed on a 2 % agarose gel (Roche diagnostics, Mannheim, Germany) supplemented with Gelred™ (Biotium, Hayward, CA). Products were purified with 1.25 U of Exonuclease I and 1 U Shrimp Alkaline Phosphatase (Fermentas Life Sciences, Pittsburgh, PA) to digest excess primers and dNTPs.

Purified PCR products were sequenced with the Big DyeH Terminator 3.1 cycle sequencing premix kit (Applied Biosystems, Foster City, CA), employing the same forward and reverse primers as used in PCR. Sequencing PCR conditions consisted of an initial denaturation step at 96 °C for 2 min, followed by 35 cycles of 96 °C for 10 s, 55 °C annealing for 5 s, 60 °C extension for 2 min, and a final extension step at 72 °C for 8 min. Purified sequencing PCR products were separated on an ABI PRISIMH 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). All sequences were checked manually and consensus sequences were constructed with MEGA 5.05 (Tamura et al. 2011).

### Phylogenetic analyses

BLAST searches using the BLASTn algorithm were performed to retrieve similar sequences from GenBank. Accession numbers of these sequences are presented in the corresponding phylogenetic trees (Figs. 1, 2, 3, and 4). Data sets were compiled in MEGA 5.0.5. Alignments were made online in MAFFT 7 (Katoh 2013) using the E-INS-i strategy and default settings. *Ophiostoma s. str.* was analyzed separately according to *BT* intron arrangement (−/−/5 for *O. pluriannulatum* complex and 3/4/- for the remainder of species). All sequences generated in this study were deposited in GenBank (Table 1). Heuristic searches with 10,000 replicates of random addition of sequences and tree bisection and reconnection (TBR) branch swapping were carried out. Gaps were considered as 'missing characters'. Maximum parsimony (MP) analyses were performed using PAUP* v4.0 (Sinauer Associates, Sunderland, MA). Support at each node was evaluated by 1,000 bootstrap replications. Maximum likelihood analysis (ML) was conducted using PhyML v3.0 (Guidon and Gascuel 2003). Substitution models were selected using the Akaike information criterion (AIC) in ModelTest v3.7 (Posada and Crandall 1998). For Bayesian Inference (BI) analyses, four MCMC chains were run simultaneously in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) from a random starting tree for 1,000,000 generations. Trees were sampled every 100th generation. Trees sampled at burn-in (15 %) were discarded, and posterior probabilities were calculated from a majority rule consensus tree regenerated from the remaining trees.

**Fig. 2** ML trees for *Ophiostoma s. str.* and three species complexes in the genus, based on ITS (left) and *BT* sequences (right). Novel sequences obtained in this study are printed in bold type. MP and ML bootstrap support values (1,000 replicates) above 75 % are indicated at the nodes as MP/ML. Posterior probabilities (above 90 %) obtained from BI are indicated by bold lines at the relevant branching points. * = bootstrap values lower than 75 %. T = ex-type isolates. Scale bar = total nucleotide difference between taxa


ITS
CIEFAP440 O. quercus
O. tasmaniense GU797211 T
O. australiae EF408603 T
O. tsotsi FJ441287 T
O. quercus AY466626
O. undulatum GU797218 T
O. catonianum AF198243 T
O. borealis EF408593 T
O. bacillisporum AY573258
O. novo-ulmi AF198235
O. himal-ulmi AF198233
O. denticiliatum FJ804490 T
O. ulmi AF198232
O. karelicum EU443762 T
O. triangulosporum AY934525 T
CIEFAP461
CIEFAP442
CIEFAP452
CIEFAP449
CIEFAP462
CIEFAP463
CIEFAP431 T
CIEFAP454
CIEFAP460
CIEFAP465
O. patagonicum sp. nov.
O. tetropii AY934524
O. rachisporum HM031490 T
O. subalpinum AB096211
O. floccosum AF198231 T
O. setosum AF128929 T
O. canum-like CMW29495
O. piceae AF198226 T
CIEFAP450 Ophiostoma sp. 1
O. breviusculum AB200423 T
O. canum-like FJ804493
O.flexuosum AY924387 T
O. canum HM031489 T
O. cupulatum AF198230 T
O. arduennense AY573241 T
O. distortum AY924386 T
O. ssiori AB096209 T
O. kryptum AY304436 T
O. minus HM031497
O. allantosporum AY934506
O. nikkoense AB506674
O. ainoae HM031495
O. tapionis HM031493 T
O. longiconidiatum EF408558 T
O. conicolum AY924384 T
O. multiannulatum AY934512 T
O. subannulatum AY934522 T
CIEFAP447 Ophiostoma sp. 2
O. sparsiannulatum FJ906817 T
O. pluriannulatum AY934517
CIEFAP423
UAMH9559 T
CIEFAP439
O. novae-zelandiae
Ophiostoma sp. 3
O. japonicum GU134169 T
O. fuscum HM031504 T
O. montium AY546711
O. bicolor DQ268604 T
O. pulvinisporum AY546714 T
O. adjuncti AY546696 T
O. ips AY546704 T
O. piliferum AF221070
O. stenoceras AF484462 T
O. abietinum AF484453 T
O. lunatum AY280485 T
O. fusiforme AY280481 T
0.02
BT
A
O. ulmi complex
CIEFAP440
O. quercus AY466648
O. quercus FJ804501
O. quercus AY466647 T
O. quercus
O. tasmaniense GU797186 T
O. tsotsi FJ441277
O. tsotsi FJ441280 T
O. australiae EF408605
O. australiae EF408606 T
O. denticiliatum FJ804502
O. denticiliatum GQ249321 T
O. bacillisporum JQ925320
O. catonianum AY466653 T
O. borealis EU443779
O. borealis GQ249317 T
O. novo-ulmi FJ430505
O. novo-ulmi FJ430509
O. ulmi DQ296094
O. himal-ulmi FJ430504
O. karelicum FJ804495
O. karelicum EU443776
O. karelicum FJ804498
O. karelicum EU443771
O. karelicum EU443773 T
O. triangulosporum AY305713 T
CIEFAP465
CIEFAP460
CIEFAP462
CIEFAP449
CIEFAP454
CIEFAP461
CIEFAP436
CIEFAP431 T
CIEFAP442
CIEFAP452
O. patagonicum sp. nov.
0.02
B
O. piceae complex
O. piceae FJ230886
O. piceae FJ430512
O. piceae AY305696
O. piceae AY789152
O. subalpinum AB200429
O. subalpinum AB200430
O. breviusculum AB200427 T
O. breviusculum HM031517
O. breviusculum HM031516
O. breviusculum AB200427
O. flexuosum DQ296090
O. flexuosum FJ269204
O. canum-like FJ804505
CIEFAP450
Ophiostoma sp. 1
O. rachisporum HM031514
O. rachisporum HM031512
O. rachisporum HM031513 T
O. canum AY305699
O. canum AY305700
O. canum AY305699
O. canum DQ296092
O. canum HM031518 T
O. canum-like HM031530
O. canum-like HM031531
O. canum-like HM031529
O. setosum AY789159
O. setosum FJ430516
O. setosum FJ455578
0.01
C
O. pluriannulatum complex
UAMH 9559 T
CIEFAP423
O. novae-zelandiae
O. sparsiannulatum FJ907176
O. sparsiannulatum FJ907178
O. sparsiannulatum FJ907177 T
CIEFAP439
Ophiostoma sp. 3
CIEFAP447
Ophiostoma sp. 2
O. multiannulatum DQ296086
O. subannulatum DQ296084
O. carpenteri DQ296083
O. pluriannulatum DQ296085
0.1

## Results

### Collection of fungi and morphology

A total of 361 samples were processed and 216 isolates were obtained. Of these, 101 isolates belonged to the Ophiostomatales, representing eight morphological groups (Groups A-G, Table 1). Eighty-seven isolates produced ascomata bearing orange-section-shaped ascospores and pesotum/sporothrix-like asexual states, being assigned to *Ophiostoma s. l.* (Groups A-D). These fungi were isolated from stained wood of *Nothofagus* spp., *Schinus patagonicus* (Phil.) I.M. Johnst., and *Tepualia stipularis* (Hook. & Arn.) Griseb., as well as bark and ambrosia beetle galleries and from nitidulid beetles. Four isolates with unknown sexual states resembled *Sporothrix* spp. (Groups E, F), and 10 isolates were grouped in *Leptographium s. l.* (Group G). All *Sporothrix* and *Leptographium* taxa were isolated from ambrosia beetle galleries on recently cut or declining *Nothofagus* trees. Altogether, 22 isolates were selected for DNA sequencing representing the different morphological groups, host associations, and geographic areas of occurrence (Table 1).

### DNA sequence comparisons

LSU sequences of 12 isolates from this study, representing groups A to G, were compared with 103 sequences retrieved from GenBank, representing all major groups in the Ophiostomatales (De Beer and Wingfield 2013). After alignment, a 452-character matrix was obtained. Maximum likelihood and BI analyses were performed assuming a transitional model (TIM1 + I + G). Topologies of trees generated under M, L, MP or BI were congruent. LSU analyses placed six isolates in different lineages within *Ophiostoma s. str.* (Fig. 1, groups A, B, C). The group D isolate positioned peripheral to the species representing the *S. schenckii-O. stenoceras* complex, closest to *O. nigricarpum* (R.W. Davidson) de Hoog, while group E isolates positioned within the latter complex (Fig. 1, groups D, E). One isolate was close to *Sporothrix lignivora* (Fig. 1, group F) and two isolates grouped within *Leptographium s. l.* (Fig. 1, group G).

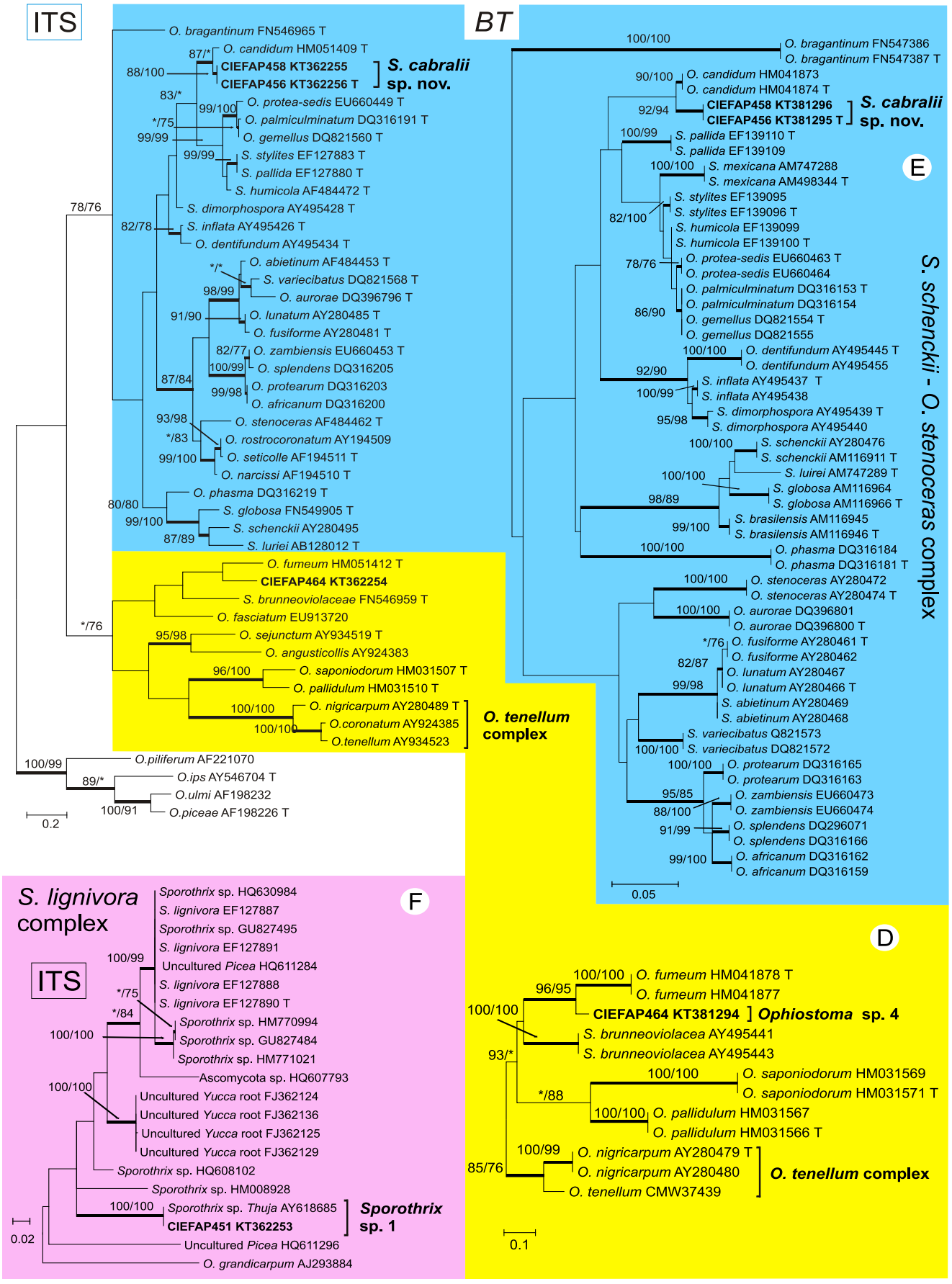
Fifteen *Ophiostoma s. str.* isolates from Patagonia were included in the ITS analyses (Fig. 2). The final matrix was composed of 69 sequences and 722 characters. For ML and BI searches, a transitional substitution model (GTR + I + G) was selected. Argentinean isolates were segregated into three species complexes (Groups A, B, and C). In group A, one isolate was included in the *O. ulmi* complex and 10 isolates formed a well-supported cluster peripheral to the *O. ulmi* (Buisman) Nannf. and *O. quercus* (Georgev.) Nannf. groups (Fig. 2, group A). The single isolate in group B was positioned near *O. piceae* and other species with similar sequences (Fig. 2, group B). ITS data placed three of our isolates and the *O. novae-zelandiae* (L.J. Hutchison & J. Reid) Rulamort isolate in the *O. pluriannulatum* species complex (Fig. 2, group C).

**Fig. 3** ML trees obtained from ITS (left) and *BT* (right) sequences of the *Sporothrix schenckii-Ophiostoma stenoceras* complex and related species, as well an ITS-derived tree for the *S. lignivora* complex (bottom left). Novel sequences obtained in this study are printed in bold type. MP and ML bootstrap support values (1,000 replicates) above 75 % are indicated at the nodes as MP/ML. Posterior probabilities (above 90 %) obtained from BI are indicated by bold lines at the relevant branching points. * = bootstrap values lower than 75 %. T = ex-type isolates. Scale bar = total nucleotide difference between taxa

*BT* sequences of *Ophiostoma s. str.* where analyzed in three separate data sets (Fig. 2) because the data for different species complexes vary too inordinately to be reliably aligned. To compare *BT* sequences of the *O. ulmi* species complex, a matrix of 35 sequences and 452 characters was constructed. ML and BI searches were performed assuming a transversional model (TVM + G). Results confirmed that the one Argentinean isolate CIEFAP440 is *O. quercus* and that the other several isolates, represented by CIEFAP431, are clearly a new species (Fig. 2, group A). To compare *BT* sequences representing the *O. piceae* complex, a matrix including 28 sequences and 278 characters was compiled. A transitional substitution model (GTR + I + G) was used to run ML and BI analyses. These trees supported the identification of our isolate as the same as an undescribed species from birch in Norway, referred to by Linnakoski (2009) as *O. canu*m-like (Fig. 2, group B). The *O. pluriannulatum* complex *BT* dataset included 11 sequences and 338 characters. ML and BI analyses were carried out assuming a transversional model (TVM + G). One isolate in this complex (CIEFAP423) was identical to *O. novae-zelandiae*, while the other two, *Ophiostoma* sp. 2 (CIEFAP447) and *Ophiostoma* sp. 3 (CIEFAP439), did not group with any species in the complex (Fig. 2, group C).

The ITS matrix representing the *S. schenckii-O. stenoceras* complex (Fig. 3) and related species consisted of 45 sequences and 726 characters. A transitional substitution model (GTR + I + G) was assumed to run ML and BI searches. The group D isolate (Fig. 3) positioned in *Ophiostoma s. l.*, peripheral to the *S. schenckii-O. stenoceras* complex, in a lineage between *O. fumeum* Kamgan, Jol. Roux & Z.W. de Beer and *S. brunneoviolaceae*. De Beer and Wingfield (2013) related these latter species to the *O. tenellum* complex. ITS sequence data of isolates assigned to group E (Fig. 3) formed a well-supported lineage closest to *O. candidum* Kamgan, Jol. Roux & Z.W. de Beer within the *S. schenckii-O. stenoceras* complex.

The *BT* dataset of the *O. tenellum* complex included 12 sequences and 384 characters. A transitional (TIM3 + G) substitution model was selected for ML and BI analyses. *BT* trees confirmed the placement of the group D isolate distinct from, but near *O. fumeum* (Fig. 3, group D). The *BT* dataset of species closest to *O. candidum* (De Beer and Wingfield 2013) consisted of 55 sequences and 320 characters. A


ITS
O. bragantinum FN546965 T
87/* O. candidum HM051409 T
88/100 CIEFAP458 KT362255
CIEFAP456 KT362256 T
S. cabralii sp. nov.
83/*
99/100 O. protea-sedis EU660449 T
*/75 O. palmiculminatum DQ316191 T
99/99 O. gemellus DQ821560 T
99/99 S. stylites EF127883 T
S. pallida EF127880 T
S. humicola AF484472 T
78/76 S. dimorphospora AY495428 T
82/78 S. inflata AY495426 T
O. dentifundum AY495434 T
*/* O. abietinum AF484453 T
S. variecibatus DQ821568 T
98/99 O. aurorae DQ396796 T
91/90 O. lunatum AY280485 T
O. fusiforme AY280481 T
82/77 O. zambiensis EU660453 T
100/99 O. splendens DQ316205
87/84 99/98 O. protearum DQ316203
O. africanum DQ316200
93/98 O. stenoceras AF484462 T
*/83 O. rostrocoronatum AY194509
99/100 O. seticolle AF194511 T
O. narcissi AF194510 T
O. phasma DQ316219 T
80/80 S. globosa FN549905 T
99/100 S. schenckii AY280495
87/89 S. luriei AB128012 T
O. fumeum HM051412 T
CIEFAP464 KT362254
S. brunneoviolaceae FN546959 T
O. fasciatum EU913720
*/76 95/98 O. sejunctum AY934519 T
O. angusticollis AY924383
96/100 O. saponiodorum HM031507 T
O. pallidulum HM031510 T
100/100 O. nigricarpum AY280489 T
100/100 O.coronatum AY924385
O.tenellum AY934523
O. tenellum complex
100/99 O.piliferum AF221070
89/* O.ips AY546704 T
O.ulmi AF198232
100/91 O.piceae AF198226 T
0.2
BT
100/100 O. bragantinum FN547386
O. bragantinum FN547387 T
90/100 O. candidum HM041873
O. candidum HM041874 T
92/94 CIEFAP458 KT381296
CIEFAP456 KT381295 T
S. cabralii sp. nov.
100/99 S. pallida EF139110 T
S. pallida EF139109
100/100 S. mexicana AM747288
S. mexicana AM498344 T
E
S. stylites EF139095
82/100 S. stylites EF139096 T
S. humicola EF139099
S. humicola EF139100 T
78/76 O. protea-sedis EU660463 T
O. protea-sedis EU660464
O. palmiculminatum DQ316153 T
O. palmiculminatum DQ316154
86/90 O. gemellus DQ821554 T
O. gemellus DQ821555
100/100 O. dentifundum AY495445 T
O. dentifundum AY495455
92/90 S. inflata AY495437 T
100/99 S. inflata AY495438
S. dimorphospora AY495439 T
95/98 S. dimorphospora AY495440
100/100 S. schenckii AY280476
S. schenckii AM116911 T
100/100 S. luirei AM747289 T
S. globosa AM116964
98/89 S. globosa AM116966 T
S. brasilensis AM116945
99/100 S. brasilensis AM116946 T
100/100 O. phasma DQ316184
O. phasma DQ316181 T
100/100 O. stenoceras AY280472
O. stenoceras AY280474 T
100/100 O. aurorae DQ396801
O. aurorae DQ396800 T
*/76 O. fusiforme AY280461 T
82/87 O. fusiforme AY280462
O. lunatum AY280467
99/98 O. lunatum AY280466 T
S. abietinum AY280469
S. abietinum AY280468
S. variecibatus Q821573
100/100 S. variecibatus DQ821572
100/100 O. protearum DQ316165
O. protearum DQ316163
95/85 O. zambiensis EU660473
88/100 O. zambiensis EU660474
91/99 O. splendens DQ296071
O. splendens DQ316166
99/100 O. africanum DQ316162
O. africanum DQ316159
0.05
S. schenckii - O. stenoceras complex
S. lignivora complex
F
ITS
100/99 Sporothrix sp. HQ630984
S. lignivora EF127887
Sporothrix sp. GU827495
S. lignivora EF127891
Uncultured Picea HQ611284
*/75 S. lignivora EF127888
S. lignivora EF127890 T
*/84 Sporothrix sp. HM770994
100/100 Sporothrix sp. GU827484
Sporothrix sp. HM771021
Ascomycota sp. HQ607793
100/100 Uncultured Yucca root FJ362124
Uncultured Yucca root FJ362136
Uncultured Yucca root FJ362125
Uncultured Yucca root FJ362129
Sporothrix sp. HQ608102
Sporothrix sp. HM008928
100/100 Sporothrix sp. Thuja AY618685
CIEFAP451 KT362253
Sporothrix sp. 1
0.02
Uncultured Picea HQ611296
O. grandicarpum AJ293884
D
100/100 O. fumeum HM041878 T
96/95 O. fumeum HM041877
100/100 CIEFAP464 KT381294
Ophiostoma sp. 4
S. brunneoviolacea AY495441
93/* S. brunneoviolacea AY495443
100/100 O. saponiodorum HM031569
*/88 O. saponiodorum HM031571 T
100/100 O. pallidulum HM031567
O. pallidulum HM031566 T
100/99 O. nigricarpum AY280479 T
85/76 O. nigricarpum AY280480
O. tenellum CMW37439
O. tenellum complex
0.1

transitional TIM3 + I + G substitution model was assumed to perform ML and BI searches. Group E isolates were distinct from isolates representing *O. candidum*, and are considered as a new, undescribed species (Fig. 3, group E).

The ITS dataset for the *S. lignivora* complex (Fig. 3) included 21 sequences and 635 characters. ML and BI analyses were performed assuming a 'Tamura & Nei' substitution model (TrN + G). All analyses showed a clear match between our isolate and an undescribed *Sporothrix* sp. isolated from *Thuja* in Canada (Lim et al. 2005) (Fig. 3, group F).

After final alignment of the 73 ITS2-LSU sequences representing *Leptographium s. l.*, including the group G isolates from Argentina, a 629 character matrix was obtained. A transversional model was used to run ML and BI analyses. Isolates from this study were positioned in a clearly distinct lineage closest to *L. pruni* Masuya & M.J. Wingf. and *Grosmannia grandifoliae* (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. (Fig. 4). *BT* sequence comparisons of group G isolates and related taxa included 34 sequences. After alignment, a 390-character matrix was obtained. A 'Tamura & Nei' substitution model (TrN + I + G) was selected to run ML and BI analyses. The *EF* matrix consisted of 26 sequences and 707 characters. A transitional model (TIM2 + G) was used. Both the *BT* and *EF* results confirmed that our group G isolates (Fig. 4, group G) were a distinct taxon, peripheral to the *G. penicillata* complex.

**Fig. 4** Phylograms obtained from the ITS2-LSU sequences (left) for *Leptographium s. l.*, as well as *EF* and *BT* sequences of *Leptographium* and *Grosmannia* spp. related to the Argentinian isolates from Patagonia. Novel sequences obtained in this study are printed in bold type. * The *Grosmannia penicillata* complex. MP and ML bootstrap support values (1,000 replicates) above 75 % are indicated at the nodes as MP/ML. Posterior probabilities (above 90 %) obtained from BI are indicated by bold lines at the relevant branching points. * = bootstrap values lower than 75 %. T = ex-type isolates. Scale bar = total nucleotide difference between taxa

## Taxonomy

Based on phylogenetic analyses, the 22 representative isolates from Argentina selected for DNA sequence analyses in this study could be assigned to 10 different species. Only two of these represented known species, namely *O. quercus* and *O. novae-zelandiae*. Of the remaining eight species, we only had sufficient material to formally describe three as new species below, respectively as *Ophiostoma patagonium* sp. nov., *Sporothrix cabralii* sp. nov. and *Leptographium gestamen* sp. nov. The five single-isolate taxa probably also represent new species, but additional isolates would be needed to confirm their phylogenetic position and taxonomic status. Sequences produced in this study of the ex-type of *O. novae-zelandiae* confirmed, in contrast to previous suggestions, that this species is distinct from *O. pluriannulatum* (Hedgc.) Syd., and thus the name is re-instated as distinct here.

***Ophiostoma patagonicum*** de Errasti & Z.W. de Beer, ***sp. nov.*** – Mycobank MB 814175

Fig. 5

Asexual states: pesotum-like and sporothrix-like

Etymology. The epithet *patagonicum* refers to the geographical area where this species was collected.

*Sexual state. Ascomata* developing after 21 days on MEA superficial or partly embedded on media. *Bases* dark brown 7.5 YR 3/3, globose, (110)114–174(218) μm diam; ornamented with brown hyphal hairs up to 160 μm long. *Ascomatal necks* dark brown (7.5 YR 3/3), lighter brown at the apex (7.5 YR 6/8), (530)1064–1376(1655) μm long, (23)30–42(48) μm wide at the base, (8)10–22(24) μm wide at the apex. *Ostiolar hyphae* divergent, tapering towards the apex, hyaline, (12)23–43(59) × (1)1.5–1.8(2) μm. *Asci* not observed. *Ascospores* accumulating in a clear, white mucilaginous mass at the ascomatal apex, turning light yellow (10YR 8/8) with age. *Ascospores* 1-celled, orange-section-shaped in side view, hyaline, (3)4.4–5.2(6) × (1)1.4–1.8(2) μm. *Asexual state* macronematal, pesotum-like, with hyaline synnemata, pigmented at the base, (170)330–480(595) μm long; conidia 1-celled, oblong, hyaline, (2)3.1–4.2(5) × 1–2 μm. Sporothrix-like micronematal state present. *Conidiogenous cells* arising directly from hyphae or as distinct, unbranched conidiophores, (9)15.5–27.2(34) × 1–1.5 μm. *Conidia* hyaline, smooth, obovoid (3.5)3.9–7.1(11) × (1)1.4–1.9(2) μm. Secondary conidia often produced. *Mycelium* aerial, superficial, and embedded in the agar. *Colonies* slow-growing, 27 mm diam. in 7 days at 25 °C, white (2.5YR 8/1), presenting irregular margin. Brown stripes (10YR 4/4) appearing after 10 days with the formation of synnemata. Culture turning dark brown (10YR 2/2) after 21 days.

Host range: Found on *Nothofagus pumilio* (Poepp. & Endl.) Krasser, *N. obliqua* (Mirb.) Oerst., *N. antarctica* (G. Forst.) Oerst., *N. dombeyi* (Mirb.) Oerst., and *Schinus patagonicus*. Also isolated from nitidulid beetles on these trees.

Distribution: ARGENTINA, Andes region from Tierra del Fuego to Neuquén provinces.

Specimens examined: ARGENTINA, Chubut Province, Depto. Futaleufú, Cordón Rivadavia, on *N. pumilio*. Andrés de Errasti, 9. 2009, holotype BAFC 52418, living culture CIEFAP431, CMW38089.

Additional specimens: ARGENTINA, Tierra del Fuego province, Depto. Río Grande, Lake Fagnano on *N. pumilio*. Andrés de Errasti, 12. 2011, living culture CIEFAP452, CMW38086. Neuquén province, Lanín National Park, Lake Lácar, Nonthué area on *N. obliqua*. Andrés de Errasti, 5. 2010, living culture CIEFAP449, CMW38085. Chubut province, Los Alerces National Park, Lake Verde on *N. dombeyi*. A. de Errasti/B. Hurley/J. Roux, 11. 2011, living culture CIEFAP454, CMW38090.


ITS2-LSU
L. wageneri var. wageneri AF343707
L. douglasii AY553381
G. wageneri AF343708
L. reconditum AF343690
L. neomexicanum AY553382
G. serpens JN135314 T
L. castellanum JN135317 T
L. yamaokae JN135315 T
G. alacris JN135313 T
L. gibbsii JN135316 T
L. wageneri var. pseudotsugae AF343706
L. pini-densiflorae AY707199 T
L. celere HQ406834 T
L. sinoprocerum EU296773 T
L. bhutanense EU650187 T
L. gracile HQ406840
L. procerum EU244638 T
L. profanum DQ354944 T
L. latens HQ406845 T
L. manifestum HQ406839 T
L. truncatum DQ062052 T
L. pinicola DQ062060
G. koreana AB222065 T
L. wingfieldii AY553398
G. robusta AY544619 T
G. aurea AY544610 T
L. terebrantis EU296777 T
L. longiclavatum AY816686 T
G. clavigera AY544613 T
L. pyrinum DQ062072 T
L. albopini AF343695
L. lundbergii DQ062068 T
L. yunnanense AY553415 T
L. conjunctum HQ406832
G. huntii AY544617
G. laricis DQ062074
L. aenigmaticum AY553389 T
G. piceaperda AY707209
L. pineti DQ062076
G. cainii AY744548 T
G. leptographioides AF343710
G. sagmatospora AY744546
G. davidsonii EU879126 GU13418
G. cucullata AJ538335 T
G. olivaceapini AJ538336 T
G. olivacea AJ538337 T
L. pityophilum AF343679 T
G. radiaticola AY744551 T
G. galeiformis AY744552 T
G. crassivaginata AF343673
L. eucalyptophilum AF343703 T
L. hughesii AF343700
L. abieticolens AF343701 T
L. pistaciae HQ406846 T
L. bistatum AY348305 T
L. chlamydatum EU979333 T
L. americanum DQ062079 T
L. abietinum AF343670
L. euphyes AF343686
L. alethinum AF343685
L. altius HQ406851 T
L. curviconidium HQ406850 T
G. penicillata DQ097851 T
G. abiocarpa AJ538339 T
L. fructetum DQ097847 T
L. curvisporum EU979328 T
G. dryocoetidis AJ538340 T
CIEFAP453 KT362234 T
CIEFAP459 KT362236
CIEFAP457 KT362235
Leptographium gestamen sp. nov.
L. pruni AB091219 AB104900
G. grandifoliae AF343711
Sporothrix schenckii JQ070113/JQ070139
EF
L. chlamydatum EU979349 T
L. chlamydatum EU979351
L. chlamydatum EU979350
L. altius HQ406877
L. altius HQ406877 T
L. curviconidium HQ406873
L. curviconidium HQ406874 T
L. curvisporum EU979345
L. curvisporum EU979344 T
L. curvisporum EU979347
G. americana DQ062045
G. americana DQ062046 T
L. abietinum DQ062048
L. abietinum DQ062047
L. fructetum HQ848407
L. fructetum HQ848408
L. bistatum HQ848406
L. bistatum HQ848405
L. pistaciae HQ406871
L. pistaciae HQ406870 T
L. alethinum EU502830
CIEFAP457 KT381301
CIEFAP453 KT381300 T
CIEFAP459 KT381302
Leptographium gestamen sp. nov.
L. pruni HQ848411
L. pruni HQ848412
G. penicillata complex
0.05
BT
L. chlamydatum EU979343
L. chlamydatum EU979342
L. clamydatum EU979341 T
G. penicillata DQ097860
G. penicillata DQ097861 T
L. abietinum AY263183
L. abietinum DQ268627
L. curvisporum EU979336 T
L. curvisporum EU979340
L. fructetum DQ097854 T
L. fructetum DQ097855
L. fructetum HQ848399
G. abiocarpa DQ097858
G. abiocarpa DQ097857 T
L. pistaciae HQ406894 T
L. pistaciae HQ406895
L. bistatum AY348306 T
L. bistatum HQ848397
L. bistatum AY348307
L. abieticolens AY348310
L. alethinum EU502813
L. alethinum AY534940
G. grandifoliae DQ296119
L. pruni HQ848404
L. pruni HQ848403
CIEFAP457 KT381298
CIEFAP453 KT381297 T
CIEFAP459 KT381299
Leptographium gestamen sp. nov.
G. americana DQ062012
G. americana DQ062013 T
L. curviconidium HQ406898 T
L. curviconidium HQ406897
L. altius HQ406900
L. altius HQ406899 T
G. penicillata complex
0.05
0.02

**Table 1** Representative isolates from Patagonia, Argentina, included in the phylogenetic analyses

| Group | Species name | Isolate | Tree host | Substrate | Province | LSU | ITS2-LSU | ITS | BT | EF |
|---|---|---|---|---|---|---|---|---|---|---|
| A | *Ophiostoma quercus* | CIEFAP440 | *N. dombeyi* | Dead fallen wood | Río Negro | KT362222 | | KT362237 | KT381278 | |
| A | *O. patagonicum* | CIEFAP449 | *N. obliqua* | Dead fallen wood | Neuquén | | | KT362238 | KT381282 | |
| A | *O. patagonicum* | CIEFAP463 | *S. patagonicus* | Nitidulid beetle | Chubut | KT362224 | | KT362240 | KT381288 | |
| A | *O. patagonicum* | CIEFAP460 | *N. pumilio* | Nitidulid beetle | Chubut | | | KT362243 | KT381279 | |
| A | *O. patagonicum* | CIEFAP452 | *N. pumilio* | Dead fallen wood | T. d/Fuego | | | KT362241 | KT381284 | |
| A | *O. patagonicum* | CIEFAP461 | *N. pumilio* | Nitidulid beetle | T. d/Fuego | | | KT362239 | KT381286 | |
| A | *O. patagonicum* | CIEFAP462 | *N. pumilio* | Nitidulid beetle | Chubut | | | KT362242 | KT381287 | |
| A | *O. patagonicum* | CIEFAP431 | *N. pumilio* | Dead fallen wood | Chubut | KT362223 | | KT362244 | KT381280 | |
| A | *O. patagonicum* | CIEFAP454 | *N. dombeyi* | AB gallery dying tree | Chubut | | | KT362245 | KT381283 | |
| A | *O. patagonicum* | CIEFAP442 | *N. antarctica* | Dead fallen wood | Neuquén | | | KT362246 | KT381281 | |
| A | *O. patagonicum* | CIEFAP465 | *N. pumilio* | AB gallery dead tree | Chubut | | | KT362247 | KT381285 | |
| B | *Ophiostoma* sp1 | CIEFAP450 | *N. dombeyi* | BB gallery dead tree | Chubut | KT362225 | | KT362248 | KT381289 | |
| C | *O. novae-zelandiae* | CIEFAP423 | *N. obliqua* | Dead fallen wood | Neuquén | KT362226 | | KT362249 | KT381292 | |
| C | *Ophiostoma* sp2 | CIEFAP447 | *T. stipularis* | Dead fallen wood | Río Negro | KT362227 | | KT362250 | KT381290 | |
| C | *Ophiostoma* sp3 | CIEFAP439 | *N. dombeyi* | Dead fallen wood | Chubut | | | KT362251 | KT381291 | |
| D | *Ophiostoma* sp4 | CIEFAP464 | *N. pumilio* | AB gallery dead tree | Chubut | KT362228 | | KT362254 | KT381294 | |
| E | *Sporothrix cabralii* | CIEFAP456 | *N. pumilio* | Dead fallen wood | T. d/ Fuego | KT362229 | | KT362256 | KT381295 | |
| E | *S. cabralii* | CIEFAP458 | *N. pumilio* | AB gallery dead tree | T. d/Fuego | KT362230 | | KT362255 | KT381296 | |
| F | *Sporothrix* sp1 | CIEFAP451 | *N. dombeyi* | AB gallery dead tree | Chubut | KT362231 | | KT362253 | | |
| G | *Leptographium gestamen* | CIEFAP453 | *N. dombeyi* | AB gallery dying tree | Chubut | KT362232 | KT362234 | | KT381297 | KT381300 |
| G | *L. gestamen* | CIEFAP457 | *N. pumilio* | AB gallery dead tree | T. d/Fuego | KT362233 | KT362235 | | KT381298 | KT381301 |
| G | *L. gestamen* | CIEFAP459 | *N. pumilio* | AB gallery dead tree | T. d/Fuego | | KT362236 | | KT381299 | KT381302 |

*AB* ambrosia beetle, *BB* bark beetle, *N Nothofagus*, *S Schinus*, *T Tepualia*

Notes: This species groups peripheral to other hardwood-infesting species in the *O. ulmi* complex within *Ophiostoma s. str.*, and its orange-section-shaped ascospores, and synnematous and sporothrix-like asexual states correspond well to those of other species in the complex (De Beer and Wingfield 2013). It is thus appropriate to treat the species in *Ophiostoma*.

***Sporothrix cabralii*** de Errasti & Z.W. de Beer, ***sp. nov.*** – Mycobank MB 814176;

Fig. 6

Etymology: the species is named for Dr. Daniel Cabral, acknowledging his contributions to mycology in Argentina.

*Sexual state* not known. *Asexual state* young conidiophores short (8)16.6–32.5(42) × (1)2.5–3.8(4.5), slightly asymmetrical, consisting of a short basal cell and a conidiogenous cell bearing prominent denticles. *Conidiophores* sometimes absent and conidia arising directly from undifferentiated hyphae. Mature conidiophores unbranched, usually of considerable length, (10)23–65(170) μm long. *Conidiogenous cells* with denticles, discrete, cylindrical, tapering towards the apex, (5)6.2–9.5(15) × (1.5)2.1–2.9(3) μm. *Conidia* oblong with truncate bases, hyaline, (3)4.4–7.9(13.5) × (1)1.2–1.7(2.5) μm. Secondary conidia often produced. *Mycelium* superficial, hyaline; aerial mycelium abundant, floccose. Colony slow-growing, 15 mm diam. in 7 days at 25 °C. *Colonies* white (2.5YR 8/1) with irregular margin.

Host range: Found in the galleries of the ambrosia beetles *Gnathotrupes* spp. and on dead wood of *Nothofagus pumilio*.

Distribution: ARGENTINA, Tierra del Fuego province.

Specimens examined: ARGENTINA, Tierra del Fuego province, Depto. Río Grande, Lake Fagnano on *N. pumilio*. A. de Errasti, 12. 2011, holotype BAFC52420, living culture CIEFAP456, CMW38098.

Additional specimens examined: ARGENTINA, Tierra del Fuego province, Depto. Río Grande, Lake Fagnano on *N. pumilio*. A. de Errasti, 12. 2011, living culture CIEFAP458, CMW38099.

Notes: This species, known only by its sporothrix-like asexual state, groups closest to *Ophiostoma candidum* in the *Sporothrix schenckii-Ophiostoma stenoceras* complex. *O. candidum* has similar asexual morphology when compared to *S. cabralii*. The former species has been isolated from wounds on *Eucalyptus* trees in South Africa (Kamgan Nkuekam et al. 2012). The generic status of this species complex is currently being re-considered, and at the present time, asexual species in this group are best treated in the genus *Sporothrix* (De Beer and Wingfield 2013; De Beer et al. 2013b).

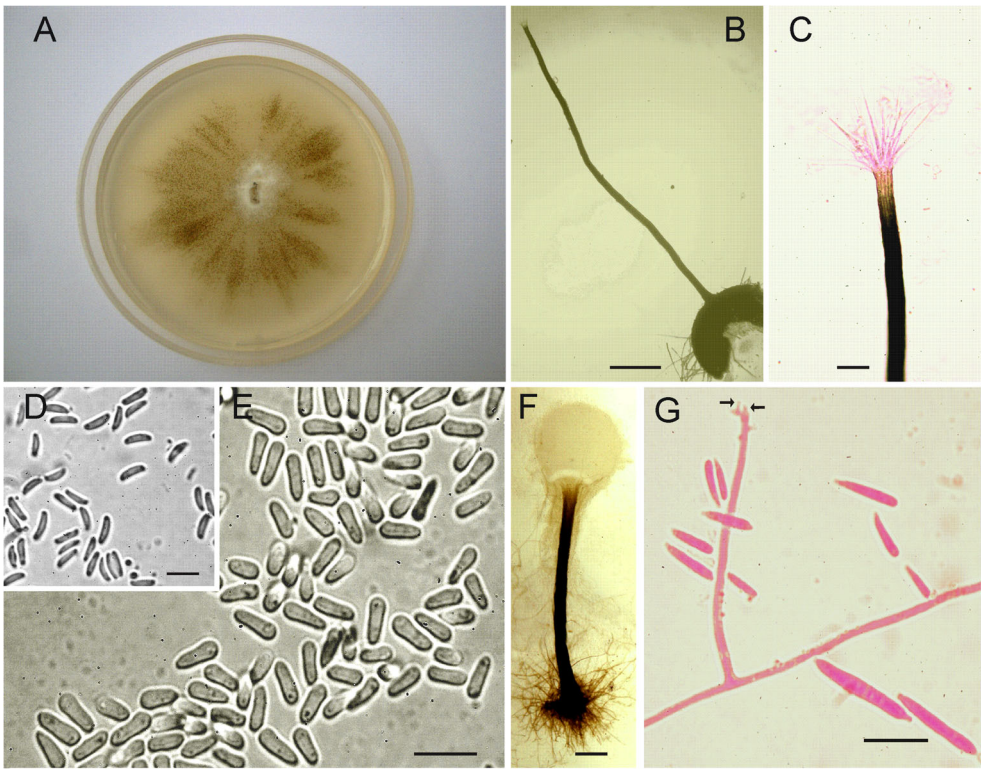



**Fig. 5** ***Ophiostoma patagonicum sp. nov.*** **a** CIEFAP431 holotype culture on MEA, **b** long-necked ascomata on MEA, **c** divergent ostiolar hyphae, **d** ascospores, **e**, **f** conidia and synnemata of pesotum-like asexual state, and **g** sporothrix-like asexual state, black arrows indicate denticles of conidiogenous cell. Scale bars: B = 100 μm; C, F, G = 10 μm; D, E = 5 μm

***Leptographium gestamen*** de Errasti & Z.W. de Beer, ***sp. nov.*** – Mycobank MB 814177;

Fig. 7

Etymology: from Latin 'gestamen' something worn or carried on the body, referring to this species always being found in association with ambrosia beetles.

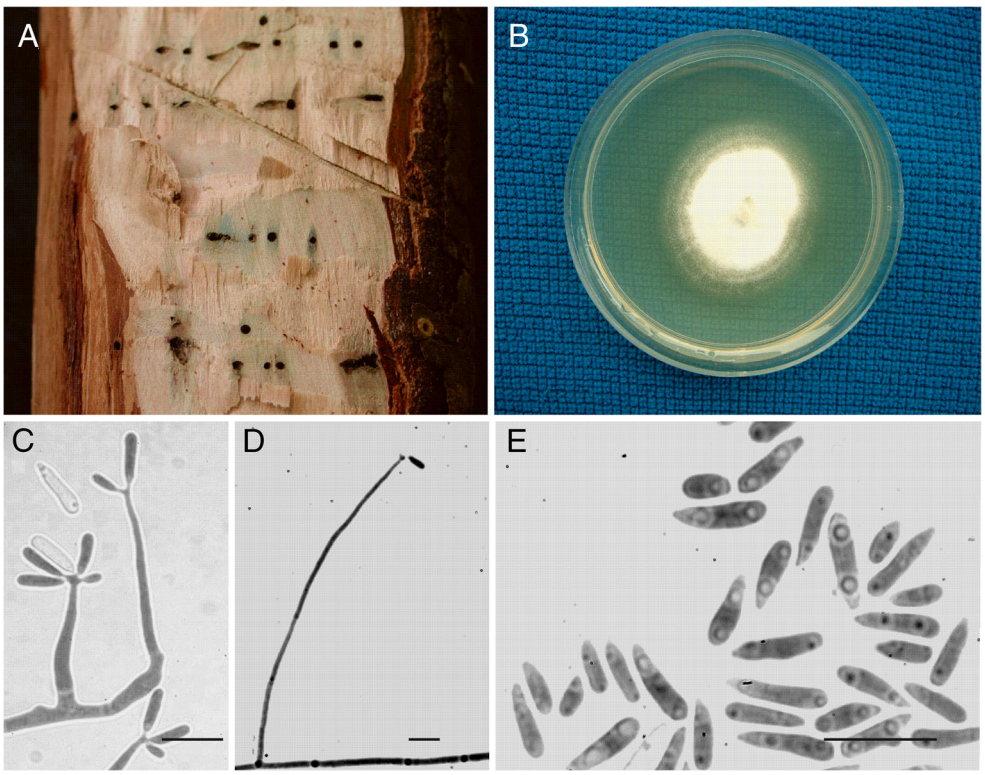



**Fig. 6** ***Sporothrix cabralii sp. nov.*** **a** Galleries of *Gnathotrupes* (ambrosia beetles) species on *Nothofagus* colonized by *S. cabralii*, **b** CIEFAP458 holotype culture on MEA, **c** young conidiophore with denticulate conidiogenous cells, **d** mature conidiophore, and **e** conidia. Scale bars: C, D, E = 10 μm

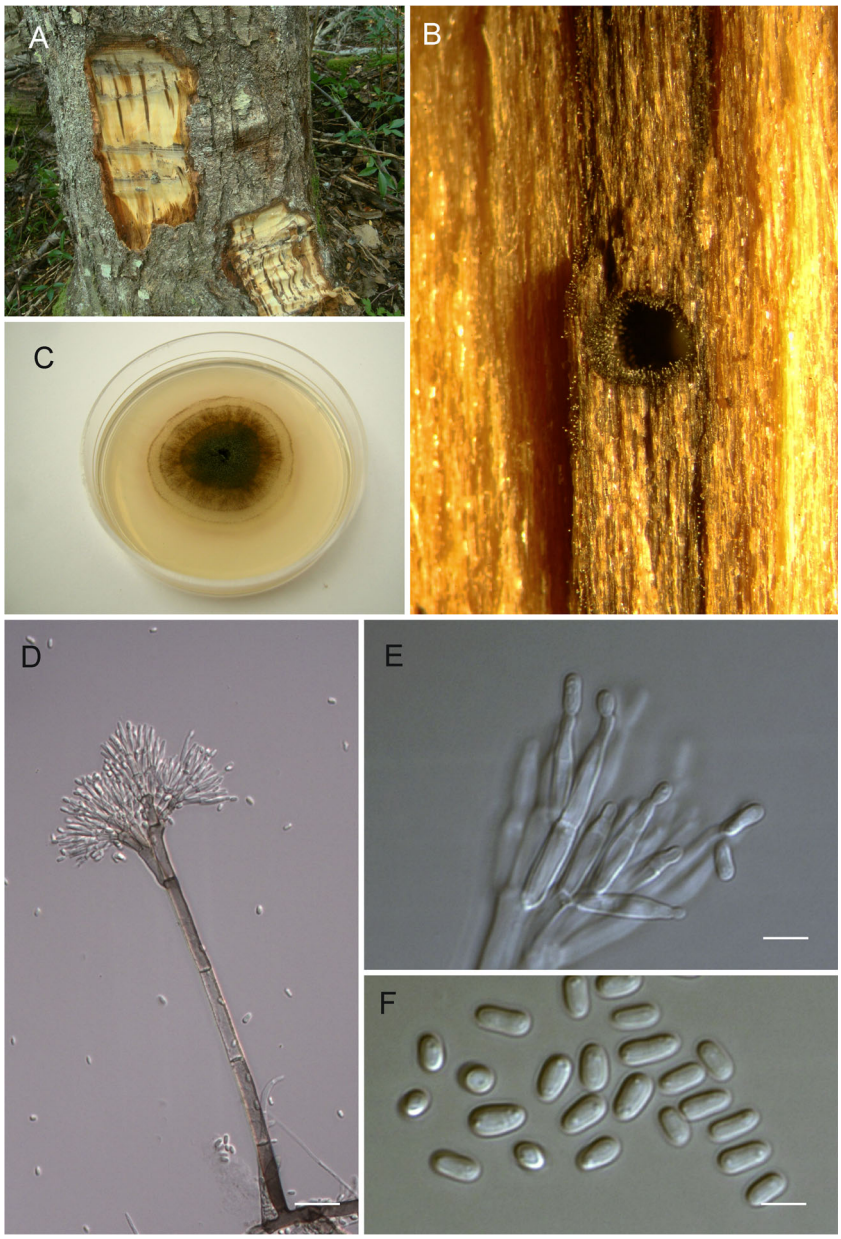



**Fig. 7** ***Leptographium gestamen sp. nov.*** **a** Ambrosia beetle-infested *Nothofagus* tree. Dark streaks of *Leptographium* colonization associated with the galleries of the beetles. **b** Galleries of ambrosia beetles (*Gnathotrupes* spp.) with *L. gestamen* conidiophores on its surface. **c** BAF453 holotype culture on MEA, **d** conidiophore, **e** conidiogenous cells, and **f** conidia. Scale bars: D = 10 μm; E, F = 5 μm

*Sexual state* not known. *Asexual state* conidiophores occurring singly, arising directly from the mycelium, erect, macronematous, mononematous, (80)147–203(235) μm long, rhizoid-like structures absent. *Stipes* light brown (10YR 7/6) to dark brown (10YR 3/3) towards the base, not constricted, cylindrical, simple, 3–8-septate. Branching pattern Type A (Jacobs and Wingfield 2001), two primary branches arising from the main stype. *Conidiogenous apparatus* (28)33.1–42.4(53) μm long, consisting of 3–5 series of branches. *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering at the apex, (7)8.1–11.3(13.5) × (1.5)1.7–2.7(3) μm. *Conidia* aseptate, oblong with truncate bases and rounded apices, hyaline (3)3.5–4.8(6) × (1)1.7–2.6(3) μm. *Mycelium* superficial and submerged in agar, no aerial mycelium. Colonies very slow growing, 12 mm diam. in 7 days at 25 °C. *Colonies* greenish brown (5Y 5/4), colony margin effuse.

Host range: Found specifically in the galleries of *Gnathotrupes* species, a group of ambrosia beetles with more than 15 species infesting *Nothofagus* (Aguayo Silva et al. 2008).

Distribution: ARGENTINA, Andes region, from Tierra del Fuego to Río Negro provinces.

Specimens examined: ARGENTINA, Chubut province, Los Alerces N.P., Lake Verde on *N. dombeyi*. A. de Errasti/B. Hurley/J. Roux, 11.2010, holotype BAFC52421, living culture CIEFAP453, CMW38096.

Additional specimen examined: ARGENTINA, Tierra del Fuego province, Depto. Río Grande, Lake Fagnano on *N. pumilio*. A. de Errasti, 12. 2011, living culture

CIEFAP459 = CMW38095. Depto. Río Grande, Lake Fagnano on *N. pumilio*. A. de Errasti, 12. 2011, living culture CIEFAP457, CMW38097.

Notes: This species, known only by its leptographium-like asexual state, does not group in any of the ten species complexes defined by De Beer and Wingfield (2013a) in *Leptographium s. l.*, but rather between the *G. penicillata* complex and *L. pruni* and *G. grandifoliae*, which also do not form part of any currently defined species complex. Because the generic boundaries of *Grosmannia* and *Leptographium* are still under reconsideration, we have followed the recommendations by De Beer and Wingfield (2013) and treat the new species in the genus *Leptographium*.

***Ophiostoma novae-zelandiae*** (L.J. Hutchison & J. Reid) Rulamort Bull. Soc. Bot. Centre-Ouest, n.s. 21: 512. 1990.

≡ *Ceratocystis novae-zelandiae* Hutchison & J. Reid, N. Z. J. Bot. 26: 70. 1988.

Host range: Found on dead wood of *Nothofagus obliqua*, and bark beetle-infested wood of *Podocarpus* spp.

Distribution: ARGENTINA, NEW ZEALAND.

Specimens examined: NEW ZEALAND, Manawatu-Wanganui Region, Taupo, Urewera National Park, on *Podocarpus* sp., J. Reid, 06.1982, ex-holotype culture UAMH9559. ARGENTINA, Neuquén province, Lanín National Park, Lake Láccar, Nonthue area, on *Nothofagus obliqua*, A. de Errasti, 05. 2009, living culture CIEFAP423, CMW38107.

Note: This species has been reduced to synonymy with *O. pluriannulatum* based on ITS sequences (Thwaites et al. 2005). However, it is known that sequence comparisons of the ITS region have failed to distinguish satisfactorily between closely related species in this complex (Zanzot et al. 2010). Our phylogenetic analyses based on *BT* do not support that synonymy (Fig. 2). Furthermore, *O. novae-zelandiae* has a *BT* intron arrangement of −/−/5, different from *O. pluriannulatum* (3/4/-). We thus consider *O. novae-zelandiae* to be a valid species, distinct from all other species in the *O. pluriannulatum* complex. The presence of pesotum-like synnemata in the original description by Hutchison and Reid (1988) was neither observed in the present study nor by Thwaites et al (2005). The latter authors suggested that the original collection consisted of a mixed culture and our results concur with that view. An emended diagnosis will be provided for *O. novae-zelandiae* in an upcoming revision of all the species in the *O. pluriannulatum* complex (De Beer, personal communication).

## Discussion

This study represents the first detailed consideration of species of Ophiostomatales from Argentina. Although it clearly does not reflect the results of a comprehensive collection of these fungi, the surveys conducted were reasonably thorough and covered a number of years. In all, 361 samples were assembled and 101 isolates of Ophiostomatales were obtained. These were then identified based primarily on DNA sequence comparisons as representing 10 taxa, two of known species and eight representing novel taxa. Of the new species, three included a sufficient number of isolates to justify providing names for them.

Of the new species described in this study, *Ophiostoma patagonicum*, *Leptographium gestamen*, and *Sporothrix cabralii*, are most likely native to Patagonia. *O. patagonicum* is closely related to *O. triangulosporum* Butin, in the *O. ulmi* complex. *O. triangulosporum* was first isolated in Brazil on *Araucaria angustifolia* (Bertol.) Kuntze (Butin 1978) and it is defined by ascospores with triangular sheaths and raffaelea-like conidiophores. This is different from *O. patagonicum* and the other species included in the *O. ulmi* complex, which occur mostly on hardwood tree species and have orange-section-shaped ascospores and pesotum-like synnemata. *O. patagonicum* was isolated from *Nothofagus* and *Schinus* species, from dead wood, declining trees, ambrosia beetle galleries, and nitidulid beetles. This fungus was found in Neuquén, Río Negro, Chubut, and Tierra del Fuego provinces, areas approximately 2,000 km apart, and this suggests that *O. patagonicum* is a widely distributed species, which is neither host- or insect-vector-specific. Reports from *O. piceae* on native hardwoods from Chile have been based on morphology (Billings 1993; Butin and Aquilar 1984) and could represent *O. patagonicum*, since the two species have similar morphology, and *O. piceae* is not commonly associated with hardwoods (De Beer et al. 2003).

Based on DNA sequence comparisons, *Sporothrix cabralii* described in this study is closely related to *O. candidum*, a species recently described from *Eucalyptus* trees in South Africa (Kamgan Nkuekam et al. 2012). The asexual state of *O. candidum* produced white cultures with abundant aerial mycelia, elongated conidiophores, and conidiogenous cells with prominent denticles, similar to those of *S. cabralii*. The latter, however, differs from *O. candidum*, in having longer conidiophores and larger conidia. It was isolated only in Tierra del Fuego Pprovince, associated with galleries of the ambrosia beetle *Gnathotrupes* (Coleoptera: Scolytinae) on dead *Nothofagus pumilio* trees.

*Leptographium gestamen* is not closely related to any species complex in *Leptographium s. l.* Its closest relatives were shown to be *L. pruni* and *Grosmannia grandifoliae*, although the three species did not form a monophyletic group with statistical support. All these species occur on hardwoods, and morphological differences between these taxa include conidiophore branching patterns as defined by Jacobs and Wingfield (2001): type A in *L. gestamen* and type B in *L. pruni* and *G. grandifoliae*. Other differences are the presence of a sporothrix-like asexual state in *L. pruni* and the presence of rhizoids at the bases of the stalks in

*G. grandifoliae*; *L. gestamen* lacks both these attributes. *L. gestamen* was invariably isolated from ambrosia beetle galleries on dead or declining *Nothofagus* trees. Trees showing declining symptoms presented dark streaks beneath the bark, as a consequence of *Leptographium* colonization in the sapwood. This new species was collected in Tierra del Fuego, Chubut, and Río Negro provinces and its distribution probably includes the whole Andes region of Patagonia.

Some species collected in this study, and specifically *L. gestamen* and *S. cabralii*, were obtained from dead or declining *Nothofagus* trees, associated with galleries of ambrosia beetles in the genus *Gnathotrupes*. Although pathogenicity was not tested in this study, this has previously been considered for ophiostomatoid fungi-associated *Platypus* spp. found in wilted, ambrosia beetle-infested *Nothofagus* trees in New Zealand (Faulds 1973, 1977). This fact raises the interesting question as to whether *L. gestamen* and *S. cabralii* could contribute to *Nothofagus* decline (Aguayo Silva et al. 2008; Kirkendall 2011) in Patagonia.

The present study confirmed the occurrence in Argentina of *O. quercus* and *O. novae-zelandiae. O. novae-zelandiae* is reported for the first time outside New Zealand. This species has been synonymized with *O. pluriannulatum* based on ITS sequence comparisons and mating experiments (Thwaites et al. 2005). Our findings do not support the status of *O. novae-zelandiae* as a synonym of *O. plurianulatum*.

This work represents the most extensive and intensive survey of Ophiostomatales on native trees from the Argentinean Patagonia. This is a region previously unexplored for these fungi, and it revealed the existence of several novel taxa, most of which are probably endemic. Three new species were described, two species were reported from Argentina for the first time and five taxa remain to be fully characterized once additional strains become available to do so. Clearly, further sampling on native forests in Patagonia will be necessary to fully characterize the diversity of the Ophiostomatales in the area. Furthermore, it will be interesting to consider the presence of this group of fungi on non–native trees such as the exotic conifers planted extensively in the region. This would also provide interesting data relating the interactions between native and exotic trees, their fungal associates, and the movement of these organisms globally.

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