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Short title: *Huntiella decorticans* in Patagonia

# *Huntiella decorticans* sp. nov. (Ceratocystidaceae) associated with dying *Nothofagus* in Patagonia

A. de Errasti[1]

*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Centro Forestal CIEFAP, Ruta 259 km 4 Esquel, Chubut, CP 9200, Argentina*

Z.W. de Beer

*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Pretoria, 0028, South Africa*

M. Rajchenberg

*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Centro Forestal CIEFAP, Ruta 259 km 4 Esquel, Chubut, CP 9200, Argentina*

M.P.A. Coetzee

*Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Pretoria, 0028, South Africa*

M.J. Wingfield

J. Roux

*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Pretoria, 0028, South Africa*

***Abstract:*** During a survey of ophiostomatoid fungi in native forests of southern Argentina, several isolates of *Huntiella* species were obtained from *Nothofagus* trees. Sequences of multiple gene regions were used to identify these fungi, and their pathogenicity was tested

on *N*. *pumilio* and *N*. *dombeyi*. Phylogenetic analyses revealed a novel taxon described here as *H*. *decorticans* sp. nov. Inoculations on *N*. *dombeyi* and *N*. *pumilio* in the forest showed that *H*. *decorticans* is able to produce localized lesions on healthy *Nothofagus* trees.

***Key words:*** canker, *Ceratocystis*, nitidulid beetles, pressure pad

## INTRODUCTION

The temperate forests of southern South America are dominated by species of *Nothofagus*. These forests are restricted to Argentina and Chile, occurring on both sides of the Patagonian Andes. Relative to their biological and ecological importance (Donoso 1994, Armesto et al. 1995, Veblen et al. 1996), little is known regarding the pathogens of these iconic trees (Gamundi and Lederkremer 1989, Cwielong and Rajchenberg 1995, Minter et al. 1998, Hansen et al. 2007). This is equally true for the "ophiostomatoid fungi", a polyphyletic group of genera sharing a common morphology, taxonomic history and similar ecology (de Beer et al. 2013). These fungi include some of the most important pathogens of trees and agents of blue stain in timber globally (Wingfield et al. 1993).

A small number of ophiostomatoid fungi have been described from *Nothofagus* species (Butin and Aquilar 1984, Billings 1993), but these were studied mostly in a period when the genera *Ceratocystis* and *Ophiostoma* were confused, before the major revisions of the taxonomy of these fungi (Hausner et al. 1993, Wingfield et al. 1993, Spatafora and Blackwell 1994, Paulin-Mahady et al. 2002, Massoumi Alamouti 2009, Reblova et al. 2011, de Beer et al. 2013). Most recently *Ceratocystis* (Ascomycota, Microascales, Ceratocystidaceae) was redefined to represent species only in what had been referred to as the *C*. *fimbriata* sensu lato species complex (de Beer et al. 2014). Other species complexes in the genus were classified into separate genera, namely *Chalaropsis* Peyronel,

*Davidsoniella* Z.W. de Beer, T.A. Duong & M.J. Wingf., *Endoconidiophora* Münch, *Huntiella* Z.W. de Beer, T.A. Duong & M.J. Wingf. and *Thielaviopsis*. Went (de Beer et al. 2014). All share a similar morphology adapted for insect dispersal, with long-necked ascomata producing sticky spore masses at their apices and a thielaviopsis-like asexual state (Wingfield et al. 1993, de Beer et al. 2014).

There are only two species of Ceratocystidaceae species reported to be associated with *Nothofagus* trees. These are *Huntiella moniliformis* (Hedgc.) C. Moreau (= *Ceratocystis moniliformis*) (Billings 1993) and *Davidsoniella australis* (J. Walker & Kile) Z.W. de Beer, T.A. Duong & M.J. Wingf. (= *Thielaviopsis australis*) (Kile and Walker 1987). Of these, *H. moniliformis* is reported as the causal agent of sap-stain on *N. dombeyi* and *N. pumilio* in Chile (Billings 1993) and *D. australis* causes an important wilt disease of native *N. cunninghamii* in Tasmania (Kile and Walker 1987, Hosking 1989).

Several species in the Ceratocystidaceae are primary pathogens causing vascular wilts, stem canker and rot of root crops (Kile 1993). Others are weakly pathogenic or saprophytic and many are responsible for blue stain of logs and lumber (Hedgcock 1906, Davidson 1976). Among the best known and most destructive pathogens of broadleaf trees are *Ceratocystis fagacearum*, the cause of oak wilt in North America (Henry et al. 1944), *C. platani* (J.M. Walter) Engelbr. & T.C. Harr., which causes death of *Platanus* species in Europe (Panconesi 1999), *C. albifundus* M.J. Wingf., de Beer & M.J. Morris, the wilt pathogen of Australian *Acacia* species in Africa (Roux and Wingfield 2009) and various species of *Ceratocystis* that cause canker and wilt diseases on a large number of forest and fruit trees (van Wyk et al. 2004a, 2007, 2012; Engelbrecht et al. 2007; Tarigan et al. 2011).

During a recent survey of ophiostomatoid fungi occurring on *Nothofagus* species in southern Argentina, several isolates of fungi in the Ceratocystidaceae were obtained from dying and dead *Nothofagus* trees. The aims of this study were to identify these isolates using phylogenetic data generated from different loci and to test their pathogenicity on *N. dombeyi* and *N. pumilio*.

## MATERIALS AND METHODS

*Collection of isolates*.—Three national parks (Los Alerces, Nahuel Huapi, Lanín) in Neuquén, Río Negro and Chubut provinces of Argentina were surveyed for ophiostomatoid fungi every autumn 2009–2011. Two sites where harvesting occurs, in Chubut (Corcovado) and Tierra del Fuego Provinces (Tolhuin), were surveyed in spring or summer over the same period. Declining, dead and fallen trees showing symptoms of infection by ophiostomatoid fungi, including wood staining and the presence of typical fruiting bodies, were selected for sampling. Samples collected from trees, consisting of sections of wood and bark, were taken to the laboratory in plastic bags to maintain moisture. When samples exhibited fruiting structures, fungi were isolated by lifting spore masses from the apices of ascomata or conidiogenous cells. These were transferred to 2% (w/v) malt extract agar (MEA; 20 g Difco agar, 20 g Difco malt extract). When no fruiting bodies were observed, wood tissue was incubated in sealed moistened plastic bags 5–25 d until sporulation was observed and isolations were then made in the same way.

In addition stained wood chips were surface-disinfested with 70% ethanol for 2 min and rinsed with sterile distilled water and plated onto MEA. Pure cultures were obtained by transferring single hyphal tips onto fresh media.

Isolates used in this study are maintained in the culture collection of the Centro Forestal CIEFAP, Argentina. Duplicates of the ex-type and other cultures were deposited in the Culture Collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina (BAFCcc), and in the Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), Pretoria, South Africa (CMW), for taxonomic purposes only.

*DNA extraction, PCR and DNA sequencing*.—Seven representative isolates of the unknown Ceratocystidaceae were used for molecular characterization. DNA was extracted from ca.100 mg fungal mycelium grown in malt extract broth (2% malt extract, Difco) using an Ultraclean® Microbial DNA extraction KIT (Mo Bio Laboratories, Carlsbad, California).

Four gene regions were amplified for sequencing and phylogenetic analyses. Part of the 5′ end of the 28S gene region of the nuclear rDNA (28S) was amplified with primers LROR and LR5 (Vilgalys and Hester 1990). The nuclear rDNA internal transcribed spacer 1 (ITS1), 5.8S gene, internal transcribed spacer 2 (ITS2) region, collectively referred to as the ITS region, was amplified with primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Partial sequences of the β-tubulin (BT) and transcription elongation factor 1-α (*TEF*1α) gene regions were generated with primers βt1a/βt1b (Glass and Donaldson 1995) and EF1F/EF2R (Jacobs et al. 2004) respectively. Reaction mixtures, 25 mL total volume, consisted of 2.5 mL PCR reaction buffer, 2.5 mM $MgCl_2$, 200 mM of each dNTP, 0.2 mM of each primer, 1 U FastStartTaq DNA Polymerase (Roche Applied Science, Mannheim, Germany) and 2 mL diluted genomic DNA solution. Amplifications were performed in an Eppendorf Master Cycler H gradient (Eppendorf, Hamburg, Germany) with 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 by electrophoresis on a 2 % agarose gel (Roche Diagnostics, Mannheim, Germany) with Gelred™ (Biotium, Hayward, California) as the DNA staining reagent. Products were purified with 1.25 U exonuclease I and 1 U shrimp alkaline phosphatase (Fermentas Life Sciences, Pittsburgh, Pennsylvania) 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, California) employing the same forward and reverse primers used for 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 4 min and a final extension step at 72 C for 8 min. Purified PCR products were separated on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California). All sequences were assessed manually and consensus sequences were constructed with MEGA 5.05 (Tamura et al. 2011).

*Phylogenetic analyses*.—To examine the relatedness of the Argentinean isolates with other species of Ceratocystidaceae, 28S, ITS, *BT* and *TEF*1α sequences were compared with sequences of representative taxa obtained from GenBank. Datasets were compiled in MEGA 5.0.5. Alignments were done with the online version of MAFFT 7 (Katoh 2013), applying the E-INS-i and FFT-NS-i strategies. Sequence alignments were inspected manually in MEGA 5.05. All sequences, matrices and trees included in this study were deposited in GenBank (FIG. 1) and TreeBASE (submission ID 16456). Phylogenetic trees were generated based on maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). MP analyses were performed with PAUP* 4.0b10 (Sinauer Associates, Sunderland, Massachusetts). Heuristic searches with 10 000 replicates of random addition of sequences and tree bisection and reconnection (TBR) as the branch-swapping algorithms were carried out to search for the most parsimonious tree(s). All characters were unordered and of equal weight, with gaps considered as missing characters. Heuristic searches with 1000 replicates of simple sequence addition were performed to estimate bootstrap support at each node. Tree length (TL), consistency index (CI) and retention index (RI) were calculated for the resulting trees. ML analyses were conducted with PhyML 3.0 (Guidon and Gascuel 2003). Substitution models were selected for each dataset with the Akaike information criterion (AIC) in jModelTest 3.7 (Posada and Crandall 1998). Confidence supports for the tree nodes were estimated with 1000 replication bootstrap analyses. For Bayesian Inference (BI), four MCMC chains were run simultaneously from a random starting tree for 5 000 000 generations. Trees were sampled every 100th generation. Trees sampled during the MCM analyses were discarded with burn-in set to 15%, and posterior probabilities were calculated from a majority rule consensus tree generated from the remaining trees.

*Morphology and culture characters*.—Morphology of the sexual state of the isolates from *Nothofagus* was assessed with dry specimens of purified cultures. Culture characters and asexual morphology were studied from 21 d old cultures on MEA. Plates were incubated at 25 C in the dark. Conidiophores and ascomata were mounted on microscope slides in distilled water and phloxine. Fifty measurements of each relevant microscopic structure were made for each isolate. Averages (mean), standard deviation (sd) and minimum (min) and maximum (max) measurements are presented for each structure as follows: (min-) mean minus sd – mean plus sd (-max). The culture colors were described with the charts of Munsell (1912).

*Pathogenicity trials*.—On 29 Oct 2012 pathogenicity trials were initiated at the Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB) experimental station, Chubut province (1006 masl, 1050 mm/y precipitation, 42°12′45″S; 72°32′44″W). A uniform stand of *N*. *pumilio* (DBH = 13.5 ± 4.6; 30-40 y old) was selected to randomly inoculate 10 trees per isolate, including 10 trees as controls. The same procedure was replicated on 31 Oct 2012 at Reserva Provincial ex-concesión Vicente Robles, Chubut Province (693 masl, 1430 mm/y of precipitation, 42°12′45″S; 72°32′44″W) in a mixed stand of *N*. *dombeyi* (DBH = 12,3 ± 6.9; uneven-aged stand, 45–50 y old) and *Austrocedrus chilensis*.

Two isolates (BAFCcc4492, BAFCcc4493) were tested for pathogenicity at each site. Inoculations were made by removing a bark plug from the tree trunk with a 6 mm cork borer to expose the phloem and inserting inoculum into the wound, with the mycelium facing the phloem. The bark plug was replaced and the inoculated area was covered with moist sterile tissue, aluminum foil and tape. Inoculum consisted of actively growing mycelium of the test fungus on 2% MEA or sterile agar plugs for the controls.

Ten weeks after inoculation (4, 5 Feb 2013) the outer bark was removed and the length of the necrotic lesions in the cambium and outer sapwood were measured. Small pieces (2–5 × 5–20 cm) of symptomatic sapwood were cut from lesions approximately 10–15 cm from the inoculation points on five representative trees inoculated with each strain of the test fungi or the controls. These were placed in moist chambers to induce sporulation and to confirm that the lesions were caused by the inoculated fungi. Data were tested with analyses of variance (ANOVA) in the program SPSS17.0 (IBM software). Isolates were used as the independent variable and the lesion length as the dependent variable.

## RESULTS

*Collection of isolates and morphological characters*.—Seven isolates with cultural characters, sexual and asexual morphology typical of fungi in the Ceratocystidaceae were isolated from *Nothofagus* trees. Isolates BAFCcc4492 and BAFCcc4493 were obtained from declining trees with thinning canopies and dead branches (TABLE 1). These trees (n = 2) had cracks in their bark, under which thick, black, mycelial mats (FIG. 2), which are referred to as “pressure pads” (Juzwik et al. 2011), were found. These pressure pads were

infested with nitidulid beetles (Coleoptera, Nitidulidae). Galleries of ambrosia beetles, identified as *Gnathotrupes* species (Coleoptera, Scolytinae), were found in the same trees. In contrast, isolates BAFCcc4488, BAFCcc4489, BAFCcc4490, BAFCcc4491 and BAFCcc4494 were recovered from felled (n = 2) and fallen trees (n = 3) that also had pressure pads beneath their bark. All isolates had perithecioid ascomata with long necks, conical spines on their bases, divergent ostiolar hyphae at the apices of their necks, hat-shaped ascospores and a thielaviopsis-like asexual state. These features are typical of species in the genus *Huntiella* (Hunt 1956, de Beer et al. 2014).

*Phylogenetic analyses*.—Ceratocystidaceae isolates from *Nothofagus* trees in Argentina generated amplicons of 870, 579, 576 and 884 bp for the 28S, ITS, *BT* and *TEF*1α regions respectively. These sequences were compared with representative sequences of closely related species retrieved from GenBank. Sequences were analyzed in four different datasets, one for each locus. The final 28S matrix contained 587 characters including gaps, of which 71 characters were parsimony informative. From the MP analysis, 132 trees were obtained (CI = 0.59, RI = 0.86). A transitional model (TIM3+I+G) was selected for this dataset and incorporated in the ML and BI analyses. Phylogenies obtained from MP, ML and BI were congruent, indicating that the representative isolate from Argentina is related to *H. moniliformis*, *C. fagacearum*, *C. adiposa* and *Ambrosiella* species (FIG. 1).

The ITS dataset consisted of 418 characters including gaps. In total 355 uninformative characters were excluded before the phylogenetic analysis based on parsimony, which resulted in 52 trees of equal length (CI = 0.76, RI = 0.94). For ML and BI analyses a transversional substitution model was selected (TVM+I+G) in jModelTest. Topologies of MP, ML and BI trees were concordant. Phylogenetic trees generated from

the ITS dataset clustered all isolates from Argentina in a well-defined group. The closest relative however could not be identified with certainty because of low posterior probability values and bootstrap support (FIG. 1).

Alignment of *BT* sequences resulted in a matrix of 525 characters, of which 418 were considered uninformative for parsimony analysis. The most parsimonious tree had a CI = 0.85 and RI = 0.96. For ML and BI analyses, the TrN+G model was incorporated based on the results from jModelTest. Topologies of trees resulting from MP, ML and BI were similar, although ML and BI had higher resolution. *BT* results showed that the Argentinean isolates considered in this study reside in a distinct clade within *Huntiella* (FIG. 1).

The alignment of *TEF*1α sequences consisted of 379 characters, and 231 of them were parsimony informative. The most parsimonious tree had a CI of 0.89 and an RI of 0.97. The best fitting substitution model selected for the ML and BI analyses was a general time reversible model (GTR+G). The tree topologies obtained from the BI, ML and MP analyses were congruent, but higher resolution was achieved with MP. *TEF*1α results were in agreement with ITS and *BT* results.

Based on DNA sequence comparisons of four regions it is clear that the isolates from *Nothofagus* species in Argentina represent an undescribed taxon. It therefore is described below.

TAXONOMY

**Huntiella decorticans** de Errasti, Z.W. de Beer & Jol. Roux sp. nov. FIGS. 2, 3

MycoBank MB809115

*Typification:* ARGENTINA, CHUBUT: Los Alerces National Park, 42°43′00.55″S, 71°45′06.77″W (735 masl). On *Nothofagus dombeyi*, 19 Nov 2011, *A. de Errasti*/*M.*

*Rajchenberg/B. Hurley/J. Roux*, (**holotype** BAFC 52365). Ex-type cultures BAFCcc4492 = CMW38106.

*Etymology:* Refers to the ability of the fungus to break the bark of *Nothofagus* trees with pressure pads.

Ascomata superficial. Ascomatal bases dark brown to black, globose to subglobose, (260–)283.2–345.1(–410) µm diam, ornamented with conical, thick-walled, dark brown spines, (–14)19.1–26.4(30–) × (5–)6.6–8.9(–10) µm. Ascomatal necks dark brown to black, (490–)533.4–695.2(–920) µm long, (44–)48.4–52.8(–56) µm wide at the base, (16–)17.7–18.3(–19) µm wide at the apex. On the natural substrate, ascomatal necks ex-centric and sometimes lateral. Ostiolar hyphae divergent, hyaline, (18–)23.5–27.6(–32) µm long. Asci globose, evanescent. Ascospores accumulating in a slime drop at the apex of each ascomatal neck, light yellow, turning amber in 10–14 d. Ascospores hat-shaped in side view, aseptate, hyaline, invested in a sheath, (4–)4.3–5.2(–5.5) × (2.5–)2.7–3.1(–3.5) µm, including the sheath.

Colonies white at first, turning gray to dark brown (10YR 3/3) in 14 d; producing abundant white aerial mycelium. Growth 32 mm diam. in 7 d. Hyphae light brown (10YR 4/6), smooth or granulated, not constricted at septa. Asexual state thielaviopsis-like. Conidiophores occurring singly on mycelium, swollen at the bases, tapering toward the apex, (23–)45.2–74(–92.) µm × (3–)4.2–7.8(–8) µm, (3–)3.3–5.3(–7) µm wide at the apex. Conidia of two types: primary conidia oblong, aseptate, hyaline, catenate, (8–)14.7–17.1(–19) × (2.5–)3.4–4.3(–5) µm. Secondary conidia barrel-shaped, aseptate, hyaline, catenate, (4–)7.5–8.6(–10) × (4)4.2–5.4(–7) µm. Chlamydospores absent.

*Host range:* Associated with *Nothofagus pumilio* and *N. dombeyi*.

*Distribution:* Argentina, Andes region, from Tierra del Fuego to Neuquén Provinces.

*Additional specimens examined:* ARGENTINA: TIERRA DEL FUEGO; Dto. Río Grande, Lago Fagnano on *N. pumilio*, Dec 2011, *A. de Errasti*, BAFCcc4493 = CMW38104. RÍO NEGRO; Parque Nacional Nahuel Huapi, Pto Blest on *N. dombeyi*, Apr 2011, *A. de Errasti*, BAFCcc4491. NEUQUÉN; Parque Nacional Lanín, Lago Correntoso on *N. dombeyi*, May 2010, *A. de Errasti* BAFCcc4490.

*Notes*: *Huntiella decorticans* has wider ascomata bases than *H. moniliformopsis* (Z.Q. Yuan & Mohammed) Z.W. de Beer, T.A. Duong & M.J. Wingf. (van Wyk et al. 2004b) and longer conical spines than those of *H. inquinans* (Tarigan, M. van Wyk & M.J. Wingf.) Z.W. de Beer, T.A. Duong & M.J. Wingf. (Tarigan et al. 2010), the two species with the largest such structures. Other morphological characters were very similar to those of *H. oblonga* (R. N. Heath & Jol. Roux) Z.W. de Beer, T.A. Duong & M.J. Wingf., except for ascospores which are larger in *H. oblonga* (2–3 × 7–8 μm) (Heath et al. 2009) than *H. decorticans* (4.3–5.2 × 2.7–3.1 μm). Culture characters in *H. decorticans* were similar to those in *H. oblonga*, *H. moniliformis* and *H. moniliformopsis*, with white aerial mycelium that becomes gray/black after a few weeks, and smooth, granulated hyphae. Growth of *H. decorticans* at 25 C was slower than for the species mentioned above (van Wyk et al. 2004b, Heath et al. 2009).

*Pathogenicity tests*.—Both *H. decorticans* strains (BAFCcc4492, BAFCcc4493) produced distinct lesions in the cambium and outer sapwood of the inoculated trees. The bark above the lesions was depressed and the infected areas resemble annual cankers (FIG. 4). Beneath the bark, the cambium was extensively discolored with streaking typical of some species of Ceratocystidaceae (FIG. 4). No lesions were found in control inoculations and callus formed around the wound sites. Re-isolations from the affected tissue consistently yielded the inoculated fungus. Mean lesion length values produced by isolates BAFC4492cc and

BAFCcc4493 were statistically different from controls, and similar results were obtained for both *N. pumilio* and *N. dombeyi* trials (FIG. 5).

## DISCUSSION

This study led to the discovery of a new species of *Huntiella* from native *Nothofagus* trees in Patagonia, described here as *H. decorticans*. The fungus was collected from diseased and dying trees and from the logs of harvested and felled trees. It has the capacity to cause distinct lesions in the sapwood when inoculated into healthy trees.

*Huntiella decorticans* has typical morphological characters of the genus, most notably spines on the bases of the ascomata, divergent ostiola hyphae and hat-shaped ascospores. Based on morphology *H. decorticans* could be differentiated easily from other species of *Huntiella* by its larger ascomata bases and longer ornamenting spines. Growth in culture at 25 C was slower than for other species in this group.

DNA sequence comparisons for a suite of loci clearly showed that *H. decorticans* represent a unique, previously unknown species most closely related to *Huntiella*. Although it is morphologically similar to other *Huntiella* species, isolates consistently resided in a distinct cluster. Of note, for every region analyzed the sequence divergence between *H. decorticans* and *H. moniliformis* was greater than the difference between *H. moniliformis* and other species in the genus. This could reflect geographical isolation after introduction, but it also could indicate an ancient divergence between the two species groups.

Sánchez (2011) reported *H. moniliformis* (as *C. moniliformis*) on *Nothofagus* in the same stand where the holotype of *H. decorticans* was collected but was unable to isolate it. It is probable that these represent the same species. Similarly the fungus reported as *C.*

*moniliformis* by Billings (1993) on *Nothofagus* from Chile was probably also *H. decorticans*.

The physical manifestation of *H. decorticans* on *Nothofagus* trees in Argentina is intriguing and suggests that the fungus could be a primary pathogen. The distinct lesions on living trees with pressure pads cracking the bark to expose infectious propagules to the outside is reminiscent of the symptoms of oak wilt caused by *Ceratocystis fagacearum* (Fergus et al. 1957). The fact that the fungus was able to cause similar lesions in inoculated trees supports the view that it is a pathogen in nature. However infected trees did not exhibit wilting such as those infected by *C. fagacearum*, suggesting a comparatively low level of virulence. Certainly there was no indication that *H. decorticans* is a wilt pathogen and it appears to result in localized cankers on tree stems.

The occurrence of pressure pads and nitidulid beetles within cankers caused by *H. decorticans* suggests that these structures are involved in attracting insect vectors to the fungus. This is the case with *C. fagacearum* (Gibbs and French 1980, Appel et al. 1990, Juzwik et al. 1998, Cease and Juzwik 2001) and *C. manginecans* M. van Wyk, Al-Adawi & M.J. Wingf. (syn. *C. acaciivora* Tarigan & M. van Wyk) (van Wyk et al. 2007, Tarigan et al. 2011).

Species of *Huntiella* are generally regarded as nonpathogenic saprobes (Davidson 1935, van Wyk et al. 2006). With the growing number of species recognized in this complex, it would be reasonable to expect that exceptions may exist. Various species in the complex that have been tested for pathogenicity have produced lesions on the stems of inoculated trees (Tarigan et al. 2010), but it is unknown whether this reflects an ability to cause disease under natural conditions. *H. bhutanensis* (M. van Wyk, M.J. Wingf. & T.

Kirisits) Z.W. de Beer, T.A. Duong & M.J. Wingf. (van Wyk et al. 2004b, Kirisits et al. 2013) is the only species of *Huntiella* closely associated with a conifer-infesting insect, producing an odor different to that of other species in this group. *Huntiella decorticans* likewise appears to have a very different biology from other species in *Huntiella* and this is clearly worthy of further investigation.

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## LEGENDS

FIG. 1. Phylograms obtained from ML analyses of 28S, ITS, *BT* and *TEF*1α regions of *Huntiella decorticans* sp. nov. and related taxa. MP and ML bootstrap support values above 70% are indicated at each node as MP/ML. Posterior probabilities above 90% (obtained from BI) are indicated by bold lines at the relevant nodes. * = bootstrap values lower than 75%. Bar = total nucleotide difference between taxa.

FIG. 2. Pressure pad of *Huntiella decorticans* on *Nothofagus dombeyi*. A. Broken bark of a declining tree. B. Pressure pad beneath bark infested with nitidulid beetles.

FIG. 3. *Huntiella decorticans* sp. nov., morphological characters from type specimen (BAFCcc4492). A. Perithecioid ascomata on natural substrate. B. Perithecioid ascoma on MEA. C. 7 d old culture on MEA. D. 14 d old culture on MEA. E, Conical spines. F. Divergent ostiolar hyphae. G. Ascospores. H. Thielaviopsis-like conidiophore. I. Barrel-shaped conidia. J. Cylindrical conidia. Bars: B = 100 µm; E,F,G,H,I,J = 10 µm.

FIG. 4. Lesions on *Nothofagus* following inoculation trials with *Huntiella decorticans* (BAFCcc4492). A. Control. B. Tree with evidence of a canker 10 wk after inoculation. C. Lesion on cambial area. D. Lesion on outer sapwood. Bars: A, B, C = 1 cm; D = 10 cm.

FIG. 5. Results of field inoculations with *Huntiella decorticans* sp. nov. (isolates BAFCcc4492 and BAFCcc4493) and a negative control. Letters above bars indicate statistical differences between isolates ($P < 0.05$).

## FOOTNOTES

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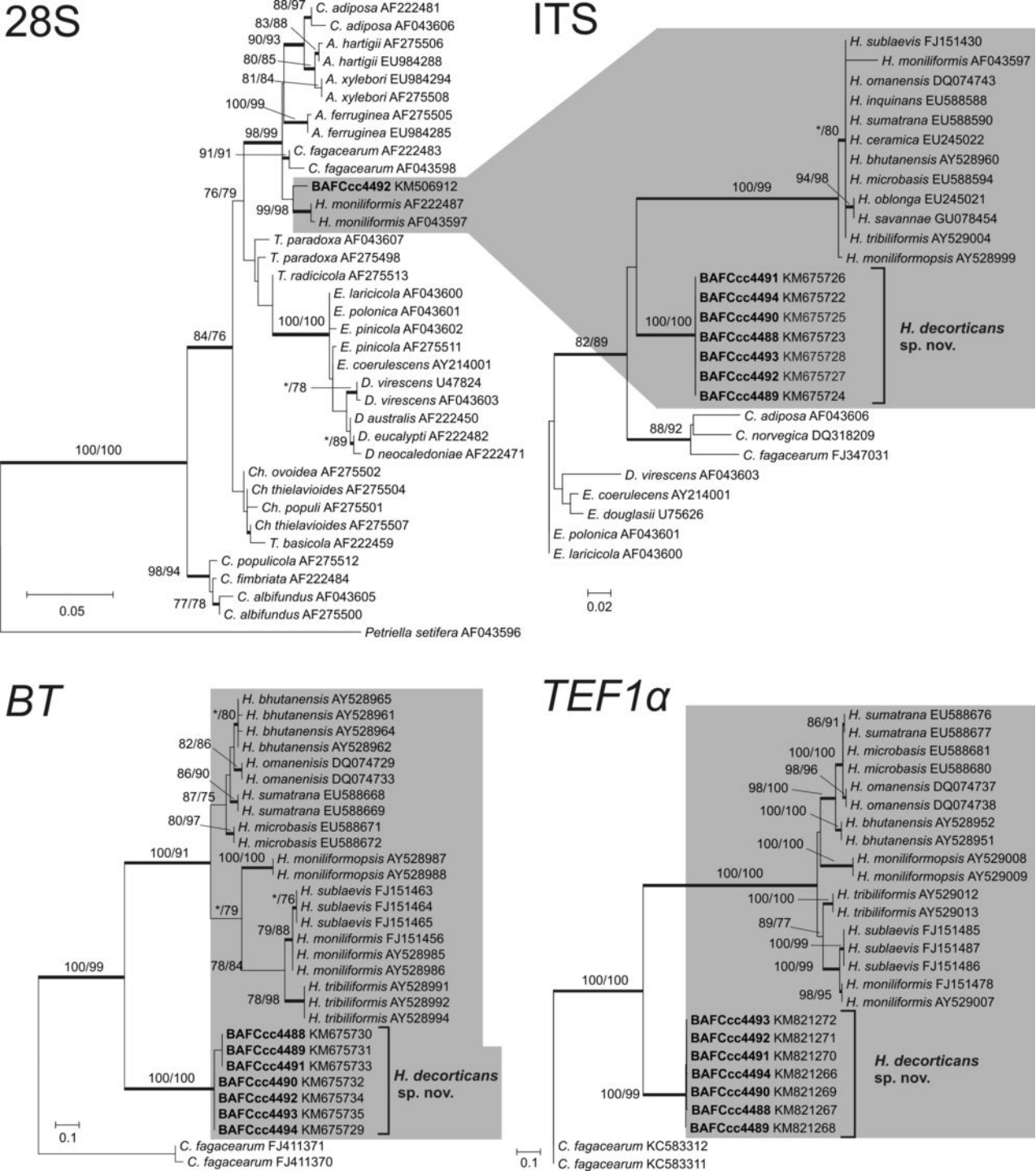
[1]Correspondig author. E-mail: aderrasti@ciefap.org.ar

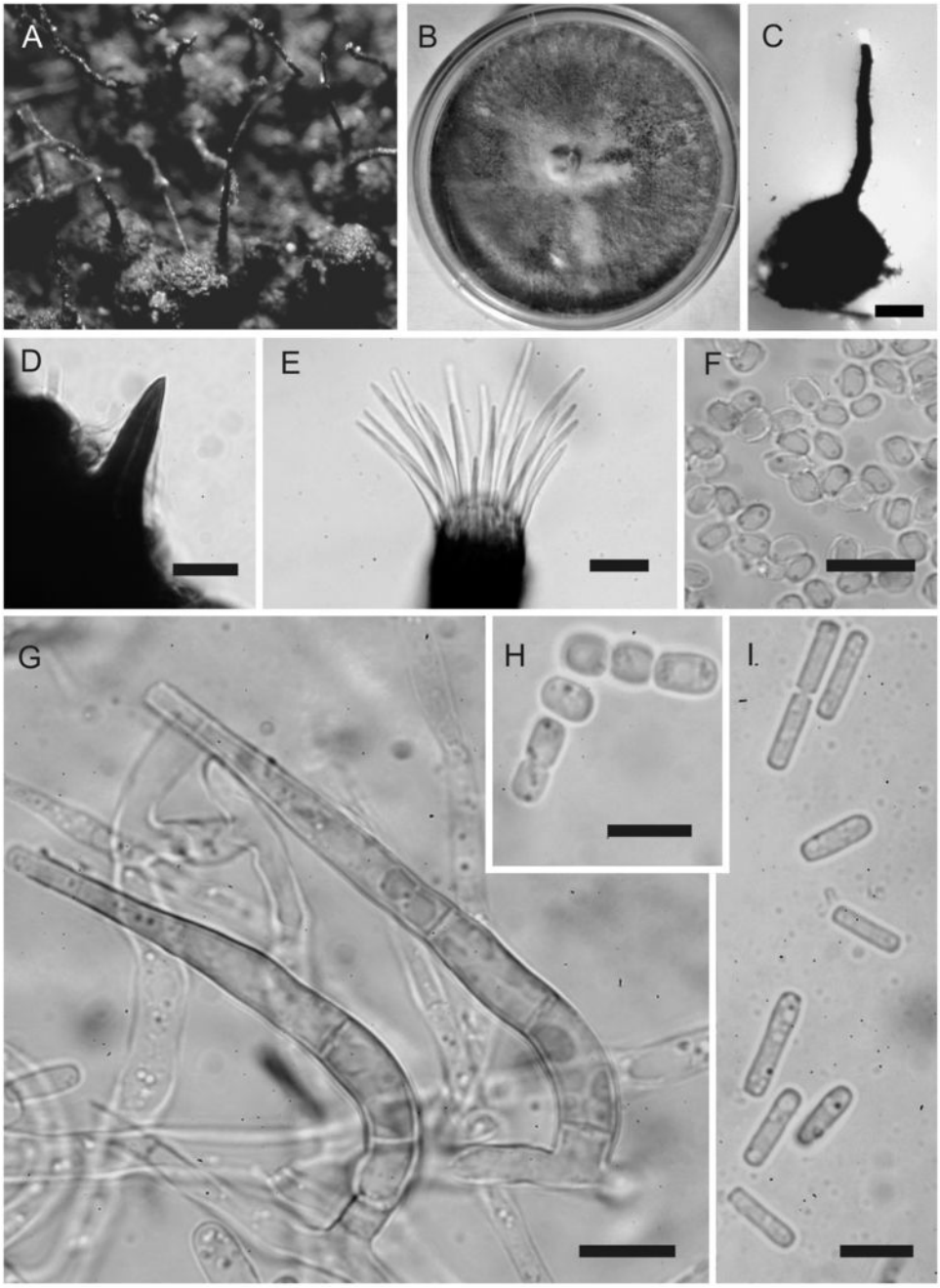
TABLE I. *Huntiella decorticans* isolates from southern Argentina, host, geographical occurrence and associated insects when known

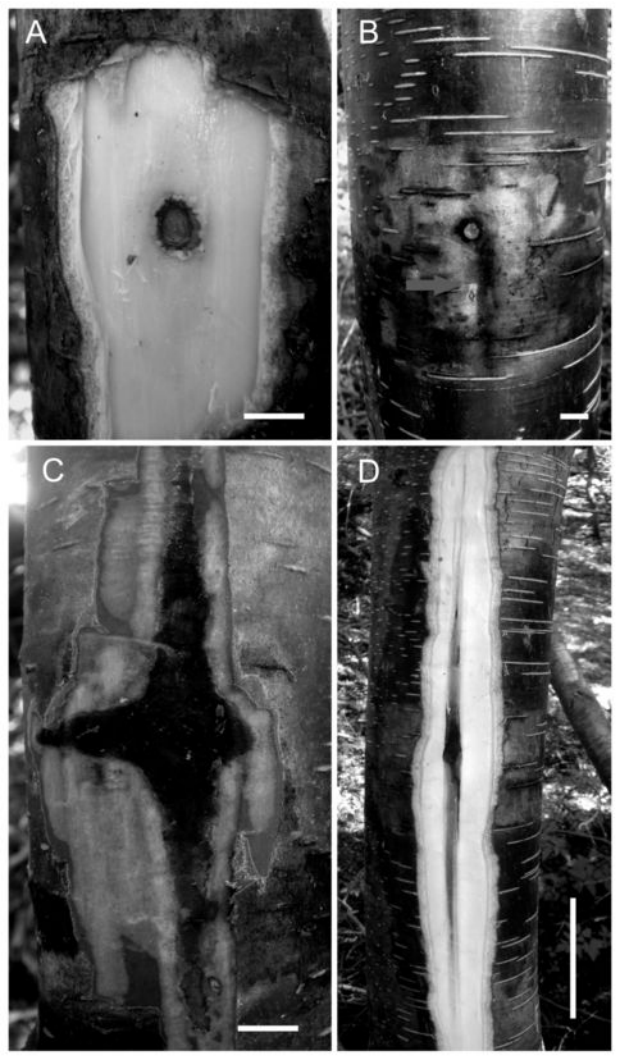
| Isolate | Province | Host | Observations |
|---|---|---|---|
| BAFCcc4494 | Chubut | *N. pumilio* | Recently felled tree |
| BAFCcc4488 | Chubut | *N. pumilio* | Recently felled tree |
| BAFCcc4489 | R. Negro | *N. dombeyi* | More than 1 y on the ground |
| BAFCcc4490 | Neuquén | *N. dombeyi* | Recently felled tree |
| BAFC4cc4491 | R. Negro | *N. dombeyi* | Recently felled tree |
| **BAFCcc4492**[a] | Chubut | *N. dombeyi* | Declining tree. Nitidulids, ambrosia beetle galleries |
| **BAFCcc4493** | T. del Fuego | *N. pumilio* | Dead, standing tree. Ambrosia beetle galleries |

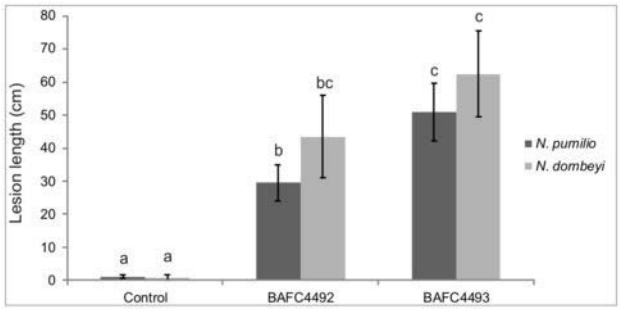
Isolates in boldface were used in pathogenicity trials.
[a] Ex-type culture.


28S
C. adiposa AF222481
C. adiposa AF043606
A. hartigii AF275506
A. hartigii EU984288
A. xylebori EU984294
A. xylebori AF275508
A. ferruginea AF275505
A. ferruginea EU984285
C. fagacearum AF222483
C. fagacearum AF043598
BAFCcc4492 KM506912
H. moniliformis AF222487
H. moniliformis AF043597
T. paradoxa AF043607
T. paradoxa AF275498
T. radicicola AF275513
E. laricicola AF043600
E. polonica AF043601
E. pinicola AF043602
E. pinicola AF275511
E. coerulescens AY214001
D. virescens U47824
D. virescens AF043603
D australis AF222450
D. eucalypti AF222482
D neocaledoniae AF222471
Ch. ovoidea AF275502
Ch thielavioides AF275504
Ch. populi AF275501
Ch thielavioides AF275507
T. basicola AF222459
C. populicola AF275512
C. fimbriata AF222484
C. albifundus AF043605
C. albifundus AF275500
Petriella setifera AF043596
0.05
ITS
H. sublaevis FJ151430
H. moniliformis AF043597
H. omanensis DQ074743
H. inquinans EU588588
H. sumatrana EU588590
H. ceramica EU245022
H. bhutanensis AY528960
H. microbasis EU588594
H. oblonga EU245021
H. savannae GU078454
H. tribiliformis AY529004
H. moniliformopsis AY528999
BAFCcc4491 KM675726
BAFCcc4494 KM675722
BAFCcc4490 KM675725
BAFCcc4488 KM675723
BAFCcc4493 KM675728
BAFCcc4492 KM675727
BAFCcc4489 KM675724
H. decorticans sp. nov.
C. adiposa AF043606
C. norvegica DQ318209
C. fagacearum FJ347031
D. virescens AF043603
E. coerulecens AY214001
E. douglasii U75626
E. polonica AF043601
E. laricicola AF043600
0.02
BT
H. bhutanensis AY528965
H. bhutanensis AY528961
H. bhutanensis AY528964
H. bhutanensis AY528962
H. omanenisis DQ074729
H. omanenisis DQ074733
H. sumatrana EU588668
H. sumatrana EU588669
H. microbasis EU588671
H. microbasis EU588672
H. moniliformopsis AY528987
H. moniliformopsis AY528988
H. sublaevis FJ151463
H. sublaevis FJ151464
H. sublaevis FJ151465
H. moniliformis FJ151456
H. moniliformis AY528985
H. moniliformis AY528986
H. tribiliformis AY528991
H. tribiliformis AY528992
H. tribiliformis AY528994
BAFCcc4488 KM675730
BAFCcc4489 KM675731
BAFCcc4491 KM675733
BAFCcc4490 KM675732
BAFCcc4492 KM675734
BAFCcc4493 KM675735
BAFCcc4494 KM675729
H. decorticans sp. nov.
0.1
C. fagacearum FJ411371
C. fagacearum FJ411370
TEF1α
H. sumatrana EU588676
H. sumatrana EU588677
H. microbasis EU588681
H. microbasis EU588680
H. omanensis DQ074737
H. omanensis DQ074738
H. bhutanensis AY528952
H. bhutanensis AY528951
H. moniliformopsis AY529008
H. moniliformopsis AY529009
H. tribiliformis AY529012
H. tribiliformis AY529013
H. sublaevis FJ151485
H. sublaevis FJ151487
H. sublaevis FJ151486
H. moniliformis FJ151478
H. moniliformis AY529007
BAFCcc4493 KM821272
BAFCcc4492 KM821271
BAFCcc4491 KM821270
BAFCcc4494 KM821266
BAFCcc4490 KM821269
BAFCcc4488 KM821267
BAFCcc4489 KM821268
H. decorticans sp. nov.
0.1
C. fagacearum KC583312
C. fagacearum KC583311


A
B


A
B
C
D
E
F
G
H
I


A
B
C
D


Lesion length (cm)
80
70
60
50
40
30
20
10
0
a
a
b
bc
c
c
Control
BAFC4492
BAFC4493
N. pumilio
N. dombeyi