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OCORRÊNCIA DE *Pseudomerulius curtisii*, *Gelatoporia subvermispora* E *Sacoporia polyspora* NO SUL DO BRASIL

DISSERTAÇÃO DE MESTRADO

Daiana Bortoluzzi Baldoni

Santa Maria, RS, Brasil

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OCORRÊNCIA DE *Pseudomerulius curtisii*, *Gelatoporia subvermispora* E *Sarcoporia polyspora* NO SUL DO BRASIL

Daiana Bortoluzzi Baldoni

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Orientador: Prof[a]. Dr[a]. Zaida Inês Antoniolli

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OCORRÊNCIA DE *Pseudomerulius curtisii*, *Gelatoporia subvermispora* E *Sarcoporia polyspora* NO SUL DO BRASIL

elaborado por
Daiana Bortoluzzi Baldoni

como requisito parcial para obtenção do grau de
Mestre em Ciência do Solo

COMISSÃO EXAMINADORA:

Zaida Inês Antoniolli, Drª. (Presidente/Orientadora)

Gilberto Coelho, Dr. (UFSM)

Diego Pascoal Golle, Dr. (UNICRUZ)

Santa Maria, 20 de Julho de 2012.

Aos meus amados pais, Iracema e Valter
exemplos de perseverança e honestidade.
Ao meu avô José Valentin "in memoriam".
Ao meu querido André.

Dedico.

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"A mente que se abre a uma nova idéia, jamais

voltará ao seu tamanho original."

(Albert Einsten)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciência do Solo
Universidade Federal de Santa Maria

OCORRÊNCIA DE *Pseudomerulius curtisii*, *Gelatoporia subvermispora* E *Sarcoporia polyspora* NO SUL DO BRASIL

AUTORA: DAIANA BORTOLUZZI BALDONI
ORIENTADORA: ZAIDA INÊS ANTONIOLLI
Data e Local da Defesa: Santa Maria, 20 de julho de 2012

O Pampa é o mais novo dos biomas brasileiros, sendo o menos estudado e um dos mais explorados comercialmente. Dentre estas formas de exploração, destaca-se o plantio de grandes áreas com espécies florestais exóticas. Os estudos envolvendo espécies animais e vegetais têm revelado grande biodiversidade, mas ainda são escassos para microrganismos. O objetivo deste trabalho foi coletar, isolar e analisar morfológica e molecularmente três fungos decompositores de madeira de *Pinus* sp. no bioma Pampa: *Pseudomerulius curtisii* (Berk.) Redhead & Ginns, *Gelatoporia subvermispora* (Pilát) Niemelä e *Sarcoporia polyspora* P. Karsten. Efetuou-se a caracterização morfológica e o sequenciamento da região ITS1-5.8S-ITS2 das espécies, permitindo suas identificações e comparações filogenéticas com a literatura. *P. curtisii* chama atenção pela raridade e beleza dos basidiomas e similaridade morfológica com os espécimes do Hemisfério Norte e Oceania, mas com uma distância genética, podendo mostrar o isolamento geográfico e novo taxon a ser descrito. *G. subvermispora*, amplamente estudado pelo elevado potencial biotecnológico e industrial, apresenta alta similaridade morfogenética com espécimes dos EUA, porém com divergência genética dos espécimes da Eurásia. *S. polyspora* apresenta alta similaridade morfológica com os espécimes do hemisfério Norte, mas há falta de sequências disponíveis para comparação filogenética. Essas espécies foram registradas pela primeira vez na América do Sul. *P. curtisii*, *G. subvermispora* and *S. polyspora* (Basidiomycota) apresentam grande potencial para estudos de ciclagem de nutrientes e formação do solo pela capacidade de degradação de lignina e/ou celulose.

Palavras-chave: Fungos degradadores. Morfologia. nrDNA. Região ITS1-5.8S-ITS2.

ABSTRACT

Master Dissertation
Graduate Program in Soil Science
Federal University of Santa Maria

OCORRENCE OF *Pseudomerulius curtisii, Gelatoporia subvermispora* AND *Sarcoporia polyspora* ON SOUTHERN BRASIL

AUTHOR: DAIANA BORTOLUZZI BALDONI
ADVISOR: ZAIDA INÊS ANTONIOLLI
Date and Local of the Defense: Santa Maria, july 20th 2012.

The Pampa is the newest Brazilian biomes. It is one of the lest studied and the one of the most economically exploited. Among these forms of exploitation, there is the cultivation of great dominated grassy fields with exotic forest species. Researches involving animal and plant species have revealed great biodiversity, however, they are still rare for microorganisms. The aim of the study was to collect, to isolate and to analyze morphologically and molecularly three wood-decaying fungi growing on *Pinus* sp. in Pampa biome: *Pseudomerulius curtisii* (Berk.) Redhead & Ginns, *Gelatoporia subvermispora* (Pilat) Niemelä and *Sarcoporia polyspora* P. Karsten. It was conducted the morphological characterization and the sequencing of ITS1-5.8S-ITS2 of species allowing their identification and phylogenetic comparisons with the literature. *P. curtisii* calls attention to the rarity and beauty of the basidiome and morphological similarities with specimens of the Northern Hemisphere and Oceania, but with a genetic distance, and may show the geographic isolation and the new taxon to be described. *G. subvermispora*, widely studied by the high biotechnology and industrial potential. It has high similarity with morphogenetic U.S. specimens, but with genetic divergence of Eurasian specimens. *S. polyspora* shows high morphological similarity with the specimens of the northern hemisphere, but there is a lack of sequences available for phylogenetic comparison. These species were registered for the first time in South America. *P. curtisii*, *G. subvermispora* and *S. polyspora*, fungi Basidiomycota show high potential for nutrient cycling and soil formation by the capacity or lignin and cellulose degradation.

Keywords: Degradation fungi. Morfology. nrDNA. ITS1-5.8S-ITS2 region.

SUMÁRIO

INTRODUÇÃO

O bioma Pampa abrange uma área compartilhada pelos países Argentina, Brasil e Uruguai. Sua representação no território brasileiro encontra-se na metade sul do Estado do Rio Grande do Sul, abrangendo cerca de 176.000 km², equivalendo a 64% do território gaúcho e a 2,07% do território brasileiro. Este bioma, até o ano de 2004, não esteve introduzido como uma unidade de conservação e, a partir deste ano foi considerado como um dos seis biomas brasileiros (IBGE, 2004). O Pampa destaca-se dos outros biomas por ser o único cuja ocorrência é restrita a somente a um Estado do Brasil e por ser o menos estudado dos biomas brasileiros (BOLDRINI et al., 2010; IBGE, 2004). A demora para inclusão desse bioma como uma unidade de conservação implicou na destruição e fragmentação do ecossistema (TILMAN et al., 2001).

Segundo Boldrini et al. (2010), este bioma pode ser dividido em fitofisionomias, distinguíveis pelas espécies vegetais predominantes como: os campos de barba-de-bode, os campos de solos rasos, os campos de solos profundos, os campos dos areais, a vegetação savanóide, os campos do centro do Estado e os campos litorâneos. Essa diferenciação se deve as condições geomorfológicas únicas impostas nessa região. Os raros estudos de biodiversidade realizados no bioma Pampa apresentaram elevada riqueza de espécies, estimada em 2.200 espécies vegetais, 385 espécies de pássaros (SANTOS e SILVA, 2011) e cerca de 90 espécies de mamíferos terrestres (BILENCA e MIÑARRO, 2004) e tais estimativas estão longe de estarem completas.

Atualmente o bioma Pampa vem passando pela mudança do uso do solo com a implantação de um novo modelo de desenvolvimento, ancorado no plantio de grandes áreas com espécies florestais exóticas (SANTOS e TREVISAN, 2009). O Rio Grande do Sul já possui 280.198 hectares com plantio de *Eucalyptus* sp. e mais de 164.000 hectares de *Pinus* sp. com crescimento médio anual de 3% na expansão das áreas de plantio (ABRAF, 2012). Com o intuito industrial de produzir um milhão de toneladas de celulose por ano, estima-se que em breve o Estado terá um milhão de hectares florestados com estas espécies exóticas (SALLABERRY, 2009). Adicionalmente, nessas áreas de plantio no bioma Pampa, pouco se conhece sobre a diversidade de microrganismos do solo, principalmente dos fungos degradadores que habitam esses locais, sua identificação e preservação genética para demais estudos.

Os solos predominantes da região da campanha são os Argissolos, os Planossolos, os Gleissolos e os Neossolos. Alguns desses solos, principalmente os mais arenosos como os Neossolos, são considerados solos frágeis e passíveis de degradação, se não impostas práticas conservacionistas para seu manejo (STRECK et al., 2008).

De acordo Webster e Webster (2007) 80.000 a 120.000 espécies de fungos foram descritas até este ano, embora o número total estimado de espécies seja cerca de 1,5 milhões. Os pesquisadores enfatizam a importância de se conhecer a diversidade dos fungos nos países tropicais e subtropicais, onde acredita-se que pode ser encontrado grande número das espécies ainda desconhecidas pela ciência (RINALDI et al., 2008). Segundo Hawksworth (2004) os estudos em regiões neotropicais estão comprovando que os fungos degradadores corticióides e poliporóides são menos conhecidos do que se estimava pela ciência.

Os microrganismos do solo desempenham inúmeros processos essenciais ao funcionamento dos ecossistemas naturais e manejados (JESUS e MOREIRA, 2008). Dentre as muitas atividades desenvolvidas pelos microrganismos neste ambiente destaca-se a decomposição da matéria orgânica, a participação no ciclo biogeoquímico de vários elementos, a mineralização de nutrientes, a fixação biológica de nitrogênio (ALVES et al., 2006), a agregação das partículas do solo (PASSARIN et al., 2007), a degradação de xenobióticos (KRISTANTI et al., 2011) e o controle biológico de pragas e doenças (SOUZA e DUARTE, 2007).

Os fungos degradadores são organismos de suma importância para a ciclagem de nutrientes no solo, principalmente na degradação de polímeros complexos, como a lignina e a celulose, por possuírem capacidade genética para a produção de enzimas que decompõem ou que modificam esses resíduos (FERRAZ, 2010). Muitas vezes em consequência dessa modificação, esses compostos se tornam passíveis decomposição por outros microrganismos do solo até a sua mineralização (MOREIRA e SIQUEIRA, 2006). Poucos microrganismos possuem essa capacidade, o que torna o metabolismo desses organismos extremamente importante para o ambiente e para a manutenção do equilíbrio do ecossistema (KIRK e CULLEN, 1998). Dessa forma, é de grande interesse científico a bioprospecção e a identificação de espécies de fungos degradadores, assim como a preservação desses recursos genéticos do Pampa Sulino.

De acordo com o tipo de degradação, os fungos degradadores podem ser classificados em fungos causadores da decomposição ou podridão branca (*White-rot-fungi*) que atuam simultaneamente sobre a celulose, hemicelulose e lignina; e em fungos causadores da decomposição ou podridão castanha (*Brown-rot-fungi*), efetivos na degradação dos

polissacarídeos celulose e hemicelulose, mas pouco eficientes na degradação de lignina (ALEXOPOULUS et al., 1996). Existe ainda, um terceiro grupo, os fungos de decomposição branda (*Soft-rot-fungi*), que atuam sobre a lignina e os polissacarídeos celulose e hemicelulose, entretanto, com velocidade de degradação inferior às das podridões branca e castanha (KIRK e CULLEN, 1998).

Os sistemas degradativos de espécies de podridão branca têm sido intensivamente estudados, em razão do elevado potencial de utilização em aplicações biotecnológicas, como na biodegradação de compostos xenobióticos (RABINOVICH et al., 2004), na biorremediação do solo (AFRIDA et al., 2009), na decomposição de resíduos madeireiros (ALONSO et al., 2007; VAN HEERDEN et al., 2008), na biopolpação da madeira (FERRAZ et al., 2008; GULSOY e EROGLU, 2011) na produção enzimática e no biobranqueamento de polpas celulósicas (AFRIDA et al., 2009).

Nos estudos de aplicações biotecnológicas, os fungos de podridão castanha são utilizados na biorremediação do solo (PURMONO et al., 2011) na biodegradação de compostos xenobióticos (MONRROY et al., 2006), na sacarificação enzimática (LEE et al., 2008), na biodegradação e na produção enzimática (VALASKOVA e BALDRIAN, 2006). Portanto, o conhecimento das espécies de fungos degradadores presentes no bioma Pampa é de grande importância, pois pode colaborar para alavancar inúmeros estudos de aplicações biotecnológicas. Todavia, para que ocorram estes estudos, existe a necessidade da correta taxonomia por meio da identificação morfológica e molecular dessas espécies.

Contudo, além do crescente interesse em aplicações biotecnológicas, também são realizados estudos sobre a diversidade, a distribuição geográfica e a filogenia dessas espécies, na tentativa de juntar as peças de um quebra cabeças na evolução dos fungos, como em estudo realizado por Skrede et al. (2011).

Corroborando para os estudos evolutivos, estão os trabalhos taxonômicos baseados em características morfológicas e em ferramentas de biologia molecular para o conhecimento da diversidade desses microrganismos no ambiente, como em estudos de Coelho (2005) e Binder et al. (2010).

Diante da carência de estudos de diversidade de fungos degradadores em áreas de plantio de espécies exóticas no bioma Pampa, esse trabalho visou isolar, identificar e caracterizar algumas espécies de fungos de decomposição branca e marrom presentes em uma área de plantio de *Pinus* sp. em Santa Maria, Rio Grande do Sul, Brasil, (Fig. 1), para posterior realização de estudos de aplicações biotecnológicas desses organismos.

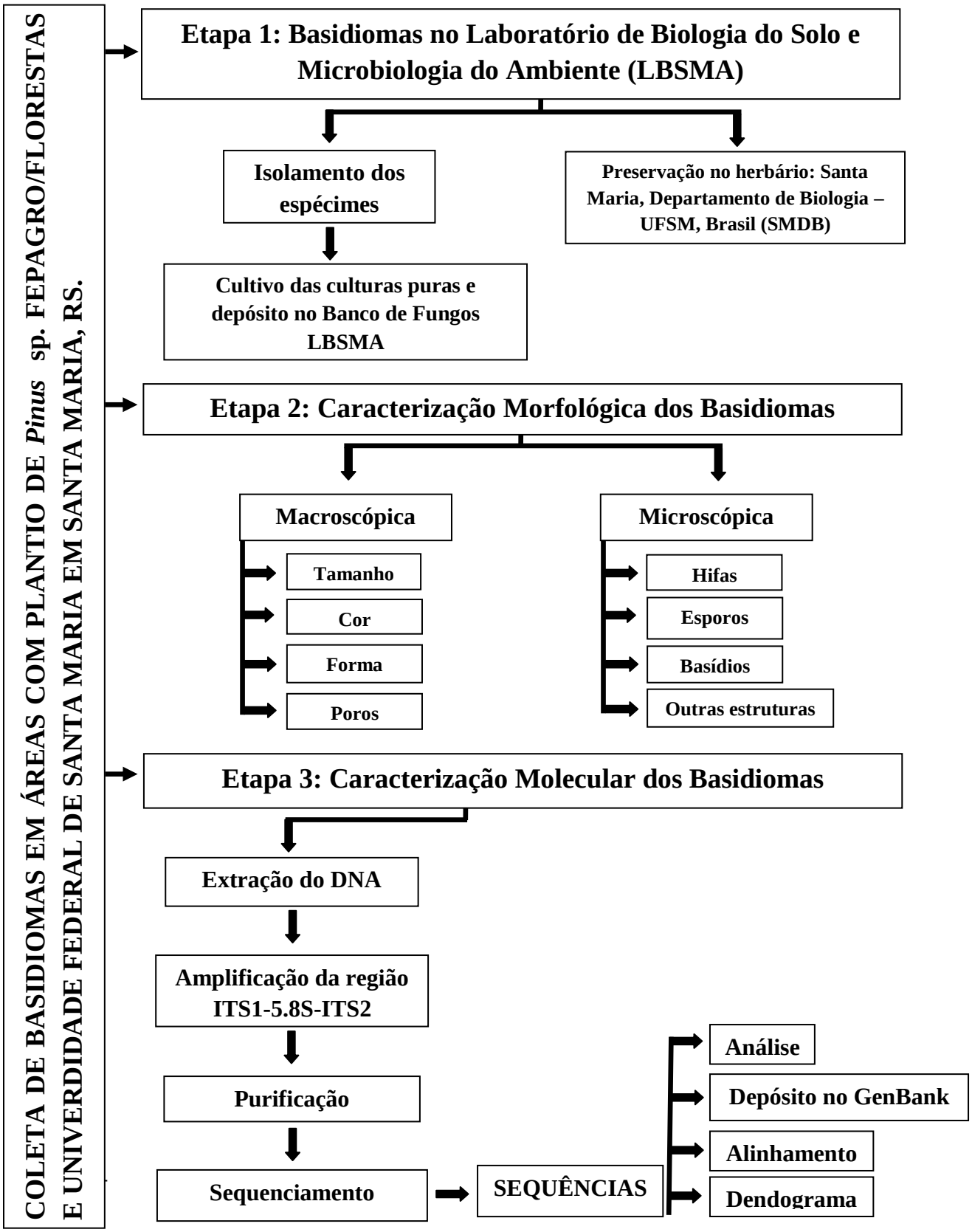

Figura 1 – Esquema do trabalho realizado com os basidiomas coletados na área de plantio de *Pinus* sp. da FEPAGRO/Florestas em Santa Maria, RS.

Brown rotting fungus closely related to *Pseudomerulius curtisii* (Boletales) recorded for the first time in South America.

Baldoni DB[1*], Coelho G[2], Jacques RJS[3], Silveira RMB[4], Grebenck T[5] and Antoniolli ZI[6]

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Abstract
In the region of Santa Maria, Southern Brazil, we have analyzed morphologically and molecularly some interesting brown-rotting mushroom specimens closely related to *Pseudomerulius curtisii*. Except for minor differences in morphology and ITS sequence similarity, collections have corresponded to *P. curtisii* by basidiospore size and shape, the kind of hyphal system, the macromorphology, the slightly unpleasant pungent spicy smell turning stronger upon drying, and, particularly, by the highly supported and closely related clade after phylogenetic analysis. Perhaps due the rarity in nature, morphological data are not abundant in literature and appears to be somewhat incomplete to discordant for the species, so we provide a more detailed description and illustrations from collected specimens.

Key words – Basidiomycetes – brown-rot – ITS region – *Pinus elliottii* – saprophytic fungi – Tapinellineae.

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Introduction

Forest environments require a complex set of organisms ensuring decomposition of available organic substrates, where fungi play an important decomposition and nutrient translocation role (Watkinson et al. 2006). Basidiomycota are the most important woody biomass decomposers. White-rot fungi have high potential for enzyme degradation of lignin and cellulose while brown-rot fungi attack preferentially cellulose compounds. Both groups were considered as important organisms in recycling carbon of coniferous wood and also the main cause of decay in wooden structures (Gilbertson & Ryvarden 1986, Zabel & Morrell 1992, Wei et al. 2010). Brown-rot fungi were estimated as being about 6% of wood-decaying fungi with 70% members belonging to Polyporaceae *s. l.*, and 85 % associated with gymnosperm hosts (Gilbertson 1981, Nakasone 1993).

The brown wood rot fungus *Pseudomerulius* has been positioned into the order Boletales Gilbert, tribe Tapinellineae Agerer, and family Tapinellaceae Ch. Hahn based on

recent molecular studies (Binder & Hibbett 2004, Binder et al. 2010, Skrede et al. 2011). The genus *Pseudomerulius* was published by Jülich in 1979 comprising two species - *P. aureus* (Fr.) Jülich and *P. elliottii* (Massee) Jülich (Jülich 1979) and a third species, *P. curtisii* (Berk.) Redhead & Ginns, was later included (Redhead & Ginns 1985). These species are closely related to *Leucogyrophana* and *Serpula*, but differed from both genera in having much smaller and narrower spores and basidia. *P. curtisii* is difficult to miss due to its vivid golden-yellow colour. The geographic distribution ranges from North and Central Americas - USA, Canada, Hawaii, Mexico, Dominican Republic (Ginns & Lefebvre 1993, Gilbertson & Hemmes 1997, Mora & Garza 1997); East Asia - Korea, Japan, Thailand (Takahashi et al. 2005, Quang et al. 2006, Chandrasrikul et al. 2011); Oceania - Australia and Tasmania (Ginns 1971, Fuhrer 2005, Ratkowsky & Gates 2005). Previous phylogenetic analyses indicated its close relation to *Pseudomerulius aureus* (Fr.) Jülich, *Tapinella panuoides* (Batsch) E. - J. Gilbert and *Bondarcevomyces taxi* (Bondartsev) Parmasto (Larsson et al. 2004, Binder & Hibbett 2006) although the low number of available sequences did not enable any intraspecific variation study up to date.

In general, wood-decaying fungi have great potential in biotechnology, industry, and pharmaceutical uses. Species of *Pseudomerulius* are known to have medicinal properties such as producing a series of p-terphenyl derivatives that act as free-radical scavengers (Quang et al. 2006, Zhou et al. 2010). In addition, extracts can protect cultured neuronal cells against glutamate neurotoxicity, oxidative damage of supercoiled DNA or act as general antioxidant (Lee et al. 2003, Liu 2006, Zhou et al. 2010).

Pseudomerulius curtisii is unreported from South America. However, basidiomes suspected to be this species were recently found in Southern Brazil. The present study aims to elucidate the taxonomic position of the Brazilian material from a morphological study and from molecular data.

Methods

Fungal material collections and morphological characterization

Basidiomes with poroid or similar hymenophores (merulioid, irpicoid or poroid-raduloid) have been regularly collected in years 2007 - 2012 from native and cultivated ornamental/experimental areas with *Pinus* spp. or *Eucalyptus* spp. in the municipality of Santa Maria, central region of Rio Grande do Sul State, Southern Brazil. Main collection sites were the cultivated areas at FEPAGRO/Florestas - Fundação Estadual de Pesquisa Agropecuária and Campus of UFSM (Federal University of Santa Maria) following other local Basidiomycota studies (Coelho et al. 2006, Andreazza et al. 2008, Sulzbacher et al. 2010).

Each collection was briefly described in situ, photographed, and carefully collected in plastic pots or absorbent paper for preservation, isolation, and laboratory analysis. The type of wood rot was inferred from the substrate. Diploid mycelium was isolated from sporocarps and cultivated according to Brundrett et al. (1996) and is stored in the Bank of Fungi of Prof. Marcos Rubens Fries Soil Biology and Environmental Microbiology Laboratory, CCR/UFSM; under the voucher numbers given in Table 1. Preserved specimens are deposited at ICN (Universidade Federal do Rio Grande do Sul, Brazil) and SMDB (Universidade Federal de Santa Maria, Brazil) Herbaria. Morphological features were assessed from fresh basidiomes through handmade sections, under the stereoscopic microscope (up to 40x magnification) or Hund H500 microscope (magnification up to 1000x). A Munsell Soil Color Chart (1994) was used as reference to the colour names. KOH 5% plus aqueous phloxine or drops of Melzer's reagent were used for liquid mounting media and chemical tests. Measurements were statistically analyzed using EXCEL® (Microsoft Office® 2003) and abbreviations presented follow Coelho (2005): $D_{(m)}$= diameter (average); $L_m \times W_m$= av. Length

× av. width ± standard degree; *Q*= quotient of length/width; Q_m= av. quot.; *Qr*= quot. range; *n/n* = n measurements from/n basidiomes. Basidiospore shapes are classified based on the *Q* range intervals of Stalpers (2007).

Molecular analysis

DNA was extracted from parts of the basidiomes using a DNeasy Plant Mini Kit (Qiagen, São Paulo, Brazil). The complete ITS region in nrDNA (ITS1-5.8S-ITS2) was amplified with primers ITS1 and ITS4 (White et al. 1990). Reaction were adapted for optimal amplification as follows: initial denaturation cycle at 94 °C for 2 min, 40 cycles of denaturation at 94 °C for 1 min., annealing at 50 °C for 1 min., and extension at 72 °C for 1 min. and 30 s., plus one final extension cycle at 72 °C for 7 min. Reactions were performed in a total volume of 25 µL, with the components: 10 ng of template DNA, 1 µmol of each primer, 20 mM Tris-HCl (pH 8.4) and 50 mM reaction buffer, 2 mM $MgCl_2$, 0,2 mM dNTP, 2,5 unit of Taq DNA polymerase (Invitrogen, São Paulo, Brazil) and H_2O miliqui.

After the amplification, electrophoresis was performed to check the amplification in 1.5% agarose gel and 1X TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.0). DNA was stained with Blue green Loading Dye I® (LGC Biotecnologia, Cotia, Brazil) and observed in ultraviolet light. PCR products were purified with the Gen Elute PCR clean-up Kit (Sigma, Saint Louis, USA) following manufacturer's instructions and sequencing was carried out in Mega BACE sequencer 500 (Amersham Biosciences).

Sequenced fragments were analyzed using the program Staden Package 2.0.0b (Staden et al. 2003). Sequences were deposited in a public nucleotide database (GenBank) under the accession numbers given in Table 1.

Selected closely related sequences for phylogenetic relationship analysis from the Boletales were retrieved from the GenBank database (Table 1) on 11 March 2012. Sequences were aligned with MAFFT 6.0 program (Katoh et al. 2002) using L-ins-i algorithm.

A GTR+I nucleotide substitution model was used after the ModelTest (Posada 2006) run. The Maximum Likelihood (ML) method analyses were performed using PhyML 2.45 program (Guindon & Gascuel 2003) with 1000 bootstraps, I and G invariants were estimated. Tree was drawn and modified in Mega 5.0 (Tamura et al. 2011).

The Neighbor Joining (NJ) and Maximum Parsimony (MP) (established with the same model used to construct the ML tree) methods analyses were performed in MEGA 5.0 (Tamura et al. 2011) and 1000 bootstrap replicates were used in all reconstructions. Sequences from *Athelia arachnoidea* (GU187504), *A. epiphylla* (GU187501), *Jaapia argillacea* (GU187524) were used as outgroup.

Table 1 Specimens of fungi included in this study. In bold the accession Genbank number referred to the sequences obtained from *Pseudomerulius curtisii*, Santa Maria, RS, Brazil.

Species	**Strain**	**Locality**	**GenBank accession number**
Athelia arachnoidea (Berk.) Jülich	CBS: 418.72	Netherlands	GU187504
Athelia epiphylla Pers.	CFMR: FP-100564	USA	GU187501
Bondarcevomyces taxi (Bondartsev) Parmasto	Dai2524	China	DQ534575
Coniophora arida (Fr.) P. Karst.	CFMR: FP-104367	USA	GU187510
Coniophora cerebella (Pers.) Pers.	8	USA	GU187513
Coniophora marmorata Desm.	P 307	United Kingdom	AJ518880
Coniophora marmorata Desm.	MUCL: 31667	Belgium	GU187515

Coniophora olivacea (Fr.) P. Karst.	MD-264	USA	AM747534
Coniophora prasinoides (Bourdot & Galzin) Bourdot & Galzin	MA-Fungi 19417	USA	AJ419197
Coniophora puteana (Schumach.) P. Karst.	MUCL:1000	Germany	GU187521
Jaapia argillacea Bres.	CBS:252.74	Netherlands	GU187524
Leucogyrophana arizonica Ginns	CFMR:RLG-9902	USA	GU187527
Leucogyrophana mollusca (Fr.) Pouzar	CFMR:L-10277	USA	GU187525
Leucogyrophana mollusca (Fr.) Pouzar	P 263 (14167)	Sweden	AJ419914
Leucogyrophana mollusca (Fr.) Pouzar	P 265 (G 201)	-	AJ419915
Leucogyrophana olivascens (Berk. & M.A. Curtis) Ginns & Weresub	CFMR:HHB-11134	USA	GU187532
Leucogyrophana pinastri (Fr.) Ginns & Weresub	MA-Fungi 7924	Spain	AJ419214
Leucogyrophana pinastri (Fr.) Ginns & Weresub	P 273 (G 117)	Germany	AJ419916
Leucogyrophana pinastri (Fr.) Ginns & Weresub	P 275 (G 202a)	-	AJ419917
Leucogyrophana romellii Ginns	DAOM 148653	USA	GU187530
Leucogyrophana romellii Ginns	CFMR:T-547	Canada	GU187529
Pseudomerulius aureus (Fr.) Jülich	CFMR:FP-103859	USA	GU187534
Pseudomerulius curtisii (Berk.) Redhead & Ginns	REH8912	Australia	GU187533
Pseudomerulius curtisii (Berk.) Redhead & Ginns	DJL-DR-4	Dominican Republic	GU187536
Pseudomerulius curtisii (Berk.) Redhead & Ginns	**DBB1**	**Brazil**	**JN974314**
Pseudomerulius curtisii (Berk.) Redhead & Ginns	**DBB34**	**Brazil**	**JX157585**
Serpula lacrymans (Wulfen) P. Karst.	REG 383	-	GU187542
Serpula himantioides (Fr.) P. Karst.	CFMR: RLG-12941	USA	GU187547
Tapinella atrotomentosa (Batsch) Šutara	122/98	USA	GU187550
Tapinella atrotomentosa (Batsch) Šutara	78/97	USA	GU187549
Tapinella panuoides (Fr.) E.-J. Gilbert	JLM 1752	USA	GU187551
Tapinella panuoides (Fr.) E.-J. Gilbert	MB05-019	USA	GU187548

Results

We collected seven samples belonging to the genus *Pseudomerulius*. All collections showed high morphological similarity with *Pseudomerulius curtisii* as described in 1985.

Summary description of morphological characters of *Pseudomerulius curtisii* related collections from Brazil:

(Figs. 1-2)

Basidiome annual, effuse-reflexed to pileate, gregarious, flexible, fleshy, watery, breakable, firm upon drying, up to 65 × 52 × 12 mm, with an unpleasant, pungent and spicy smell resembling pet food or a strong odor of cinnamon that intensify after desiccation. **Pileus** dimidiate, flabelliform to spatulate, fleshy; pilear surface yellow (8/6-7/8 2.5Y), with shades of olive brown (4/3–4/4 2.5Y) when bruised; pileus surface cottony to felty, wavy; margin indistinct, rounded, lobed. **Hymenophore** irregularly lamellate to almost merulioid, formed by strongly corrugated gills, waxy, vivid coloured, yellow (8/6-7/8 10YR) to brownish yellow (6/6-6/8 10YR) to dark olive brown (3/3 2.5Y) when bruised; folds as corrugated or irregular lamellae, radially oriented, occasionally forked, more straight and shallow next to the margin,

soon becoming irregular to wavy and deeper at maturity, thicker next to the substrate, thinning
g towards the margin, (0.5–)1(–2)/mm in width, P_m= 1.21, *n*= 61/1; dissepiments thick, glabrous, smooth; margin yellow (8/6-7/8 10YR), forming a sterile growing zone, felty, slightly incurved on hymenophore, fragile, easily bruised. **Fold layer** concolorous to the hymenophore, up to 3 mm thick. **Context** yellow (8/6-8/8 2.5Y), paler than the folds, thick, up to 10 mm thick, homogeneous, fleshy, easily to macerate, with a slightly darker cortex formed by the felty, pilear surface.

Fig. 1 – Basidiomes of *Pseudomerulius curtisii* related specimens on decayed wood of *Pinus* sp. **A** Pileus, upper view (DBB 1 SMDB 13.701), Scale bar = 2 cm. **B** Pileus, lower view showing folded lamellae (ICN 139784), bar = 0,5 cm. This picture is copyright of Gilberto Coelho.

Hyphal system monomitic. **Tramal generative hyphae** clamped, whitish opaque, thin to usually thick-walled, with a narrow, sinuous, and discontinuous lumen, often branched, sinuous in outline, swelling in KOH, (1.8-)2.8-6.8(-8.4) µm diam., D_m= 4.2, *n*= 62/1. **Contextual generative hyphae** clamped, whitish opaque, thick-walled, sinuous in outline, almost solid, with a narrow, sinuous, and with a discontinuous lumen, sometimes very enlarged, often branched, (4-)4.4-10.8(-12) µm diam., D_m= 6.8, *n* = 62/1, narrowing and elongating to form a felty pileus surface.

Hymenophore with **basidia** clavate, four-sterigmate, (12.8–)14.4-17.6(-20) × (3.2-)3.6-4(-4.4) µm, L_m × W_m= 16.1 ± 1.91 × 3.67 ± 0.35, Q_r= 2.91-6.00, Q_m= 4.40 ± 0.62, n= 61/1. **Basidiospores** ellipsoid, narrowly ellipsoid to subcylindrical, abundant, moderately thick-walled, indextrinoid, often guttulate, 3.2-4(-4.4) × 1.6-1.8(-2) µm, L_m × W_m= 3.6 ± 0.38 × 1.79 ± 0.11, Q_r= 1.74-2.50, Q_m= 2.04 ± 0.20, n= 60/1; **hyphidia and cystidia** not seen.

Associated wood rot: brown.

Known geographic distribution: From North and Central Americas to East Asia, and Oceania, (Fig. 3).

Substrate: growing on fallen decayed wood of *P. elliottii* and other *Pinus* spp. over forest soil; more common in autumn, sometimes in wet summer; rare in occurrence in the study area.

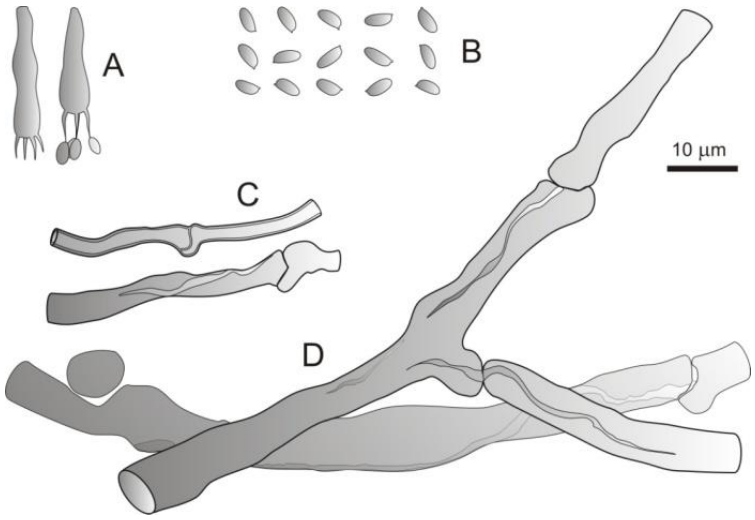

Fig. 2 – Microscopical structures of *Pseudomerulius* collections from Brazil. **A** Basidia. **B** Ellipsoid basidiospores. **C** Tramal generative hyphae presenting thin to thickened walls with irregular lumen. **D** Contextual generative hyphae (ICN 139783).

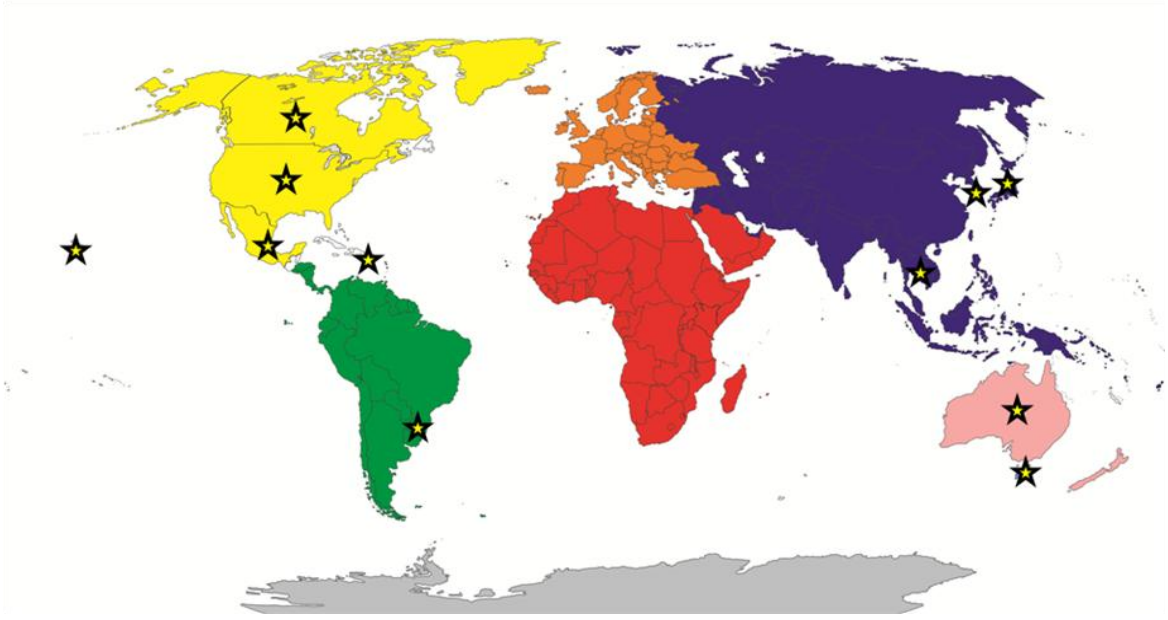

Fig. 3 – Geographical distribution of *Pseudomerulius curtisii*. The stars mark the occurrence of the species on continents, according to a literature review.

Specimens examined – Brazil, Rio Grande do Sul, Santa Maria, Campus/UFSM, on *Pinus elliottii*, 17 Dec 2007, leg. G. Coelho, **NºGC 653-4 (ICN 139783)**; on *Pinus* sp., 21 Dec 2007, **NºGC 654-3 (ICN 139784)**; on *P. elliottii*, 15 Jan 2008, **NºGC 662-2 (ICN 139785)**; on *P. elliottii* 19 Jan 2008, **NºGC 663-2 (ICN 139786)**; on *Pinus* sp., 29 Jan 2008, **NºGC 665-1 (ICN 139787)**; FEPAGRO, on *Pinus* sp., 03 Jan 11, leg. G. Coelho and D.B. Baldoni, **N°DBB 1 (SMDB 13.701);** on UFSM, *Pinus* sp., leg. G. Coelho and D.B. Baldoni, 05 Jan 2012, **N° DBB 34 (SMDB 13.702)**.

The NJ, MP and ML analyses of the same sequence dataset yielded similar phylogenetic trees (Fig. 4). The analyzed sequences from Brazil formed a well-supported uniform clade, closely related to *P. curtisii* (GU187533), from Australia).

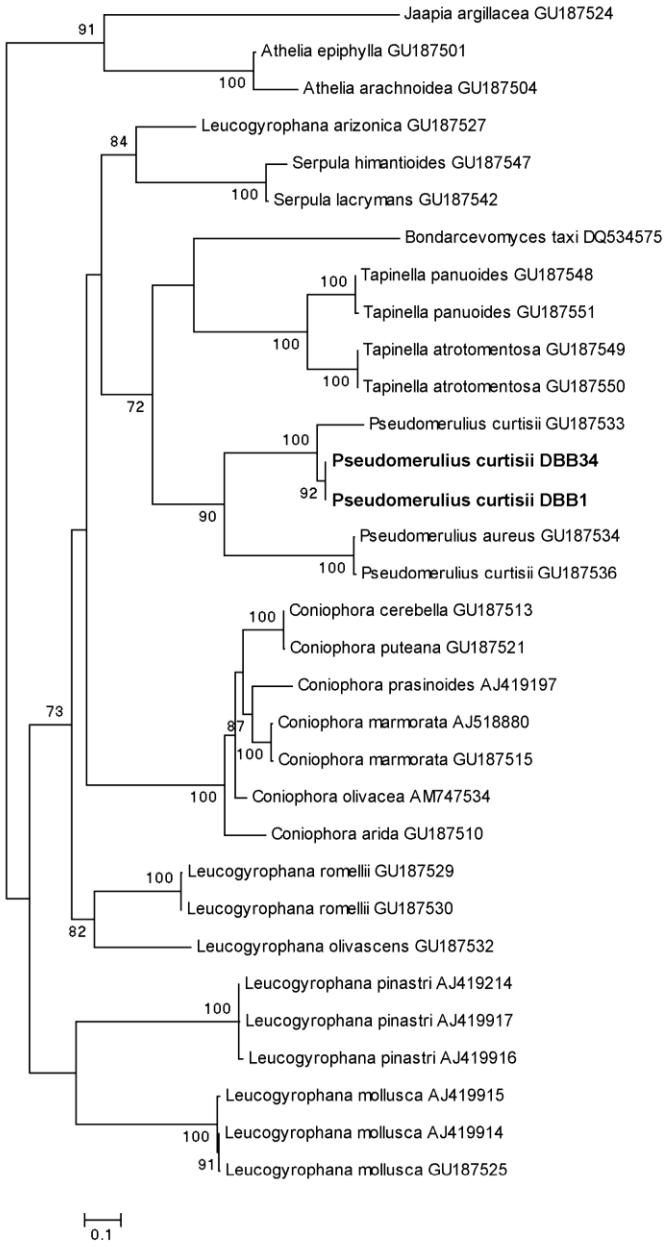

Fig. 4 – Phylogenetic reconstruction of the *Pseudomerulius curtisii* obtained from ITS1-5.8S-ITS2 sequences. Bootstrap values (in %) are from maximum likelihood (ML) analyses (1000 bootstraps). Only topologies with bootstrap values of at least 50% are shown. Sequences from *Athelia arachnoidea*, *A. epiphylla* and *Jaapia argillacea* were used as outgroup.

Discussion

As shown by Binder et al. (2010) *Pseudomerulius* is in family Tapinellineae with Coniophoraceae as the sister clade and Serpulaceae, represented in our tree by *Serpula lacrymans* and *S. himantioides*, as a basal group. We have confirmed the close relation of *Pseudomerulius* with *Leucogyrophana*. The latter genus appeared polyphyletic.

The *Pseudomerulius* collections from Brazil showed very similar morphology when compared with the original description of *P. curtisii* and those of its synonyms in literature, but with some minor differences depending on the author, in particular: the thickness of the basidiospore and hyphae walls, hyphae diameter, and the presence of slightly unpleasant pungent spicy smell turning stronger upon drying. Our mature fresh collections were microscopically mainly built by a monomitic hyphal system with clamped and almost solid hyphae usually having a sinuous lumen; they were also characterized by producing hyaline, indextrinoid, and narrowly-ellipsoid to subcylindrical basidiospores. The macromorphological features including yellow- lemon to golden yellow vivid colours in cartilaginous, pileate basidiomes and the strong radially folded hymenophores (lamellar-corrugated to merulioid type) were shared, making *P. curtisii* and our collections readily recognizable in nature and very attractive. Other macromorphologically related species can be separated from our collections and original *P. curtisii*, namely: *P. aureus* (Fr.) Jülich, by presenting effuse to a few reflexed basidiomes and hyphae relatively narrower, even also presenting merulioid hymenial surface (Ginns 1998), and *Tapinella panuoides*, by having paler (whitish to brownish yellow) true lamelar basidiomes, yet reflexed to pileate (Gilbertson 1981). Bessete et al. (2000) reported *P. curtisii* under the nomenclatural synonym *Meiorganum curtisii* (Berk.) Singer, Garcia & Gomez among the North American boletes as having dextrinoid basidiospores. Presence of dextrinoid reaction of basidiospores was not confirmed in our analysis of fresh mature specimens and that of some other authors under the same name (Gilbertson & Hemmes 1997, Gilbertson et al. 2002) or under different names (Ginns 1969, as *Merulius crassus*).

Molecular analysis of species within *Pseudomerulius* separated well the analyzed collections from Brazil. A well supported clade and only 92% similarity with the Australian *P. curtisii* sample (GU187533) may indicate the presence of a cryptic species or highly separated geographic variety existing in South America (Brazil). The collection of *Pseudomerulius curtisii* GU187536 clustering in *P. aureus* clade may represent a misidentification.

Pseudomerulius curtisii, to which the Brazilian collections fit best, usually grows hidden at the base or along the sides of decayed logs in the study area. Perhaps due this fact, it has been considered as rare in the world, even though obvious in appearance (with bright yellow basidiomes and minutely rugose gills) and strikingly in its odor of cinnamon (Lee et al. 2009). The species was previously unknown in South America and our analysis indicate a close relationship of Brazilian collections with *P. curtisii*, yet with some minor differences, which would need further detailed analysis and may potentially result in a new, yet undescribed taxon from this continent.

Based on literature and internet reviews, we have found a scarcity and slowing increasing of biogeographical, molecular, and biochemical data about the species in focus; the present study represents a contribution to the better knowledge of the species and to local future studies on soil formation and decomposition by wood-decomposing agents of fungal diversity in cultivated and native forests ecosystems.

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Gelatoporia subvermispora, a rare soil-forming fungi growing on decayed Pine wood in Southern Brazil

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Abstract

Fungi, in especial Basidiomycota, are important carbon recyclers and humic agents in forest ecosystems. It was analyzed morphologically and molecularly a polypore collected in cultivated pine forests in Southern Brazil. The species is known for selective lignin degradation prior to cellulose being industrially useful for biopulping and other enzymatic processes. Based on nrDNA ITS region, Brazilian specimen has shown to be conspecific with *Gelatoporia subvermispora* specimens cladding together with a strong bootstrap value; higher BLAST similarity was presented in relation to North American specimens and somewhat lower to Eurasian specimens. Even presenting a wide geographic distribution (Nordical-Australian), the species could be evolving into distinct taxa. The species is reported for the first time in South America.

Keywords: Basidiomycota; selective degradation; *Pinus* sp.; white-rot-fungi.

Introduction

Wood consists of cellulose, hemicellulose and lignin as the major components; due to these biopolymers, wood and other lignocellulosic resources represents the major organic carbon source and the most renewable resource for energy and human activities (Tanaka et al. 2009; Dashtban et al. 2010). Biodegradation of these materials are mainly executed by fungal organisms, Basidiomycota in special; among the latter, brown rot fungi are able to degrade only carbohydrates leaving brown-colored lignin residue mostly intact, whilst white-rot fungi usually degrade lignin and carbohydrates with efficiency (Mendonça et al. 2008; Lundell 2010). This simultaneous degradation of all wood polymers is the main type of white-rot decay. However, the selective degradation of lignin represents a secondary and interesting

type by its potential economical uses, in which lignin or phenolic compounds must be altered or removed (Otjen and Blanchette 1987; Blanchette et al. 1988; Kirk and Cullen 1998). In this much rarer process, fungal enzymes remove lignin and non-cellulosic polysaccharides without extensive degradation of cellulose (Agosin et al. 1990; Ferraz et al. 2003).

Gelatoporia subvermispora exhibits a substantial ability for selective removal of a large fraction of lignin without attacking the valuable cellulose (Messner and Srebotnik 1994; Fackler 2007). By producing ceriporic acids, it strongly inhibits the Fenton reaction to suppress the formation of OH, which the low molecular mass oxidant are able to erodes wood cell walls enhancing the accessibility of fungal extracellular enzymes (Teranishi et al. 2003; Schimidt 2006). The seletive degrading capability becomes it the best among several hundred species of fungi examinated for biopulping, so generating a great economical interest and extensive study (Ferraz et al. 2008).

Originally described as *Poria subvermispora* by Pilát in 1940, the species was subsequently rearranged under several genera; but notably recombined as *Ceriporiopsis subvermispora* (Pilát) Gilbertson and Ryvarden (1985), it has been mostly referred so in taxonomical to biodegrading studies. Also in 1985, Niemelä proposed the new genus *Gelatoporia* for accommodating *G. pannocincta* (Romell) Niemelä and *G. subvermispora* (Pilát) Niemelä, which remained underused until recently. *Gloeoporus pannocinctus* (Romell) J. Erikss. differs from *G. subvermispora* by presenting tetrapolar sexuality and heterocytic nuclear behaviour in opposition to bipolar sexuality (nuclear behavior unknown) from the latter (Rajchenberg 2011).

Ceriporiopsis subvermispora was placed in the family Meruliaceae based on morphological characters (Nakasone and Burdsall 1984; Burdsall 1998; Kirk et al. 2008; Moreno et al. 2011). Through the study of its evolutionary relationships, the species was formerly referred to phlebioid clade in Polyporales (Binder et al. 2005), however subsequent phylogenetic analysis have shown that it appeared well-supported as a separate monotypic genus; then reappraised under *Gelatoporia* since *Ceriporiopsis* was shown to be markedly polyphyletic (Tomsovsky et al. 2010; Miettinen and Larson 2011). Today, the genus are found to form a monophyletic clade called *Cinereomyces* with uncertain position in Polyporales along with other genera, namely: *Obba*, *Sebipora* and *Cinereomyces* (Miettinen and Rajchenberg 2012).

This soil-forming Basidiomycota species is widely recognized as industrially important mainly for biopulping and bioremediation, but potentially for other enzymatic processes. The aims of this study are to present taxonomical and molecular data about a rare

fungi growing on decayed wood of cultivated *Pinus* spp. and to extend its distribution up to Southern Brazil - hitherto restricts to North America and Eurasia.

Materials and Methods

Fungal material collections and morphological characterization

Basidiomes were collected in cultivated *Pinus* sp. forest of FEPAGRO/Florestas (Fundação Estadual de Pesquisa Agropecuária) in Santa Maria, Rio Grande do Sul State, Brazil. In site each collection was briefly described, photographed, carefully collected in plastic pots or absorbent paper for preservation, isolation and laboratorial analysis. The type of wood rot was inferred from the substrate. Diploid mycelium was isolated from sporocarps and cultivated according to Brundrett et al. (1996) and is stored in the Bank of Fungi of Prof. Marcos Rubens Fries Soil Biology and Environmental Microbiology Laboratory, CCR/UFSM; under the voucher numbers given in Table 1. Preserved specimens and culture collections are deposited at ICN (Universidade Federal do Rio Grande do Sul, Brasil) and SMDB (Universidade Federal de Santa Maria, Brasil) Herbaria. Herbarium abbreviations are according to (http://sweetgum.nybg.org/ih/).

Morphological features were assessed from fresh basidiomes through handmade sections, under the stereoscopic microscope (up to 40x magnification) or Hund H500 microscope (magnification up to 1000x). A Munsell Soil Color Chart (1994) was used as reference to the color names. KOH 5% plus aqueous phloxine or drops of Melzer's reagent were used for chemical tests. Measurements were statistically analyzed using EXCEL® (Microsoft Office® 2003) and abbreviations presented follow Coelho (2005): $D_{(m)}$= diameter (average); $L_m \times W_m$= av. Length × av. width ± standard degree; Q= quotient of length/width; Q_m= av. quot.; Qr= quot. range; n/n = n measurements from/n basidiomes. Basidiospore shapes are classified based on the Q range intervals conform Stalpers (2007).

Fungal names authorities follow the original description or the IndexFungorum (Kirk et al. 2008). Wood substrates species are abbreviated, as follows: *P. elliottii* Engelm. (*Pe*); unidentifyed *Pinus* sp. (*Psp*).

Molecular analysis

DNA was extracted from parts of the basidiomes using a DNeasy Plant Mini Kit (Qiagen®, São Paulo, Brazil). The complete ITS region in nrDNA (ITS1-5.8S-ITS2) was amplified with primers ITS1 and ITS4 (White et al. 1990). Reaction were adapted for optimal amplification as follows: initial denaturation cycle at 94 °C for 2 min, 40 cycles of denaturation at 94 °C for 1 min., annealing at 50 °C for 1 min., and extension at 72 °C for 1 min. and 30 s., plus one final extension cycle at 72 °C for 7 min. Reactions were performed in a total volume of 25 µL, with the components: 10 ng of template DNA, 1 µmol of each primer, 20 mM Tris-HCl (pH 8.4) and 50 mM reaction buffer, 2 mM $MgCl_2$, 0,2 mM dNTP, 2,5 unit of Taq DNA polymerase (Invitrogen®, São Paulo, Brazil) and H_2O miliqui.

After the amplification, electrophoresis was performed to check the amplification in 1.5% agarose gel and 1X TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.0). DNA was stained with Blue green Loading Dye I® (LGC Biotecnologia, Cotia, Brazil) and observed in ultraviolet light. PCR products were purified with the Gen Elute PCR clean-up Kit (Sigma, Saint Louis, USA) following manufacturer's instructions and sequencing was carried out in Mega BACE sequencer 500 (Amersham Biosciences).

Phylogenetic analyses

Sequenced fragments were analyzed using the program Staden Package 2.0.0b (Staden et al. 2003). A BLASTn search of the National Center for Biotechnology Information databases verified that the sequence obtained from *Gelatoporia subvermispora* DBB3 was affiliated to the Meruliaceae. Fungal sequences (ITS1-5.8s-ITS2) obtained in our laboratory were aligned with other sequences from GenBank using ClustalX (Larkin et al. 2007) and in BioEdit (Hall 1999) to obtain a final alignment (Table 1). The sequence were deposited at GenBank (Altschul et al. 1997) under the accession number JX206797.

The phylogeny was reconstructed by ITS1-5.8S-ITS2 analyses. The Tamura-Nei model nucleotide substitution model (Tamura and Nei 1993) was estimated using FindModel run in May, 11, 2012. The Maximum Likelyhood analyse was performed in MEGA 5.0 (Tamura et al. 2011) were 1000 bootstrap replicates were used in all reconstructions. Sequences of *Ceriporia lacerata* (FJ462746) and *Irpex lacteus* (AF163046) were used as outgroups (Lee et al. 2009).

Table 1 Specimens included in this study. In bold the accession Genbank number referred to the sequence obtained from *Gelatoporia subvermispora*

Species	Strain	Locality	GenBank accession number
Ceriporia lacerata N. Maek., Suhara & R. Kondo	T140	China	FJ462746
Ceriporiopsis subvermispora (Pilát) Gilb. & Ryvarden	CBS 347.63	USA	FJ349621
Ceriporiopsis subvermispora (Pilát) Gilb. & Ryvarden	KCTC6891	USA	FJ496695
Ceriporiopsis subvermispora (Pilát) Gilb. & Ryvarden	ATCC 90467	USA	FJ545252
Ceriporiopsis subvermispora (Pilát) Gilb. & Ryvarden	FP-90031	USA	FJ713106
Ceriporiopsis subvermispora (Pilát) Gilb. & Ryvarden	BRNU 592909	Czech Republic	FJ496694
Cinereomyces lindbladii (Berk.) Jülich	FBCC 117	Finland	HQ659223
Cinereomyces lindbladii (Berk.) Jülich	M113	Latvia	JF340289
Gelatoporia subvermispora (Pilát) Niemelä	FCL273	Poland	JF837189
Gelatoporia subvermispora (Pilát) Niemelä	H:Heikki Kotiranta 20822	Russia	FN907911
Gelatoporia subvermispora (Pilát) Niemelä	Juha Kinnunen 1052 (H)	Finland	HQ659225
Gelatoporia subvermispora (Pilát) Niemelä	Tuomo Niemela 5978 (H)	Poland	HQ659227
Gelatoporia subvermispora (Pilát) Niemelä	Yu-Cheng Dai 3120 (H)	China	HQ659226
Gelatoporia subvermispora (Pilát) Niemelä	Vjatcheslav Spirin 2156 (H)	Russia	HQ659228
***Gelatoporia subvermispora* (Pilát) Niemelä**	**DBB3**	**Brazil**	**JX206797**
Irpex lacteus (Fr.) Fr.	SFC 971006-12	-	AF163046
Obba valdiviana (Rajchenb.) Miettinen & Rajchenb.	Genevieve Gates FF503 (CIEFAP)	Australia	HQ659235
Obba valdiviana (Rajchenb.) Miettinen & Rajchenb.	Mario Rajchenberg 11043 (CIEFAP)	Argentina	HQ659238
Obba rivulosa (Berk. & M.A. Curtis) Miettinen & Rajchenb.	FBCC 938	Finland	HQ659233
Obba rivulosa (Berk. & M.A. Curtis) Miettinen & Rajchenb.	Reijo Penttila 15077 (H)	Finland	HQ659230
Sebipora aquosa Miettinen	Otto Miettinen 9265	Indonesia	HQ659243
Sebipora aquosa Miettinen	Otto Miettinen 8868	Indonesia	HQ659242
Wrightoporia bracei (Murrill) I. Lindblad & Ryvarden	JV100877	USA	JF692199

Results and Discussion

Gelatoporia subvermispora (Pilát) Niemelä (1985)

Mycotaxon 22(2): 364, 1985.

≡ *Poria subvermispora* Pil., Stud. Bot. Cech. 3: 2, 1940.

Basidiome annual, resupinate, reflexed in some parts, 94 × 72 × 2 mm, thick, fleshy, waxy to cartilaginous, easily to break apart, light in weight, firmer upon drying. **Hymenophore** poroid, moderately fragile, white (8/1 10YR) to very pale brown (8/4 10YR), darkening to very pale brown when bruised (7/3-7/4 10YR), not shinning; pores round, (1–)2–

4(–5)/mm, P_m= 2.97, n= 115/2; dissepiments velutinous, thin, entire; margin indistinct, formed by a flattened and fibrous to cottony mycelium, more or less 1 mm in width. **Tube layer** concolorous to the hymenophore, up to 1.5 mm thick, non-stratified. **Context** thin, less than 1 mm, concolorous to the hymenophore, homogeneous.

Hyphal system monomitic. **Tramal generative hyphae** clamped, hyaline to whitish-opaque, thin- to thick-walled, with an evident lumen to solid, smooth, branched (Fig.1c), (1.6–)2.4–4.4(–5.2) µm, D_m= 3.3, n= 85/2.

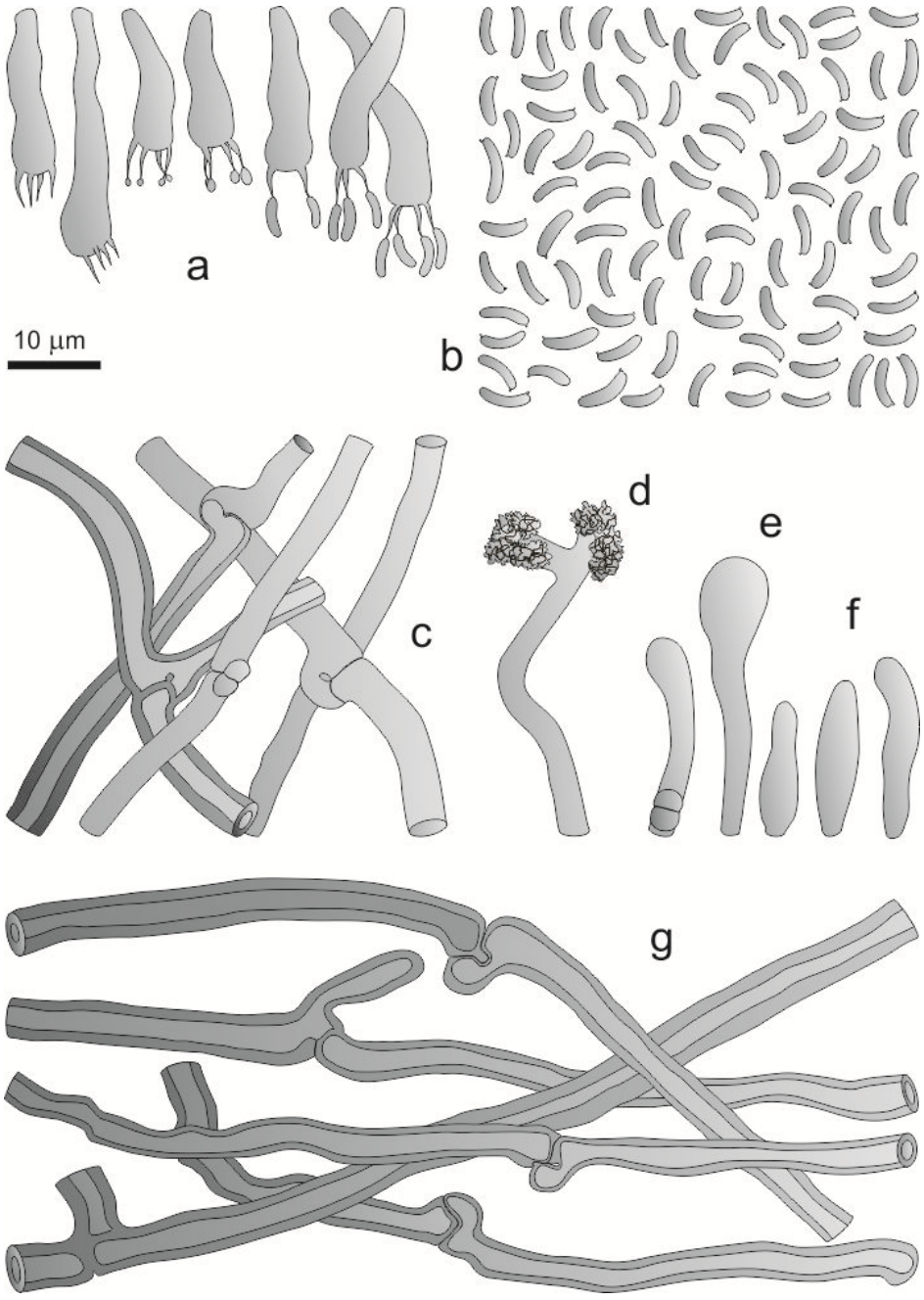

Fig 1 Microscopical characters of *Gelatoporia subvermispora*. **a** Basidia. **b** Basidiospores. **c** Tramal generative hyphae **d** Encrusted hyphae from dissepiments. **e** and **f** Few differentiated Cystidioles. **g** Contextual generative hyphae

Contextual generative hyphae clamped, hyaline to whitish-opaque, thin- to thick-walled, with evident lumen to solid, smooth, branched (Fig. 1g), (2–)2.4–5.2(–6) µm diam., D_m= 3.7, *n*= 85/2, mostly very thick-walled and with a narrow lumen. **Encrusted hyphae** present in dissepiments, with simple to branched apex (lacking septa), with small, crystalline incrustations (Fig. 1d), about 13.2–18.4 x 2.4–3.6 µm, *n*= 12/1. Hyphal pegs present on the tube wall, small, short, formed by cystidia-like hyphae enlarged at the apex.

Hymenium with **basidia** clavate, long, four-sterigmate (Fig. 1a), (12.4–)14.4–22.4(–33.6) × (4–)4.4–5.2(–7.2) µm, $L_m \times W_m$= 17.1 ± 3.33 × 4.70 ± 0.48, Q_r= 2.58–5.69, Q_m= 3.64 ± 0.62, *n*= 88/2. **Basidiospores** allantoid, thin-walled, hyaline, smooth (Fig. 1b), (4.2–)4.4–5.6(–6) × (1–)1.2–1.4(–1.6) µm, $L_m \times W_m$= 5.10 µm ± 0.47 × 1.20 ± 0.13, Q_r= 3.00–6.50, Q_m= 4.31 ± 0.52, *n*= 107/2. **Cystidioles** ventricose, thin-walled, hyaline (Fig. 1e,f), (9.2–)2.8–20(–22.4) × (2.4–)2.8–4(–4.8) µm, $L_m \times W_m$= 14.9 µm ± 2.57 × 3.27 ± 0.55, Q_r= 2.17–7.33, Q_m= 4.66 ± 1.06, *n*= 85/2.

Associated wood rot: white (Fig. 2 a-d).

Substrate: decayed wood of *Pinus elliottii* and *Pinus taeda* (Fig 2 a-d).

Fig 2 a-d Resupinate basidiomes of *Gelatoporia subvermispora* under the same decayed wood of *Pinus taeda*, photographed in different times. **a** and **b** Hymenophore detail of poroid basidiomes, Scale bars = 0,5 and 1 cm. **b** Wood detail presenting a clear fibrous white wood-rot of *G. subvermispora*. Scale bars = 5 cm

Specimens Studied: BRAZIL, Rio Grande do Sul, Santa Maria, Camobi, leg. G. Coelho, 08.XII.2000, Nº GC 266-3 (ICN 139634), *Pe*; 15.XII.200, Nº GC 267-2 (ICN 139635), *Pe*; 18.XII.2000, Nº GC 268-3 (ICN 139636), *Pe;* 29.XI.2001, Nº GC 328-1 (ICN 139637), *Pe*; Nº GC 328-2 (ICN 139638), FEPAGRO, on *Pe* 03.I.2011, leg. G. Coelho and D.B. Baldoni, **N°DBB3 (SMDB...)**.

Distribution: in Europe, it was firstly known as an eastern species, found in Finland, Poland, Czechoslovakia and Yugoslavia Niemelä (1985); also reported to Central Europe and North America, United States and Southern part of Canada (Gilbertson and Ryvarden 1986; Ryvarden and Gilbertson 1993); in East Asia, it was known from China, far East Russia, Korea and Indonesia; after, assumed as a temperate species in the Northern Hemisphere - but a rare species everywhere (Lee et al. 2009; Nuñez et al. 2001; Núnez and Ryvarden 2001; Miettinen and Rajchenberg 2012), (Fig 3).

Sexuality and Cultural characters: heterothallic bipolar; see Nakasone (1981) and Stalpers (1978).

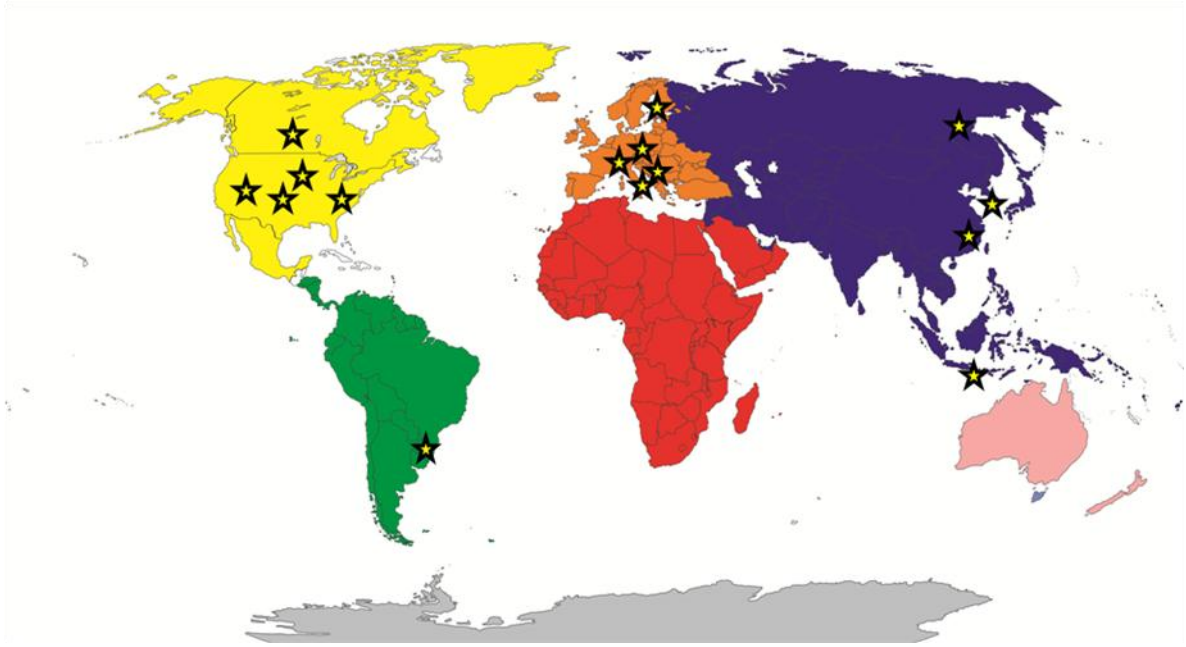

Fig 3 – Geographical distribution of *Gelatoporia subvermispora*. The stars mark the occurrence of the species on continents, according to a literature review.

Remarks: *Gelatoporia subvermispora* presents white, resupinate and somewhat cartilaginous basidiomes, middle-sized pores (1–5/mm), large allantoid basidiospores (4–6 × 1–1.5 µm), and encrusted hyphae at the pore mouths - it preferentially decays coniferous substrata in the study area. ***Sebipora lowei*** (Rajchenb.) firstly described in *Ceriporiopsis* and collect in the study area, is also a white-colored, ressupinate and aggressive white-rot causing

species; it differs from *Gelatoporia* by its smaller allantoid spores (2.5–4.5 × 0.8–1.2 µm), capitate cystidia in the trama, hymenial incrusted cystidioles, and smaller pores (6–8/mm); besides growing usually on native woods, rarely on *Pinus*. ***Ceriporiopsis pannocincta*** (Rom.) Gilbn. et Ryv. is also distinguished by producing smaller allantoids spores (3.5–4.5 × 0.7–1 µm), and fusiform to almost tubular cystidia (Gilbertson and Ryvarden 1986). ***Ceriporiopsis hyalina*** (Berk.) Quanten has smaller pores, 6–8(–12), and smaller allantoid to short cylindrical spores, 3.7–4 × 0.8–1.2 µm.

A BLASTn search revealed that ITS Brazilian sequence DBB3 was 99.0% similarity to four other *Gelatoporia subvermispora* sequences retrieved from GenBank database. Phylogenetic tree (Fig 4) performed with Maximum Likelihood approach generated a well supported clade with *G. subvermispora* DBB3 nesting among several other conspecific sequences.

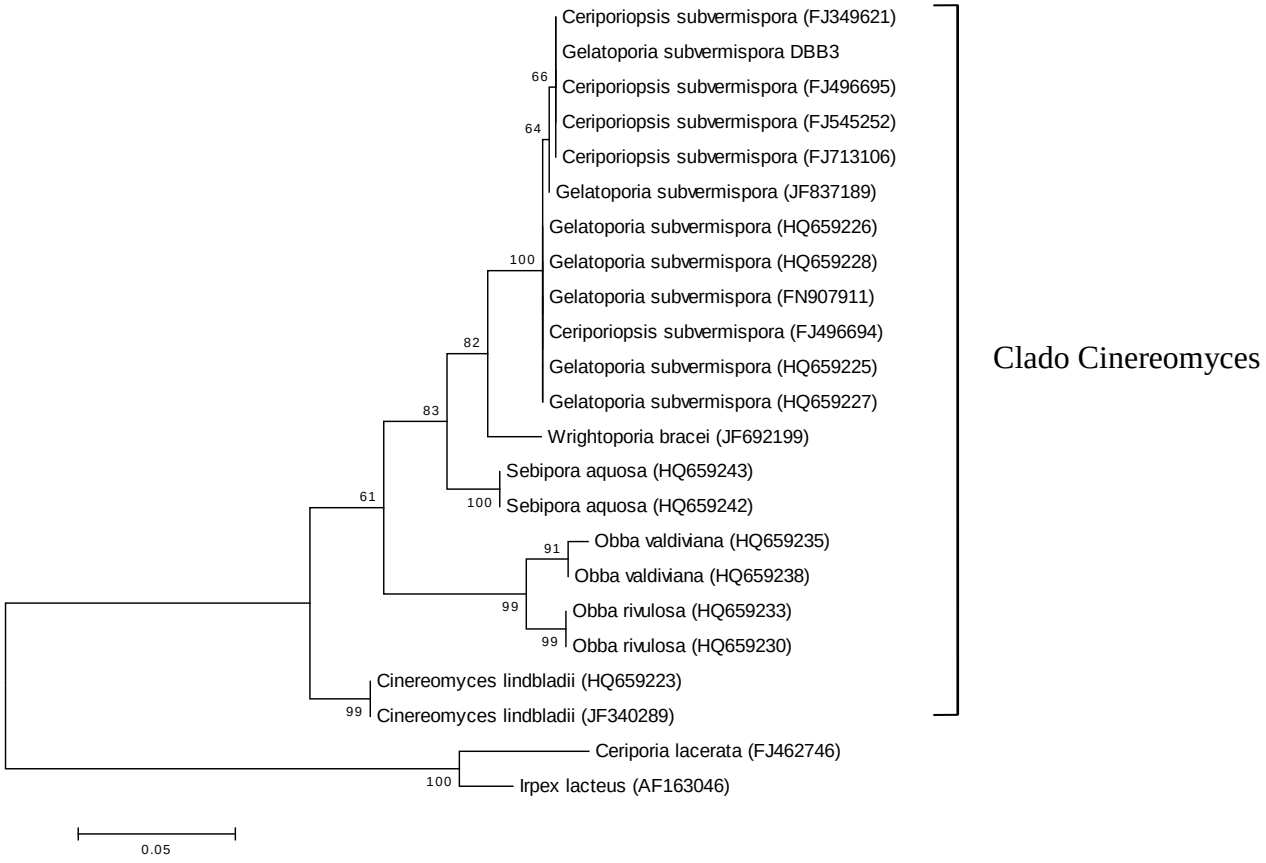

Fig 4 Phylogenetic reconstruction of the *Gelatoporia subvermispora* obtained from ITS1-5.8S-ITS2 sequences. Bootstrap values (in %) are from maximum likelihood (ML) analyses (1000 bootstraps). Only topologies with bootstrap values of at least 50% are shown. Sequences of *Ceriporia lacerata* (FJ462746) and *Irpex lacteus* (AF163046) were used as outgroup

Brazilian specimen presented higher similarity with North American species clading among then; some distinction from the other Eurasian species was supported by a strong boostrap value. Results found by Miettinen and Rajchenberg (2012) in their phylogenetic

analysis using nrDNA ITS region, suggested that geographic distribution involving different continents can cause small divergence between the species in a few base pairs and could be related to the separation in two distinct taxa; such differences being homogeneous among the species from the same continent.

Also in this analysis, we observe the formation of distinct monophyletic clades for the species *Sebipora aquosa* and *Cineromyces lindbladii*. For the genus Obba the source into two separate branches clades, one for *O. valvidiana* and one for *O. rivulosa*. Note the close relationship between these species, but are separated into four groups. These results were formerly found by Miettinen and Rajchenberg (2012) in their clade *Cinereomyces*. Finally, for instance forming an outgroup for the species *Ceriporia lacerata* and *Irpex lacteus*.

Based on morphological and phylogenetic analyses, we have confirmed the identity of DBB3 specimen within the genus *Gelatoporia* with the highest molecular proximity in relation to American specimens. *Gelatoporia subvermispora* has been included in several studies involving biotecnological applications, like: metane production from wood fermentation (Amirta et al. 2006); xenobiotic biodegrading (Krivobok et al. 1998; Mendonça et al. 2008); wood biopulping (Yaghoubi et al. 2008; Okano et al. 2009; Wan and Li 2010; Gulsoy and Eroglu 2011); enzimatic production (Harreither et al. 2009; Magalhães et al. 2006); pre-treatment for etanol producting (Sasaki et al. 2011); and biobleaching (Christov et al. 1998). Even though being subject of several biotechnological studies and great potential industrial interests, this species has a wide geographic distribution, however were not previously recorded from South America.

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Sarcoporia polyspora: a rare wood-degrading Basidiomycota is newly recorded to South America

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Abstract
Brown-rotting fungi and coniferous forests have a possible common and interesting co-evolutionary history particularly in the North Hemisphere. These fungi degrade almost exclusively wood cellulosic materials participating heavily in the carbon cycle and soil formation, although being less diverse than white-rotters, in their ecosystems and exotic forests known as important sources of energy and wood production. Studying wood-inhabiting fungi in cultivated pine forests in Southern Brazil, we have collected and analyzed morphologically and molecularly (ITS region) a rare fungus named *Sarcoporia polyspora*, which has shown to be an aggressive decompositor previously unreported from South America.

Key words – biodegradation – macrofungus – *Pinus* sp. wood – soil humus

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Introduction

Fungi are able to modify their substrates secreting enzymes that act extracellularly and other compounds; they find their niche in forests either in the leaf litter, in soil organic matter (humus) or in wood, as in the case of most Basidiomycota, which attack its primordial compounds – cellulose, hemicelluloses and lignin. Brown rotters degrade almost exclusively cellulosic materials, are not predominant in the group, but present a possible interesting co-evolutionary history particularly within coniferous forests, where they participate heavily in the carbon cycle and soil formation.

Species associated with brown rots were firstly assigned as representing about 7% of American wood-rotting Basidiomycota (120 from 1700 sp), 85% occurring on coniferous substrates, and estimated as being less than 10 % in Europe. The total number of brown-rotting polypores was reported as being 22% (77 from 322) in Europe and 18% (79 from

about 439) in North America, whereas a lower percentage of 14% (98 from 704) was found in China, for instance. In all cases, the majority of brown-rotting polypores were found on gymnosperm wood in boreal forests; except to a higher percentage of 38,7% (25 from 62) reported to Patagonian Andes (Southern Argentina) mostly on *Nothofagus* species, Fagaceae, (Gilbertson 1980, Gilbertson and Ryvarden 1986, Ryvarden & Gilbertson 1993, Rajchenberg 2006, Dai 2011).

Sarcoporia polyspora P. Karsten is an aggressive brown-rotting polypore characterized by producing dextrinoid spores. Currently included in the family Fomitopsidaceae (Kirk et al. 2008), it was originally described in 1894, but after redescribed or recombined under different names, as morphologically discussed by Vlasak and Kout (2010). The species has been most referred under the name *Parmastomyces transmutans* (Overh.) Ryvarden & Gilb. (1984, 1994), especially after Ryvarden (1991), who stated that *Sarcoporia* would be morphologically more similar to *Hapalopilus*, suggesting its synonymy with a polypore genus characterized by lacking dextrinoid reaction in spores and producing white rot.

The species has been considered as common to North America, with several records to Asia, but rare in Europe and North Africa. The aim of this study is to contribute to the knowledge about this rare and potentially useful species providing morphological and molecular insights, besides extending its geographic distributional data to cultivated pine forests in Southern Brazil.

Methods

Fungal material collections and morphological characterization

Basidiomes were collected in cultivated areas with *Pinus* spp. at FEPAGRO/Florestas (Fundação Estadual de Pesquisa Agropecuária) in Santa Maria, Rio Grande do Sul State, Southern Brazil. Specimens were observed, photographed and collected in plastic pots or absorbent paper for transporting to Prof. Marcos Rubens Fries Soil Biology and Environmental Microbiology Laboratory, CCR/UFSM; after they were analyzed and preserved. Specimens are extant at Instituto de Ciência Naturais - UFRGS, Brasil, ICN Herbarium (acronyms are according to: http://sweetgum.nybg.org/ih/). Munsell Soil Color Charts (1994) were used for naming colors. Microscopical analyses were performed through handmade sections of fresh basidiomes using a razor blade and mounting on glass slides with drops of KOH 5% plus aqueous phloxine or drops of Melzer's reagent. Statistical analysis of measurements was performed with EXCEL® (MS Office 2003), its abbreviations are presented conform Coelho (2005). Authors of fungal names follow Kirk & Ansell (2008). Coniferous substrates are abbreviated as: *Pt.* for *P. taeda* L. and *P.sp* for unidentifyed *Pinus* species.

Molecular analysis

DNA was extracted from parts of the basidiomes using a DNeasy Plant Mini Kit® (Qiagen, São Paulo, Brazil). The complete ITS region in nrDNA (ITS1-5.8S-ITS2) was amplified with ITS1 and ITS4 primers (White et al. 1990). Amplification reaction was performed conform Lupatini et al. (2008). After amplification, electrophoresis was performed to check the amplification in 1.5% agarose gel and 1X TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.0). DNA was stained with BlueGreen Loading Dye I® (LGC Biotecnologia, Cotia, Brazil) and observed in ultraviolet light. PCR products were purified with the Gen Elute PCR clean-up Kit® (Sigma, Saint Louis, USA) following manufacturer's instructions and sequencing was carried out in Mega BACE sequencer 500 (Amersham Biosciences).

Sequenced fragments were analyzed using the program Staden Package 2.0.0b (Staden et al. 2003). A BLASTn search of the National Center for Biotechnology Information

databases verified that the sequence obtained from *Sarcoporia polyspora* was affiliated to the Polyporaceae. Fungal sequences (ITS1-5.8s-ITS2) obtained in our laboratory were aligned with other sequences from GenBank using ClustalX (Larkin et al. 2007) and in BioEdit (Hall 1999) to obtain a final alignment (Table 1). The sequence was deposited at GenBank (Altschul et al. 1997) under the accession number JX268521.

The phylogeny was reconstructed by ITS1-5.8S-ITS2 analyses. The General Time Reversible model nucleotide substitution model was estimated using FindModel run in May, 11, 2012. The Maximum Likelyhood analysis was performed in MEGA 5.0 (Tamura et al. 2011) where 1000 bootstrap replicates were used in all reconstructions. *Pseudomerulius curtisii* GU187536 and GU187533 sequences were used as outgroups.

Table 1 Specimens of fungi included in this study. In bold the accession Genbank number referred to the sequences obtained from *Sarcoporia polyspora* in Santa Maria, Southern Brazil.

Species	**Strain**	**Locality**	**GenBamk acession number**
Antrodia carbonica (Overh.) Ryvarden & Gilb.	FP 105585-R	Taiwan	EU232211
Antrodia sinuosa (Fr.) P. Karst.	RLG1182R	Taiwan	AY966450
Antrodia sinuosa (Fr.) P. Karst.	155753	Sweden	JQ518273
Antrodia sp. P. Karst.	AFTOL-ID 1504	USA	DQ484059
Antrodia xantha (Fr.) Ryvarden	FCUG 100	Taiwan	EU232209
Antrodia xantha (Fr.) Ryvarden	P209 (G185)	Turkey	AJ415569
Oligoporus placentus (Fr.) Gilb. & Ryvarden	P100	Germany	AJ416069
Oligoporus placentus (Fr.) Gilb. & Ryvarden	P120 (FPRL 280)	Germany	AJ249267
Parmastomyces mollissimus (Maire) Pouzar	Dai10193	China	FJ627250
Sarcoporia polyspora P. Karst.	**DBB20**	**Brazil**	**JX268521**
Postia placenta (Fr.) M.J. Larsen & Lombard	JV0509/174	USA	JN592502
Postia placenta (Fr.) M.J. Larsen & Lombard	FPRL 280	Germany	EF524035
Pseudomerulius curtisii (Berk.) Redhead & Ginns	DJL-DR-4	Dominican Republic	GU187536
Pseudomerulius curtisii (Berk.) Redhead & Ginns	REH8912	Australia	GU187533

Results

Sarcoporia polyspora P. Karsten

= *Polyporus subcartilagineus* Overh. Overholts L.O. Mycologia 33(1), 1941. = *Polyporus transmutans* Overh. Mycologia 44: 226, 1952. = *Tyromyces kravtzevianus* Bondartsev & Parm. In Parm. Mycotheca Estonica I. No. 25. 1957. = *Tyromyces mollissimus* Maire. In Maire R. Bull. Soc. Hist. Nat. Afrique N. 36. 1945 (Figs. 1-2).

Basidiome annual, resupinate, effuse-reflexed to pileate, rarely imbricate, fragile, soft, fleshy, watery, jelly and putrescent upon aging, firm upon drying, 250 × 80 × 20 mm. **Pileus** when present broadly-attached, usually limited a small reflexed part, fleshy; pilear surface white (8/1 5YR), (Fig. 1C,D), with shades of yellowish red (5/6–4/6 5YR) to dark reddish brown (3/4 5YR) especially when bruised; pileus surface cottony to fibrous, not very regular, having wrinkles and depressions, sometimes forming discrete concentric zones. **Hymenophore** poroid, concolorous to the pileus surface; (Fig. 1A, B), pores round to polygonal, (1–)2(–3)/mm, P_m= 2.00, n= 97/3; dissepiments thin, velutinous, slightly dentate; margin of pileate basidiomes white (8/1 5YR), limited to a growing sterile zone, rounded,

margin of resupinate basidiomes more conspicuous, sterile, broad, up to 20 mm, becoming jelly on aging. **Tube layer** concolorous to the hymenophore, up to 20 mm thick, dense, forming a palisade, representing the major part of the basidiome. **Context** concolorous to the hymenophore, thin, up to 1 mm thick, homogeneous, fleshy to gelatinous, easily to macerate, sometimes difficult to distinguish from tube layer.

Hyphal system monomitic. **Tramal generative hyphae** clamped, (Fig. 2d), thin-walled to slightly thick-walled, sparsely branched, hyaline, (2–)2.4–4(–4.4) µm diam., D_m = 2.9, n = 60/1. **Gloeopleurous hyphae** wider than tramal hyphae , easily found when intensely stained in phloxine, usually observed as conspicuously hyaline wider hyphae, thin-walled to thick-walled, usually tortuous in outline, often solid, clamped, (5.6–)8.0–20 µm diam., D_m = 11.3, n = 60/1; D_m = 2.6, n = 60/1.

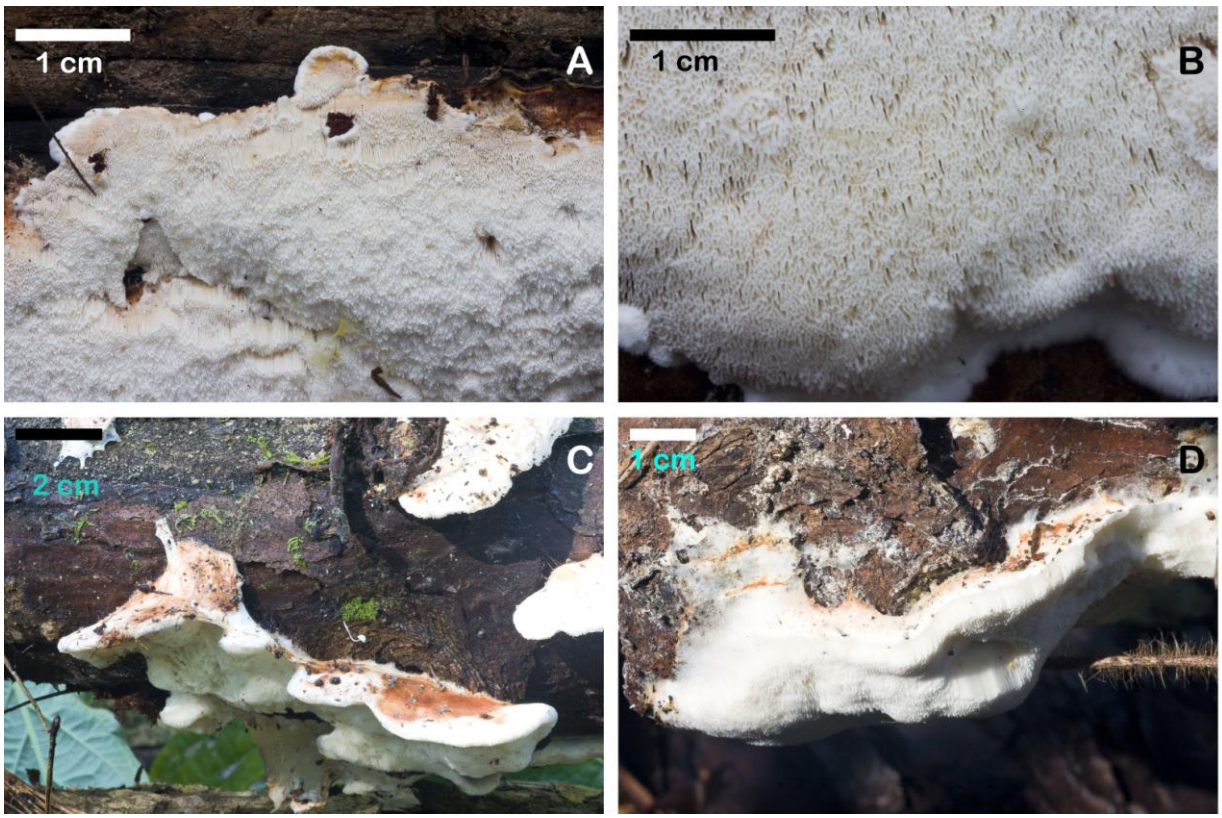

Fig. 1. Basidiomes of *Sarcoporia polyspora* P. Karsten on decayed fallen trunk of *Pinus* spp. **A** and **B** Details from white pored hymenophores. **C** and **D** Effused-reflexed basidiomes with white to reddish brown pilear surface. This picture is copyright of Gilberto Coelho.

Contextual generative hyphae clamped, thin-walled to slightly thick-walled, hyaline to whitish opaque, sparsely branched, (2.8–)3.2–5.6(–6) µm diam., D_m = 4.2, n = 60/1. **Gloeoplerous hyphae** wider than tramal hyphae, (Fig. 2e), usually with a denser contents than contextual hyphae and intensely staining in phloxine, usually observed as conspicuously hyaline wider hyphae, thin-walled to thick-walled, usually tortuous in outline, often solid, clamped, usually difficult to observe, (2.8–)4.4–12(–17.6) µm diam., D_m = 7.7, n = 60/1.

Hymenium with **basidia** clavate (Fig. 2a), hyaline, four-sterigmate, (13.6–)17.6–22.4(–25.6 × (5.2–)5.6–6.4(–7.6) µm, $L_m \times W_m$= 20.01 ± 2.33 × 5.90 ± 0.36; Q_r= 2.27–4.62; Q_m= 3.42 ± 0.44, n= 60/1. **Basidiospores** ellipsoid, (Fig. 2c), narrowly ellipsoid to subcylindrical, abundant, moderately thick-walled, strongly dextrinoid, often guttulate, (4–)4.4–5.6(–6.0) × (2.0–)2.4–2.8(–3.2) µm, $L_m \times W_m$ = 4.8 ± 0.49 × 2.49 ± 0.26, Q_r= 1.57– 2.40,

Q_m = 1.95 ± 0.21, n = 63/1. **Hyphidia** inconspicuous, long, hyaline, sometimes ventricose, narrowing towards the apex, with simple to branched apex, 11–24 x 3.5–6.5 µm, n = 8.

Associated wood rot: brown.

Substrate: on fallen coniferous wood on the forest ground, usually in advanced stages of decomposition, on *Pinus taeda* L. in the study area.

Sexuality and cultural characters: heterothallic and tetrapolar; see Nobles (1965 as *P. subcartilagineus*, see synonymy below species name); Stalpers (1978, as *P. kravtzevianus*); nuclear behavior heterocytic (Hibbett & Donoghue 2001, Rajchenberg 2011).

Specimens examined: Brazil, Santa Maria, Boca do Monte, FEPAGRO, leg. G. Coelho, 03.II.2011, **NºGC 338-9 (ICN 139765)**, on *Pinus taeda* L.

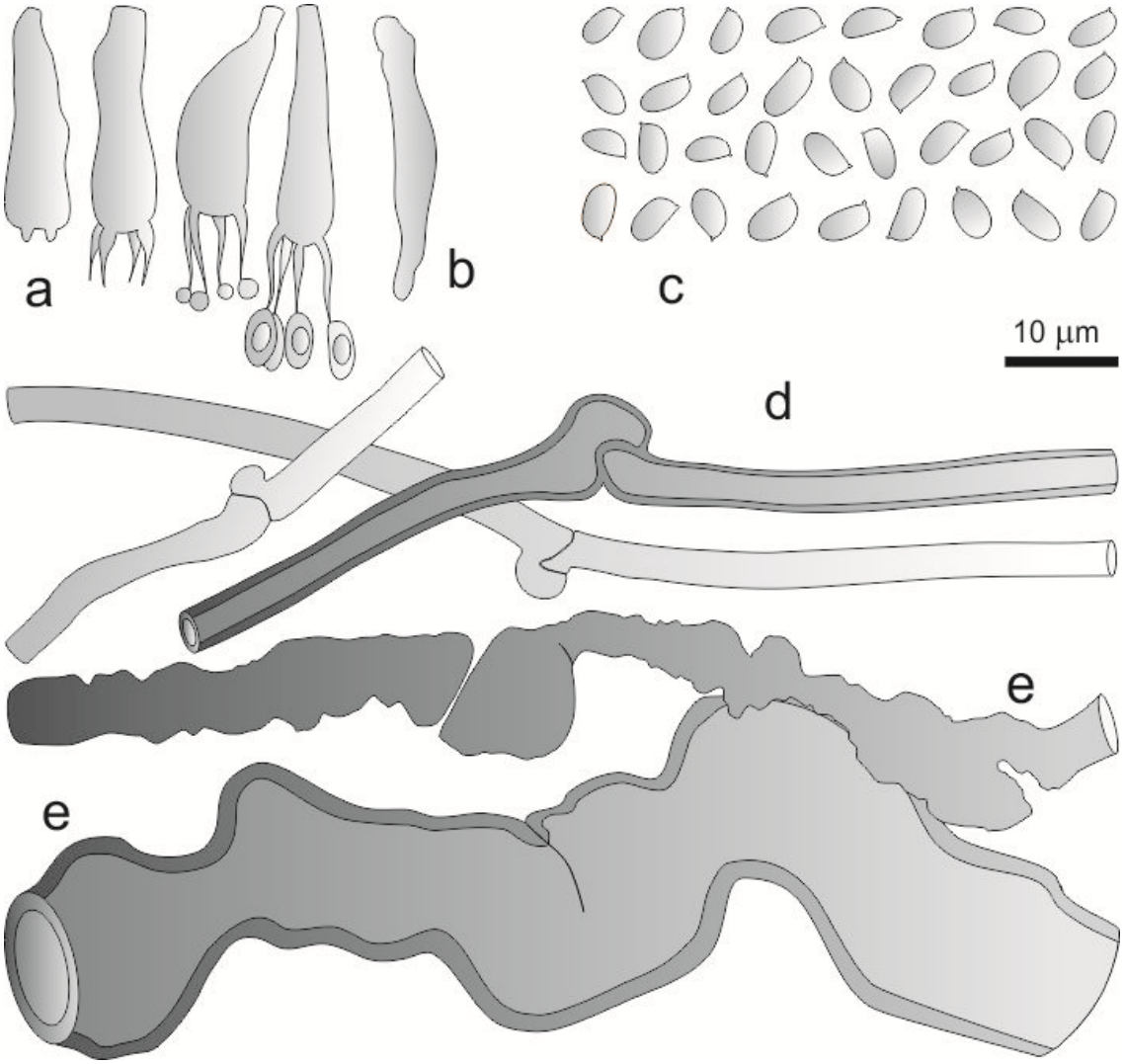

Fig. 2 - Microscopical characters of *Sarcoporia polyspora* P. Karsten. **a** Basidia. **b** Cystidiolum. **c** Basidiospores. **d** Tramal and contextual generative hyphae. **e** Gloeopleurous-hyphae from context. This picture is copyright of Gilberto Coelho.

Distribution: To a more complete distributional picture see Vlasak and Kout (2010); in summary, it was considered as rare in Europe, common in North America (usually as *Parmastomyces transmutans*), and reported several times to Asia (in Russia, as *P. kravtzevianus*, and China, as *P. molissimus*), (Fig. 3).

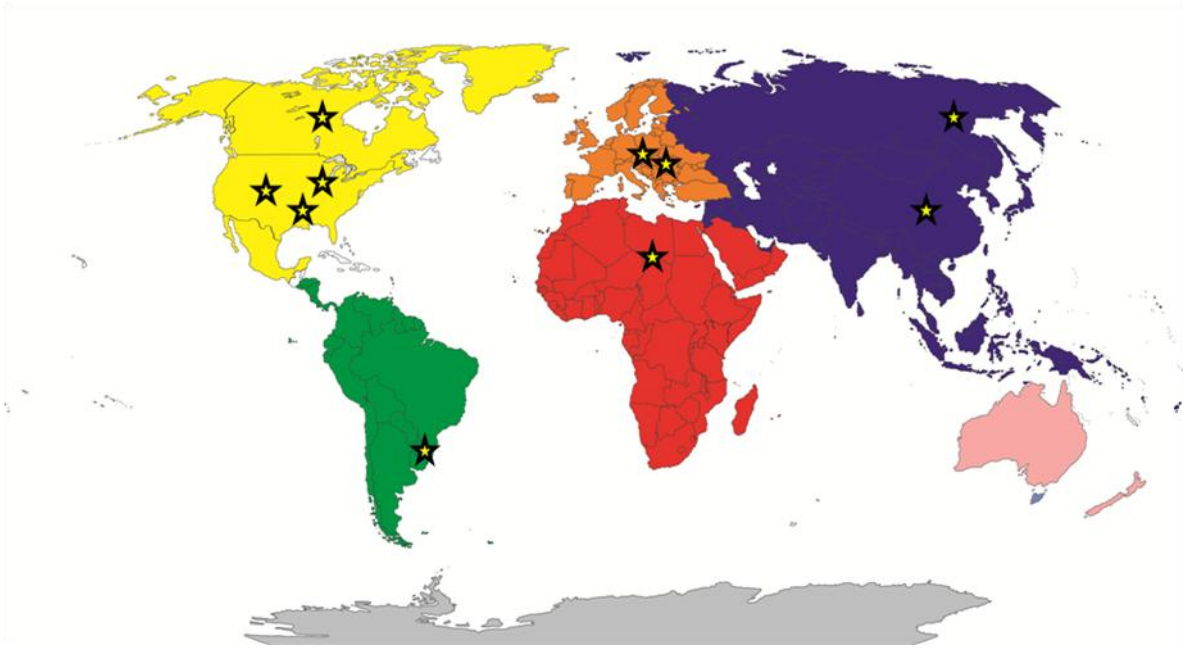

Fig. 3 – Geographical distribution of *Sarcoporia polyspora*. The stars mark the occurrence of the species on continents, according to a literature review.

Discussion

Sarcoporia polyspora is characterized by presenting basidiomes white to very pale brown, fleshy to almost gelatinous, resupinate to effuse-reflexed, not rhizomorphic, and large pores (1–3/mm). It also presents a monomitic hyphal system dominated by clamped generative hyphae throughout the basidiomes and gloeopleurous hyphae mainly in the context; a remarkable character is the abundant, ellipsoid to subcylindrical, and dextrinoid basidiospores. The latter feature differentiates it from other brown-rotting species of *Antrodia*, *Fibroporia*, *Amyloporia*, and *Postia* (*Oligoporus*) with white to brownish or yellowish white resupinate basidiomes.

Hibbett & Binder (2002) formerly found *Sarcoporia polyspora* (referred there as *P. transmutans*) grouped in a polyporoid clade in their study primarily based on 18S rDNA, but using a small species dataset. In contrast, other multi-locus study performed by Binder et al. (2005) using a larger dataset (656 species, mostly represented only by nrLSU sequences), shown *S. polyspora* (*P. transmutans*) into the *Antrodia* clade; result revisited by Lindner & Banik (2008).

The ML analysis of the same sequence dataset yielded similar phylogenetic trees (Fig. 4). The analyzed sequence from Brazil fell well-supported in an isolated position closely related to that of its synonym *P. molissimus* (FJ627250), which was elected for inclusion in this study by its recognized morphological similarity.

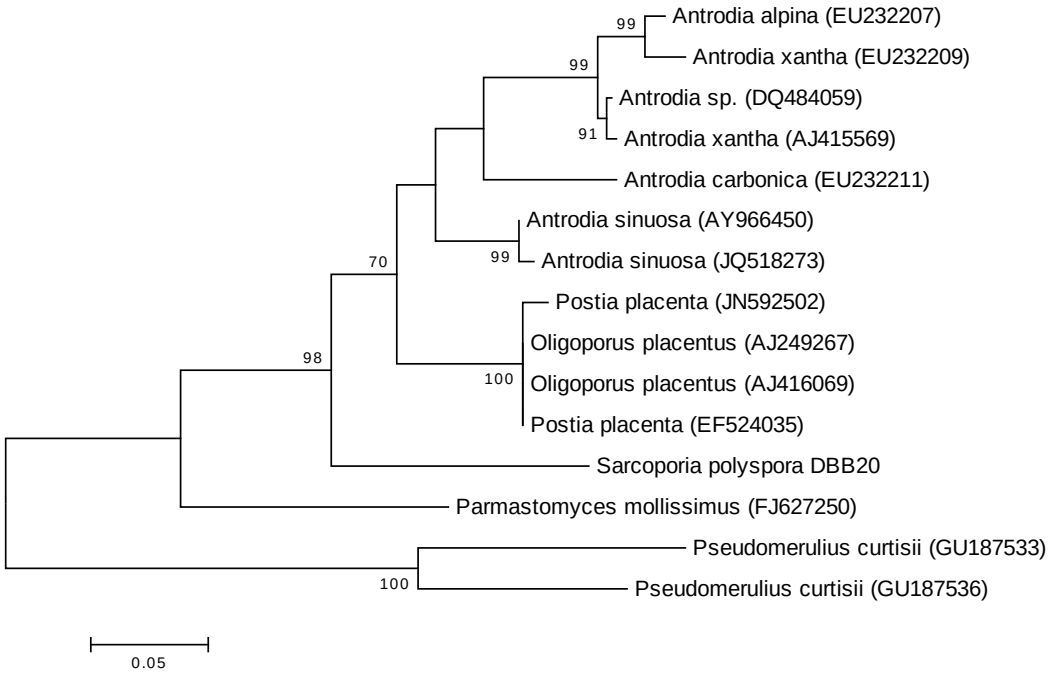

Fig. 4 – Phylogenetic reconstruction of the *Sarcoporia polyspora* obtained from ITS1-5.8S-ITS2 sequences. Bootstrap values (in %) are from maximum likelihood (ML) analyses (1000 bootstraps). Only topologies with bootstrap values of at least 50% are shown.

It was obtained in this study an evident separation between Brazilian sequence, DBB20, and that of *P. mollissimus* supported by a high boostrap value. It must be noted that it was found an evident distinction between these sequences manifested by a low similarity score of 84% in ITS1-5.8S-ITS2 region; but also between our specimen and the others from the dataset chosen among the most similar sequences retrieved with BLAST program. Furthermore, sequences of ITS1-5.8S-ITS2 region of *Sarcoporia polyspora* are almost inexistent at GenBank database, becoming difficult to get a more accurate molecular identification and phylogenetic assessment of the specimen.

This study represents a preliminary attempt to access the phylogenetic relationships of *Sarcoporia* starting from Brazilian specimens. More studies, however, using ITS and other genomic regions need to be carried out in order to elucidate phylogenetic relationships and possible internal speciation of a genus supposed to be monotypic (Vlasak & Kout 2010). Conspecificity between our *S. polyspora* and *P. mollissimus* (GenBank) sequences was not sufficiently elucidated in this study based on ITS1-5.8S-ITS2 region due the low similarity involved, so there is a need for generating more sequences in the genus and introducing larger datasets in order to obtain a better phylogenetic understand. Additional morphological, biochemical, and molecular studies are still necessary for resolving the status of specimens so widely distributed in the world. These records of *Sarcoporia polyspora* are the first one from South America.

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CONSIDERAÇÕES FINAIS

As espécies coletadas na área de plantio de pinus da FEPAGRO/Florestas representam um pouco da importância dos estudos da diversidade de espécies de fungos degradadores presentes nesses locais no bioma Pampa. Pela primeira vez foi realizado o registro das espécies *Pseudomerulius curtisii* (Berk.) Redhead & Ginns, *Gelatoporia subvermispora* (Pilát) Niemelä *e Sarcoporia polyspora* P. Karsten para a América do Sul, Brasil. Por meio desse estudo foi possível elucidar melhor a distribuição geográfica e a filogenia dessas espécies de grande importância na formação da matéria orgânica dos solos. Também obteve-se a caracterização morfológica e molecular dos espécimes contribuindo para a identificação das mesmas. Além disso, tornou-se disponível o material herborizado, as sequências (região ITS1-5.8S-ITS2) de todos os espécimes e os isolados das espécies *P. curtisii* e *G. subvermispora* depositados no Banco de fungos do Laboratório de Biologia do Solo e Microbiologia do Ambiente Prof Marcos Rubens Fries, (UFSM) no Brasil, para futuros estudos em diferentes áreas do conhecimento.

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