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

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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 important decomposition and nutrient an 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 are considered 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 have been estimated as being about 6% of wooddecaying fungi with 70% of the 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 Boletales, tribe Tapinellineae, order and family Tapinellaceae based on recent molecular studies (Binder & Hibbett 2004, Binder et al. 2010, Skrede et al. 2011). The genus Pseudomerulius was described with 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 differ 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, including 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. **Bondarcevomyces** Gilbert and taxi (Bondartsev) Parmasto (Larsson et al. 2004, Binder & Hibbett 2006) although the low number of available sequences has not enabled any intraspecific variation study 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 poroidraduloid) have been regularly collected from 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 isolation, laboratory analysis, and preservation. 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 Laboratory of Soil Biology and Environmental Microbiology Prof. Marcos Rubens Fries 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 a 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  $\times$  av. width  $\pm$  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). Reactions were adapted for

Species	Strain	Locality	GenBank
			accession number
Athelia arachnoidea	CBS: 418.72	Netherlands	GU187504
Athelia epiphylla	CFMR: FP-100564	USA	GU187501
Bondarcevomyces taxi	Dai2524	China	DQ534575
Coniophora arida	CFMR: FP-104367	USA	GU187510
Coniophora cerebella	8	USA	GU187513
Coniophora marmorata	P 307	United Kingdom	AJ518880
Coniophora marmorata	MUCL: 31667	Belgium	GU187515
Coniophora olivacea	MD-264	USA	AM747534
Coniophora prasinoides	MA-Fungi 19417	USA	AJ419197
Coniophora puteana	MUCL:1000	Germany	GU187521
Jaapia argillacea	CBS:252.74	Netherlands	GU187524
Leucogyrophana arizonica	CFMR:RLG-9902	USA	GU187527
Leucogyrophana mollusca	CFMR:L-10277	USA	GU187525
Leucogyrophana mollusca	P 263 (14167)	Sweden	AJ419914
Leucogyrophana mollusca	P 265 (G 201)	-	AJ419915
Leucogyrophana olivascens	CFMR:HHB-11134	USA	GU187532
Leucogyrophana pinastri	MA-Fungi 7924	Spain	AJ419214
Leucogyrophana pinastri	P 273 (G 117)	Germany	AJ419916
Leucogyrophana pinastri	P 275 (G 202a)	-	AJ419917
Leucogyrophana romellii	DAOM 148653	USA	GU187530
Leucogyrophana romellii	CFMR:T-547	Canada	GU187529
Pseudomerulius aureus	CFMR:FP-103859	USA	GU187534
Pseudomerulius curtisii	REH8912	Australia	GU187533
Pseudomerulius curtisii	DJL-DR-4	Dominican Republic	GU187536
Pseudomerulius curtisii	DBB1	Brazil	JN974314
Pseudomerulius curtisii	DBB34	Brazil	JX157585
Serpula lacrymans	REG 383	-	GU187542
Serpula himantioides	CFMR: RLG-12941	USA	GU187547
Tapinella atrotomentosa	122/98	USA	GU187550
Tapinella atrotomentosa	78/97	USA	GU187549
Tapinella panuoides	JLM 1752	USA	GU187551
Tapinella panuoides	MB05-019	USA	GU187548

**Table 1** Specimens of fungi included in this study. In bold, GenBank accession numbers of sequences obtained from *Pseudomerulius curtisii*, Santa Maria, RS, Brazil.

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  $\mu$ L, with the components: 10 ng of template DNA, 1  $\mu$ mol of each primer, 20 mM Tris-HCl (pH 8.4) and 50 mM reaction buffer, 2 mM MgCl<sub>2</sub>, 0,2 mM dNTP, 2,5 unit of Taq DNA polymerase

(Invitrogen, São Paulo, Brazil) and ultrapure  $H_2O$ .

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<sup>®</sup> (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



**Fig. 1** – 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).

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 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.

## Results

We collected seven samples belonging to the genus *Pseudomerulius*. All collections showed high morphological similarity with *Pseudomerulius curtisii* as described by Redhead & Ginns (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  $65 \times 52 \times$ 12 mm, with an unpleasant, pungent and spicy smell resembling pet food or a strong odor of cinnamon that intensifies 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 towards



**Fig. 2** – 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.

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.

**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,  $(1.8-)2.8-6.8(-8.4) \mu m$  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,  $(12.8-)14.4-17.6(-20) \times (3.2-)3.6-4(-4.4) \ \mu\text{m}, L_m \times W_m = 16.1 \pm 1.91 \times 3.67 \pm 0.35, Q_r = 2.91-6.00, Q_m = 4.40 \pm 0.62, n = 61/1.$  **Basidiospores** ellipsoid, narrowly ellipsoid to subcylindrical, abundant, moderately thick-walled, indextrinoid, often guttulate,  $3.2-4(-4.4) \times 1.6-1.8(-2) \ \mu\text{m}, L_m \times W_m = 3.6 \pm 0.38 \times 1.79 \pm 0.11, Q_r = 1.74-2.50, Q_m = 2.04 \pm 0.20, n = 60/1$ ; **hyphidia and cystidia** not seen. Associated wood rot: brown.

Known geographic distribution – From North and Central Americas to East Asia, and Oceania.



**Fig. 3** – 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.

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.

Specimens examined – Brazil, Rio Grande do Sul, Santa Maria, Campus/UFSM, on *Pinus elliottii*, 17 Dec 2007, leg. G. Coelho, N°GC 653-4 (ICN 139783); on *Pinus* sp., 21Dec 2007, N°GC 654-3 (ICN 139784); on *P*. *elliottii*, 15 Jan 2008, N°GC 662-2 (ICN 139785); on *P. elliottii* 19 Jan 2008, N°GC 663-2 (ICN 139786); on *Pinus* sp., 29 Jan 2008, N°GC 665-1 (ICN 139787); FEPAGRO, on *Pinus* sp., 3 Jan 2011, leg. G. Coelho & D.B. Baldoni, N°DBB 1 (SMDB 13.701); on UFSM, *Pinus* sp., leg. G. Coelho & D.B. Baldoni, 5 Jan 2012, N° DBB 34 (SMDB 13.702).

The NJ, MP and ML analyses of the same sequence dataset yielded similar phylogenetic trees (Fig. 3). The analyzed sequences from Brazil formed a well-supported uniform clade, closely related to *P. curtisii* (GU187533), from Australia.

#### 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. hvphae 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 Tapinella (Ginns 1998), and surface 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 (92%) and only 95% 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 vellow 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 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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