

Sarcoporia polyspora (Basidiomycota, Polyporales): a rare wood-decay fungus newly recorded from South America

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With 3 figures and 1 table

Abstract: Brown-rot fungi degrade almost exclusively wood cellulosic materials participating in the carbon cycle and soil formation of many forest regions, these are especially important on boreal and cultivated coniferous forests. These fungi and forests may have a common co-evolutionary history. Studying wood-inhabiting fungi in cultivated pine forests in Southern Brazil, we have analyzed morphologically and molecularly specimens of *Sarcoporia polyspora*, which has shown to be a rare and aggressive decomposer previously unreported from South America. Nuclear ribosomal ITS region sequences from North and South American specimens were compared.

Key words: macrofungus, *Pinus* spp., wood, soil humus, brown rot, polypores.

Introduction

Fungi are able to modify their substrates by secreting extracellular enzymes and thus produce significant ecological impacts; they find their niche in forests on leaf litter, soil organic matter (humus) or wood, as in the case of polypores (Basidiomycota), which attack primary wood compounds – cellulose, hemicellulose and lignin. Brownrot fungi, which degrade almost exclusively cellulosic materials, are not numerically dominant among wood decayers (Hibbett & Donoghue 2001, Baldrian & Valáškova 2008), but are positively correlated and present a possible interesting co-evolutionary

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history within coniferous forests, where they participate heavily in the carbon cycle and soil formation (Gilbertson 1980, 1981; Fukami et al. 2010).

Species associated with brown rot were firstly assigned as representing about 7% of American wood-rotting Basidiomycota (120 from 1700 spp), 85% occurring on coniferous substrates, and estimated as being less than 10% in Europe (Gilbertson 1980). The total number of brown-rot 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 was found on gymnosperm wood in boreal forests; except to a higher percentage of 38.7% (25 from 62) reported to Patagonian Andes (Southern Argentina, temperate forests) mostly on *Nothofagus* species, Fagaceae (Gilbertson 1980, Gilbertson & Ryvarden 1986, Ryvarden & Gilbertson 1993, Hibbett & Donoghue 2001, Rajchenberg 2006, Dai 2011).

Sarcoporia polyspora P.Karst. is an aggressive brown-rot polypore species observed to cause extensive degradation of wood; it is characterized by producing white to pale brown resupinate to effused-reflexed basidiomes, a monomitic hyphal system with clamp connections, dextrinoid basidiospores, tetrapolar mating system and heterocytic nuclear behavior. Currently included in the family Fomitopsidaceae Jülich (Kirk et al. 2008), this species was originally described by Karsten in 1894 from Finland and, after that, it was redescribed or recombined under different names, as detailed discussed in Vlasák & Kout (2010). This species has been mostly referred as Parmastomyces transmutans (Overh.) Ryvarden & Gilb. (Ryvarden & Gilbertson 1984, Gilbertson & Ryvarden 1987, Ryvarden & Gilberson 1994), more especially after 1991, when Ryvarden stated that Sarcoporia would be morphologically more similar to Hapalopilus P.Karst., suggesting its synonymy with a polypore genus characterized by monomitic hyphal system, but lacking dextrinoid reaction in spores and producing a white rot.

Sarcoporia polyspora has been considered as common in western North America, with several records to Asia, but rare in Europe and North Africa (Gilbertson & Ryvarden 1987, Vlasák & Kout 2010). The aim of this study is to present the finding of this species in pine forests in South Brazil, contributing to the knowledge about the distribution, morphology and molecular insights of this rare and ecologically important species.

Materials and methods

FUNGAL MATERIAL COLLECTIONS Brazilian specimens 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. MORPHOLOGICAL CHARACTERIZATION: Specimens were observed, photographed and collected in plastic bottles or absorbent paper for transporting to Prof. Marcos Rubens Fries Soil Biology and Environmental Microbiology Laboratory, CCR/UFSM. Preserved specimens 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/). Munsell Soil Color Charts (1994) were used for naming colors. Microscopic studies were performed through handmade sections of fresh basidiomes and mounting on glass slides with drops of KOH 5% plus aqueous phloxine or Melzer's reagent. Statistical analysis of measurements was performed with EXCEL* (MS Office 2003) and its abbreviations are presented following Coelho (2005): *Dm* = arithmetical mean of diameter; *Lm* × *Wm* = arithmetical

means of length and width (from where Q can be calculated, Q = Lm/Wm); Qr = quotient variation from the length/width of each measurement (L/W ratio); Qm = arithmetical mean of Qr; n = x/y, being the number (x) of a microscopic structure measured from a given number (y) of specimens. Authors of fungal names follow Kirk & Ansell (2008). Coniferous substrates are abbreviated as: Pt. for Pinus taeda P. sp. for unidentified Pinus species. Basidiospore shape classification was based on Qr defined according to resupinate Russulales species database (available at: http://www.cbs.knaw.nl/russulales/).

SEQUENCING 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 following Baldoni et al. (2012). 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 1* (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 ABI-PRISM 3100 Genetic Analyzer (Applied Biosystems).

PHYLOGENETIC ANALYSIS: 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 was used to verify and confirm that the sequences obtained from *S. polyspora* were affiliated to the Polyporaceae. Sequences were aligned with MAFFT 6.0 program (Katoh et al. 2002) using E-ins-i algorithm (Table 1). The sequences were deposited at GenBank (Altschul et al. 1997), under the accession number: KF501892, KF501893, KF501894 (Table 1).

The phylogeny was reconstructed based on analyses of the ITS region in MEGA 5.0 (Tamura et al. 2011) with Maximum Likelihood. A total of 1000 bootstrap replicates were used in all the reconstructions. The Tamura-Nei model nucleotide substitution model was estimated using ModelTest (Posada 2006) run with Uniform rates and partial deletion (95%) parameters. Sequences of *Tremella globispora* D.A. Reid (AF444432) and *Tremella cinnabarina* D.A. Reid (AF444430) were used as outgroups. Additional sequences were retrieved from GenBank, including those of isolates of *S. polyspora* from USA (collected in eastern USA), and other wood-decay fungi pertaining to the families Polyporaceae and Meruliaceae).

Results

Taxonomy

Sarcoporia polyspora P.Karst., Hedwigia 33: 15 (1894)

= Polyporus subcartilagineus Overh., Mycologia 33(1), 1941. = Polyporus transmutans Overh., Mycologia 44: 226, 1952. = Tyromyces kravtzevianus Bondartsev & Parmasto., in Parmasto. 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, effused-reflexed to pileate, rarely imbricate, fragile, soft, fleshy, watery, jelly and putrescent upon aging, firm upon drying, $250 \times 80 \times 20$ mm. PILEUS when present broadly-attached, usually limited to a small reflexed part, fleshy; pileal 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 after 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; (Figs 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

Table 1. Specimens included in this study. Accession Genbank numbers in bold referred to the ITS sequences obtained from *Sarcoporia polyspora* in Southern Brazil and United States of America.

Species	Strain	Locality	GenBamk acession Number
Amyloporia carbonica (Overh.) Vampola & Pouzar	FP 105585-R	USA	EU232211
Amyloporia sinuosa (Fr.) Rajchenb., Gorjón & Pildain	RLG1182R	USA	AY966450
Amyloporia sitchensis (D.V.Baxter) Vampola & Pouzar	164		JN182921
Amyloporia xantha (Fr.) Bondartsev & Singer ex Bondartsev	FCUG 100		EU232209
Climacocystis borealis (Fr.) Kotl. & Pouzar	5400	Estonia	JN710527
Climacocystis borealis	KHL13318	Estonia.	JQ031126
Climacocystis borealis	B27	Latvia	FJ903302
Coriolopsis caperata (Berk.) Murrill	PonSok 2-6b	Panama	EU030144
Coriolopsis rigida (Berk. & Mont.) Murrill	CECT20449		JX134278
Coriolopsis gallica (Fr.) Ryvarden	FP102596Asp	USA	JN164997
Coriolopsis trogii (Berk.) Domanski	YDHSD		EU790491
Coriolopsis trogii	XSD-37		EU273516
Grifola frondosa (Dicks.) Gray	10	Germany	FR686557
Grifola frondosa	CIRM-BRFM 1162		GU731562
Grifola gargal Singer	GG010	Chile	AY854085
Grifola sordulenta (Mont.) Singer	PDD:86931	New Zealand	GU222266
Grifola sordulenta	TENN 55054		AY854085
Parmastomyces mollissimus (Maire) Pouzar	Dai10193		FJ627250
Phlebia albomellea (Bondartsev) Nakasone	FP-101843-sp	USA	AY219369
Phlebia chrysocreas (Berk. & M.A.Curtis) Burds.	FCUG2827	USA	HQ153411
Phlebia livida (Pers.) Bres.	MG104	Russia	HQ153416
Phlebia nitidula (P.Karst.) Ryvarden	Nystroem 020830	Sweden	EU118655
Phlebia subserialis (Bourdot & Galzin) Donk	UFMGCB 1883	Brazil	HQ377286
Phlebia uda (Fr.) Nakasone	USDA Kropp-1		AB084621
Pycnoporellus fulgens (Fr.) Donk	DLL2009-158		JQ673193
Taiwanofungus camphoratus (M.Zang & C.H.Su) Sheng H.Wu, Z.H.Yu, Y.C.Dai & C.H.Su	Baolun-1106C		JN165025
Taiwanofungus camphoratus	Baolun-1106B		JN165024
Taiwanofungus salmoneus (T.T.Chang & W.N.Chou) Sheng H.Wu, Z.H.Yu, Y.C.Dai & C.H.Su	B492		EU232203
Taiwanofungus salmoneus	B147	(.*)	EU232202

Sarcoporia polyspora P.Karst.	L16072	USA	KC585394
Sarcoporia polyspora	23436	USA	KC585392
Sarcoporia polyspora	L14910	USA	KC585226
Sarcoporia polyspora	DBB29	Brazil	KF185092
Sarcoporia polyspora	DBB30	Brazil	KF185093.
Sarcoporia polyspora	DBB33	Brazil	KF185094
Tremella cinnabarina (Mont.) Pat.	CBS 8234		AF444430
Tremella globispora D.A.Reid	CBS 6972		AF444432
Trametes conchifer (Schwein.) Pilát	FP102251sp	USA	JN164987
Trametes ectypa (Berk. & M.A.Curtis) Gilb. & Ryvarden	FP103976sp	USA	JN164961
Trametes junipericola Manjón, G.Moreno & Ryvarden	HUBO 6916	Italy	AY684171
Trametes ochracea (Pers.) Gilb. & Ryvarden	LE(BIN)-093	Russia	AB158314
Trametes pubescens (Schumach.) Pilát	DLL2010-068		JQ673026
Trametes versicolor (L.) Lloyd	T-868	USA	AF139961

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- 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, frequently observed as conspicuously hyaline wider hyphae, thin-walled to thick-walled, usually tortuous in outline, often solid, clamped, (5.6-)8-20 µm diam., $D_m = 11.3$, n = 60/1; $D_m = 2.6$, n = 60/1.

Contextual generative hyphae clamped, thin- to slightly thick-walled, hyaline to whitish opaque, sparsely branched, (2.8–)3.2–5.6(–6) μ m 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) μ m 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, $L_m \times W_m = 20.01 \pm 2.33 \times 5.90 \pm 0.36$; $Q_r = 2.27–4.62$; $Q_m = 3.42 \pm 0.44$, 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) × (2–)2.4–2.8(–3.2) μm, $L_m \times W_m = 4.8 \pm 0.49 \times 2.49$

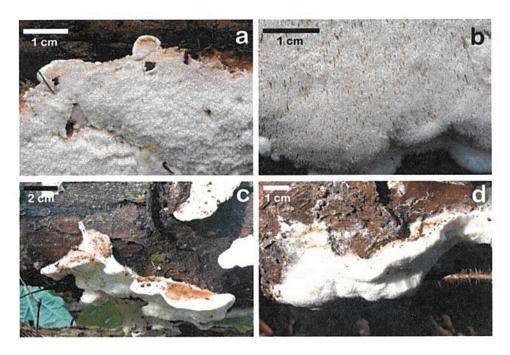


Fig. 1. Basidiomes of *Sarcoporia polyspora* on decayed trunks of *Pinus* spp. a. and b. Details from white pored hymenophores, c and d. Effused-reflexed basidiomes with white to reddish brown pileal surface.

 \pm 0.26, Q_r = 1.57– 2.40, Q_m = 1.95 \pm 0.21, n = 63/1. Hyphidia inconspicuous, long, hyaline, sometimes ventricose, narrowing towards the apex, with simple to branched apex, $11-24 \times 3.5-6.5 \mu m$, 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 studied area.

SEXUALITY AND CULTURAL CHARACTERS: Heterothallic and tetrapolar; see Nobles (1965), as *Polyporus subcartilagineus* Overh., and Stalpers (1978), as *Parmastomyces kravtzevianus* (Bondartsev & Parmasto) Kotl. & Pouzar; nuclear behavior is heterocytic (Hibbett & Donoghue 2001, Rajchenberg 2011).

Specimens examined: Brazil, Santa Maria, Boca do Monte, FEPAGRO, leg. G.Coelho, 03.II.2001, N°GC 338-9 (ICN 139765), on Pt. 03.II.2001, N°GC 338-9 (ICN 139766), Pt; 28.IV.2001, N°GC 293-1 (ICN 139766), Pt; N°GC 293-8 (ICN 139767), Pt; 24.V.2003, N°GC 388-4 (ICN 139768), Pt; leg. G.Coelho & V.G.Cortez, 15.V.2007, N°GC 631-2 (ICN 139769), Psp; N°GC 631-7 (ICN 139770), Pe; N°GC 631-8 (ICN 139771), Pt; 20.VI.2007, N°GC 638-2 (ICN 139772), Psp.

DISTRIBUTION: For a more complete distributional range see Vlasák & Kout (2010); in summary, it was considered as rare in Europe and North Africa, common in North America (usually as *Parmastomyces transmutans*), reported several times to Asian part

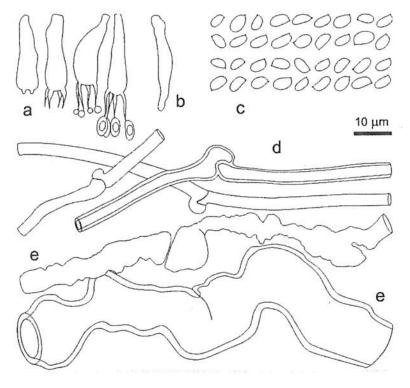


Fig. 2. Microscopic characters of *Sarcoporia polyspora* (ICN 139769). a. Basidia. b. A cystidiolum. c. Basidiospores. d. Tramal and contextual generative hyphae. e. Gloeopleurous-hyphae from context.

of Russia (as *P. kravtzevianus*), and common in northeast China (as *P. molissimus*, Dai & Penttilä 2006). Additionally from South America (present study).

Sarcoporia polyspora is characterized by presenting basidiomes white to very pale brown (sometimes with brownish red shades), fleshy to almost gelatinous, resupinate to effused-reflexed, not rhizomorphic, and with 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* P.Karst., *Fibroporia* Parmasto, *Amyloporia* Singer, *Postia* Fr. and *Oligoporus* Bref., with white to brownish or yellowish white resupinate basidiomes.

Discussion

In the present study, North American and Brazilian isolates of *S. polyspora* formed a clade with a strong bootstrap support (100%) indicating a conspecific origin and

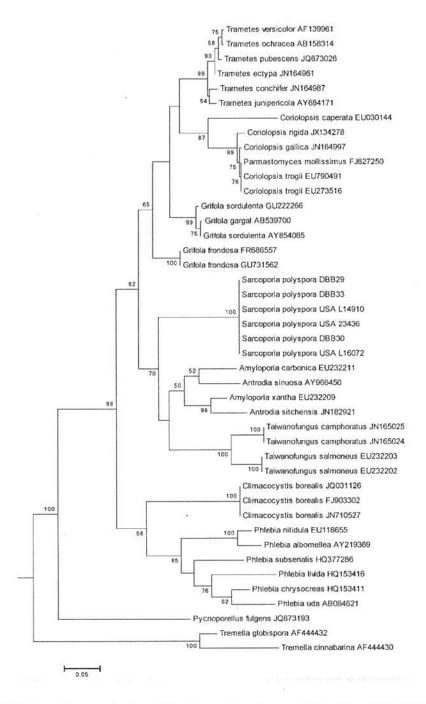


Fig. 3. Phylogenetic reconstruction of the *Sarcoporia polyspora* obtained from ITS1-5.8S-ITS2 sequences. Bootstrap values (in %) are from maximum likelihood (ML) analysis (1000 bootstraps). Only bootstrap values of at least 50% are shown.

confirming the presence of this species in South America (Fig. 3). The Brazilian material is also morphologically similar to the North American one described by Gilberston & Ryvarden (1987). There was another ITS sequence in Genbank named as *P. molissimus* (FJ62725), a currently accepted taxonomical synonym Gilbertson & Ryvarden (1987), which nested within the *Trametes-Coriolopsis* group in our phylogenetic tree (*Trametes clade* of polyporoids as treated by Justo & Hibbett 2011); a BLAST search performed on it showed the highest similarities with *Coriolopsis trogii* (Berk.) Domanski sequences indicating that this isolate was misidentified as *Sarcoporia*.

In terms of the evolutionary relationship among Sarcoporia and other wood decay fungi, several phylogenetic studies have placed S. polyspora (as Parmastomyces transmutans) within the polyporoid or antrodia clades, related to other brown rot fungi. Hibbett & Binder (2002), based on SSU-nrDNA dataset (133 species), placed it within the polyporoid clade. In a multi-locus study performed by Binder et al. (2005) using a much larger dataset (656 species, mostly represented by nrLSU sequences), S. polyspora nested within the antrodia clade. In the study of Lindner & Banik (2008) based on nrLSU sequences, it also appeared as part of the antrodia clade apparently related to Grifola frondosa (Dicks.) Gray (a white rotter). Shigel et al. (2006) performed another study based on ITS sequences nesting S. polyspora between the Antrodia and Postia-Oligoporus clades, closer to Amylocystis lapponica (Romell) Bondartsev & Singer ex Singer – a species morphologically similar to S. polyspora (Vlasák & Kout 2010), both species being exceptionally tetrapolar in mating system, whilst mostly brown rotters are bipolar (Hibbett & Donoghe 2001). In the study of Yu et al. (2010) based on partial LSU nrDNA sequences, S. polyspora appeared more closely related to the genera Auriporia Ryvarden, Amylocystis Bondartsev & Singer ex Singer, Oligoporus Bref. and Taiwanofungus Sheng H.Wu, Z.H.Yu, Y.C.Dai & C.H.Su (grouped within their clade B), than to Antrodia, Daedalea Pers., Fibroporia, Fomitopsis P.Karst., Piptoporus P.Karst., and Neolentiporus Rajchenb. (placed within clade A). More recently, in the study of Ortiz-Santana et al. (2013), using nuclear LSU and ITS rDNA sequences, isolates of S. polyspora nested with isolates of Auriporia and Taiwanofungus and appeared related to a clade containing isolates of Amylocystis Bondartsev & Singer ex Singer and Dacryobolus Fr. As mentioned above some of these genera shared the production of resupinate basidiocarps with monomitic hyphal systems with clamps, however since those groups were not always statistical supported the relationship among Sarcoporia and other brown rot fungi remains unresolved. Here isolates of S. polyspora nested with isolates of Amyloporia, Antrodia and Taiwanofungus, not related to Grifola Gray or Coriolopsis Murrill isolates; therefore this study confirms its placement within the antrodia clade as suggested in some of the studies mentioned above.

These new records of *S. polyspora* are the first ones from South America. The genus *Sarcoporia* is currently accepted as monotypic and rarely recorded on the globe, but considered as having a wide distribution (Vlasák & Kout 2010). So, additional molecular, ecological, and biochemical studies are still necessary, especially for Europe and Asia, in order to resolve potential cryptic speciation and elucidate phylogenetic and ecological relationships of the genus.

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