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Fistulinella ruschii, sp. nov., and a new record of *Fistulinella campinaranae* var. *scrobiculata* for the Atlantic Forest, Brazil

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ABSTRACT

Fistulinella is a small genus of boletoid fungi in the subfamily Austroboletoidae in the order Boletales. In this paper, *F. ruschii* from the Atlantic Forest is proposed as new to science and *F. campinaranae* var. *scrobiculata*, known from the Brazilian Amazon forest, is recorded for the first time in the Atlantic Forest. Macro- and microscopic descriptions, molecular data (nuc rDNA ITS1–5.8S–ITS2 and nuc 28S rDNA), photographs of the basidiomata, and scanning electron microscopy images of basidiospores are provided for both species. Based on sampling of six taxa, New World *Fistulinella* is found to be a strongly supported monophyletic group, but the genus at large is nonmonophyletic.

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Boletaceae; Boletales; fungal
diversity; Neotropics;
taxonomy; 1 new taxon

INTRODUCTION

Fistulinella Henn. comprises 25 species, mainly with a pantropical distribution, including Australia, Cameroon, Colombia, Costa Rica, Jamaica, Guyana, Malaysia, Martinique, Mexico, New Zealand, and Venezuela (Guzmán 1974; Singer 1978, 1986; Wolfe 1979, 1982; Pegler and Young 1981; Pegler 1983; Singer et al. 1983, 1991; Watling and Gregory 1989; Ortiz-Santana et al. 2007; Kirk et al. 2008; Watling 2008; Fulgenzi et al. 2010; Vasco-Palacios et al. 2014). *Fistulinella staudtii* Henn. from Cameroon is the type species (Hennings 1901).

Fistulinella is included in Austroboletoidae G. Wu & Zhu L. Yang (Boletaceae, Boletales) together with *Veloporphyrellus* L.D. Gómez & Singer, *Mucilopilus* Wolfe, and *Austroboletus* (Corner) Wolfe (Wu et al. 2014). *Fistulinella* is characterized by a whitish tubular hymenophore that becomes pink at maturity, a pinkish to pink-brown spore print, and microscopically by the smooth, elongate-fusoid, weakly to distinctly dextrinoid basidiospores and presence of gelatinized tissues in the pileipellis and hymenophoral trama (Singer 1986).

Most of the boletoid species known from Brazil are from Amazonian forest (Singer and Digilo 1957, 1960; Singer et al. 1983; Singer and Araujo 1986; Neves and Capelari 2007). However, a high diversity of boletes has also been observed in the coastal Atlantic Forest (Rick

1960; Oliveira and Sousa 1995, 1996, 2002; Watling and de Meijer 1997; Magnago and Neves 2014; Barbosa-Silva et al. 2017; Magnago et al. 2017, 2018). The Atlantic Forest is considered an important global biodiversity hot spot, includes many endemic species, and environmentally is highly variable along the coast and inland areas of Brazil, including coastal forests, araucaria mixed forests, deciduous and semideciduous forests, mangrove swamps, marshlands, and oceanic islands. Originally, it covered 1 300 000 km² (~15% of Brazil). However, after decades of deforestation due to harvest of valuable lumber, agroindustry, and urbanization (Dean 1932–1994), less than 8% of the original area remains preserved (SOS Mata Atlântica 2013).

The two varieties of *F. campinaranae* Singer are the only taxa in the genus known from Brazil (Singer et al. 1983). In this study, *F. campinaranae* var. *scrobiculata* Singer is recorded for the first time from the Atlantic Forest and *Fistulinella ruschii* is described as new.

MATERIALS AND METHODS

Field work.—Field expeditions were carried out in the following areas: Reserva Biológica Augusto Ruschi, municipality of Santa Teresa, Espírito Santo (19°54' 19.60"S, 40°34'8.20"W); the Universidade Federal da

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Paraíba, João Pessoa, Paraíba (7°08'18"S, 34°50'38"W); Parque Estadual da Serra do Conduru, municipality of Serra Grande, Bahia (14°26'49.5"S, 39°05'37.1"W); and the Unidade de Conservação Ambiental Desterro, Florianópolis, Santa Catarina (27°31'51.6"S, 48°30'44.1"W). The vegetation type in these areas is Atlantic Forest (dense ombrophilous forest). The climate is tropical humid with high annual rainfall (2800 mm). The mean annual temperature varies between 20 and 31 C.

Morphology.—Macro- and microscopic analyses followed established methods used for basidiomycetes (Largent et al. 1977; Largent 1986). For basidiospore quotient (Q), Qr indicates the range of Q values; Qm indicates the average of Qr; n, the number of basidiospores measured; and s, the number of specimens examined. Color codes (e.g., OAC 663) were based on the Online Auction Color Chart (Kramer 2004). Basidiomata were dried with a food dehydrator (Total Chef TCFD-05 Deluxe; Koolatron Corporation, Taiwan) at about 40 C.

For scanning electron microscopy (SEM) of the basidiospores, fragments of the hymenophore were removed from dried basidiomata, mounted directly on aluminum stubs using carbon adhesive tabs, coated with 30 nm of gold, and examined with a scanning electron microscope (JEOL JSM-6390LV), operating at 10KeV, at the Laboratório Central de Microscopia Eletrônica of Universidade Federal de Santa Catarina (LCME-UFSC). Line drawings were traced from digital photographs. Voucher material was deposited at FLOR and ICN. Duplicates are at VIES (Thiers, continuously updated).

Sequencing and phylogenetic analyses.—DNA extraction from dried basidiomata followed Góes-Neto et al. (2005). The primer pairs ITS6-R/ITS8-F and LR0R/LR7 were used to amplify the nuc rDNA ITS1-5.8S-ITS2 (internal transcribed spacer [ITS]) and nuc 28S rDNA (28S) regions following Dentinger et al. (2010). Sequencing was performed by Macrogen Korea using the polymerase chain reaction (PCR) primers. Sequence chromatograms were manually checked, and contigs were assembled and edited in Geneious 6.1.8 (Kearse et al. 2012). Alignments were generated using MAFFT 7 (Katoh and Standley 2013), using Q-INS-i and G-INS-i algorithms for ITS and 28S, respectively. The aligned sequences were cleaned using Gblocks, allowing smaller final blocks and gap positions within the final blocks (Talavera and Castresana 2007). The resulting alignments were deposited in the TreeBASE (<http://www.treebase.org/treebase/index.html>) under accession number 21027.

Phylogenetic trees were reconstructed under two criteria, maximum likelihood (ML) and Bayesian inference (BI), from the concatenated ITS+28S alignment. The data set was subdivided into four partitions: ITS1, 5.8S, ITS2, 28S. ML was carried out with RAxML-HPC 8 (Stamatakis 2014) in the CIPRES Science Gateway (Miller et al. 2010; <http://www.phylo.org/>) using GTRGAMMA as the model of evolution (Stamatakis 2006) and 1000 bootstrap replications. BI was conducted in MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) also on the CIPRES Science Gateway using four parallel Markov chain Monte Carlo (MCMC) chains, which were allowed to run for 20 million generations, sampling trees and parameters every 1000 generations. Best-fit substitution models were set to each partition calculated by MrModeltest 2.3 (Nylander 2004). The Akaike information criterion (AIC) was chosen to select the most appropriate model of DNA substitution for each data set or data partition used in the analyses. The convergence diagnostic was calculated every 100 generations, and its critical value was set in order to automatically stop the analysis when the standard deviation of the split frequencies had reached the value defined by the stopval command (stoprule = yes stopval = 0.01). In all analyses, the first 25% of trees from each run were discarded as burn-in. Resulting trees from the two independent runs were then pooled to produce one 50% majority-rule consensus tree, and Bayesian posterior probabilities (BPPs) were generated for the resulting tree. A node was considered well supported if it showed a BPP ≥ 0.96 and/or bootstrap (BS) $\geq 80\%$. The ingroup included sequences of *Fistulinella*, *Austroboletus*, *Veloporphyrellus*, and *Mucilopilus*. *Bothia* and *Soliococcus* were used as outgroups since they are the sister group to *Austroboletoidae* (Wu et al. 2014). All phylogenetic trees were visualized using FigTree (Morariu et al. 2009).

RESULTS

Molecular analysis.—Eleven new sequences of *Fistulinella* (seven ITS and four 28S) were generated during this study. ITS BLASTn queries of the Brazilian specimens of *Fistulinella* indicate the highest matches with species of *Fistulinella* and a sequence labeled *Boletellus*, but none of the searches resulted in more than 95% similarity. In the 28S BLASTn queries, the most similar taxa were sequences labeled *Fistulinella*, *Tylopilus*, and *Xerocomus*, but none resulted in more than 94% similarity. In GenBank, only four specimens named *Fistulinella* were represented by ITS sequences and five by 28S sequences.

Additional sequences from Brazilian *Austroboletus* species were generated for the analysis in order to provide additional molecular data regarding boletoid fungi from the Neotropics. These included three ITS and five 28S sequences of *A. festivus* from the Atlantic Forest studied morphologically by Magnago and Neves (2014) and one ITS sequence from the holotype (INPA78693) of *A. rionegrensis* from Amazonia (Singer et al. 1983). All sequences analyzed in this study are included in TABLE 1.

Phylogenetic analyses.—Sixty-seven sequences were combined into a single matrix (33 ITS, 34 28S) from 47 specimens representing 28 taxa, both newly generated in this study or downloaded from

GenBank. The final concatenated ITS+28S data set resulted in a matrix of 1341 sites. The best models of nucleotide substitution estimated for each partition in the data sets were the following: SYM+G for ITS1, K80+I for 5.8S, TPM1uf+G for ITS2, and TIM1+I+G for 28S. The ML tree, with BS and BPP values on branches, is shown in FIG. 1. Both phylogenetic analyses showed that specimens of *Fistulinella* sampled from the New World grouped into one strongly supported clade (BS = 100%, BPP = 1.0). The analysis showed a robust position for *F. ruschii* (BS = 100%, BPP = 1) in the New World *Fistulinella* group and as the sister group to a well-supported clade including other New World *Fistulinella* sequences used in the analyses. In addition, the Brazilian specimen of *F. campinaranae* var.

Table 1. GenBank accession numbers and voucher numbers of sequences used for the phylogenetic analysis.

Species	Collection no.	Origin	GenBank accession No.	
			ITS	LSU
<i>Austroboletus amazonicus</i>	AMV2032*	Colombia	KF937309	KF714510
<i>Austroboletus amazonicus</i>	AMV1914	Colombia	KF937308	KF714509
<i>Austroboletus amazonicus</i>	AMV1839	Colombia	KF937307	KF714508
<i>Austroboletus festivus</i>	TH8732	Guyana	KT339224	—
<i>Austroboletus festivus</i>	ACM575	Brazil	KY886203	KY888000
<i>Austroboletus festivus</i>	ACM573	Brazil	KY886202	KY888001
<i>Austroboletus festivus</i>	CHC245	Brazil	—	KY887998
<i>Austroboletus festivus</i>	ACM564	Brazil	—	KY887999
<i>Austroboletus festivus</i>	ACM574	Brazil	—	KY888002
<i>Austroboletus fusisporus</i>	HKA575207	China	JX889719	JX889720
<i>Austroboletus gracilis</i>	N. Arnold 112/96	USA	—	DQ534624
<i>Austroboletus lacunosus</i>	PDD 83019	New Zealand	KP191804	—
<i>Austroboletus lacunosus</i>	MEL2265009	Australia	KC552015	KC552057
<i>Austroboletus niveus</i>	PDD 81219	New Zealand	KP191802	—
<i>Austroboletus niveus</i>	MEL2053830	Australia	KC552016	KC552058
<i>Austroboletus niveus</i>	PDD 105246	New Zealand	KP191801	KP191673
<i>Austroboletus novaezelandiae</i>	PDD 105213	New Zealand	KP191800	KP191672
<i>Austroboletus novaezelandiae</i>	PDD 105097	New Zealand	KP191803	KP191671
<i>Austroboletus occidentalis</i>	MEL2300518	Australia	KC552017	KC552059
<i>Austroboletus rionegrensis</i>	I. Araujo 937*	Brazil	KY886201	—
<i>Austroboletus rostrupii</i>	TH8189	Guyana	JN168683	—
<i>Austroboletus</i> sp.	KD12-250	India	KM597479	—
<i>Bothia castanella</i>	MB03-053*	USA	DQ867110	DQ867117
<i>Bothia fujianensis</i>	HKA582694	China	KM269195	KM269193
<i>Fistulinella campinaranae</i> var. <i>scrobiculata</i>	ACM484	Brazil	KY886204	KY888003
<i>Fistulinella campinaranae</i> var. <i>scrobiculata</i>	AMV1980	Guyana	—	KF714520
<i>Fistulinella campinaranae</i> var. <i>scrobiculata</i>	AMV1783	Guyana	—	KJ195892
<i>Fistulinella cinereoalba</i>	TH8471*	Guyana	KT339237	GQ477439
<i>Fistulinella gloeocarpa</i>	KM162946	Guyana	GQ981503	—
<i>Fistulinella prunicolor</i>	REH9502	Australia	—	JX889648
<i>Fistulinella ruschii</i>	ACM526*	Brazil	KY886206	KY888006
<i>Fistulinella ruschii</i>	ACM491	Brazil	KY886207	—
<i>Fistulinella ruschii</i>	MAN391	Brazil	KY886210	—
<i>Fistulinella ruschii</i>	ACM485	Brazil	KY886205	KY888004
<i>Fistulinella ruschii</i>	ACM1317	Brazil	KY886208	KY888005
<i>Fistulinella ruschii</i>	CATO105	Brazil	KY886209	—
<i>Fistulinella</i> sp.	AMV511	Colombia	KF878352	—
<i>Fistulinella</i> sp.	MEL2382828	Australia	KP012703	—
<i>Fistulinella viscida</i>	238	New Zealand	—	AF456826
<i>Mucilopilus castaneiceps</i>	HKA575045	China	—	KF112382
<i>Mucilopilus castaneiceps</i>	Tsukuba 227	China	AB289669	—
<i>Veloporphyrellus pseudovelatus</i>	HKA552673	China	—	JX984541
<i>Veloporphyrellus alpinus</i>	HKA557490	China	—	JX984537
<i>Veloporphyrellus pantoleucus</i>	Gomez 21232	Costa Rica	—	JX984547
<i>Veloporphyrellus velatus</i>	HKA563668	China	—	JX984546
<i>Veloporphyrellus conicus</i>	BZ1670	Belize	—	JX984543
<i>Soliococcus polychromus</i>	Trappe 15399	Australia	JX888459	JQ287643

Note. Collections followed by an asterisk are holotypes.

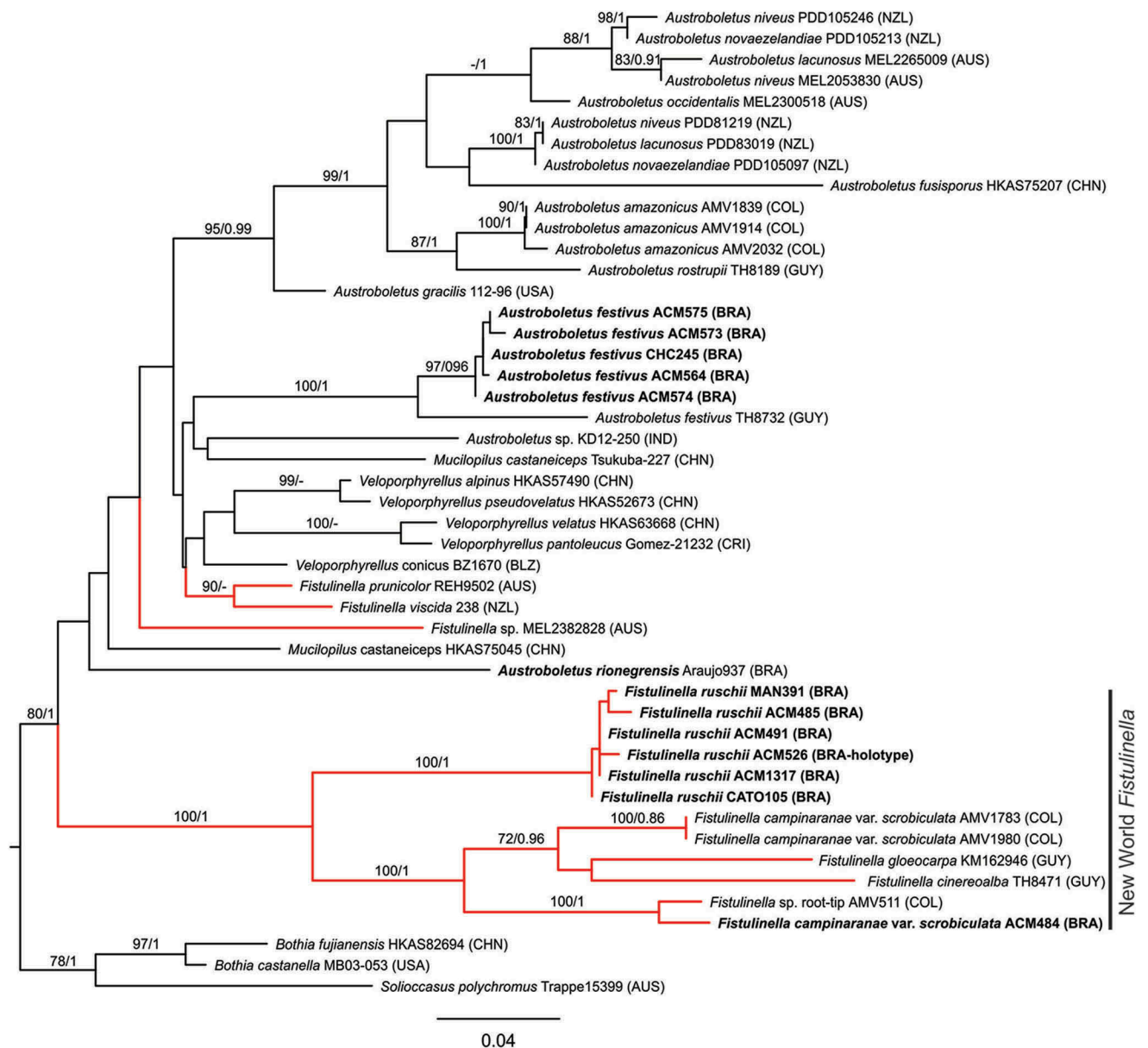


Figure 1. ML tree based on ITS and 28S sequences of selected Austroboletoidae. BS >70% and BPPs >0.90 are shown. Taxa in bold represent sequences generated in this study. Red bold lines represent *Fistulinella* specimens.

scrobiculata (ACM484) is most closely related to an ectomycorrhizal (EcM) morphotype root tip (AMV511) isolated from the tree host *Pseudomonotes tropenbosii* (Dipterocarpaceae) from Colombia (BS = 100%, BPP = 1). Sequences from four specimens of *Fistulinella* from Colombia and Guyana clustered together (BS = 72%, BPP = 0.96) apart from the Colombian root tip and Brazilian sample. The name *F. campinaranae* var. *scrobiculata* has been applied to two of the samples from Colombia, but these do not cluster with branch labeled as such from Brazil. Three Old World samples from Australasia labeled in GenBank as *F. viscida* and *F. prunicolor* clustered outside the clade

containing New World *Fistulinella* but with poor measures of branch support in the Austroboletoidae at large. *Fistulinella* is thus found to be a nonmonophyletic genus; however, the type (*F. staudtii*, described from Cameroon) has yet to be sequenced.

TAXONOMY

Fistulinella campinaranae var. *scrobiculata* Singer, Beih. Nova Hedwigia 77:148. 1983. **FIGS. 2, 4A–C**

Pileus 16–27 mm broad, parabolic when young, becoming convex when mature; grayish brown (OAC

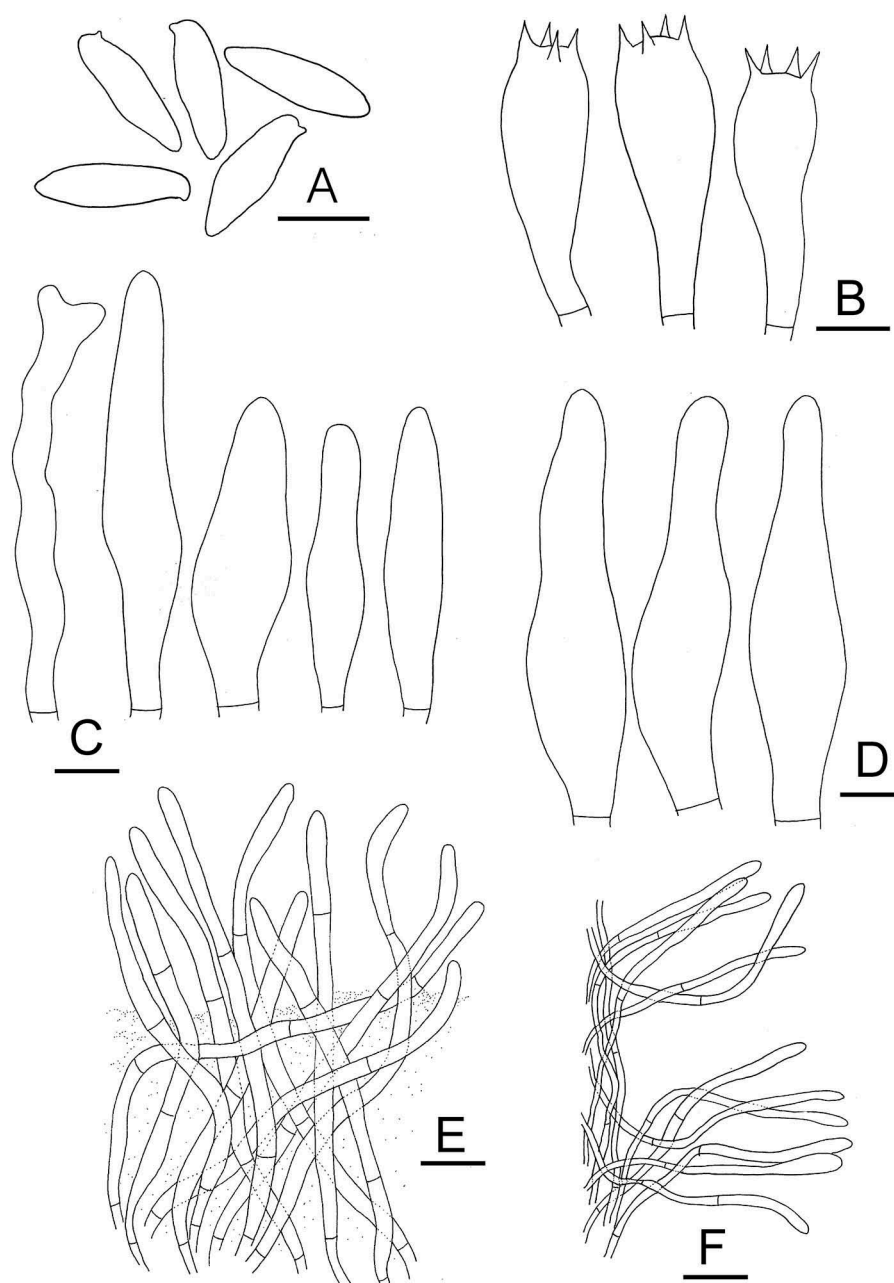


Figure 2. Anatomical features of *F. campinaranae* var. *scrobiculata* (ACM484). A. Basidiospores. B. Basidia. C. Cheilocystidia. D. Pleurocystidia. E. Upper layer of pileipellis. F. Stipitipellis. Bars: A–D = 10 μm ; E–F = 25 μm .

733) with a whitish margin; surface finely fibrillose, scrobiculate over whitish ground, margin entire, slightly viscid to almost dry; context white, unchanging. Tubes 3–6 mm long, depressed around the stipe; two pores per mm, isodiametric, whitish gray when young, pinkish gray when mature (OAC 753). Stipe 60–71 mm long, 2–3 mm wide at the apex, 3–4 mm wide at the base, central, clavate and enlarged towards the base; surface glabrous, slightly viscid whitish but browning where touched; context white, unchanging. Spore print pinkish brown. Odor and Taste not observed.

Basidiospores 14–18 \times 4–6 μm (Qr = 3.0–3.5; Qm = 3.23; n/s = 30/1), subfusiform to fusiform, hyaline to light yellow, weakly dextrinoid, multiguttulate with elongate to circular oil drops, smooth, thin-walled. Basidia 31–37 \times 10–12 μm , narrowly clavate, thin-walled, hyaline, inamyloid, 4-spored, sterigmata 4–5 μm long. Pleurocystidia 43–76 \times 10–17 μm , subventricose, lageniform or nearly fusiform, hyaline, inamyloid, smooth, thin-walled. Cheilocystidia with variable shapes: cylindrical, 24–42 \times 3–4 μm , some with bifurcate apices; otherwise fusiform, 46–52 \times 7–8 μm ; or elongate cylindrical septate, 48–73 \times 2–4 μm wide, these more frequent in young basidiomata.

Hymenophoral trama boletoide, strongly divergent, mediostratum of many narrow parallel hyphae, these 2–4 µm broad, light yellow; lateral stratum lighter in color, gelatinized, hyphae 2–6 µm wide. Pileipellis an ixotrichoderm, some hyphae with ochre content in H₂O, dextrinoid, 5–7 µm wide, terminal cells with a rounded apex, regularly septate. Pileus trama strongly interwoven, hyphae 6–9 µm wide, light yellow. Stipitipellis in two layers, outer layer with clusters of slightly interwoven projecting cylindrical hyphae, hyphae 4–6 µm wide, septate, with light yellow intracellular content in H₂O; lower layer with thin hyaline hyphae, subparallel to loosely woven, immersed in a gelatinized matrix, no caulocystidia or caulobasidia observed. Stipe trama composed of vertically arranged hyphae, 6–10 µm wide. Clamp connections absent in all tissues.

Habit, habitat, and distribution: solitary or gregarious. In Brazil, known from Amazonas in the Amazon forest on the ground in humus-sandy or decayed wood (Singer et al. 1983) and from Bahia in the Atlantic Forest (present study) on the ground close to leguminous trees (Fabaceae). Also, recorded from Colombia, frequently in the Amazon Forest area dominated by *Pseudomonotes tropenbosii* (Vasco-Palacios et al. 2014).

Material examined: BRAZIL. BAHIA: Serra Grande, Parque Estadual da Serra do Conduru, 30 Nov 2012, A. C. Magnago 484 (FLOR 51608); AMAZONAS: Estrada Manaus-Caracará, km 45, 25 Apr 1980, R. Singer B12131 (**paratype** INPA 102043); AMAZONAS: Estrada Manaus-Caracará, km 45, 12 Feb 1979, R. Singer B11491 (**paratype** INPA 106001).

Additional specimens examined: GUYANA. REGION 8 POTARO-SIPARUNI: *Fistulinella cinereoalba*, Pakaraima Mountains, Upper Potaro River Basin, vicinity of Potaro base camp, 17 Jun 2002, Henkel 8471 (**isotype** NY 1193857).

Notes: Singer et al. (1983) separated *Fistulinella campinaranae* into two varieties according to the surface texture of the pileus: var. *scrobiculata* with a scrobiculate pileus and var. *campinaranae* with a glabrous pileus. Other minor characteristics were also observed in var. *scrobiculata*; however, Singer et al. (1983) questioned how reliable these were given they only had a few specimens and that both varieties occurred in the same vegetation types (*campinarana* and *campina*). In descriptions by Singer et al., the lignicolous habit was observed for var. *campinaranae*, but occurrences on the ground on sandy humus or rotten wood for var. *scrobiculata*. Our collection from the Atlantic Forest matches best the description of var. *scrobiculata* due to the finely rugulose scrobiculate pileus surface and terricolous habit.

Fistulinella campinaranae var. *scrobiculata* is now known from the Brazilian Amazon forest, with an extension into the coastal Atlantic Forest. This variety

is morphologically similar to the Neotropical species *F. cinereoalba* and *F. gloeocarpa*, but it differs from these by the presence of cylindrical to aciculate pleurocystidia in *F. cinereoalba* and a browning reaction on the hymenophore when pressed, a feature not seen in *F. campinaranae* var. *scrobiculata*. *Fistulinella gloeocarpa* can also be distinguished from the other taxa by the presence of ampulaceous pleurocystidia and a repent ixocutis comprising a thick glutinous layer, a feature absent in *F. campinaranae* var. *scrobiculata*.

Both paratype specimens of *F. campinaranae* var. *scrobiculata* deposited at INPA (INPA 106001, INPA 102043) are in poor condition and comprise just a few fragments, making it difficult to study the morphology and to extract DNA of sufficient quality for molecular analyses. The holotype of *F. campinaranae* var. *campinaranae* (Singer B10109, INPA) was requested for examination but could not be located.

The specimens of *F. campinaranae* var. *scrobiculata* from the Brazilian Atlantic Forest and from the Colombian Amazon morphologically fit the description of the species provided by Singer et al. (1983). However, based on the molecular results presented here, it is possible that there are cryptic species hidden under this name. More collections and DNA sequencing from both *F. campinaranae* var. *scrobiculata* and var. *campinaranae* are necessary to better understand the relationships among the specimens from the Atlantic Forest and the Amazon.

Fistulinella ruschii A.C. Magnago, sp. nov. FIGS. 3, 4D–H

Mycobank MB820546

Typification: BRAZIL. ESPÍRITO SANTO: Santa Teresa, Reserva Biológica Augusto Ruschi, Trilha Casa da Pedra (19°54'19.60"S, 40°34'8.20"W), 5 Dec 2012, A. C. Magnago 526 (**holotype** FLOR 51611). GenBank: ITS = KY886206; 28S = KY888006.

Etymology: (Latin) in honor of the Brazilian naturalist Augusto Ruschi.

Diagnosis: Differs from other known Neotropical *Fistulinella* by the presence of broadly cylindrical septate pleurocystidia.

Pileus 10–40 mm broad, parabolic when young to convex or depressed, chestnut brown (OAC 657, 659) to orange-brown (OAC 645, 646); surface velutinous, dry, becoming slightly viscid, margin entire, fibrillose when mature, fibrils dark orange (OAC 629, 663) against a light orange background (OAC 652, 632); context whitish, unchanging. Tubes 4–7 mm long, strongly depressed to almost free around the stipe, whitish to cream pinkish (OAC 676, 620); pores white when young, light pink when mature (OAC 620), 1.5–2 per

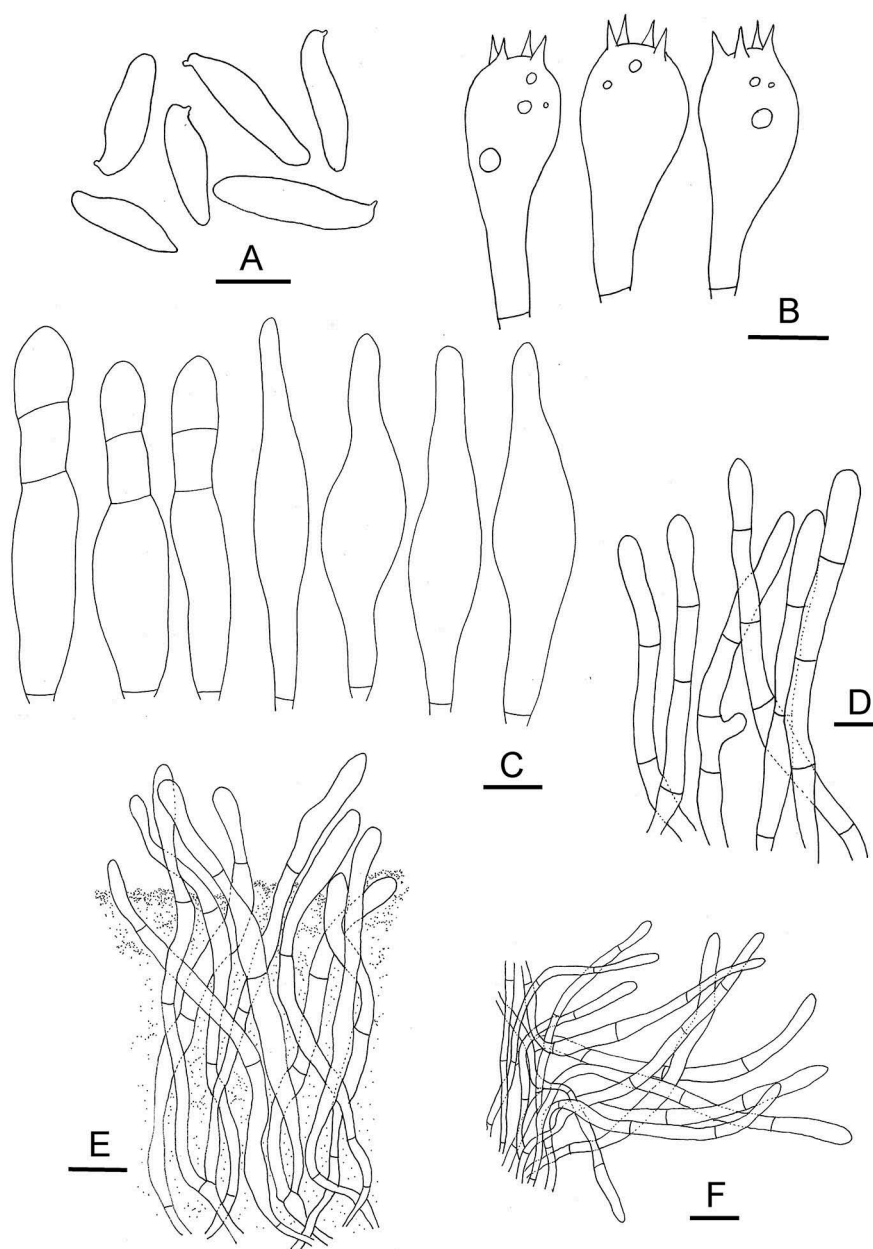


Figure 3. Anatomical features of *F. ruschii* (ACM526, holotype). A. Basidiospores. B. Basidia. C. Pleurocystidia of two types. D. Cheilocystidia. E. Upper layer of pileipellis. F. Stipitipellis. Bars: A–D = 10 μ m; E–F = 25 μ m.

mm, isodiametric. Stipe 42–80 mm long, 5–7 mm wide at the apex, 10–15 mm at base, subclavate to fusiform, surface slightly viscid, cream pinkish (OAC 655) covered by a whitish pruina, browning where touched, base with white rhizomorphs; context white, unchanging. Macrochemical reactions: NH_4OH 10% on pileus surface becoming reddish orange, on stipe surface becoming yellow. Spore print pinkish brown (OAC 605). Odor and Taste not observed.

Basidiospores 14–18(–22) \times 4–5 μ m ($Q = 3.44$; $Q_r = 3.0$ –4.4; $Q_m = 3.44$; $n/s = 30/6$), subfusiform, fusiform to slightly sigmoid, with suprahilar depression; hyaline to light pinkish, inamyloid, guttulate, smooth. Basidia

26–34 \times 10–14 μ m, broadly clavate, thin-walled, hyaline, inamyloid, 4-spored, sterigmata 2–3 μ m long. Pleurocystidia of two kinds: lanceolate to ventricose-rostrate, 41–78 \times 6–14 μ m, hyaline, inamyloid, sometimes slightly wrinkled in the middle portion, very frequent; or broadly cylindrical, septate (1–3) on the upper two thirds, 48–70 \times 7–12 μ m, projecting 26–54 μ m beyond hymenium, hyaline, inamyloid, scattered. Cheilocystidia cylindrical, septate, more frequent in young basidiomata, 4–6 μ m wide, projecting 75–86 μ m beyond hymenium. Hymenophoral trama boletoid, strongly divergent, mediostratum parallel, hyphae 3–5 μ m wide, light yellow, inamyloid; lateral stratum

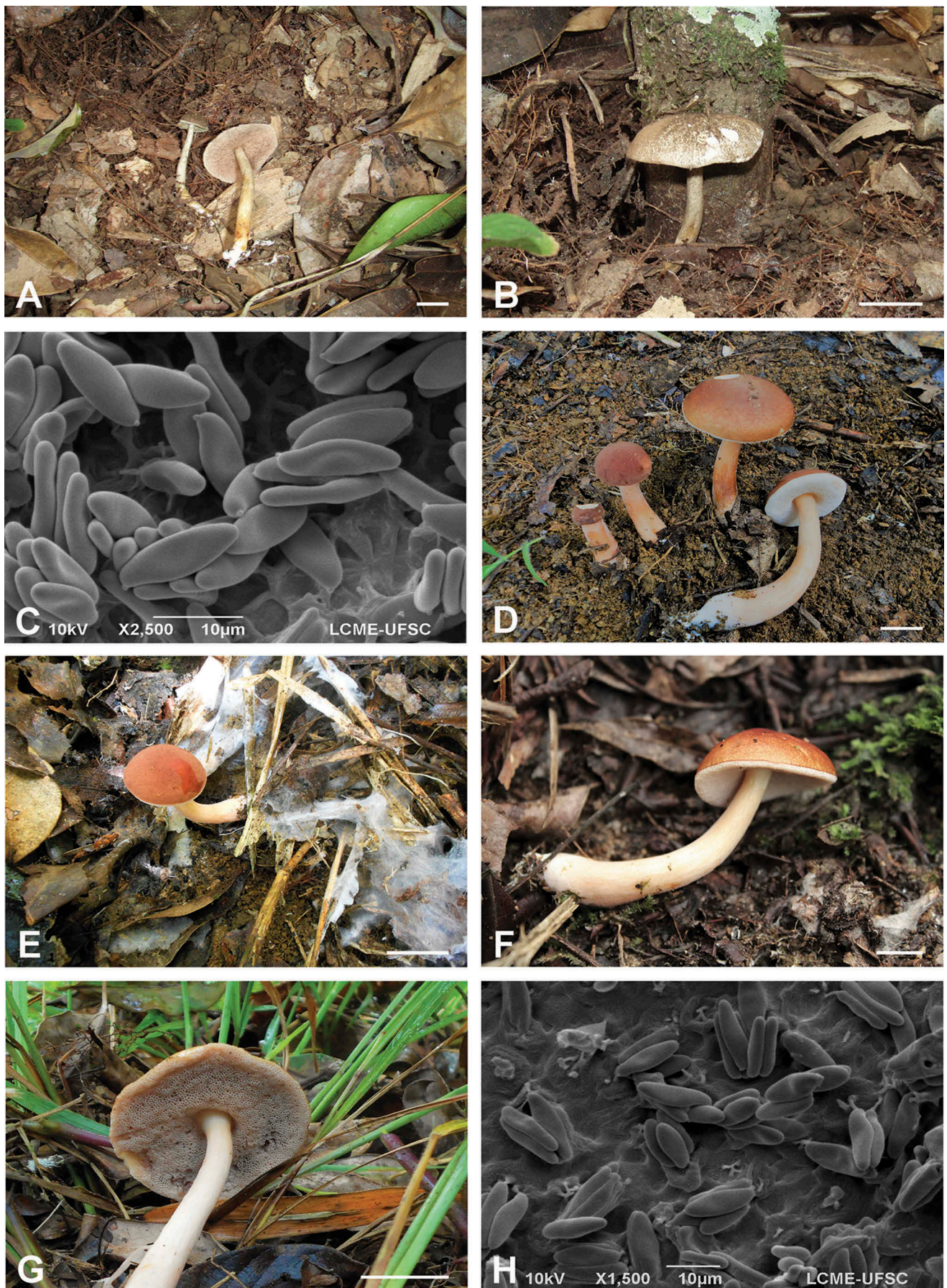


Figure 4. Basidiomata and SEM images of basidiospores. A–C. *Fistulinella campinaranae* var. *scrobiculata* (ACM484). D–H. *Fistulinella ruschii*. D, E, F. Holotype (ACM526). G. Paratype (ACM491). H. Paratype (ACM1317). Bars: A–B, D–G = 10 mm.

divergent, immersed in a gelatinized matrix, hyphae 4–7 μm wide. Pileipellis in three layers immersed in a gelatinized matrix: the suprapellis an ixotrichoderm or ixotrichodermal palisade, yellowish brown, inamyloid, regularly septate; terminal cells 4–11 μm wide with rounded apices; mediopellis interwoven to suberect, hyphae 2–4 μm wide, pale yellow; subpellis of repent hyphae 2–3 μm wide. Pileus trama interwoven to subparallel, hyphae 3–11 μm wide, light yellow. Stipitipellis in two layers, the outer layer with tufts of erect to suberect hyaline cylindrical elements, septate, 4–10 μm wide, light yellow, caulobasidia present; the lower layer with subparallel hyaline hyphae, 2–5 μm wide, immersed in a gelatinized matrix. Stipe trama parallel, hyphae 3–11 μm wide, hyaline. Clamp connections absent.

Other specimens examined: BRAZIL. ESPÍRITO SANTO: Santa Teresa, Reserva Biológica Augusto Ruschi, Trilha Casa da Pedra, (19°54'19.62"S, 40°34'8.21"W) 4 Dec 2012, A.C. Magnago 491 (FLOR 51610), 14 Dec 2016, A.C. Magnago 1317 (ICN 192819); PARAÍBA: João Pessoa, Universidade Federal da Paraíba, Mata do Biotério (7°08'18"S, 34°50'38"W), 12 Dec 2009, M.A. Neves 391 (ICN 192820); BAHIA: Serra Grande, Parque Estadual da Serra do Conduru, 30 Nov 2012, A.C. Magnago 485 (FLOR 51609); SANTA CATARINA: Florianópolis, Saco Grande, Unidade de Conservação do Desterro (27°31'51.6"S, 48°30'44.1"W), 15 Jan 2015, C. Oliveira 105 (ICN 192818).

Habit, habitat, and distribution: Solitary or growing gregarious on the ground on soil near leguminous trees (Fabaceae). In Brazil, recorded in four areas ca. 1000 km from each other along the coastal Atlantic Forest.

Notes: *Fistulinella ruschii* is the only Neotropical species of the genus that has septate pleurocystidia. *Fistulinella campinaranae* var. *campinaranae* differs by its grayish brown scrobiculate pileus and subventricose, lageniform, or fusiform pleurocystidia. *Fistulinella jamaicensis* is described with smaller basidiospores (9.5–12 \times 4.8–5.2 μm). *Fistulinella mexicana* occurs on wood, has a stipe base that is disciform covered by a transparent glutinous layer, and has smaller basidiospores (11–14.2 \times 4.3–5.3 μm) compared with *F. ruschii*. *Fistulinella venezuelae* has a pileus with an obtuse disc that is slightly umbonate and has much longer basidiospores (up to 21.5 μm) than *F. ruschii*. The pleurocystidia of *F. venezuelae* are described as elongate, fusoid, or ampullaceous.

Fistulinella mollis, which occurs widely in eucalyptus forests in Australia (trees also very abundant in Brazil), is morphologically similar to *F. ruschii* because of the orange-brown pileus and white to pink hymenophore, but it differs from *F. ruschii* by the viscid to glutinous pileus and the presence of nonseptate pleurocystidia.

Fistulinella ruschii is also similar to the Australian *F. prunicolor*; however, the latter has a dark brown to reddish to plum pileus that is viscid when wet, soft-textured white context, vinaceous pink tubes, and a white stipe with yellow stains (Pegler and Young 1981; Watling and Li 1999).

DISCUSSION

The phylogenetic analyses performed during this study corroborate the morphological placement of the new species *F. ruschii* in *Fistulinella*. However, the genus is not monophyletic, as New World samples cluster apart from Old World samples. The analyses also showed the relation of *F. campinaranae* var. *scrobiculata* with other Neotropical species such as *F. cinereoalba* and *F. gloeocarpa* from Guyana and a Colombian *F. campinaranae* var. *scrobiculata* as observed by Vasco-Palacios et al. (2014).

Most species of *Fistulinella* from the Neotropics are known from only the type specimen or few collections. In several cases, the specimens are poorly preserved, and the taxon should be re-collected to understand better the morphology and collection of molecular data. Unfortunately, the Austroboletoidae is not well represented in GenBank (Nuhn et al. 2013; Wu et al. 2014). Therefore, a reliable phylogenetic inference of *Fistulinella*, including combined analyses, is still difficult to obtain. No sequences of boletoid specimens from Brazil were available in GenBank, and there are only a few sequences from other Neotropical countries. Additional studies including more Neotropical boletoid sequences and type species should be carried out to assess the phylogenetic structure of these groups within Austroboletoidae.

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