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**Article**

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# Two new *Tylopilus* species (Boletaceae) from Northeastern Atlantic Forest, Brazil

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## Abstract

The Atlantic Forest of Brazil harbors a great diversity of boletoid fungi, many of which remain to be documented and described. Two distinct taxa of *Tylopilus* from Northeastern Atlantic Forest, *T. dunensis* and *T. pygmaeus*, are proposed as new based on evidence from both morphological and molecular data. We provide detailed macro- and microscopic descriptions of each species including scanning electron micrographs of the basidiospores.

**Keywords:** Boletales, ITS, LSU, Neotropics, Taxonomy

## Introduction

*Tylopilus* P. Karst (1881: 16) is one of the largest and most widespread ectomycorrhizal genera in the Boletaceae. It is known from Africa, Australia, Asia, Europe and the Americas. About twenty species have been described in the Neotropics, from Colombia (Halling 1989), Belize (Halling *et al*. 2008), Costa Rica (Singer *et al*. 1983, Wolfe & Bougher 1993, Amtoft *et al*. 2002, Halling *et al*. 2008, Osmundson & Halling 2010), Guyana (Henkel 1999, 2001, Fulgenzi *et al*. 2007), Honduras, Mexico, Nicaragua (Singer *et al*. 1983), and Venezuela (Singer & Digilo 1960). Four species and two varieties of *Tylopilus* are known for Brazil: *T. acutesquamosus* Singer (1983: 117), *T. arenarius* Singer (1978: 423), *T. potamogeton* Singer (1978: 433), *Tylopilus aquarius* var. *aquarius* (Singer) Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo from Amazon Forest, and *Tylopilus aquarius* var. *megistus* Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo from Atlantic Forest (Singer 1978, Singer *et al*. 1983, Barbosa-Silva *et al.* 2017).

The traditional morphological concept of *Tylopilus* s. l. includes boletoid fungi with a dry, glabrous to subtomentose pileus, pore surface that is usually whitish when young becoming pinkish to pinkish brown at maturity, and a solid, glabrous, pruinose or reticulate stipe that lacks a partial veil or glandular dots. The spores are pink, pinkish brown to vinaceous in mass (never with olivaceous hues), smooth, and fusoid to ovoid-phaseoliform. The hymenial cystidia are usually present as pseudocystidia, and clamp connections are absent (Karsten 1881, Singer 1986, Smith & Thiers 1971).

However, several recent molecular phylogenetic analyses have shown that the taxonomy of boletoid fungi is still elusive and generic circumscriptions are in a state of flux (Binder & Hibbett 2006, Dentinger *et al.* 2010a, Nunh *et al.* 2013, Wu *et al.* 2014). *Tylopilus* s. l. is considered to be a polyphyletic genus (Dentinger *et al.* 2010a, Nuhn *et al.* 2013, Wu *et al.* 2014) and many species that were traditionally classified in *Tylopilus* have been recently accommodated into new genera such as *Zangia* Yan C. Li et Zhu L. Yang (2011:129) (Li *et al.* 2011), *Australopilus* Halling & Fechner (2012: 422) and *Harrya* Halling, Nuhn & Osmundson (2012: 422) (Halling *et al.* 2012).

Recent fieldwork in the Northeastern Atlantic Forest of Brazil resulted in the collection of several tylopiloid boletes with combinations of features not reported before. Here we provide morphological and molecular phylogenetic evidence that these specimens represent two new species best classified in *Tylopilus* s. l. Molecular data for the recently described *T. aquarius* var. *megistus* are also provided.

## Material and Methods

*Collection sites and Morphology*

Collections were made between 2008 and 2013 during the rainy season in the following Brazilian Northeastern Atlantic Forest localities: Reserva Biológica Guaribas, Paraíba; Parque Estadual Dunas do Natal, Rio Grande do Norte; and Parque Estadual da Serra do Conduru, Bahia.

Macroscopic features were described from fresh basidiomes. Color codes (e.g. OAC 640) were based on the Online Auction Color Chart (Kramer 2004). Micromorphological features were examined with an Olympus CX21 microscope and the use of descriptive terms followed Largent *et al.* (1977). Fungal tissue was rehydrated and mounted in water, 3% KOH, Melzer's solution, or Congo Red. At least twenty micro structures of each structure type were measured for each collection examined. Qm refers to the mean length/width ratio of basidiospores. For scanning electron microscopy (SEM) of the basidiospores, fragments of the hymenophore were removed from dried basidiomes, 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 *Laboratório Central de Microscopia Eletrônica* of *Universidade Federal de Santa Catarina* (LCME-UFSC). Line drawings were traced from digital photographs. Voucher specimens are deposited at FLOR and HUEFS (Thiers, continuously updated).

DNA extraction, PCR amplification and sequencing

DNA extraction from dried basidiomes follows Góes-Neto *et al*. (2005). The primer pairs ITS6-R/ITS8-F and LR0R/LR7 were used to amplify the nuclear ribosomal internal transcribed spacers (ITS; ITS1-5.8S-ITS2) and the nuclear ribosomal large subunit (LSU, 28S) regions following Dentinger *et al.* (2010b) and Vilgalys & Hester (1990), respectively. PCR products were purified using PEG (polyethylene glycol) (Sambrook *et al.* 1989). Sequencing was performed with a BigDye Terminator v3.1 Cycle Sequencing Kit following the manufacturer's procedure, using the same primers cited above. Some specimens were processed from fresh tissue blotted in FTA Plant Cards as described in Dentinger *et al.* (2010b). Sequence chromatograms were manually checked and edited in Geneious 6.1.8 (Kearse *et al*. 2012).

*Sequence alignment and phylogenetic analysis*

Newly generated DNA sequences were combined with 23 ITS and 55 LSU sequences belonging to *Tylopilus* s. l. downloaded from GenBank, with sequences of *Bothia castanella* (Peck) Halling, T.J. Baroni & Manfr. Binder (2007: 311) used as an outgroup (Table 1). Alignments were generated using MAFFT v. 7 (Katoh & Standley 2013), following the Q-INS–i and G-INS-i criteria (for ITS and LSU, respectively), and then manually inspected and adjusted, as necessary, with MEGA 6 (Tamura *et al*. 2013). The indels present in both datasets were recoded as binary characters according to the 'simple indel coding method' (SIC, Simmons and Ochoterena 2000) as implemented in SeqState (Müller 2005). The resulting binary characters were joined as distinct partitions to the final matrices. The final alignments (as well the final topologies) are deposited in TreeBASE (http://www.treebase.org/treebase/index.html) under ID S21078. Maximum Likelihood (ML) and Bayesian Inference (BI) criteria were applied to the datasets, which were divided in three partitions to the ITS (ITS1+ITS2, 5.8S and indels) and two to the LSU (LSU and indels).

For ML analysis, the best fit model of nucleotide evolution to each partition was obtained according to BIC (Bayesian Information Criterion), as implemented in the software jModelTest 2.1.6 (Guindon & Gascuel 2003, Darriba *et al*. 2012). ML analysis was carried out with RAxML v. 8.2.4 (Stamatakis, 2014), available in the CIPRES science gateway (Miller *et al.* 2010, http://www.phylo.org/), using GTRGAMMA as the model of evolution (Stamatakis 2006), with branch support estimated using nonparametric bootstrapping (BS) by implementing the rapid bootstrap option in RAxML (command–f a) with a random starting tree and auto bootstopping using MRE.

BI was performed using MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) as implemented on the CIPRES Science Gateway 3.1 (Miller *et al.* 2010), with four parallel MCMC chains run for 10 million generations, sampling every 1000 generations. Four rate categories were used to approximate the gamma distribution parameter. Of all trees sampled, 20% were discarded as burn-in and checked by the convergence criterion (average standard deviation of split frequencies <0.01) with Tracer v.1.6 (Rambaut *et al.* 2014), while the remaining were used to reconstruct a 50% majority-rule consensus tree and to estimate Bayesian posterior probabilities (BPP) of the branches. A branch was considered to be strongly supported if it showed a BPP ≥ 0.95 and/or BS ≥ 90%, while moderate support was considered BPP ≥ 0.90 and/or BS ≥ 70%. *Bothia castanella* was defined as outgroup, based on previous papers (Nunh *et al.* 2013, Wu *et al*. 2014).

**TABLE 1.** Taxa, vouchers and Genbank accession numbers used in the molecular analyses.

| Species | Collection No. | Origin | GenBank accesion No. | |
|---|---|---|---|---|
| | | | ITS | LSU |
| *Bothia castellanea* | MB03-53 | USA | DQ867110 | DQ867117 |
| *Tylopilus aff. balloui* | HKAS59700 | China | □ | KF112458 |
| *Tylopilus aff. rigens* | HKAS53388 | China | □ | KF112405 |
| ***Tylopilus aquarius* var. *megistus*** | **ACM297** | **Brazil** | **MF113422** | **MF113430** |
| ***Tylopilus aquarius* var. *megistus*** | **MAN460** | **Brazil** | **MF113423** | **MF113431** |
| *Tylopilus alboater* | TH6941 | USA | □ | AY612832 |
| *Tylopilus atronicotianus* | Both s.n. | USA | EU685114 | EU685110 |
| *Tylopilus atronicotianus* | snWN | USA | □ | KF030293 |
| *Tylopilus badiceps* | 173/97 | USA | □ | DQ534628 |
| *Tylopilus badiceps* | MB03-52 | USA | □ | KF030336 |
| *Tylopilus badiceps* | 78206 | USA | □ | KF030335 |
| *Tylopilus badiceps* | NCJ20 | USA | □ | AY612833 |
| *Tylopilus balloui* | 2D7 | Japan | AB973758 | □ |
| *Tylopilus balloui* | 2D6 | Japan | AB973757 | □ |
| *Tylopilus balloui* | TH8409 | Guyana | □ | HQ161873 |
| *Tylopilus balloui* | TH8593 | Guyana | □ | HQ161872 |
| *Tylopilus balloui* | TWO1121 | Australia | □ | EU430743 |
| *Tylopilus balloui* | TWO1117 | Australia | □ | EU430741 |
| *Tylopilus balloui* | TWO1122 | Australia | □ | EU430742 |
| *Tylopilus balloui* | TWO1198 | Thaliand | □ | EU430740 |
| *Tylopilus balloui* | TWO1132 | Australia | □ | EU430739 |
| *Tylopilus balloui* | TWO1105 | Australia | □ | EU430738 |
| *Tylopilus balloui* | TWO1030 | USA | □ | EU430737 |
| *Tylopilus balloui* | REH8526 | Belize | □ | EU430736 |
| *Tylopilus balloui* | REH8521 | Belize | □ | EU430735 |
| *Tylopilus balloui* | REH8292 | USA | □ | EU430734 |
| *Tylopilus balloui* | REH9467 | Australia | □ | JX889676 |
| *Tylopilus balloui* | TH6385 | Guyana | □ | AY612823 |
| *Tylopilus balloui* | FMNH1073250 | Mexico | □ | EU430733 |
| ***Tylopilus dunensis*** | **MAN216** | **Brazil** | **MF113418** | □ |
| ***Tylopilus dunensis*** | **MAN218** | **Brazil** | **MF113419** | **MF113428** |
| ***Tylopilus dunensis*** | **MAN281** | **Brazil** | **MF113420** | □ |
| *Tylopilus exiguus* | TH9549 | Guyana | KT339205 | KT339205 |
| *Tylopilus exiguus* | TH8929 | Guyana | JN168776 | □ |
| *Tylopilus felleus* | JMP0093 | USA | EU819449 | □ |
| *Tylopilus felleus* | 17516 | Italy | JF908787 | □ |
| *Tylopilus felleus* | HA33 | Latvia | KR019864 | □ |
| *Tylopilus felleus* | KHL8542 | Sweden | □ | AY586723 |
| *Tylopilus felleus* | HKAS54926 | Germany | □ | KF112411 |
| *Tylopilus felleus* | Tf1 | Germany | □ | AF139710 |
| *Tylopilus felleus* | HKAS55832 | China | □ | HQ326934 |
| *Tylopilus ferrugineus* | 210-97 | USA | □ | AF139711 |
| *Tylopilus indecisus* | 98/98 | USA | □ | AF456820 |
| *Tylopilus intermedius* | BD277 | USA | □ | HQ161875 |
| *Tylopilus leucomycelinus* | 18463 | Guatemala | JF908789 | □ |
| *Tylopilus microsporus* | HMAS:84730 | China | KM975485 | KM975494 |
| *Tylopilus microsporus* | HKAS59661 | China | □ | KF112450 |
| *Tylopilus neofelleus* | YT20090720 | Japan | KM975489 | KM975497 |
| *Tylopilus neofelleus* | MG475a | China | KM975486 | □ |
| *Tylopilus neofelleus* | YT20121007 | Japan | □ | KM975496 |
| *Tylopilus neofelleus* | YT20120811 | Japan | □ | KM975495 |
| *Tylopilus neofelleus* | HKAS50319 | China | □ | HQ326936 |
| *Tylopilus oradivensis* | REH8187 | Costa Rica | □ | EU430732 |
| *Tylopilus oradivensis* | REH8087 | Costa Rica | □ | EU430731 |
| *Tylopilus orsonianus* | TH8926 | Guyana | JN168777 | □ |
| *Tylopilus otsuensis* | HKAS53401 | China | □ | KF112449 |

*...continued on the next page*

**TABLE 1.** (Continued)

| Species | Collection No. | Origin | GenBank accesion No. | |
|---|---|---|---|---|
| | | | ITS | LSU |
| *Tylopilus pakaraimensis* | TH8965 | Guyana | JN168778 | □ |
| *Tylopilus pernanus* | 8061 | Indonesia | □ | JX889644 |
| *Tylopilus pernanus* | 8066 | Indonesia | □ | JX889645 |
| *Tylopilus plumbeoviolaceoides* | GDGM:32630 | China | □ | □ |
| *Tylopilus plumbeoviolaceoides* | CHU25 | China | DQ407261 | □ |
| *Tylopilus plumbeoviolaceoides* | GDGM:42624 | China | □ | KM975498 |
| *Tylopilus plumbeoviolaceoides* | HKAS50210 | China | □ | HQ326937 |
| *Tylopilus porphyrosporus* | GO-2009-237 | Mexico | KC152268 | □ |
| *Tylopilus porphyrosporus* | 17898 | Italy | JF908788 | □ |
| *Tylopilus porphyrosporus* | HKAS76671 | China | □ | KF112482 |
| *Tylopilus potamogeton var. irengensis* | TH8801 | Guyana | JN168779 | JN168779 |
| ***Tylopilus pygmaeus*** | **ACM486** | **Brazil** | **MF113421** | **MF113429** |
| *Tylopilus rhoadsiae* | RV98-261 | USA | □ | AY612836 |
| *Tylopilus rubrobrunneus* | 1504-Q-6072 | Canada | KM248939 | □ |
| *Tylopilus rubrobrunneus* | BD329 | USA | □ | HQ161876 |
| *Tylopilus rubrobrunneus* | 152/98 | USA | □ | DQ534629 |
| *Tylopilus rufonigricans* | TH8925 | Guyana | KC155380 | □ |
| *Tylopilus rufonigricans* | TH6376 | Guyana | □ | AY612835 |
| ***Tylopilus sp*** | **MAN217** | **Brazil** | **MF113424** | **MF113432** |
| ***Tylopilus sp*** | **MAN282** | **Brazil** | **MF113425** | □ |
| ***Tylopilus sp*** | **MAN288** | **Brazil** | **MF113426** | □ |
| ***Tylopilus sp*** | **MAN215** | **Brazil** | **MF113427** | □ |
| *Tylopilus sp* | TH9198 | Guyana | KT339204 | KT339204 |
| *Tylopilus tabacinus* | HN2295 | USA | □ | AY612837 |
| *Tylopilus variobrunneus* | snHor02 | USA | □ | KF030316 |
| *Tylopilus variobrunneus* | 9306tv | USA | □ | KF030315 |
| *Tylopilus vinaceipallidus* | TH8859 | Guyana | JN168780 | □ |
| *Tylopilus violatinctus* | HKAS50208 | China | □ | KF112472 |
| *Tylopilus violatinctus* | HKAS50279 | China | □ | HQ326935 |

## Results

*Molecular analysis*

Fifteen new sequences of *Tylopilus* s. l. (10 ITS, 5 LSU) were generated during this study. The ITS dataset resulted in an aligned matrix of 862 bp (including gaps). For this dataset, the best fit models of nucleotide substitution estimated for each partition were TPM1uf+I+G (ITS1, ITS2) and TrNef+G (5.8S). The LSU dataset resulted in an aligned matrix of 933 bp (including gaps). The TrN+I+G model was chosen as the best fit model of nucleotide substitution implemented in MrBayes. For the ITS dataset, automatic bootstopping terminated after 300 pseudoreplicates. Both ML and Bayesian analyses resulted in very similar topologies, either for the ITS and LSU. The respective ML trees showing BS and BPP values on branches are shown in Figs. 1 and 2.

In the ITS analysis, the sequence of *Tylopilus pygmaeus* sp. nov. is closely related to an unidentified *Tylopilus* (TH9198) from Guyana (BS=80, BPP=0.94), and three sequences of *Tylopilus dunensis* sp. nov. clustered in a well-supported clade (BS=100, BPP=1), close to an unidentified (and possibly yet undescribed) *Tylopilus* species from Brazil (MAN215) (Fig. 1). In the LSU analysis, *T. pygmaeus* is closely related (BS=90, BPP=1) to an undescribed *Tylopilus* species (MAN217), together forming a clade sister to *T. dunensis* (Fig. 2).

Two sequences representing *Tylopilus aquarius* var. *megistus* clustered in the ITS analysis in a well-supported clade (BS=100, BPP=1), sister to a sequence identified as *T. potamogeton* var. *irengensis* T.W. Henkel (1999: 656) (TH8801) from Guyana. This clade (BS=94, BPP=1), represents sect. Potamogetones sensu Singer, including species with pileus and stipe more or less tomentose, not reticulate, short basidiospores, a bluing $NH_4OH$ reaction, and bitter taste (Singer *et al.* 1983). In the LSU analyses, the two sequences of *T. aquarius* var. *megistus* clustered in a well-supported clade (BS=100, BPP=1), embedded within a poorly supported clade composed of several species of *Tylopilus* from the Paleotropics and Neotropics (Fig. 2).

The molecular phylogenies presented in Figs 1-2 provide strong evidence that, alongwith our morphological studies, indicate *T. dunensis* and *T. pygmaeus* are distinct taxa that should be recognized at the species level.

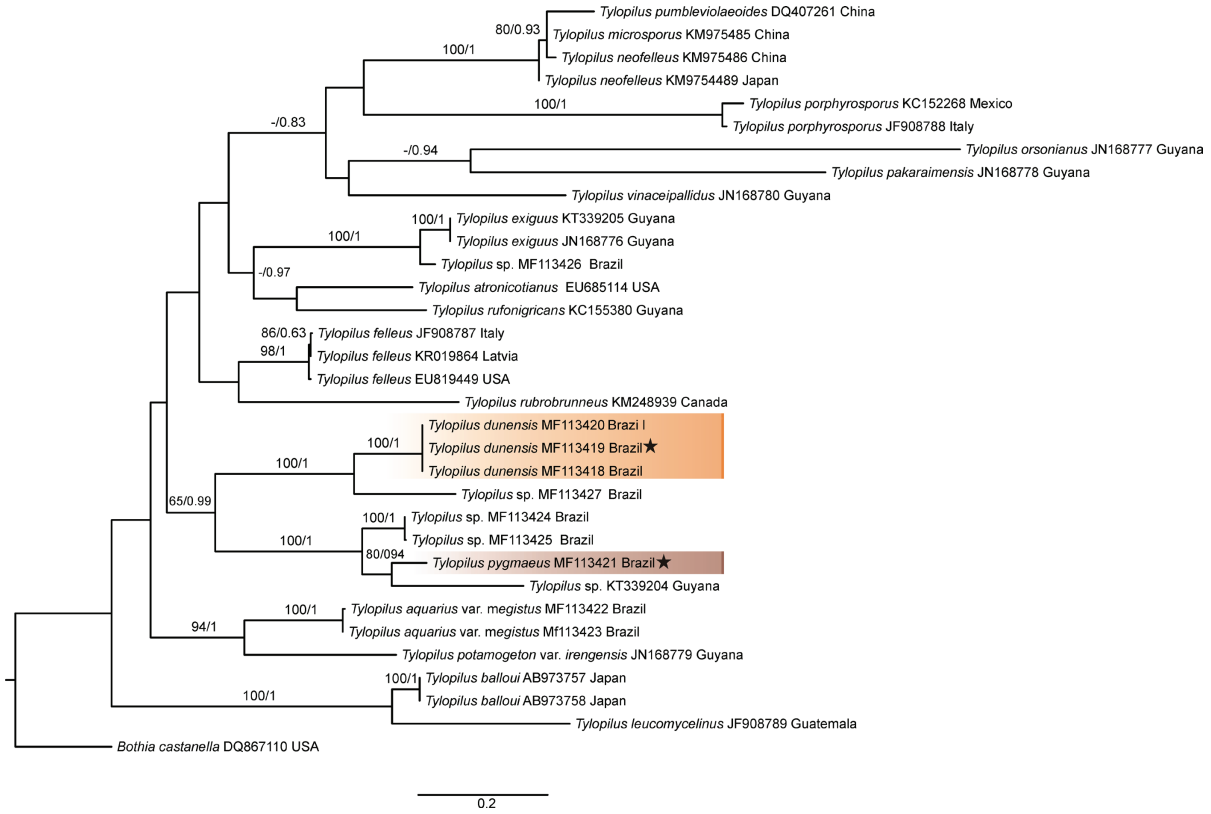


**FIGURE 1.** Maximum likelihood (ML) tree of *Tylopilus* from dataset of 33 ITS sequences rooted with the outgroup (*Bothia castanella*). Bayesian posterior probability above 0.7 and nonparametric bootstrap values above 50% are shown.

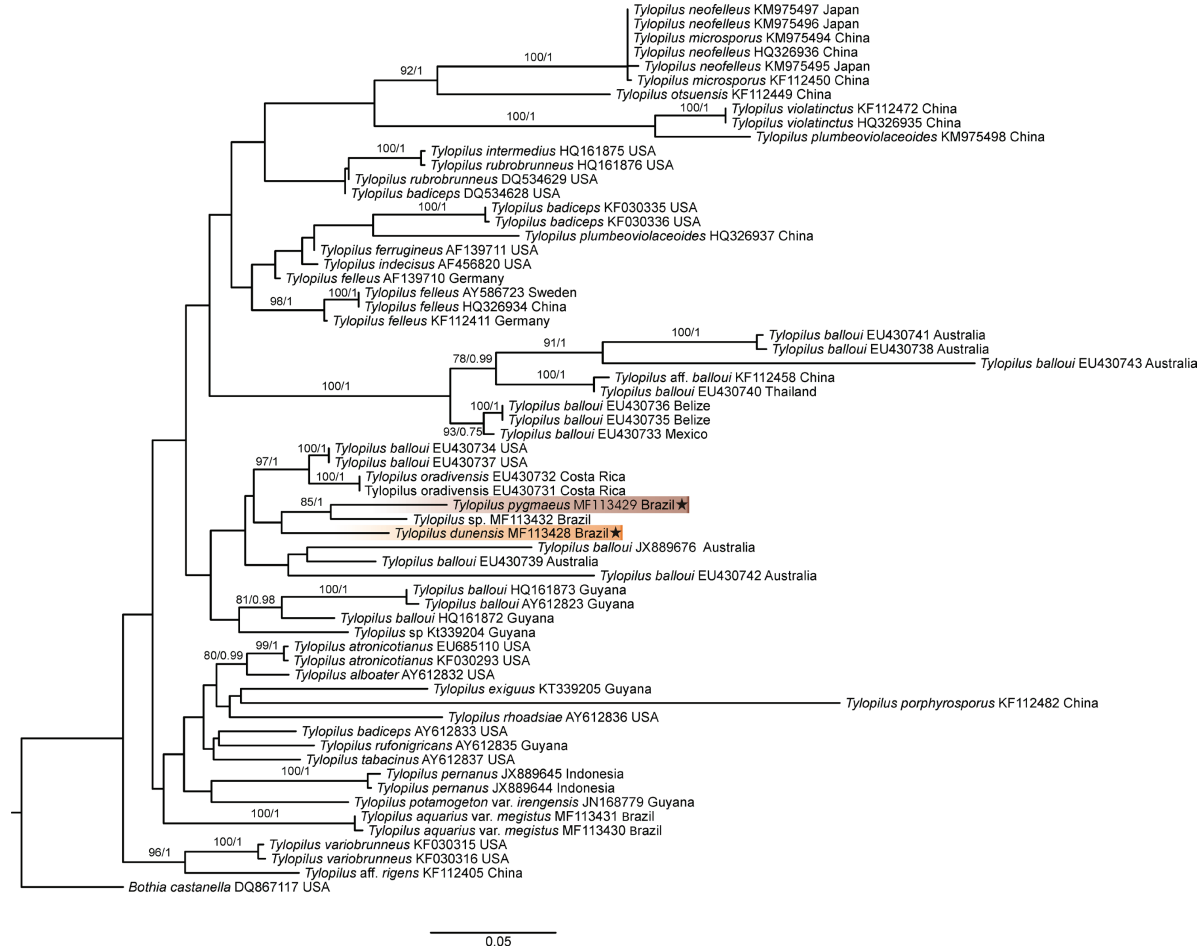


**FIGURE 2.** Maximum likelihood (ML) tree of *Tylopilus* from dataset of 63 LSU sequences rooted with the outgroup (*Bothia castanella*). Bayesian posterior probability above 0.7 and Bootstrap values above 50% are shown.

## Taxonomy

***Tylopilus dunensis*** A.C. Magnago & M.A. Neves, *sp. nov.* Fig. 3.

Mycobank: MB 819523

Type:—BRAZIL, Rio Grande do Norte, Natal, Parque Estadual Dunas do Natal, Trilha da Geografia, growing on white sand soil on dunes, 24 April 2008, *Neves MA 218* (holotype: HUEFS138281!; isotype: FLOR51718!) GenBank accession: ITS = MF113419, 28S = MF113428.

Etymology:—from the Latin *dunensis* = referring to the habitat (white sand dune area) where the new species was collected.

*Pileus* 25−115 mm broad, at first convex, with age becoming plano-convex to plano-depressed, yellow orange (OAC 789), to bright orange (OAC 644) with some red tones (OAC 670), finely velutinous under lens, dry, margin slightly inrolled and entire; context 5−14 mm centrally, whitish to yellowish (OAC 815), unchanging when exposed. *Tubes* 4−13 mm long, whitish to cream (OAC 816), slightly decurrent; pores 1−2 mm broad, whitish, staining light orange brown upon pressure. *Stipe* 30−65 × 8−28 mm, central to eccentrical, subequal, whitish to cream yellow (OAC 814); smooth, context cream (OAC 815), unchanging; extreme base with white mycelium. *Macrochemical reactions*: $NH_4OH$ unchanging on pileus and stipe surfaces.. *Spore print* pinkish.

*Basidiospores* 6−9 × 3−4 µm (Qm=1.91), ellipsoid to elongate in frontal view, the inner side applanate to bean shaped (phaseoliform) in side-view, in mass light yellow, inamyloid to weakly dextrinoid, smooth, thin walled; hilar appendage 0.5−1 µm long. *Basidia* 27−40 × 6−10 µm, clavate, thin walled, hyaline, many with granular contents; 4-sterigmate, 4−6 µm long. *Cystidia* abundant both on pores (cheilocystidia) and tubes (pleurocystidia), similar in size and shape, 32−82 x 6−13 µm, fusoid, ventricose, some clavate, hyaline, but also many with golden contents, dextrinoid. *Hymenophoral trama* boletoid, mediostratum of subparallel to interwoven hyphae, 3−5 µm wide, lateral stratum hyphae 5−8 µm wide, divergent, inamyloid. *Pileipellis* a trichodermium, interwoven in a gelatinized matrix, hyphae 2−5 µm wide, light brown with golden brown contents, dextrinoid, hyphae regularly septate. *Pileus trama* interwoven to subparallel, partly gelatinized, light yellow, with dextrinoid contents. *Stipitipellis* in two layers, external layer hymeniform, with terminal cells 5−13 µm wide, hyaline, some with golden contents; presence of caulobasidia and caulocystidia in variables shapes, including clavate, cylindrical, fusoid, ventricose, and capitate; lower layer with narrow hyphae with golden brown contents as observed in $H_2O$, 2−4 µm wide, interwoven vertically arranged in a gelatinized matrix. *Stipe trama* parallel to subparallel, hyphae 4−15 µm broad, hyaline, inamyloid, smooth and thin walled. *Clamp connection* absent.

Habit and habitat:—Solitary to scattered, sometimes caespitose, growing on white sand dunes in *restinga* vegetation in the far north of costal Atlantic Forest.

Specimens examined (paratypes):—BRAZIL, Rio Grande do Norte, Natal, Parque Estadual Dunas do Natal, 5°51'S, 35°11'W, 24 April 2008, 216 (HUEFS138279!, FLOR51716!) GenBank accession: ITS = MF113418; 29 April 2008, *Neves MA* 255 (HUEFS138318!), 256 (HUEFS138319!), 258 (HUEFS138321!), 24 May 2008, *Neves MA* 281 (HUEFS138368!) GenBank accession: ITS = MF113420.

Additional specimens examined:—AUSTRALIA, Queensland, *Tylopilus balloui* Fraser Island, road from Central Station to Eurong. 25°29'6''S, 153°5'18''E, 11 February 2009, *Halling RE* 9053 (NY!). BELIZE, Cayo District, *Tylopilus balloui* Mountain Pine Ridge: Douglas Da Silva, British Military Swamp. 16°58'9''N, 88°59'38''E, 6 October 2003, *Halling RE* 8526 (NY!). COSTA RICA, Cartago, *Tylopilus oradivensis*, Guarco, Palo Verde. +/_ 4.5 km E of km 31 of Interamerican Highway. 9°46'34''N, 83°56'42''W, 1 June 2001, *Halling RE* 8087 (NY!).

Comments:—*Tylopilus dunensis* is morphologically similar to the North American *Tylopilus balloui* Peck (1912: 157). Both have a yellow-orange to orange-red pileus, white to cream hymenophore, cream to pale yellow stipe and do not change color when in contact with ammonium; basidiospores are shorter than 10.5 µm long and the pileipellis is trichodermium. *Tylopillus balloui*, however, does not have a gelatinized pileipellis, the trama has gloeopleurous hyphae, the pseudocystidia are fusoid ventricose to broadly-ventricose to ventricose-mammilate and abundant, the cheilocystidia are spheropedunculate to broadly spheropedunculate, and the caulocystidia are clavate to fusoid ventricose with staining contents restricted to the cytoplasm and interrupted by hyaline vacuoles, as observed by Wolfe (1981) in the type studies of *Tylopilus* described by Charles H. Peck.

Osmundson & Halling (2010) described *Tylopilus oradivensis* Osmundson & Halling (2010: 476) from Costa Rica as part of the *balloui* complex based on morphological and molecular data. This species differs from *T. dunensis* by having a reddish orange to red pileus and stipe and larger (8.2−12 × 3−4 µm) subfusiform to fusiform basidiospores.

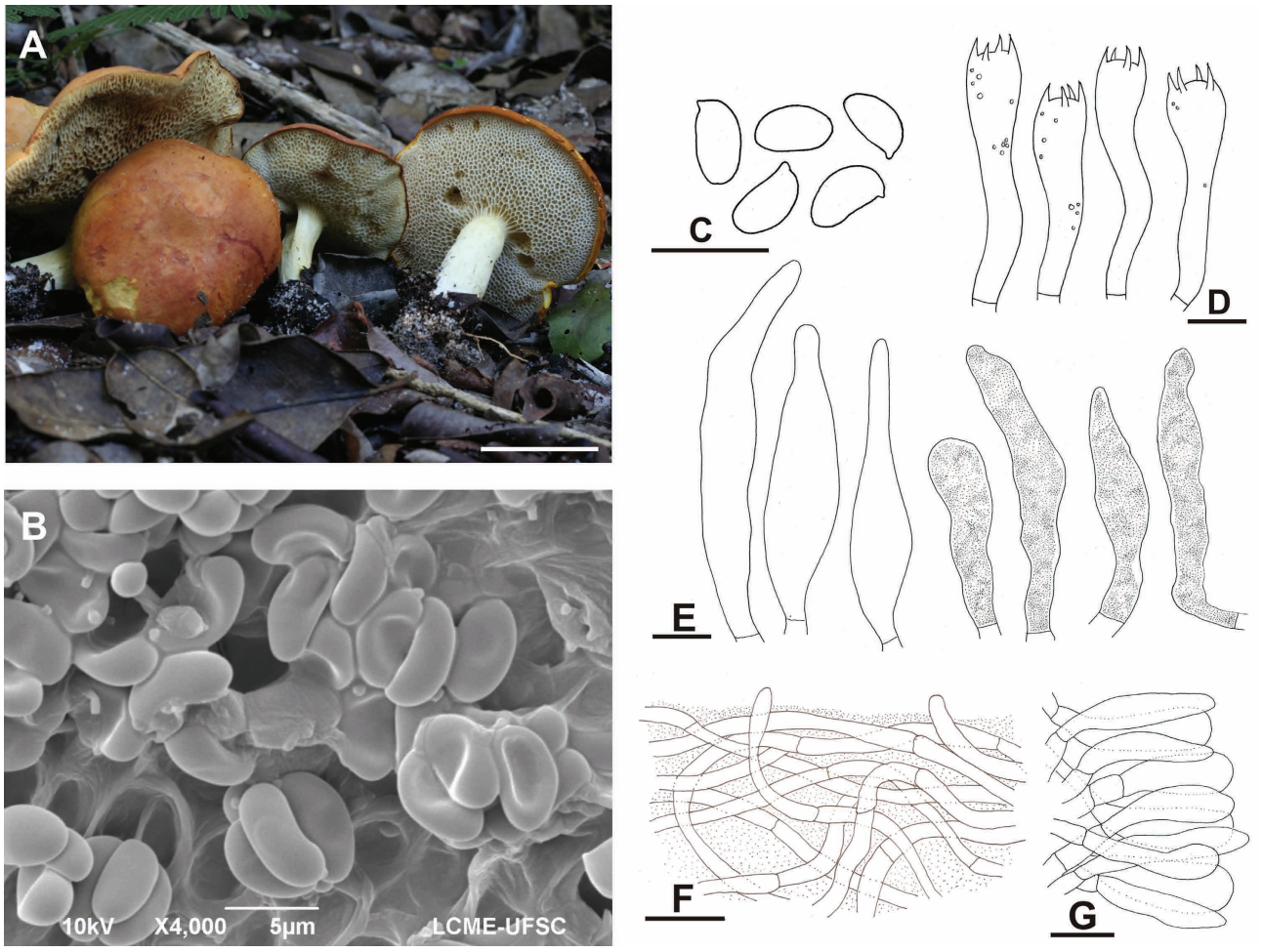


**FIGURE 3.** *Tylopilus dunensis*. A—Photographs of fresh basidiomes in the field. B-C—Basidiospores; D—Basidia; E—Cystidia; F—Pileipellis; G—Stipitipellis. Bar 10 µm.

***Tylopilus pygmaeus*** A.C. Magnago & R.M. Silveira *sp. nov.* Fig. 4.

Mycobank: MB 819524

Type:—BRAZIL, Bahia, Itacaré, Parque Estadual da Serra do Conduru, 30 November 2012, *Col. Rezende DHC & Montoya CAS*, *Magnago AC* 486 (holotype: FLOR51612!) GenBank accession: ITS = MF113421, 28S = MF113429.

Etymology:—from the Latin *pygmaeus* = small, short; referring to the small size of the basidiomes.

*Pileus* 11−26 mm broad, at first parabolic, becoming plano-convex with age, brown (OAC 638) to tannish brown (OAC 721), velutinous, dry, becoming dark brown when bruised, margin slightly inrolled and entire when young; context 3−7 mm, cream (OAC 683). *Tubes* 3−6 mm long, adnate, slightly depressed around stipe, whitish then pale pinkish; pores 2−4 per mm, angular, staining light brown under pressure. *Stipe* 22−35 × 4−8 mm, central, sub-equal, glabrous to velutinous, cream to light pinkish brown; context cream (OAC 683); extreme base with white mycelium. *Basidiospores* 7−9 × 4−5 µm (Qm=1.69), ellipsoid in frontal view, the inner side applanate to phaseoliform in side-view, hyaline, inamyloid, smooth, thin walled, hilar appendage 0.5−1 µm long. *Basidia* 25−35 × 8−10 µm, clavate, thin walled, hyaline, inamyloid; 4-sterigmate, 3−4 µm long. *Cystidia* abundant on pores (cheilocystidia) and tubes (pleurocystidia), not differentiated from each other, projecting slightly or not, 24−39 × 8−11 µm, ventricose-rostrate to lageniform, the majority with golden brown contents, strongly dextrinoid but some hyaline and without contents. *Hymenophoral trama* boletoid in a gelatinized matrix, mediostratum of many narrow parallel to interwoven hyphae, 3−5 µm wide, these yellow to light yellow, lateral stratum hyphae 3−11 µm wide, hyaline, strongly divergent. *Pileipellis* a trichodermium consisting of erect to sub-erect terminal hyphae, cylindrical to fusoid, 28−73 × 8−10 µm, differentiated like pileocystidia, with golden brown contents and dextrinoid. *Pileus trama* interwoven; hyphae 4−6 µm wide, light yellow, some with granular dextrinoid contents. *Stipitipellis* hymeniform, terminal hyphae 23−42 × 7−11 µm, clavate to ventricose, with golden brown contents, strongly dextrinoid; caulobasidia present. *Stipe trama*

subparallel to interwoven hyphae, vertically arranged, hyphae 3−10 µm wide, light yellow. *Clamp connections* absent. *Macrochemical reactions*: not observed. *Spore print* pinkish.

Habit and habitat:—Gregarious on sandy soil under broadleaf trees in Northeastern Atlantic Forest.

Additional specimens examined:—BRAZIL, Amazonas, *Tylopilus arenarius* Sing. Estrada Manaus-Caracaraí, km 45, 3 February 1978, *Singer B10590* (INPA-*type*!); *Tylopilus potamogeton* Sing. Rio Negro, 20 km ca. de São Gabriel da Cachoeira, 20 January 1978, *Araujo, I.* 938 (INPA!); *Tylopilus aquarius* var. *aquarius*, Igarapé do Tarumãzinho, 14 December 1978, *Singer B* 11433 (INPA-*type*!).

Comments:—*Tylopilus potamogeton* is morphologically similar to *T. pygmaeus* by having small basidiomes, a velutinous brownish pileus, and a whitish to pinkish hymenophore. *Tylopilus potamogeton* differs mainly by its cinnamon to fuscous umber stipe that is densely fibrillose and tomentose at the base, and the hymenophore does not turn brown when bruised. Microscopically the arrangement and appearance of cystidia, pileipellis and stipitipellis are similar, however, *T. potamogeton* has longer basidiospores (9−12 × 6−8 µm), cystidia that are hyaline, fusoid and mucronate. *Tylopilus aquarius* var. *aquarius* can be differed by the dimorphic basidiospores (8−11.5 × 5−7.5 and 11−16 × 5−6 µm), and versiforme cystidia. *Tylopilus arenarius* differs by the whitish to slightly lilac pileus, reticulation on the upper third of the stipe, larger basidiospores (7−9 × 4−5 µm) and cystidia that are fusoid to ampullaceous (Singer *et al.* 1983, Barbosa-Silva *et al.* 2017).

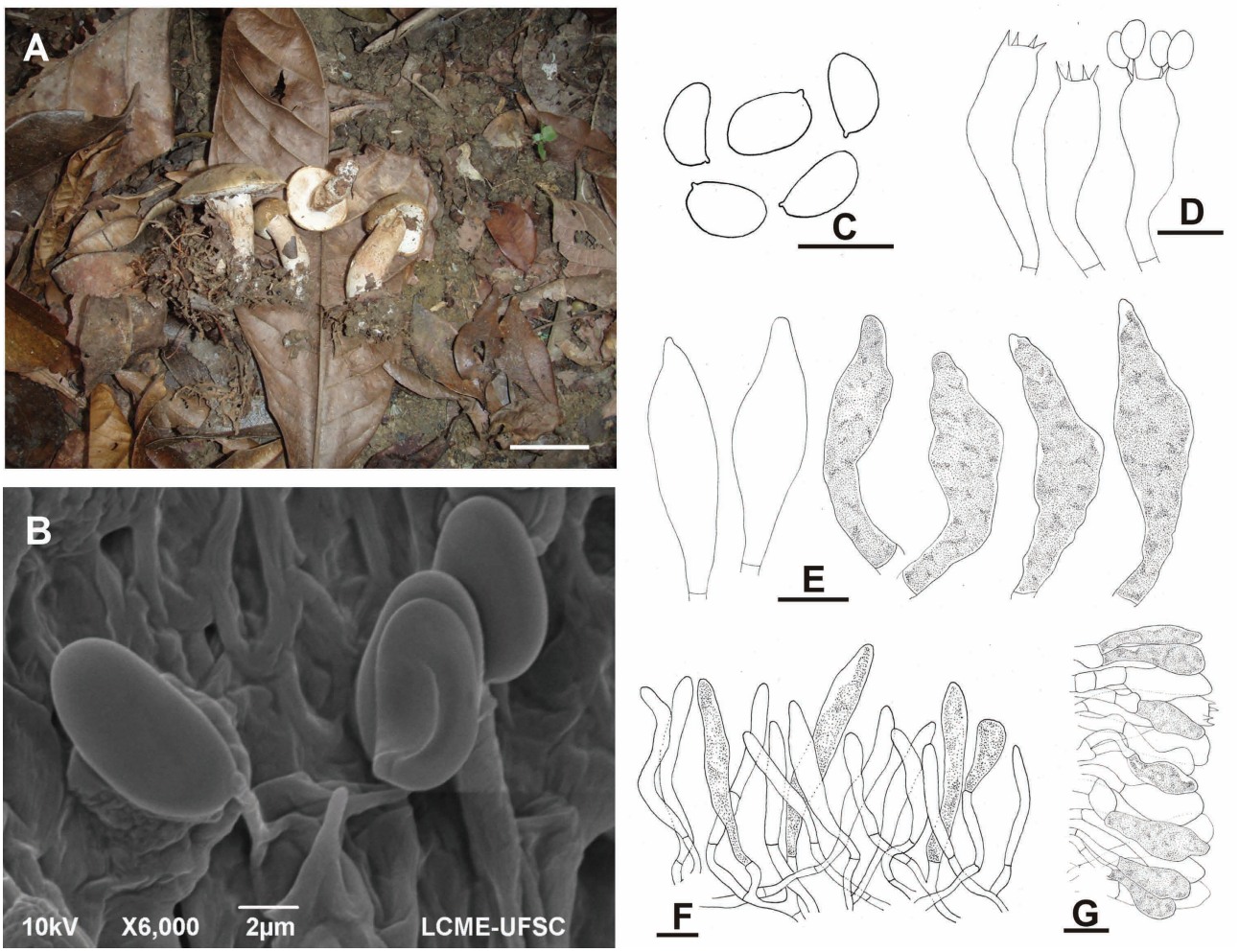


**FIGURE 4.** *Tylopilus pygmaeus.* A—Photographs of fresh basidiomes in the field. B-C—Basidiospores; D—Basidia; E—Cystidia; F—Pileipellis; G—Stipitipellis. Bar 10 µm.

## Discussion

The Northeastern Atlantic Forest of Brazil is a habitat well-known for its unique flora but its fungal diversity has been thus far largely neglected. Our discoveries illustrate that these forests harbor a rich diversity of macrofungi that have

never been documented. White sand dune environments, in particular, may be a reservoir for a specialized community of ectomycorrhizal species that have evolved in these distinct soils, as suggested by Singer *et al.* (1983), Sulzbacher *et al.* (2013) and Roy *et al.* (2016). For example, many other putatively ectomycorrhizal macrofungi have been recorded by us at Parque Estadual das Dunas, including other species belonging to the well-known ectomycorrhizal families Boletaceae, Amanitaceae, Cantharellaceae, Russulaceae and Sclerodermataceae. The woody vegetation in the park is mainly composed of species belonging to the Fabaceae, Myrtacaceae and Sapotaceae, which are candidates for ectotrophic mutualists (Sulzbacher *et al.* 2013). Further survey work is needed to better document both the ectotrophic status of the native woody vegetation and further document the extensive diversity of ectomycorrhizal fungi that remain unknown.

*Tylopilus* s. str. was recovered as monophyletic by Nuhn *et al.* (2013) and Wu *et al.* (2014) including the type species, *T. felleus*, using three molecular markers (LSU, tef1, and rpb1). Although the phylogenetic analyses shown here (Figs 1-2) are based on two markers, which are not sufficient for resolving persistent infrageneric ambiguities in the Boletaceae, they do allow us to confirm the distinctiveness of two new taxa among related *Tylopilus* s.l. using a molecular phylogenetic approach.

Halling *et al.* (2008) indicated that the current circumscription of the type species of *Tylopilus balloui* represents a species complex rather than a single species. Our phylogenetic reconstruction based on LSU supports this view since specimens identified as *T. balloui* cluster in two distinct, unrelated clades. The first clade includes specimens from Australia, China, Thailand, Mexico, and Belize. The second includes specimens from the USA (type locality), Australia, and Guyana. In the second clade are included specimens initially identified as *T. balloui*, suggesting that other specimens originating outside of the USA that are currently assigned to *T. balloui* should also be recognized as distinct taxa at the species rank.

These results suggest that there is a hidden diversity of species under the name *T. balloui* and reinforce the need for a comprehensive taxonomic review of the complex. Other unique sequences included in the molecular phylogenetic datasets presented here are from *Tylopilus* specimens collected in Northeastern Brazil (MAN215, MAN217, MAN282, MAN288), that probably also represent new species. However, for the time being, we do not possess enough material of good quality to definitively assess them. This highlights the fact that the Atlantic Forest of Brazil harbors many species of fungi yet to be described.

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