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Taxonomy and phylogeny of *Macrolepiota*: two new species from Brazil

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ABSTRACT

Macrolepiota is a poorly known genus in the Neotropics. In order to increase knowledge about this group, we collected specimens from the Atlantic Forest in southern and northeastern Brazil. *Macrolepiota cyanolamellata* and *M. sabulosa* from subtropical and tropical regions, respectively, are proposed as new species. We performed molecular phylogenetic analyses of the nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS) and the combined data set ITS + nuclear large subunit rDNA (28S) + RNA polymerase II second largest (*RPB2*), as well as morphological analyses. Two lineages with unique morphotypes were found. The species proposed were strongly supported as the sister lineage closely related to *M. clelandii* and *M. subcitrophylla*. Detailed descriptions and illustrations of their macro- and microscopic characters are provided.

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KEYWORDS
Agaricaceae; ITS; Neotropical fungi; *RPB2*; 28S; 2 new taxa

INTRODUCTION

Macrolepiota, a genus within Agaricaceae (Agaricales, Basidiomycota), was established by Singer (1948). Currently, there are about 35 species worldwide (Kirk et al. 2008; Ge et al. 2010, 2012; Lebel and Syme 2012) and the genus is classified into three sections: *Macrolepiota* (Singer 1948), *Macrosporae* (Bon 1981), and *Volvatae* (Ge et al. 2010). *Macrolepiota* contains secotioid species, although most are agaricoid (Lebel and Syme 2012). Several *Macrolepiota* species are used for cooking, such as *M. procera* (Ayaz et al. 2011), *M. bonaerensis* (Wright and Albertó 2002), and *M. kerandi* (Wright and Albertó 2002). The agaricoid species are macroscopically characterized by medium- to large-sized, fleshy basidiomata, free lamellae remote from the stipe, pileus covered by trichodermic scales, spore print white to cream, a prominent, simple to complex annulus, which is eventually mobile, stipe often covered by colored bands in full-grown specimens, and volva present in some species.

Microscopically, they have large basidiospores, generally larger than 10 µm, thick-walled, with germ pore covered by a hyalinous cap (Vellinga 2003b), congophilous, metachromatic, and dextrinoid; clamp connections present in most species; with cheilocystidia, but never with pleurocystidia.

Although *Macrolepiota* is well known in Europe, China, and Australia, the genus has been scarcely studied in tropical and subtropical areas, especially in South America, where there are few well-defined species that lack molecular studies. Currently, 15 *Macrolepiota* species have been recorded from South America: *M. bonaerensis* (Rick 1961; Wright and Albertó 2002; Rosa and Capelari 2009; Suaza Blandón 2016); *M. brasiliensis* (Rick 1961; Raithelhuber 1988); *M. brunnescens* (Heinemann and de Meijer 1996; Vellinga and Yang 2003); *M. colombiana* (Franco-Molano 1999; Ferreira and Cortez 2011); *M. dolichaula* (Grandi et al. 1984); *M. excoriata* (Rick 1907); *M. fornica* (Raithelhuber 1988); *M. fuligineosquarrosa* (Alves et al. 2016); *M. gracilenta* (Capelari et al. 2015); *M. kerandi* (Wright and Albertó 2002; Putzke et al. 2014; Suaza Blandón 2016); *M. mastoidea* (Grandi et al. 1984; Rosa and Capelari 2009); *M. procera* (Rick 1961; Bononi et al. 1984); *M. pulchella* (Vellinga and Yang 2003); *M. stercoraria* (Rick 1961; Raithelhuber 1988); and *M. zeyheri* (Rick 1961). Brazil holds records for all of the aforementioned species, except for *M. fornica*. However, most of these records use European or Asian names, and thus far such records have not been reviewed and have very little molecular information.

In order to better understand the *Macrolepiota* species from Brazil, we made several field collections from

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Supplemental data for this article can be accessed on the publisher's Web site.

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Atlantic Forest areas. As a result, two new species of *Macrolepiota* are proposed herein, based on morphological and molecular phylogenetic analyses.

MATERIALS AND METHODS

Sampling and morphological analysis.—The materials examined were collected from Rio Grande do Sul State, southern Brazil, and Rio Grande do Norte State, northeastern Brazil, between May 2015 and June 2016, and were deposited in the herbaria ICN and UFRN. The herbarium codes followed Thiers (continuously updated). Color notations in the descriptions followed Kornerup and Wanscher (1978). Macromorphological descriptions were based on field notes and color slides of the material. For micromorphological observations, free-hand sections of the basidiomata were mounted in 5% KOH and Congo red. Pileal/stipe structure, cheilocystidia, basidiospores, and basidia were observed under a light microscope at 1000× magnification. Melzer's reagent was used to test the dextrinoid reaction of the basidiospores. We also checked spore wall reactions to cresyl blue and Congo red. The abbreviation codes used [n/m/p] represent n basidiospores measured from m basidiomata of p collections in 5% KOH solution. Dimensions of basidiospores are presented in the form (a)b–c(d). The range b–c contains a minimum of 90% of the measured values. Extreme values (a and d) are given in parentheses. Q refers to the length/width ratio of a basidiospore in side view; avQ means average Q of all basidiospores ± sample standard deviation.

DNA isolation and amplification.—Genomic DNA was extracted from dried specimens. We amplified the following regions: nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS) using primers ITS1F and ITS4 (White et al. 1990); nuclear large subunit 28S nuc rDNA (28S) using primers LR0R and LR7 (Vilgalys and Hester 1990); and the region located between domains 6 and 7 of the RNA polymerase II second largest (*RPB2*) using primers bRPB2-6F and bRPB2-7.1R (Frøslev et al. 2005; Matheny 2005).

Polymerase chain reaction (PCR) was performed with a total volume of 30 µL containing 1 unit Taq DNA polymerase, 3.0 µL of 10× Taq polymerase reaction buffer (Applied Biological Material, Vancouver, Canada), 3.0 µL of bovine serum albumin solution (Sigma-Aldrich, St. Louis, Missouri), 50 µM of dNTP mix, 0.75 µL of 10 µM each of the two primers, and 1–2 µL of total DNA. PCR amplification of ITS was performed with 2 min initial denaturation at 95 C, followed by 35 cycles of 30 s at 95 C, 1 min and 30 s at 57 C, and 30 s at 72 C, with a final extension of 10 min at 72 C following the last cycle. PCR amplification of 28S and *RPB2* followed Vilgalys and Hester (1990) and Matheny (2005), respectively. Purification and DNA sequencing were performed by Macrogen (Geumcheon-gu, Korea). For *RPB2* and ITS, we used the same primers as for amplification, and for 28S, LR0R and LR5.

Alignment and phylogenetic reconstructions.—Sequences were assembled and manually corrected with Geneious 9.1.4 (Kearse et al. 2012), then automatically aligned with MAFFT 7 (Katoh and Standley 2013) under the auto mode for strategy. When necessary, the alignment was manually adjusted with MEGA 7.0.20 (Kumar et al. 2016). Potential ambiguously aligned segments of ITS1-ITS2 were detected by Gblocks 0.91b (Castresana 2000) through block parameters: the minimum number of sequences for conserved positions was 53% from total sequences, the minimum number of sequences for flank positions was 55% from total sequences, the maximum number of contiguous nonconserved positions was 8, the minimum length of a block was 2, and the allowed gap position was half. The data set in the ITS analyses was subdivided into three data partitions: ITS1, 5.8S, and ITS2, and combined analyses were subdivided into seven data partitions: ITS1, 5.8S, ITS2, 28S, and *RPB2*-1st, -2nd, and -3rd codon positions; the *RPB2* intron (the fourth) was excluded. The alignments were deposited into TreeBASE (submission ID: S21091).

We carried out single-gene and combined data set phylogenetic analyses with ITS and ITS+28S+*RPB2* sequences. A total of 97 specimens (two outgroup species) were included in the ITS phylogenetic analyses; of these, 30 were included in the combined data analysis. The 28S matrix was built up to domain D3 (LR5 primer). All *Macrolepiota* sequences used in this study are available in GenBank and were primarily taken from Vellinga et al. (2003) and Johnson (1999). *Leucoagaricus barssii* and *L. meleagris* were designated as outgroup based on previous studies (Ge et al. 2010, 2012). All materials and sequences used in this study are listed in SUPPLEMENTARY TABLE 1. Country abbreviations follow International Organization for Standardization (ISO) 3166 code.

All phylogenetic analyses were performed online using the CIPRES Science Gateway (Miller et al. 2010). We analyzed the data sets separately with maximum likelihood and Bayesian inference. Maximum likelihood (ML) analysis was carried out in RAxML 8.2.9 (Stamatakis 2014). The analysis first involved

100 ML searches, each starting from one randomized stepwise addition parsimonious tree under a GTRGAMMAI model, with all parameters estimated by the software. We provided a partition file to force RAxML software to search for a separate evolution model for each data set. To access the reliability of the nodes, we computed the rapid bootstrapping replicates under the same model, allowing the program to halt bootstrapping automatically by extended majority rule (MRE)-based bootstopping criterion (Pattengale et al. 2010). Bootstrap (BS) values above 80 were considered significant (high support), and above 70 were considered moderately supported.

Bayesian inference (BI) was performed in MrBayes 3.2.6 (Ronquist et al. 2012), and evolutionary models for BI were estimated using the Akaike information criterion (AIC) for each partition, as implemented in MrModeltest 2.3 (Nylander 2004). The best-fit models for each partition were implemented as partition-specific models within partitioned mixed-model analyses (HKY+G for ITS1; JC for 5.8S; GTR+G for ITS2 and 28S; SYM+G for *RPB2*-1st, -2nd, and -3rd). We set Bayesian analyses with two independent runs, each with four simultaneous chains for 10^7 generations, sampling trees at every 1000th generation. The convergence diagnostic was calculated every 10^4 generation, and its critical value was set to stop the analysis automatically when the standard deviation of the split frequencies 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 BPP value above 0.99 was considered significant (high support), and above 0.95 was considered moderately supported.

RESULTS

Phylogenetic analyses.—*DNA sequence details*. In this study, we provided 32 new sequences of *Macrolepiota* from Brazil, of which 14 were from ITS, 12 from 28S, and 6 from *RPB2* (SUPPLEMENTARY TABLE 1). The ITS ranged from 333 (*M. eucharis*, ITS1 only, AF482854) to 747 (*M. sabulosa*, KY927715) bp. The final aligned matrix of the ITS1-5.8S-ITS2 (ITS) region was 742 bp long; of these, 76 bp were excluded by Gblocks. Contrary to the other lineages, the *Macrosporae* shared the 463–473 positions (TCTAACTTTTG) in the ITS2 region. In the concatenated matrix, the 5′ region of the 28S, including domains D1, D2, and D3, ranged from 791 (*M. orientiexcoriata*, JN940278) to 820 (e.g., *M. clelandii*, AF482882) bp. In alignment, the partial *RPB2* region was 587 and 590 bp long, corresponding to nucleotide positions 1050–1639 from *M. dolichaula* AFTOL-ID 481 complete *RPB2* (DQ385886). Additionally, the ITS was from 502 (*Macrolepiota* sp. 1, Faz643) to 652 (*Macrolepiota* sp. 2, Rother126) bp long. The final aligned matrix of the combined data set was 2063 bp long.

Single-gene analysis. In the RAxML analysis, ITS alignment had 313 distinct patterns, with a proportion of gaps and undetermined characteristics of 3.6%; the bootstopping criteria indicated that 408 replicates were sufficient to estimate the internal branch support, and the final ML optimization likelihood was −lnL = 3475.294357. The two Bayesian runs converged to stable likelihood values (−lnL = 3673.86, 3675.91) after 510 000 generations, and 3826 (about 75% of the sampled trees) stationary trees from each analysis were used to compute a 50% majority-rule consensus tree to calculate posterior probabilities. The best-scoring ML tree and 50% majority-rule consensus tree did not show major conflicts in the tree topology and were mostly congruent, which allowed us to combine them (FIG. 1).

Three main clades were recovered, namely, clade 1: *Volvatae*; clade 2: *Macrosporae*; and clade 3: *Macrolepiota*. Clade 1 had ML BS/BPP 100/1 and included three lineages: *M. eucharis, M. velosa*, and an undescribed species. In clade 2 (86/1), three subclades were recovered: *M. mastoidea* lineages, *M. excoriata* lineages, and *M. orientiexcoriata*. In clade 3 (89/1), the following species were retrieved: *M. clelandii, M. colombiana, M. detersa, M. dolichaula, M. fuliginosa, M. permixta, M. procera, M. rhodosperma, M. subcitrophylla, M. turbinata*, and four undescribed species. Two of the four undescribed species are described below as *M. cyanolamellata* (99/–) and *M. sabulosa* (100/1).

Combined data set analysis. In the Bayesian analysis, after 180 000 generations, runs converged to stable likelihood values (−lnL = 6904.33, 6904.73) and 2702 stationary trees were used to compute a 50% majority-rule consensus tree and to estimate BPPs of the branches. In the RAxML, combined alignment presented 508 distinct patterns, with a proportion of gaps and undetermined characters of 24.39%; the bootstopping criteria indicated 252 replications as sufficient to access the internal branch support, and the final ML optimization likelihood was −lnL = 6757.679061. No conflict involving significantly supported nodes was found; therefore, the best-scoring ML tree and the 50% majority-rule consensus tree were combined (SUPPLEMENTARY FIG. 1).

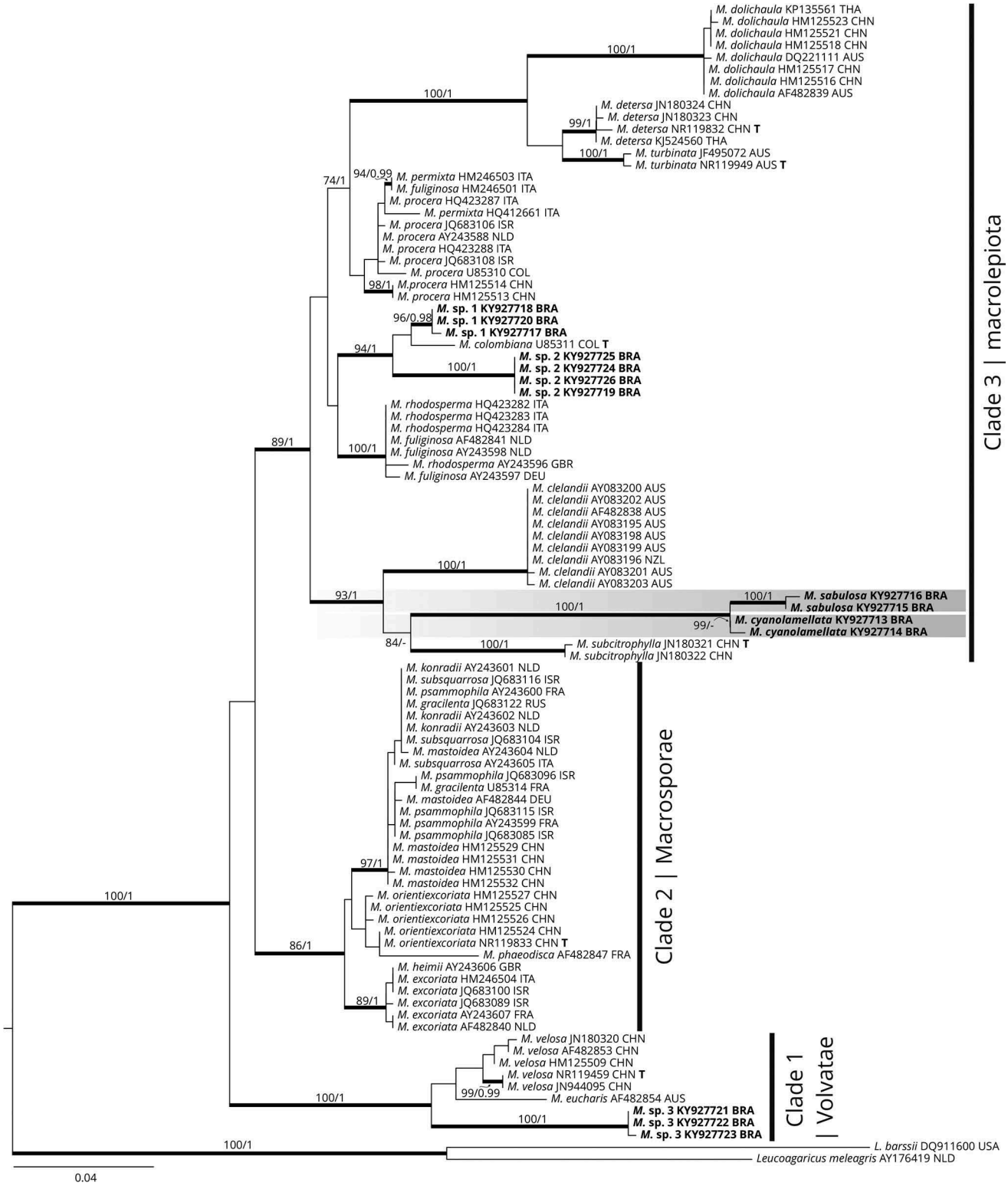

Figure 1. Phylogram showing the relationships of *Macrolepiota* based on nuc rDNA ITS sequences, inferred by maximum likelihood analysis (log likelihood −lnL = 3475.3). The numbers above and below branches are ML BS/BPP = 80/0.99 or higher (high support) and ML BS/BPP of 70/0.95 or higher (moderate support), respectively. Strict consensus tree resulted in the same topology. Support in nodes is indicated by thickened branches when bootstrap values are ≥80% and posterior probabilities are ≥0.95. New sequences generated in this paper are marked in bold.

Of the three clades recovered in the single analysis, two (*Volvateae* and *Macrosporae*) were also retrieved in the combined analysis. *Macrolepiota clelandii* was placed as sister to the remaining species in the ML tree and was recovered as closely related to *Macrosporae-Macrolepiota* clade in the Bayesian tree (data not shown). *Macrolepiota cyanolamellata* and *M. sabulosa* were retrieved as sister to the remaining *Macrolepiota* species in the BI tree (data not shown), and as closely related to *Volvateae* (SUPPLEMENTARY FIG. 1) in the ML tree. All these aforementioned phylogenetic relationships were not supported.

Morphological analysis.—In this study, we collected and examined 67 specimens, preliminarily identified as *M. bonaerensis, M. kerandi, Macrolepiota* sp. 1, *Macrolepiota* sp. 2, and *Macrolepiota* sp. 3, as well as the two new species described herein. Furthermore, we examined the type material of *M. bonaerensis* (LPS 15287), as *Agaricus bonaerensis*, Buenos Aires, Argentina, 1880 (Spegazzini 1880); *M. brasiliensis* (PACA 17151), as *Lepiota permixta* var. *brasiliensis*, São Leopoldo, Rio Grande do Sul, Brazil, 1907 (Rick 1907); *M. colombiana* (Franco-M 1636), La Ceja, Colombia, 1998 (Franco-Molano 1999); *M. pulchella* (PACA 13897), as *Lepiotella brunnea*, São Leopoldo, Rio Grande do Sul, Brazil, 1933 (Rick 1938); and *M. stercoraria* (PACA 17177), as *Lepiota stercoraria*, São Leopoldo, Rio Grande do Sul, Brazil, 1933 (Rick 1937). Unfortunately, we did not successfully extract DNA from the type materials due to their preservation and contamination conditions.

Given the results of the phylogenetic inferences that placed two unknown lineages as closely related to *M. clelandii* and *M. subcitrophylla*, and the morphological data that defined them as unique morphotypes, these lineages represent two distinct undescribed species. Therefore, these species are proposed as follows.

TAXONOMY

Macrolepiota cyanolamellata Fazolino, Lechner & Suaza Blandón, sp. nov. FIGS. 2A–C, 3
MycoBank MB821449

Typification: BRAZIL. RIO GRANDE DO SUL STATE: Porto Alegre, Morro Santana, in the soil among dry leaves, 30°03′56.9″S, 51°7′28.9″W, 5 May 2015, *Fazolino 000516* (**holotype** ICN 187662).

Diagnosis: Macrolepiota cyanolamellata is characterized by the bluish coloration of the lamellae, basidiospores with wide variation in size and shape, and the brown to reddish brown coverage of the pileus and lightly striate edges.

Etymology: "*cyanolamellata*" refers to the coloration of the lamellae in mature basidiomata.

Macroscopic features: Basidiomata medium-sized to large. Pileus 8.5–10.0 cm diam, fleshy, convex when young, plano-convex when mature with a distinct umbo at disk, dark brown (6F2, 6F3) on reddish gray background (10C2), at first smooth and continuous, then gradually breaking up into irregular patches covered with reddish brown to brown (8D3, 8E3) squamules scattered toward the margin; margin slightly striate and appendiculate. Lamellae free, crowded, white to grayish white (1A2, 1B1), becoming bluish (21B1, 21B2) with age, mainly at the edges, with lamellulae. Stipe 9.0–9.8 × 0.9–1.0 cm, cylindrical, central, attenuating upwards, grayish (21C1), covered with tiny brownish grey to reddish brown squamules (8E2, 8E3, 8E4); base slightly enlarged with white mycelium. Context whitish (1B1), does not change color when cut. Annulus ascending, simple, fixed, membranous, with the lower border the same color as the scales of the stipe. Fungal odor.

Microscopic features: Basidiospores [92/2/2], (7.5) 9.0–17.0(19.5) × (4.5)6.0–9.0(11.0) µm, Q = 1.38–2.27, avQ = 1.68 ± 0.28 variable in size and shape, ellipsoid to oblong, and some elongated and tapered, thick-walled, smooth, hyaline, dextrinoid, congophilous, metachromatic, with a germ pore covered with a hyaline cap in KOH. Basidia 26.0–38.0 × 10.0–13.0 µm, clavate, thin-walled, hyaline to pale orange, 4-spored. Cheilocystidia 19.0–53.5 × 6.5–11.5 µm, clavate, some with secondary septa, pale brownish pigmentation in KOH and small incrustations at the apex, in bunches, forming a sterile edge. Pleurocystidia absent. Squamules on pileus trichodermal, formed by cylindrical hyphae, pale brownish pigmentation in KOH; terminal elements 11.5–75.5 × 7.5–12.5 µm, cylindrical to narrowly clavate, thin-walled. Squamules on stipe similar to those on the pileus, terminal elements 29.5–71.0 × 6.5–10.5 µm. Clamp connections observed at the hyphae of the stipe context and in base of basidia, basidiole, and cheilocystidia.

Habitat and known distribution: Terrestrial and saprotrophic, solitary, growing on soil, inside the forest among dead leaves. Atlantic Forest, southern Brazil.

Comments: Macrolepiota cyanolamellata is characterized by medium-sized basidiomata with reddish gray pileus and brown to reddish brown squamules and, mainly, by the bluish coloration of the lamellae when mature. Microscopically, it is characterized by dextrinoid, congophilous, and metachromatic basidiospores of variable size and irregular shape, basidia 4-

Figure 2. A–C. *Macrolepiota cyanolamellata*, from holotype (ICN 187662). A. Pileus. B. Annulus. C. Lateral view. D–F. *M. sabulosa*, from holotype (UFRN 2693). D. Pileus. E. Annulus. F. Lateral view. Bars = 25 mm.

spored, clavate, with clamp connections, cheilocystidia clavate, some with pale brownish pigmentation and clamp connections. It resembles *M. sabulosa* in general appearance and size of basidiomata but differs from this species in the brown to reddish brown squamules on the pileus and stipe, lamellae that are whitish to grayish in *M. sabulosa*, and in the size and shape of basidiospores. *Macrolepiota brasiliensis, M. bonaerensis* (Rick 1907; Singer and Digilio 1952; Raithelhuber 1988; Wright and Albertó 2002), *M. excoriata* (Rick 1907;

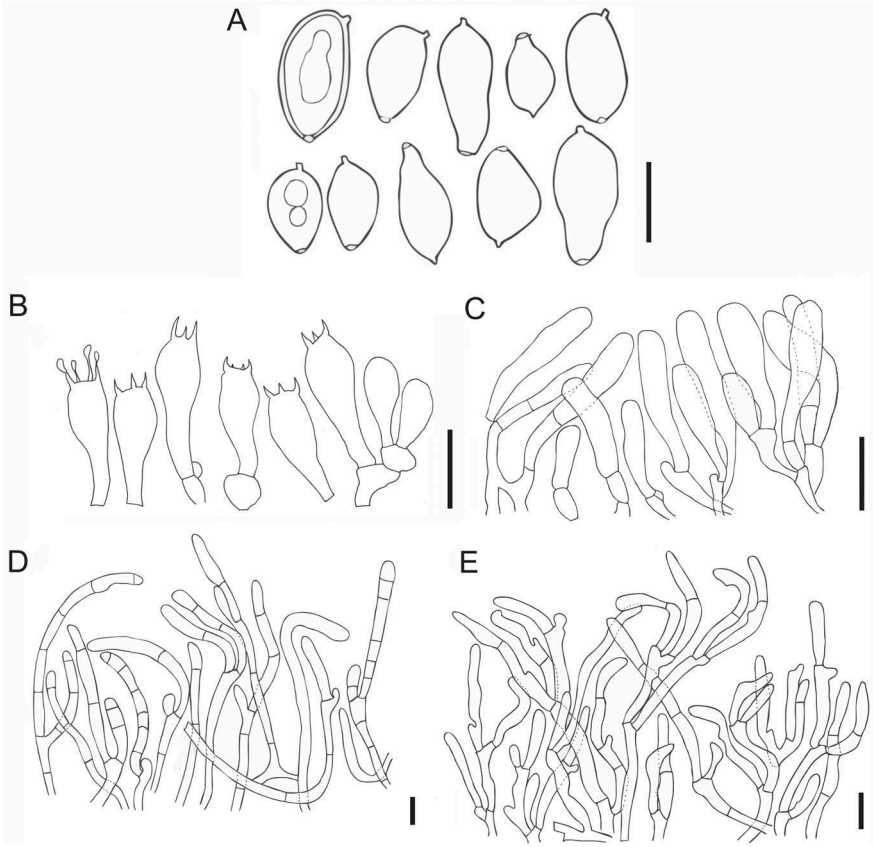

Figure 3. Microscopic features of *Macrolepiota cyanolamellata* from holotype (ICN 187662). A. Basidiospores. B. Basidia. C. Cheilocystidia. D. Squamules on pileus. E. Squamules on stipe. Bars: A = 10 µm; B–E = 20 µm.

Raithelhuber 1988), *M. fornica* (Rick 1907; Raithelhuber 1988), and *M. kerandi* (Raithelhuber 1988; Wright and Albertó 2002) differ by their whitish pileus and stipe. Furthermore, *M. stercoraria* (Rick 1907; Raithelhuber 1988) differs by its brownish to ochraceous pileus covered and yellowish annulus and *M. zeyheri* (Rick 1907; Raithelhuber 1988) by its brownish pileus covered with flakes and ocher-yellowish lamellae. Phylogenetically, *M. cyanolamellata* is closely related to *M. clelandii* and *M. subcitrophylla*, which are morphologically separated due to their whitish and yellowish basidiomata, respectively. *Macrolepiota cyanolamellata* has a simple annulus like *M. pulchella*, but the latter has a volva and yellowish lamellae (Rick 1938; Heinemann and de Meijer 1996; Vellinga and Yang 2003) and does not have clamp connections. *Macrolepiota colombiana* has brown squamules on white background, double annulus, squamules on the pileus with hyphae ± catenulate, and cheilocystidia clavate to cylindrical with pale brownish pigmentation (Franco-Molano 1999; Ferreira and Cortez 2011).

Additional specimen examined: BRAZIL. RIO GRANDE DO SUL STATE: Porto Alegre, Morro Santana, in the soil among dry leaves, 30°03′56.5″S, 51°07′28.3″W, 2 May 2015, *A.C. Magnago 1165* (**paratype** ICN 187663).

Macrolepiota sabulosa Fazolino & R.M. Silveira, sp. nov. FIGS. 2D–F, 4
MycoBank MB825230

Typification: BRAZIL. RIO GRANDE DO NORTE STATE: Natal, Parque Estadual Dunas de Natal, Trail of Geologia, in the sandy soil among dry foliage, 05°50′ 28.0″S, 35°11′35.8″W, 6 Jun 2016, *Fazolino 000689* (**holotype** UFRN 2693).

Diagnosis: Macrolepiota sabulosa has pileus coverage with a distinct dark brown umbo at disc on brownish gray background, covering formed by tiny scales with the same brown to light brown squamules and a lightly striate border, whitish to grayish lamellae, and cheilocystidia clavate to cylindrical with pale brownish pigmentation.

Etymology: The name refers to *sabulosus*: growing in sandy places.

Macroscopic features: Basidiomata medium-sized. Pileus 13.0–14.0 cm diam, fleshy, convex when young,

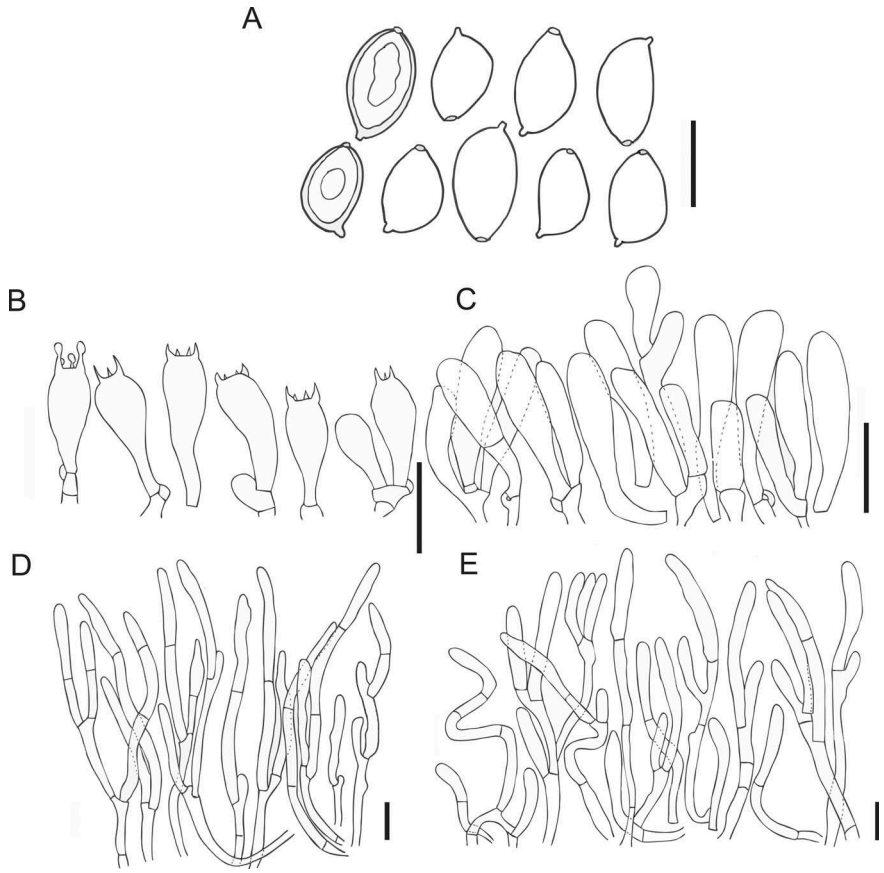

Figure 4. Microscopic features of *Macrolepiota sabulosa* from holotype (UFRN 2693). A. Basidiospores. B. Basidia. C. Cheilocystidia. D. Squamules on pileus. E. Squamules on stipe. Bars: A = 10 µm; B–E = 20 µm.

plano-convex when mature with a distinct dark brown (6F4, 6F5) umbo at disc on brownish gray (5C3) background, at first smooth and continuous, then gradually breaks up into irregular patches covered with brown (6E7, 6F7) to light brown (5D4, 5E4) squamules, scattered toward the margin, margin slightly striate and appendiculate. Lamellae free, crowded, white to grayish white (1A2, 1B1) with lamellae. Stipe 16.5–17.5 × 1.1–1.2 cm, cylindrical, central, attenuating upwards, grayish white (1B2), covered with tiny light brown (6D4) squamules; base slightly enlarged. Context grayish white (1B2), not changing color when cut. Annulus ascending, simple, fixed, with the lower border in the same color as the scales of the stipe, membranous. Fungal odor.

Microscopic features: Basidiospores [80/2/2], (8.5) 9.5–13.0(15.0) × (5.0)5.5–7.5(11.5) µm, Q = 1.49–1.98 µm, avQ = 1.16 ± 0.15 ellipsoid to oblong in side view, ellipsoid in front view, thick-walled, smooth, hyaline, dextrinoid, congophilous, metachromatic, with a germ pore, covered with a hyaline cap in KOH. Basidia 25.5–37.5 × 9.5–13.0 µm, clavate, thin-walled, hyaline to pigmented, 4-spored. Cheilocystidia 21.5–47.5 × 6.0–10.5 µm, clavate to cylindrical, some with secondary septa, pale brownish pigmentation in KOH, in bunches, forming a sterile edge. Pleurocystidia absent. Squamules on pileus trichodermal, formed by cylindrical hyphae, pale brownish pigmentation in KOH; terminal elements 28.0–63.5 × 5.5–8.5 µm, cylindrical, thin-walled. Squamules on stipe similar to those on the pileus, terminal elements 28.5–82.5 × 5.5–9.5 µm. Clamp connections observed at the base of basidia, basidiole, and cheilocystidia, also at the hyphae of the stipe context.

Habitat and known distribution: Seasonal semideciduous lowland forest. Terrestrial and saprotrophic, solitary. In the sandy soil among dry foliage. Distributed in northeastern Brazil.

Comments: Macrolepiota sabulosa is characterized by medium-sized basidiomata, dark brown umbo on brownish gray background, with brown to light brown squamules from center to margin. Microscopically, it is characterized by dextrinoid, congophilous, and metachromatic basidiospores of elliptic to oblong shapes, basidia 4-spored, clavate, cheilocystidia clavate, some with pale brownish pigmentation and clamp

connections. It resembles *M. cyanolamellata*, mainly by the shape of its basidiomata, annulus with the lower border the same color as the scales of the stipe. However, *M. sabulosa* differs by its brown to light brown squamules on brownish gray background and whitish to grayish lamellae. *Macrolepiota bonaerensis, M. brasiliensis, M. excoriata* (Rick 1907; Raithelhuber 1988), *M. fornica* (Rick 1907; Raithelhuber 1988), and *M. kerandi* differ by the whitish pileus and stipe (Rick 1907; Raithelhuber 1988; Wright and Albertó 2002). *Macrolepiota stercoraria* (Rick 1907; Raithelhuber 1988) can be separated from *M. sabulosa* by its brownish to ochraceous pileus cover and yellowish annulus, and *M. zeyheri* (Rick 1907; Raithelhuber 1988) differs by its brownish pileus covered with flakes and ocher-yellowish lamellae. Phylogenetically, *M. sabulosa* is the sister lineage of *M. cyanolamellata* and closely related to *M. clelandii* and *M. subcitrophylla*. Morphologically, *M. sabulosa* can be easily separated from *M. clelandii* and *M. subcitrophylla* by their whitish and yellowish basidiomata, respectively. *Macrolepiota sabulosa* has a simple annulus like *M. pulchella*, but the latter has a volva and yellowish lamellae (Rick 1938; Heinemann and de Meijer 1996; Vellinga and Yang 2003) and does not present clamp connections. *Macrolepiota colombiana* has brown squamules on a white background, a double annulus, squamules on the pileus with hyphae ± catenulate, and cheilocystidia clavate to cylindrical with pale brownish pigmentation (Franco-Molano 1999; Ferreira and Cortez 2011).

Additional specimen examined: BRAZIL. RIO GRANDE DO NORTE STATE: Natal, Parque Estadual Dunas de Natal, Ubaia Doce Trail, in sandy soil among dry foliage, 05°48′41.3″S, 35°11′17.5″W, 20 Jan 2016, *Xavier M.D. 70* (**paratype** UFRN 2694).

DISCUSSION

The two new species described herein are highly supported by morphological and molecular data. They are easily recognized in the field due to the particular color characteristics of their pileus and stipe, which separate them from other species in the genus.

In our study, the ITS data set was more inclusive, whereas the ITS+28S+*RPB2* data set represented a narrower sampling. However, two distinct clades (*Volvateae* and *Macrosporae*) were recovered in both analyses. The topology of our trees corroborated with previous studies (Vellinga 2003a; Ge et al. 2010, 2012). Ge et al. (2010) found the *Macrolepiota* clade with no support (MP BS = 51) and that the first splitting in this clade was *M. clelandii*, as previously evidenced by Vellinga (2003a, 2003b) and Vellinga et al. (2003), where *M. clelandii* was recovered as sister to the *M. dolichaula–M. colombiana* clade. Our analyses supported the *Macrolepiota* clade (89/1) and the first splitting was *M. clelandii* (93/1), along with three other lineages (*M. cyanolamellata, M. sabulosa*, and *M. subcitrophyla*).

The sequences provided in this study are the first for *Macrolepiota* from Brazil and were placed as five distinct lineages. Two new species are described here: *M. cyanolamellata* and *M. sabulosa*. Another species was recovered in the clade *Volvateae*, and the last two species were closely related to *M. colombiana*. Many specimens collected from Brazil resemble *M. colombiana*, as published by Ferreira and Cortez (2011). In GenBank, only *M. colombiana* and *M. procera* represent *Macrolepiota* species from South America. *Volvolepiota albida* (AF482858) cannot represent *M. brunnescens*, as we expected, but represents a *Leucoagaricus* specimen (BLAST sequence matching).

The type material of *M. bonaerensis* and that of *M. kerandi* were very degraded; it was possible to analyze only the basidiospores. However, Singer's descriptions (Singer and Digilio 1952) confirm the differences between these two aforementioned species and our new species. The type material of *M. brasiliensis* and that of *M. stercoraria* were very old and also could not be recovered. The basidiospores were observed and were smaller than those in the new species. Furthermore, Rick's original descriptions (Rick 1907, 1937) also differ in color and aspects of the pileus covering. The type of *M. pulchella* was also very degraded, but it was obviously different due to the absence of volva in *M. cyanolamellata* and *M. sabulosa*. Additionally, the basidiospores are smaller and the lamellae have a yellowish coloration (Rick 1938; Raithelhuber 1988). Sequences from the type material of *M. colombiana* and our material were clearly separated. In addition, *M. colombiana* differs by the white background of the pileus, white lamellae, and the double annulus, as well as by the format of the cheilocystidia and pileipellis elements (Franco-Molano 1999).

Neotropical and Paleotropical regions are highly diverse and unexplored areas. In this study, we described two new species of *Macrolepiota* from Brazil. In addition, we have several collections from Argentina and Brazil with interesting and distinct morphological features that lack molecular data. Therefore, there are many species still to be described. In our study, we found that Brazilian species of *Macrolepiota* clearly differ from the European species, even though they share morphological characteristics. Therefore, we must be careful when studying Neotropical species, since they represent different evolutionary histories. In order to increase knowledge about *Macrolepiota*

species, we need to apply morphological, molecular, and ecological approaches, conduct extensive sampling, and make critical morphological revisions, especially regarding taxonomic misidentifications caused by the application of species names from the Northern Hemisphere and Oceania to Neotropical specimens.

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