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Agaricus globocystidiatus: a new neotropical species with pleurocystidia in *Agaricus* subg. *Minoriopsis*

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Abstract

Agaricus is a monophyletic genus with a worldwide distribution and more than 400 described species. The genus grows on soil and can be easily recognized by the presence of an annulus on the stipe and free lamellae which become dark brown with spore maturation. Although *Agaricus* is easily recognized in the field because of its macroscopic characters, identification at the species level is difficult. Based on specimens collected in the states of Paraná and Santa Catarina, in the south of Brazil, we propose a new species *Agaricus globocystidiatus*. The new taxon is distinguished mainly by the presence of pleurocystidia, a rare morphological character in *Agaricus*. Molecular analyses based on nuc rITS1-5.8-ITS2 (ITS) barcode sequences show that *A. globocystidiatus* belongs to *Agaricus* subg. *Minoriopsis*.

Keywords: Agaricaceae, Brazil, phylogeny, systematics

Introduction

The genus *Agaricus* L. (1753:1171) (Basidiomycota) is monophyletic (Vellinga 2004) and comprises approximately 400 species worldwide (Karunarathna *et al.* 2016). Linnaeus (1753) was the first to describe the genus, in a very broad sense, including all mushrooms with lamellae and a stipe. Due to this, many species of mushroom-forming fungi were first described as *Agaricus* and have this genus name as their basionym. The representatives of *Agaricus* are saprobic and usually have the following features: large and fleshy basidioma, with a white, yellow, brown or sometimes blackish or purple pileus, which is easily separable from the stipe; lamellae free, white when young, changing to pink and finally becoming brown to dark brown when the basidiospores are mature; and an annulus that is always present and usually membranous, white, and sometimes double (Heinemann 1956; Singer 1986; Zhao *et al.* 2016). Macroscopic characters for the genus can be easily recognized in the field but species delimitation is very complex. Environmental factors, intraspecific variability and lack of characters to differentiate species are some of the problems found in the taxonomy of this genus (Zhao *et al.* 2011). *Agaricus* is microscopically characterized by the pileipellis being a cutis, hymenophoral trama regular to slightly interwoven, hyphae without clamp connections, brown basidiospores without a true apical pore, often the absence of pleurocystidia and presence or absence of cheilocystidia (Heinemann 1956; Singer 1986; Zhao *et al.* 2016). In addition to macroscopic and microscopic features, the Schäffer reaction and organoleptic characters (e.g. odor) are useful to identify subgenera, sections and species (Heinemann 1956; Zhao *et al.* 2016; Chen *et al.* 2017).

Molecular data and improved phylogenetic studies have helped clarify relationships among *Agaricus* taxa since Mitchell & Bresinsky (1999) produced the first phylogenetic analysis of the genus using ITS sequence data, based on ITS2 and 28S rDNA. Zhao *et al*. (2011) were the first to study molecular data from tropical specimens and observed that only about one-third of tropical species belong to the classical sections historically proposed based on

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temperate species, which suggests that the systematics of the genus need to be expanded. Zhao *et al.* (2016) analyzed morphological and molecular data from more than 700 samples and proposed a reconstruction of *Agaricus* based on divergence time to define taxonomic ranks. They produced a multi-gene phylogeny combining ITS, LSU, *tef-1α* and *rpb2* sequences of samples from tropical and temperate areas, and segregated the genus into five subgenera and twenty sections. Zhao *et al.* (2016) included in *Agaricus* subg. *Minores* (Fr.) R.L. Zhao & Moncalvo *Agaricus* sect. *Minores* (Fr.) Henn. and *Agaricus* sect. *Laeticolores* Heinem.

Recently, Chen *et al.* (2017) studied *Agaricus* subg. *Minores* and allied clades. According to Chen *et al.* (2017) the clade representing *Agaricus* sect. *Laeticolores* Heinem. in Zhao *et al*. (2016) corresponds to a new subgenus because *Agaricus* sect. *Laeticolores* is in fact a synonym of *Agaricus* sect. *Minores* (Fr.) Henn. since the type species of *Agaricus* sect. *Laeticolores*, *Agaricus laeticulus* Callac, L.A. Parra, Linda J. Chen & Raspé (2017:182), belongs to *Agaricus* sect. *Minores*. In agreement with the taxonomic system based on divergence time (Zhao *et al*. 2016) Chen *et al*. (2017) proposed *Agaricus* subg. *Minoriopsis* Linda J. Chen, L.A. Parra, Callac, Angelini & Raspé and ranked as subgenus because it has a stem age of 31.02 Ma. *Agaricus* subg. *Minoriopsis* is more related to *Agaricus* subg. *Flavoagaricus* Wasser and *Agaricus* subg. *Minores* (Fr.) R.L. Zhao & Moncalvo. *Agaricus* subg. *Minores* includes *Agaricus* sect. *Minores* (Fr.) Henn, *Agaricus* sect. *Leucocarpi* Linda J. Chen & Callac, and one unnamed section 1 (Chen *et al.* 2017).

The specimens of *Agaricus* subg. *Minoriopsis* are characterized by having a dark reddish purple, rarely reddish brown Schäffer's reaction; a positive KOH reaction; an odor of anise or almonds; a superous annulus that is thick at the margin, double, fibrillose-squamose, or sometimes with squamules radially arranged on the lower surface; and cheilocystidia that are clavate, pyriform, more or less globose or fusiform (Chen *et al.* 2017).

The main studies that describe species of *Agaricus* from Brazil were published by Pegler (1990), Heinemann (1993) and Meijer (2008). Pegler (1990) revised some specimens of agaricoid fungi from Brazil, collected by J.P.F.C. Montagne in 1843, and reported three species of *Agaricus* with holotypes deposited in the herbarium of the Muséum National d'Histoire Naturelle in France. Heinemann (1993) studied the tribe Agariceae in Brazil and described a new species, *A. meijeri* Heinem. (1993:368), collected in the state of Paraná, and Meijer (2008) described another *Agaricus* from Paraná, *A. stijvei* de Meijer (2008:302). Unfortunately, there are no available sequences from these specimens. To better understand the species distribution and infrageneric relationships in the genus it is important to collect and review specimens from neotropical regions that have been under studied (Zhao *et al.* 2011). In this paper a new species of *Agaricus* subg. *Minoriopsis* is described based on morphological and molecular data.

Materials and methods

Sampling and morphological study

The specimens studied were collected in the states of Paraná and Santa Catarina, in the south of Brazil, and were described macroscopically following Largent (1986) and Vellinga (1988). The chemical reactions were performed with 3% KOH (tissue turns yellow when positive) and 10% NaOH (tissue turns orange-red when positive) on the pileus and stipe surface of fresh or dried basidiomata. The color code is based on the *Online Auction Color Chart* (Kramer 2004). Micromorphological features were observed from dried specimens following the nomenclature terms of Largent & Thiers (1977) and Vellinga (1988). The notation [a/b/c] at the beginning of a set of basidiospores represents the following: (a) basidiospores were measured from (b) basidiomata taken from (c) collections. Basidiospore measurements were based on at least 20 structures per basidioma, including the Q-value (quotient of length to width), Q_m (the mean of the Q-value), L_m (the mean of length), and W_m (the mean of width). Basidiospore shape was based on the Q-value (Bas 1969).

DNA extraction, amplification and sequencing

DNA was extracted from dried pileus and lamellae fragments, following a modified CTAB extraction method (Góes-Neto *et al.* 2005). The nuc rITS1-5.8S-ITS2 (ITS) sequences were generated using the primer pair ITS8F and ITS6R (Dentinger *et al.* 2010). The PCR mix was prepared using4 µL genomic DNA (1:10), 10 µL water, 10 µL Master mix (Promega) and 0.5 µL each primer (10pmol/L). The PCR program was according to Zhao *et al.* (2011): 5 min at 95 °C; 35 cycles (1 min. at 94 °C, 1.5 min. at 55 °C, 1.5 min. at 72 °C); and 5 min at 72 °C. The PCR products were purified with 20% PEG and sequenced at Centro de Pesquisas René Rachou (Fiocruz Minas, Brazil) using the same primer pair.

Phylogenetic analyses

The generated sequences were manually checked and edited with Geneious v.8.1 (Kearse *et al.* 2012). Our dataset comprises sequences from eight newly collected *Agaricus* specimens from Brazil, 29 sequences of *Agaricus* subg. *Minores* and ten sequences of *Agaricus* subg. *Minoriopsis* taxa from GenBank (Table 1).The sequence of *A. albosquamosus* L.J. Chen, K.D. Hyde & R.L. Zhao (2016:46) (LD2012192), which is part of *Agaricus* subg. *Spissicaules* (Heinem.) R.L. Zhao & Moncalvo, was used as the outgroup. Newly obtained sequences were deposited in GenBank and accession numbers and country of origin are provided in Table 1. Sequences were aligned in MAFFT v7 (Katoh 2013) following the Q-INS-I criteria and then manually corrected using MEGA v7 (Tamura *et al.* 2013). To identify the best nucleotide evolution model, we used the AIC criterion (Akaike Information Criterion) in jModelTest v2.1.6 (Guindon *et al.* 2010; Darriba *et al.* 2012). The ITS region was partitioned into ITS1, 5.8S and ITS2, and the evolution model was estimated for each partition. The maximum likelihood (ML) analysis was performed in RAxML v8.2.8 (Stamatakis 2014) using the GTRGAMMA model with a rapid bootstrap analysis with 1,000 replicates and search for the best-scoring ML tree, and other parameters estimated by the software. The Bayesian inference (BI) analysis was performed with Mr.Bayes v3.2.6 (Ronquist & Huelsenbeck 2003) using three partitions (ITS1, 5.8S, ITS2), with two independent runs, four simultaneous independent chains and 20,000,000 generations with a sample frequency every 1,000 generation. RAxML, jModelTest, and Mr.Bayes were used in the CIPRES Science Gateway 3.1 (Miller *et al.* 2010). The outputs of the analyses were displayed with FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

TABLE 1. Specimens and sequences used for the molecular analyses. Sequences in **bold** were generated during this work.

Taxon	Collection	ITS	Location	Reference
Agaricus* subg. *Minores				
Agaricus kerriganii	LAPAG808	KT951306	Spain, Madrid	Zhao *et al.* (2016)
Agaricus kerriganii	AH-44509	KF447893	Spain, León	Parra (2013)
Agaricus edmondoi	LAPAG412	KT951326	Spain, Segovia	Zhao *et al.* (2016)
Agaricus matrum	LAPAG855	KT951310	Spain, Asturias	Zhao *et al.* (2016)
Agaricus matrum	AH-44506	KF447896	Spain, La Rioja	Parra (2013)
Agaricus friesianus	LAPAG592	KT951316	France, Pyrénées Atlantiques	Zhao *et al.* (2016)
Agaricus sp.	CA848	JF727864	Thailand	Zhao *et al.* (2011)
Agaricus sp.	CA921	KT951323	-	Zhao *et al.* (2016)
Agaricus sp.	ZRL2011156	KT951352	China, Yunnan	Zhao *et al.* (2016)
Agaricus sp.	ZRLLD013	KT951384	Thailand	Zhao *et al.* (2016)
Agaricus sp.	ZRLWXH3076	KT951388	China, Fujian	Zhao *et al.* (2016)
Agaricus sp.	ZRLWXH3150	KT951390	China, Guangdong	Zhao *et al.* (2016)
Agaricus comtulus	LAPAG724	KT951332	Spain, Burgos	Zhao *et al.* (2016)
Agaricus gemlii	AH-44510	KF447891	Spain, Canarias	Parra (2013)
Agaricus pallens	LAPAG926	KT951315	Sweden, Lapland	Zhao *et al.* (2016)
Agaricus sp.	CA846	JF727865	Thailand	Zhao *et al.* (2011)
Agaricus sp.	ZRL2012357	KT951369	China, Yunnan	Zhao *et al.* (2016)
Agaricus sp.	ZRLWXH3067	KT951387	China, Jiangxi	Zhao *et al.* (2016)
Agaricus sp.	ZRL2012576	KT951372	China, Tibet	Zhao *et al.* (2016)
Agaricus sp.	ZRL2012714	KT951381	China, Tibet	Zhao *et al.* (2016)
Agaricus sp.	ZRL2011039	KT951351	China, Yunnan	Zhao *et al.* (2016)
Agaricus aridicola	LAPAG589	KT951331	Spain, Castellón	Zhao *et al.* (2016)
Agaricus pseudolutosus	LAPAG454	KT951329	Spain, Burgos	Zhao *et al.* (2016)
Agaricus sp.	ZRL2012012	KT951359	China, Yunnan	Zhao *et al.* (2016)
Agaricus sp.	ZRL2012199	KT951367	China, Yunnan	Zhao *et al.* (2016)
Agaricus callacii	AH-42929	KF447899	Spain, Canarias	Parra (2013)
Agaricus sp.	ZRLWXH3161	KT951391	China, Guangdong	Zhao *et al.* (2016)
Agaricus sp.	LAPAM14	KT951312	Dominican Republic, Sosúa	Zhao *et al.* (2016)
Agaricus candidolutescens	LD2012129	KT951335	Thailand	Zhao *et al.* (2016)
Agaricus* subg. *Minoriopsis				
Agaricus cf. *globocystidiatus*	MATA816	JF727870	Mexico, Veracruz	Zhao *et al.* (2011)
Agaricus globocystidiatus	**MPD02**	**MF188244**	**Brazil, Santa Catarina**	
Agaricus globocystidiatus	**MPD03**	**MF188248**	**Brazil, Santa Catarina**	
Agaricus globocystidiatus	**MPD04**	**MF188245**	**Brazil, Santa Catarina**	
Agaricus globocystidiatus	**MPD29**	**MF188247**	**Brazil, Santa Catarina**	
Agaricus globocystidiatus	**MPD128**	**MF188249**	**Brazil, Santa Catarina**	

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TABLE 1. (Continued)

Taxon	Collection	ITS	Location	Reference
Agaricus globocystidiatus	**MPD19**	**MF188253**	**Brazil, Santa Catarina**	
Agaricus globocystidiatus	**MPD71**	**MF188251**	**Brazil, Paraná**	
Agaricus globocystidiatus	**EC31**	**MF188252**	**Brazil, Santa Catarina**	
Agaricus sp.	F1779	JF727853	France, Martinique	Zhao *et al.* (2011)
Agaricus sp.	RMC-1257	KM349612	USA, Arizona	Kerrigan (2016)
Agaricus sp.	RMC-1256	KM349611	USA, Arizona	Kerrigan (2016)
Agaricus rufoaurantiacus	LAPAM15	KT951313	Dominican Republic, Sosúa	Zhao *et al.* (2016)
Agaricus aff. *rufoaurantiacus*	CL/GUAD05.099	JF727857	France, Guadeloupe	Zhao *et al.* (2011)
Agaricus sp.	HAI0386	AJ884624	USA	Didukh *et al.* (2005)
Agaricus martinicensis	F2815	JF727855	France, Martinique	Zhao *et al.* (2011)
Agaricus martinicensis	LAPAM16	KX671699	Dominican Republic	Chen *et al.* (2017)
Agaricus sp.	LAPAM28	KX671700	Dominican Republic	Chen *et al.* (2017)
Agaricus* subg. *Spissicaules				
Agaricus albosquamosus	LD2012192	KT951394	Thailand	Zhao *et al.* (2016)

Results and Discussion

Phylogeny

The final ITS alignment had a total of 689 characters (gaps included) with 47 ingroup sequences, including eight generated in this study. The best models of nucleotide substitution estimated for each partition in the datasets were: TVM+G to ITS1, JC to ITS 5.8S, and HKY+I+G to ITS2. Both ML and BI tree topologies were the same, with differences only among sequences within *Agaricus* subg. *Minores*. The BI obtained by the analysis is presented in Figure 1, with posterior probability (PP) and bootstrap (BS) values above the branches.

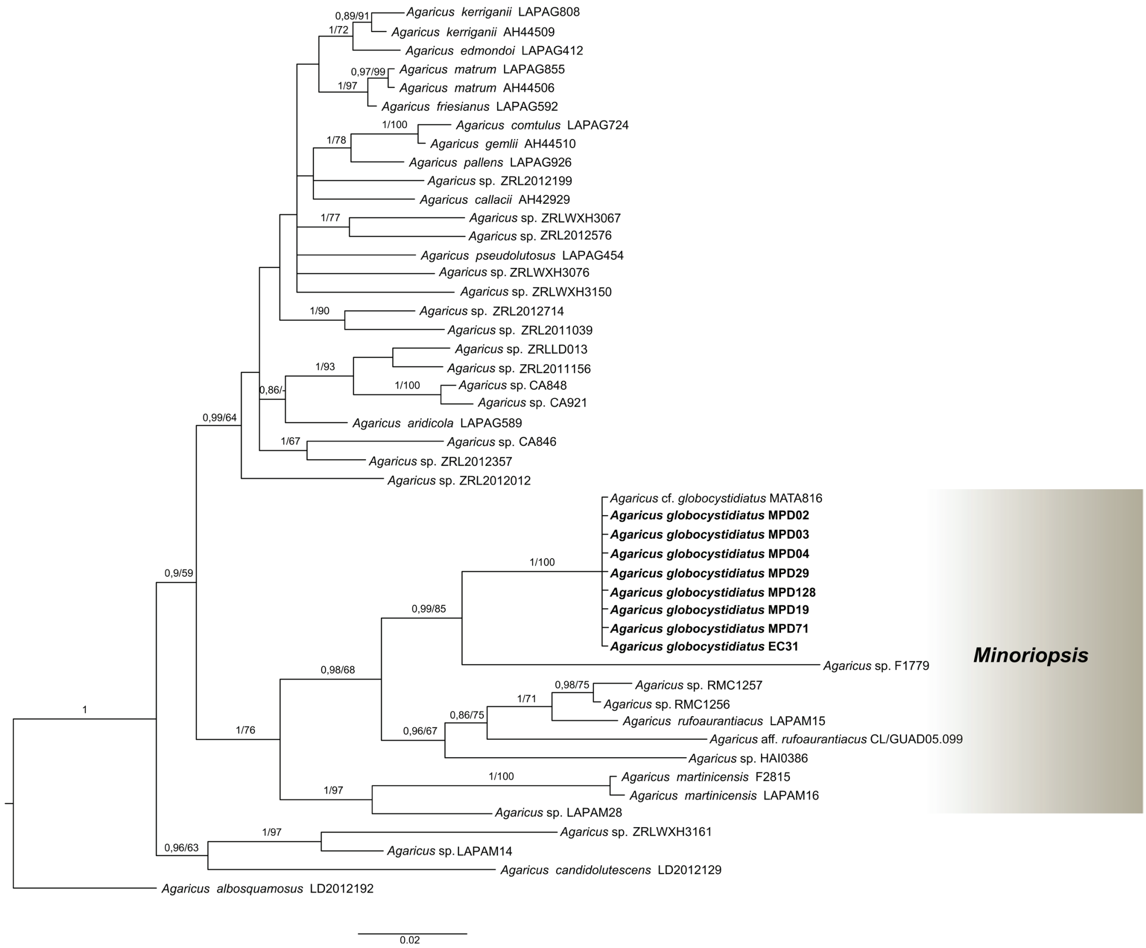

FIGURE 1. Bayesian inference tree of *Agaricus* subg. *Minoriopsis* and *Agaricus* subg. *Minores* based on ITS data and rooted with *Agaricus albosquamosus*. The sequences generated in this work are in **bold**. Posterior probability (PP) (above 0.7) and bootstrap (BS) (above 50%) support values are shown above the branches (PP/BS).

Sequences of the new species clustered in a well-supported clade with one sequence from Mexico (JF727870), which probably also represents *A. globocystidiatus*, and other sequences of *Agaricus* subg. *Minoriopsis*. The sequence JF727870 from Mexican material (MATA816) clustered in the clade TRII of Zhao *et al.* (2011) with sequences of five other specimens, but the authors did not assign a taxonomic name to MATA816 and it was not included in further analyses by Zhao *et al.* (2016).

Our analysis focused on the phylogenetic relationships between the new species and closely related species. All known members of *Agaricus* subg. *Minoriopsis* were included and the analysis clearly indicated that the new species belongs to this subgenus. However, in our tree, phylogenetic relationships between taxa of higher rank are not well supported. In our analysis, *Agaricus* subg. *Minoriopsis* appears as a sister group to *Agaricus* sect. *Minores*, and *Agaricus* subg. *Minores* appears polyphyletic, differing to what Chen *et al.* (2017) found in a multi-gene phylogenetic analysis. Chen *et al.* (2017) reported eight species in *Agaricus* subg. *Minoriopsis* and we add *A. globocystidiatus* in the present study. All species known to *Agaricus* subg. *Minoriopsis* are from the Americas (Chen *et al.* 2017).

Taxonomy

Agaricus globocystidiatus Drewinski & M.A.Neves *sp. nov.* (Figs. 2, 3)
Mycobank: MB821296

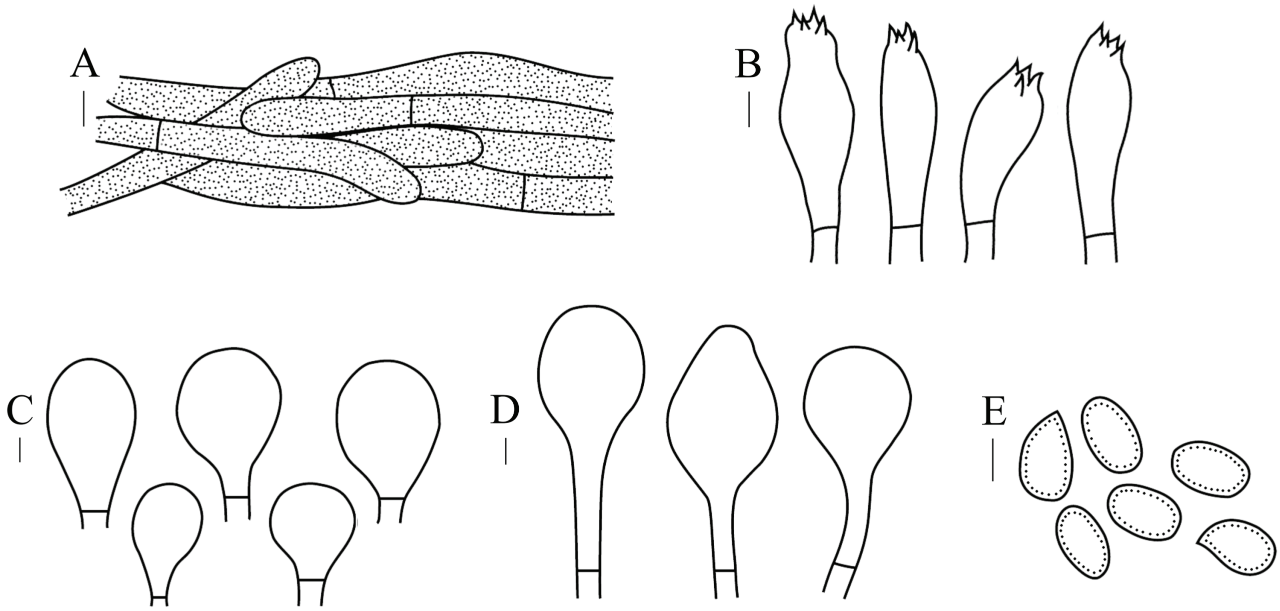

FIGURE 2. Microcharacters of *Agaricus globocystidiatus* FLOR 61594 (*MPD29*–Holotype). a. Pileipellis; b. Basidia; c. Cheilocystidia; d. Pleurocystidia; e. Basidiospores. Scale bars: 5μm. Drawings by M.P.Drewinski.

Diagnosis:—Similar to *Agaricus pleurocystidiatus* by the presence of pleurocystidia, but differing in the pileus surface covered by concentrically arranged purple scales, regular pileus margin, and stipe with brown scales and slightly yellowish on some parts when exposed.

Etymology:—*glob*-(L.) refers to the main shape of the pleurocystidia and *cystidium* (L.) refers to the presence of pleurocystidia, a rare morphological character in this genus.

Holotype:—BRAZIL. Santa Catarina: Florianópolis, Universidade Federal de Santa Catarina, near to the Department of Botany, 10 December 2015, *M.P. Drewinski MPD29* (FLOR61594).

Description:—*Pileus* 30–122 mm diam., at first parabolic, then conical truncated to convex and finally expanding to plano-convex, slightly depressed, covered by concentrically arranged purple (oac527) scales on beige (oac780) background, darker at center with concentrated scales that are scattered towards the margin, margin regular. *Pileus context* approx. 5mm thick at center and 1mm at margin, whitish or slightly yellowish in some parts when exposed, fleshy. *Lamellae* free, white at first, then pinkish and finally brown when mature, margin regular, crowded, with lamellulae. *Stipe* 102–120 × 9–20 mm, central, tapering at base, fistulose, beige pearly (oac767), smooth above annulus, with brown scales below, changing to yellow in some parts including the context of the base. *Annulus* superous, pendant, membranous, floccose on lower surface, whitish.

Basidiospores [200/10/8] (4.0–)5.0–6.2(–7.5) × (3.7–)5.0 µm, [Q= 1.25–1.66; Q_m= 1.42; L_m=5.3µm; W_m=4.2µm], broadly ellipsoid to ellipsoid, dark brown, smooth, thick-walled, apiculus conspicuous, without a germ pore. *Basidia* (16.2–)21–37(–40) × 6.2–10.0(–11.2) µm, narrowly utriform to clavate, hyaline, smooth, 4-spored. *Pleurocystidia* (25–)26–43 × (16.2–)18.7–23 µm, globose, subglobose, ovoid to broadly clavate, hyaline, smooth, thin-walled. *Cheilocystidia* (11.2–)13.7–45(–47) × (7.5–)10–21(–23) µm, clavate, subglobose to obovoid, hyaline, thin-walled, usually covering all the lamellae edge. *Lamellar trama* regular, with abundant oleiferous hyphae, up to 6.5(–8.7) µm diam. *Pileipellis* a cutis composed of cylindrical and thin- to rather thick-walled hyphae, (3.7–)5.0–11.2µm diam., branched, with purple internal plasmatic pigments. *Pileus context* composed of cylindrical and thin-walled hyphae, (5.0–)6.2–12.5 µm diam., with abundant oleiferous hyphae. *Stipitipellis* a cutis composed of cylindrical and thin-walled hyphae, 5.0–10.0 µm diam., sometimes inflated. *Macrochemical reactions* yellow in 3% KOH (positive), yellow in 10% NaOH (positive). Schaffer's reaction was unclear, but the pileus turned purple with citric acid. *Odor* sweet almond.

Habit, habitat and distribution:—solitary to gregarious, terrestrial, growing on grass in urban areas and areas of the Atlantic Forest domain; Brazil, Santa Catarina and Paraná states.

Specimens examined:—BRAZIL. Santa Catarina: Florianópolis, at the University Campus near the Botany Department, 30 July 2015, *M.P. Drewinski MPD02* (FLOR61586–Paratype); 05 October 2015, *M.P. Drewinski MPD03* (SP466715–Paratype); 07 October 2015, *M.P. Drewinski MPD04*;16 March 2016, *M.P. Drewinski MPD128* (FLOR61612–Paratype); 09 February 2017, *M.P. Drewinski MPD145* (FLOR61620–Paratype); Parque Ecológico do Córrego Grande, 11 February 2016, *E.Copini EC31* (FLOR61583); Cacupé, *Tim Brightwell MPD19* (FLOR61582); Paraná: Guarapuava, Parque Municipal das Araucárias, 12 January 2016, *M.P. Drewinski MPD71*(FLOR61599).

Notes:—The presence of pleurocystidia is a rare morphological character in *Agaricus.* Although those pleurocystidia are large and stand out from the other structures in the hymenium, they are not abundant and may go unnoticed if the material is not carefully studied (Heinemann 1980). *Agaricus globocystidiatus* is similar to *A. pleurocystidiatus* Heinem. (1980:12), from Singapore, mainly due to the presence of pleurocystidia and because of the similar annulus that is described as membranous, pendant, white and with flakes on the lower side (Heinemann 1980). However, *A. pleurocystidiatus* differs by the following: the pileus surface, which has dark brown scales on a light brown background; the pileus margin, which is appendiculate; and the fibrillose light brown stipe. Furthermore, *A. pleurocystidiatus* changes to reddish brown when cut and is known only from Singapore (Heinemann 1980).

Agaricus sinodeliciosus Z.R. Wang & R.L. Zhao (2015:192) from China also has pleurocystidia (Wang *et al.* 2015). However, *A. sinodeliciosus* differs by its semi-hypogeous habit (growing buried in sandy soil), pileus surface covered with light brown or buff brown squamules on a dirty background, involute margin, smooth or fibrillose white stipe that becomes reddish brown when bruised, negative Schäffer and KOH reactions, inferior annulus forming a broad sheath, and context that turns reddish brown when cut (Wang *et al.* 2015).

Agaricus martinicensis Pegler (1983:446) is the type species of *Agaricus* subg. *Minoriopsis*. *Agaricus globocystidiatus* is similar to *A. martinicensis* by the presence of cheilocystidia, by the purplish brown contents of the pileipellis, both species have the stipe covered with squamules below the annulus and a thin, membranous, pendent, white annulus that has floccose remnants of the universal veil at the lower surface. *Agaricus globocystidiatus* differs from *A. martinicensis* by the larger and beige pileus with purple scales and the presence of pleurocystidia.

The basidiomata of *Agaricus globocystidiatus* collected in Brazil share some characters attributed to *Agaricus* subg. *Minoriopsis* (Chen *et al.* 2017), including an annulus that is superous, a positive reaction to KOH, and the presence of clavate, subglobose to obovoid cheilocystidia. The other two species in the genus known to have pleurocystidia, *A. pleurocystidiatus* and *A. sinodeliciosus*, change to reddish brown upon cutting and belong to *Agaricus* subg. *Pseudochitonia* sect. *Sanguinolenti* Schaeff. & Moll. and sect. *Bivelares* (Kauffman) L.A. Parra, respectively (Heinemann 1980; Wang *et al.* 2015).

Agaricus globocystidiatus is a new species in *Agaricus* subg. *Minoriopsis* supported by morphological and molecular data. Although it is necessary to revise the morphology of the collection MATA816 (JF727870) to confirm the presence of pleurocystidia, our molecular results indicate that it is most likely *A. globocystidiatus*, which suggests a possible neotropical distribution for the new species here described. All sequences in *A. globocystidiatus* clade are 98.8–100% identical, with MATA816 presenting levels of sequence divergence between 1.2% and 0.1% compared to Brazilian sequences (MPD02 and MPD19, respectively). This work increases the knowledge of taxa within this subgenus and of *Agaricus* species that occur in America, which is important to better understand the phylogenetic relationships and the biogeographic patterns in the genus.

FIGURE 3. *Agaricus globocystidiatus* a–b. Basidiomata; a. FLOR61620 (*MPD145*); b. FLOR 61594 (*MPD29*–Holotype); c. Pileus surface FLOR 61599 (*MPD71*); d. Stipe with details of the scales and annulus with floccose lower surface FLOR61620 (*MPD145*); e. Pleurocystidia FLOR 61594 (*MPD29*–Holotype); f. Pleurocystidia (*MPD04*). Photos by M.P.Drewinski.

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