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Marasmius magnus (Marasmiaceae), a new species from the southern Atlantic Forest of Brazil

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Abstract

Marasmius magnus, is herein described as a new species from the southern Atlantic Forest, Brazil. It is characterized by the very large basidiomata, small and ellipsoid basidiospores, absence of cheilocystidia and rarely projecting pleurocystidia. The uniqueness of the species is confirmed by ITS data and further supported by a complete morphological description, taxonomic comments, illustrations and photographs.

Keywords: Agaricales, Marasmiineae, Neotropics, sect. Globulares, taxonomy

Introduction

Marasmius Fries (1835: 339) (Marasmiaceae) is a common and widespread genus with members that are found in mesophytic to xerophytic ecosystems and play an important role in the decomposition of plant debris and in the balance of nutrient cycling (Singer 1976, 1986, Desjardin 1989). In Brazil, 63 species are known to occur in the coastal Atlantic Forest (Capelari *et al.* 2015) from Rio Grande do Sul, in the South Region, to Rio Grande do Norte, in the Northeast Region (Batista & Silva 1951, Bononi *et al.* 1981, Grandi *et al.* 1984, Pegler 1997, Sobestiansky 2005, de Meijer 2006, Puccinelli & Capelari 2006, 2007, 2009a, 2009b, Oliveira & Capelari 2012, Oliveira *et al.* 2014).

Based on results from previous studies in *Marasmius* including molecular phylogenetic analyses (Wilson & Desjardin 2005, Wannathes *et al.* 2009, Tan *et al.* 2009), Antonín & Noordeloos (2010) emended section *Globulares* to encompass sect. *Globulares* Kühner (1933: 100) *sensu* Singer (1986) and sect. *Sicci* Singer (1958: 106.) However, sect. *Sicci* was kept as an informal group characterized by the presence of *Siccus*-type broom cells in the pileipellis, the morphological feature that distinguishes this group from the species having only *Globulares*-type smooth cells in the pileipellis, that formed sect. *Globulares* according to Singer (1986).

In this article *Marasmius magnus* is described for the first time and a morphological description, line drawings, taxonomic discussion and ITS data are presented. *Marasmius magnus* is one of the species in the genus that produce large basidiomata. In the traditional concept of the genus (Singer 1976, 1986) *M. magnus* would be included in sect. *Sicci* ser. *Haematocephali*. However, regarding the current sectional concept in *Marasmius* (Wilson & Desjardin 2005, Tan *et al.* 2009, Wannathes *et al.* 2009, Antonín & Noorderloos 2010), *M. magnus* belongs to sect. *Globulares* Kühner emend. Antonín & Noorderloos. Molecular ITS (internal transcribed spacer) sequence data confirm the uniqueness of *M. magnus* relative to the other *Marasmius* species for which ITS sequence data are available and were used to verify the taxon placement within a phylogenetic tree including members of sect. *Globulares*.

Material and Methods

Sampled areas

The specimens were collected during the summer (rainy season), from January to March (2011-2015), at Morro da Lagoa

da Conceição and Parque Municipal da Lagoa do Peri, Florianópolis, Santa Catarina (27°35'04.5"S, 48°28'29.0"W and 27°44'38.5"S 48°31'05.5"W), and Morro Santana, Porto Alegre, Rio Grande do Sul (30°03'59.4"S, 51°07'25.3"W). These areas are remnants of Atlantic Forest, a biome that harbours a high diversity of fungi.

Morphological description

The macromorphology of the specimens is based on fresh basidiomata. Color codes (e.g., OAC 642) are based on the Online Auction Color Chart (Krammer 2004). For microscopic observations, sections of dried material were rehydrated in 70% ethanol followed by 5% KOH or Melzer's reagent. The dimensions of the basidiospores included the range of length × width. Further measurements were based on multiple collections and the following parameters were determined: X_{mr} , the range of spore means of length × width; X_{mm} , the mean of spore means (± standard deviation, SD); Q_m , the mean of the quotient of the length by the width in each specimen; Q_{mr} , the range of the means of Q_m ; Q_{mm} , the mean of Q_m values (± SD); n, the number of basidiospores measured per specimen; and s, the number of specimens studied. The collections were deposited at ICN, FLOR and SP (Thiers, continuously updated). Fragments of the specimens were preserved in silica for DNA extraction.

Sequencing and phylogenetic analyses

DNA was extracted following Doyle & Doyle (1987) adapted by Góes-Neto *et al.* (2005). The primer pairs ITS6-R-ITS8-F were used to amplify the ITS (ITS1-5.8S-ITS2) region, following the cycles parameters from Dentinger *et al.* (2010). Sequencing was performed with BigDye Terminator 3.1 Cycle Sequencing Kit following manufacturer procedures, using the same primers cited above. The generated sequences and chromatograms were manually checked and edited with Geneious 6.1.8 (Kearse *et al.* 2012).

To produce the dataset for the analyses, BLAST searches (Altschul *et al.* 1990) were conducted in the NCBI database, retrieving the *Marasmius* ITS sequences closest to the query (if possible, over 90% of similarity and *e*-value equal or close to zero). The selected sequences were preferably from previous publications presenting taxonomic discussion (ex. Wannathes *et al.* 2009, Antonín *et al.* 2010, 2012, 2014). The dataset is composed of two lots of sequences assigned to members of sect. *Globulares* to form the ingroup: 1) closest taxa according to the BLAST searches; 2) distant taxa within the section for better sorting of a comprehensive sampling, spanning all infrasectional groups according to the traditional view. The outgroup was composed of taxa belonging to sect. *Marasmius* subsect. *Sicciformes*, sect. *Leveilleani* and sect. *Neosessiles*. Dataset information is included in Table 1. The alignment was done using MUSCLE (Edgar 2004) online in EMBL-EBI (http://www.ebi.ac.uk/Tools/msa/muscle/). The model GTR+G+I was selected using MrModeltest 2.3. (Nylander 2004), based on Akaike Information Criterion (AIC).

Sect./Subsect./Ser.	Species	Coll. N°	Herb. Voucher	GenBank accession N°.
Globulares	M. albimyceliosus	NW422	CMU, SFSU	EU935544 ^δ
Globulares	M. grandiviridis	NW152	CMU, SFSU	EU643514 ⁸
Globulares	M. laticlavatus	NW293	CMU, SFSU	EU643512 ⁸
Globulares	M. laticlavatus	NW412	CMU, SFSU	EU643511 ⁸
Globulares	M. maximus	V.Ant.*08.56	BRNM 714571	FJ904977 ⁿ
Globulares	M. maximus	R. Ryoo KG 224	BRNM 714672	FJ904974 ⁿ
Globulares	M. nigrodiscus		TENN 49976	KF774138 γ
Globulares	M. nigrodiscus		TENN 59556	KF774139 ^y
Globulares	M. nigrodiscus		TENN 049828	KF774140 ^γ
Globulares	M. nivicola	V.Ant.*07.53	BRNM 714573	FJ904971 ⁿ
Globulares	M. nivicola	-	KPM-NC0006038	FJ904973 ⁿ
Globulares	M. pellucidus	NW321	CMU, SFSU	EU935508 ⁸
Globulares	M. pellucidus	NW342	CMU, SFSU	EU935509 ^δ

TABLE 1. ITS dataset of Marasmius used in the phylogenetic analysis, itemized by infrageneric group.

...Continued on next page

TABLE 1. (Continued)

Sect./Subsect./Ser.	Species	Coll. Nº	Herb. Voucher	GenBank accession N°.
Globulares	M. pseudopurpureostriatus	NW286	CMU, SFSU	EU643513 ⁸
Globulares	M. purpureostriatus	V.Ant.*08.57	BRNM 714566	FJ904978 ⁿ
Globulares	M. wynneae	-	HCCN - G86	FJ904979 ⁿ
Leveilleani	M. leveilleanus	NW248 CMU, SFSU		EU935566 ⁸
Leveilleani	M. leveilleanus	NW268 CMU, SFSU		EU935567 ⁸
Marasmius/Sicciformes	M. curreyi	DED5142		FJ431237 ^o
Marasmius/Sicciformes	M. graminum	NN005953		JN943595 ^β
Marasmius/Sicciformes	M. nigrobrunneus	NW162	CMU, SFSU	EU935570 ⁸
Marasmius/Sicciformes	M. nigrobrunneus	NW416	CMU, SFSU	EU935571 ⁸
Marasmius/Sicciformes	M. ruforotula	V.Ant.*07.158	BRMN 714674	FJ936150 ^k
Marasmius/Sicciformes	M. ruforotula	-	BRMN 714679	FJ936149 ^k
Neosessiles	M. tenuissimus	NW192	CMU, SFSU	EU935568 ⁸
Neosessiles	M. tenuissimus	NW199	CMU, SFSU	EU935569 ⁸
Sicci/Atrorubentes	M. araucariae var. siccipes	NW364	CMU, SFSU	EU935511 ⁸
Sicci/Atrorubentes	M. auratus	NW076	CMU, SFSU	EU935501 ⁸
Sicci/Atrorubentes	M. auratus	NW175	CMU, SFSU	EU935502 ⁸
Sicci/Atrorubentes	M. jasminodorus	NW294	CMU, SFSU	EU935513 ^δ
Sicci/Atrorubentes	M. jasminodorus	NW414	CMU, SFSU	EU935515 ⁸
Sicci/Atrorubentes	M. iras	NW276	CMU, SFSU	EU935486 ⁸
Sicci/Atrorubentes	M. iras	NW375	CMU, SFSU	EU935487 ⁸
Sicci/Atrorubentes	M. ochroleucus	NW299	CMU, SFSU	EU935503 ⁸
Sicci/Atrorubentes	M. pseudopellucidus	NW186	CMU, SFSU	EU935504 ⁸
Sicci/Atrorubentes	M. pseudopellucidus	NW305	CMU, SFSU	EU935505 ⁸
Sicci/Atrorubentes	M. strobiluriformis		BRNM 714914	GU266263 ^µ
Sicci/Atrorubentes	M. strobiluriformis		BRNM 714915	GU266264 ^µ
Sicci/Atrorubentes	M. xestocephalus	JFK69	CMU, SFSU	EU935488 ⁸
Sicci/Atrorubentes	M. xestocephalus	NW344	CMU, SFSU	EU935489 ⁸
Sicci/Haematocephali	M. bondoi	NW011	CMU, SFSU	EU935472 ⁸
Sicci/Haematocephali	M. bondoi	NW386	CMU, SFSU	EU935476 ⁸
Sicci/Haematocephali	M. brunneoolivascens	NW112	CMU, SFSU	EU935516 ⁸
Sicci/Haematocephali	M. brunneoolivascens	NW373	CMU, SFSU	EU935517 ⁸
Sicci/Haematocephali	M. conchiformis	JO117	SP 417467	JX424038 ^{<i>π</i>}
Sicci/Haematocephali	M. confertus var.	V.Ant.*09.151	BRNM 718808	HQ607374 ^µ
	tenuicystidiatus			
Sicci/Haematocephali	M. crinipes	V.Ant.*07.150	BRNM 714684	FJ917627 ^µ
Sicci/Haematocephali	M. crinipes	R. Ryoo	BRNM 714694	FJ917629 ^µ
Sicci/Haematocephali	M. ferrugineus	R. Ryoo KG 226	BRNM 724480	HQ616662 ^µ
Sicci/Haematocephali	M. graminipes	NW078	CMU, SFSU	EU935479 ^δ

...Continued on next page

TABLE 1. (Continued)

Sect./Subsect./Ser.	Species	Coll. N°	Herb. Voucher	GenBank accession N°.
Sicci/Haematocephali	M. linderioides	JO286	SP 417469	JX424037 ^π
Sicci/Haematocephali	M. haematocephalus	NW434	CMU, SFSU	EU935529 ⁸
Sicci/Haematocephali	M. haematocephalus "f. luteocephalus"	NW310	CMU, SFSU	EU935534 ⁸
Sicci/Haematocephali	M. haematocephalus "f. variabilis"	NW430	CMU, SFSU	EU935535 ⁸
Sicci/Haematocephali	M. magnus	ACM1001	FLOR 55963	KX228846
Sicci/Haematocephali	M. magnus	ACM1002	ICN 179251	KX228847
Sicci/Haematocephali	M. magnus	ACM1128	ICN 179252	KX228848
Sicci/Haematocephali	M. magnus	MJ48	FLOR 55928	KX228849
Sicci/Haematocephali	M. magnus	NPS514	FLOR 55929	KX228850
Sicci/Haematocephali	M. siccus	DED5255	-	FJ431272 ^o
Sicci/Haematocephali	M. siccus	-	BRNM 552709	HQ607384 ^µ
Sicci/Haematocephali	M. sullivantii	TFB13629	TENN 63063	HQ665549 ^ç
Sicci/Haematocephali	M. suthepensis	TYS280	CMU, SFSU	EU935520 ⁸
Sicci/Leonini	M. bambusiniformis	NW329	CMU, SFSU	EU935521 ⁸
Sicci/Leonini	M. bambusiniformis	NW368	CMU, SFSU	EU935522 ⁸
Sicci/Leonini	M. corneri	NW269	CMU, SFSU	EU935482 ⁸
Sicci/Leonini	M. corneri	TYS274	CMU, SFSU	EU935483 ⁸
Sicci/Leonini	M. cupreostipes	NW150	CMU, SFSU	EU935485 ⁸
Sicci/Leonini	M. graminicola	V.Ant.*07.61	BRNM 714685	FJ917617 ^µ
Sicci/Leonini	M. graminicola	R. Ryoo KG 113	BRNM 714696	FJ917618 ^µ
Sicci/Leonini	M. graminicola	R. Ryoo KG 124	BRNM 714687	FJ917621 ^µ
Sicci/Leonini	M. hypochroides	NW405		EU935545 ⁸
Sicci/Leonini	M. imitarius	NW423	CMU, SFSU	EU935497 ⁸
Sicci/Leonini	M. imitarius	NW425	CMU, SFSU	EU935498 ⁸
Sicci/Leonini	M. koreanus	R. Ryoo KG 222	BRNM 714700	FJ917619 ^µ
Sicci/Leonini	M. koreanus	R. Ryoo KG 225	BRNM 714701	FJ917620 ^µ
Sicci/Leonini	M. plicatulus	NW439	-	EU935480 ⁸
Sicci/Spinulosi	M. coarctatus	NW315	CMU, SFSU	EU935541 ⁸
Sicci/Spinulosi	M. coarctatus	NW385	CMU, SFSU	EU935542 ⁸
Sicci/Spinulosi	M. cohaerens	LE7646	-	KF774175 γ
Sicci/Spinulosi	M. cohaerens	-	TENN 061237	KF774177 ^γ
Sicci/Spinulosi	M. longisetosus	JO248	SP 417470	JX424040 ^π
Sicci/Spinulosi	M. nummularius	NW266	CMU, SFSU	EU935492 ⁸
Sicci/Spinulosi	M. nummularius	NW396	CMU, SFSU	EU935493 ⁸
Sicci/Spinulosi	M. trichotus	NW262	CMU, SFSU	EU935490 ⁸
Sicci/Spinulosi	M. trichotus	NW263	CMU, SFSU	EU935491 ⁸

Published sequences are found in Antonín *et al.* (2010 ^η, 2012 ^μ, 2014 ^κ), Hughes & Petersen (GenBank) ^ς, Kiyashko *et al.* (2014) ^γ, Oliveira *et al.* (2014) ^π, Schoch *et al.* (2012) ^β, Tan *et al.* (2009) ^φ, Wannathes *et al.* (2009) ^δ and new sequences provided by this study in bold.

For Maximum Likelihood (ML), $GTR+\Gamma+I$ model plus fast-bootstrapping was set for 1,000 pseudoreplicates and full ML optimization for the final tree, all conducted in RaxML 7.0.4 (Stamatakis 2006). By implementing CAT approximations, all free model parameters (25 per site rate categories) were estimated by RAxML algorithm. GAMMA Model parameters were estimated up to an accuracy of 0.100000000 Log Likelihood units, with which the final tree was evaluated and optimized. MC³ Bayesian analyses (B) were performed with MrBayes 3.2.1 (Ronquist *et al.* 2012). Implementing GTR+G+I (6 Nst with flat Dirichlet), B consisted of two independent runs of 5,000,000 generations, sampling frequency every 500 generations, in 6 independent chains and allowing 2 swaps. The burn-in was set at 10%. Final trees were summarized by the 50% majority-rule consensus method. Branch lengths were summarized across the 95% highest posterior density trees. The tree generated by Bayesian analysis was chosen to display the phylogenetic relationships among the taxa of the final datasets.

Results

Taxonomy

Marasmius magnus A.C. Magnago & J.S. Oliveira, *sp. nov.* Figs. 1–2. MycoBank:—MB 816824

- Diagnosis:—Pileus large (31–122 mm diam.), fulvous to rusty orange, center reddish brown, with cream margin. Lamellae free to adnexed, 18–22 per cap. Stipe 70–94 × 4–6 mm, light reddish brown. Basidiospores (4.8–)5.2–8.8 × 2.5–3.8 μm. Pleurocystidia 30–87.5 × 5–7.5 μm, rarely projecting. Cheilocystidia absent. Pileipellis composed of *Siccus*-type broom cells. Caulocystidia cylindrical to bilobed.
- Type:—BRAZIL. Santa Catarina: Florianópolis, Morro da Lagoa, Trilha do Jipe, growing on decomposing leaf litter in Atlantic Forest, 27°35'04.5"S, 48°28'29.0", 20 March 2014, *Magnago AC* 1001 (FLOR 55963!). GenBank accession: ITS = KX228846.



FIGURE 1. *Marasmius magnus*. A. Basidiomata in the field. B. Detail of pileus surface. C. Detail of hymenophore. Bar = 5 cm. Photos by: Altielys C Magnago.

Etymology:---from Latin magnus (large, great); referring to the large size of the basidiomata.

Description:—*Pileus* 31–122 mm wide, paraboloid when young, broadly convex to nearly plane when mature, dry, glabrous to velutinous in the center, opaque, slightly sulcate towards the margin, fulvous to rusty orange (OAC 642), center reddish brown (OAC 656), extreme margin generally whitish cream. *Lamellae* free to adnexed, not collariate, 18–22 per cap, centrally broad, 5–13 mm wide, whitish to creamy buff (OAC 815), entire, not intervenose, distant;

lamellulae present, in 4–5 series. *Stipe* 70–94 mm long, 4–6 mm thick, cylindrical, equal, cartilaginous to fibrous, hollow, glabrous to slightly pruinose, light reddish brown (OAC 728); with whitish, tomentose basal mycelium.



FIGURE 2. *Marasmius magnus.* A. Basidiospores. B. Pleurocystidia. C. Elements of the stipitipellis. D. *Siccus*-type broom cells of pileipellis. Bar = 10 µm. Drawings by: Altielys C. Magnago.

Basidiospores (4.8–)5.2–8.8 × 2.5–3.8 µm [$X_{rm} = 6.3-7 \times 3-3.2$ µm, $X_{mm} = 6.6$ (± 0.4) × 6.6 (± 0.1), $Q_{rm} = 2-2.3$, $Q_{mm} = 2.1$ (± 0.2), n/s = 30, s = 3], ellipsoid to cylindrical, lacrimoid to short bacilliform, smooth, thin-walled, hyaline, inamyloid. Mature *basidia* not observed, *basidioles* 21–35 × 4–8 µm, clavate, hyaline. *Cheilocystidia* absent. *Pleurocystidia* 30–87.5 × 5–7.5 µm, rarely projecting over the top of the basidioles, originating deep in the subhymenium, scattered or rare, cylindrical to narrowly fusiform, apex rounded, occasionally subcapitate, hyaline, inamyloid. *Lamellae trama* loosely interwoven, hyphae 6–17 µm wide, cylindrical, hyaline, strongly dextrinoid. *Pileipellis* hymeniform, composed of *Siccus*-type broom cells, thick-walled; main body 17–28 × 6–9 µm, clavate to pyriform, ochraceous, yellowish brown to hyaline in KOH; setulae 4–21 µm long, 1.5–3 µm wide at base, rounded obtuse to tapered or acute at apex, erect, yellowish brown, dextrinoid. *Stipitipellis* composed of hyphae 2–3 µm wide, cylindrical, pale yellow, caulocystidia cylindrical to forked or bilobed, 22–18 × 4–5 µm, hyaline. *Stipe trama* composed of hyphae in parallel, 5–8 µm wide, cylindrical, hyaline, dextrinoid. Hyphae at stipe base 1–2 µm wide, cylindrical, hyaline, dextrinoid. *Clamp connections* present.



FIGURE 3. 50 % majority-rule consensus tree from Bayesian analysis of the ITS dataset. Supported nodes are depicted by thickened stems (black = strongly supported; grey = moderately supported). Statistical support values are assigned to the respective nodes (Posterior Probability/ML Bootstrap). Grey gradient shade highlights the subclade where the new species (in bold) clustered within.

Habit and habitat:--Gymnopoid habit, growing gregarious on leaf litter in coastal Atlantic Forest.

Specimens examined:—BRAZIL. Santa Catarina, Florianópolis, Morro da Lagoa, Trilha do Jipe, 20 March 2014, *Magnago AC* 1001 (holotype FLOR 55963!; isotype SP!), *Magnago AC* 1002 (ICN 179251!); 16 March 2011, *Jaeger M* 048 (FLOR 55928!), Trilha da Lagoa do Peri, 09 February 2015, *Neves MA & Smith NP* 1155 (FLOR 55930!), 13 February 2015, *Smith NP 514* (FLOR 55929!), Trilha para praia de Naufragados, 12 October 2015, *Neves MA & Smith NP* 1165 (FLOR 55830!). Rio Grande do Sul, Porto Alegre, Morro Santana, 12 January 2015, *Magnago AC* 1128 (ICN 179252!).

Phylogenetic analyses

The final matrix of the dataset consisted of 86 sequences from 52 taxa (Table 1) forming an alignment of 552 base pairs length with 291 unique site patterns, having only unambiguous blocks. The mean of the values from the runs of MC³ were: estimated marginal likelihood = -3875.33 (mean of the values of the two runs), Tree-Length (TL) = 10.980430, alpha = 0.228992, pinvar = 0.398595, rates A <=> C (0.089483), A <=> G (0.373243), A <=> T (0.093071), C <=> G (0.011757), C <=> T (0.394959), G <=> T (0.037488), frequencies pi(A): 0.249541, pi(C): 0.196779, pi(G): 0.209575, pi(T): 0.344104. For ML, the final ML Optimization Likelihood was -3722.361136; model parameters were: alpha = 0.208880, TL = 1.686086, rates A <=> C (2.133641), A <=> G (7.281515), A <=> T (2.225075), C <=> G (0.259901), C <=> T (8.856011), G <=> T (1.000000), frequencies pi(A): 0.241344, pi(C): 0.206181, pi(G): 0.221598, pi(T): 0.330877.

The 50 % majority-rule consensus tree from B is shown in Figure 3. The tree is divided into two clades: /Globulares and /Outgroup, both with high statistical support (posterior probability (PP) 1.0 and ML Bootstrap (BS) 99). /Globulares is the ingroup of taxa members of sect. *Globulares sensu* Antonín & Noordeloos (2010), with all traditional groups represented. The ITS data were informative enough to solve and provide high support for nearly all terminal nodes (species level, or a little bit higher), but many intermediary and all deep nodes are unsupported as expected. The ingroup seems to present three groups of correlated taxa that are not entirely defined by ITS. *Marasmius magnus*, represented by five samples, clustered within a distinct but unsupported subclade, which is the earliest diverging lineage within /Globulares (depicted by a gradient grey squared shade). The highly supported branch bearing *M. nivicola* Har. Takah., *M. maximus* Hongo and *M. wynneae* Berk. & Broome. Moreover, this subclade is poorly resolved, with three more branches rising from a collapsed edge. The best-scoring ML tree (Supplementary Material) presented correspondent topology, with no conflict with the Bayesian tree.

Discussion

In the current taxonomic view *Marasmius magnus* belongs to sect. *Globulares* and seems to fit well within the stirp *Pseudocorrugatus* (Singer 1976). It can be compared to *M. aztecus* Singer (1976: 235), *M. floridanus* Murrill (1940: 149), *M. glabellus* Peck (1873: 58), *M. hinnuleus* Berk. & M. A. Curtis (1868: 297), *M. hylaeae* Singer (1976: 230), *M. praeandinus* Singer (1965: 353), *M. pseudocorrugatus* Singer (1965: 340), *M. spegazzinii* (Kuntze) Sacc. & P. Syd. (1899: 117), *M. spissus* Gilliam (1975: 834), *M. sullivantii* Mont. (1856: 143) and *M. yarizae* Singer (1965: 345). These species form a group of taxa that share the following macro- and micro-morphological patterns with *M. magnus*: basidiomata often fleshy when large (otherwise, small with pileus having a narrow context that makes it almost membranous), which is similar to the large members of sect. *Globulares sensu* Singer (1986), often with a reddish brown to fulvous or ferruginous orange pileus; stipe never tending to be wiry as in *M. haematocephalus*; shortly oblong to slightly elongate basidiospores; and reduced pleurocystidia, not projecting or not very developed.

Marasmius aztecus has a pileus with very similar pigmentation, but differs by its didymous to tridymous lamellae and longer stipe (up to 106 mm long) with strigose basal mycelium. Microscopically, *M. aztecus* has larger basidiospores (8–13 µm long), shorter but distinct pleurocystidia (up to 40 µm long), differentiated cheilocystidia in the lamellae edge, a mottled pileipellis in a scalp preparation, and lacks caulocystidia (Singer 1976). *Marasmius floridanus* differs by the larger oblong basidiospores ($X_{mm} = 9 [\pm 0.5] \times 3.3 [\pm 0.1] µm$), numerous conspicuous and projecting pleurocystidia, presence of *Siccus*-type cheilocystidia, and presence of broom cells in the stipitipellis (Desjardin 1989). *Marasmius hinnuleus* differs by the much smaller basidiomata with a pileus up to 10 mm diam., longer basidiospores (9.7–13.5 µm), and shorter pleurocystidia (20–34 µm). *Marasmius hylaeae* (and var. *hypsizygus* Singer 1976: 231) differs by the pileus size (up to 30 mm diam.), larger basidiospores (10–11 × 4.3–5.5 µm), shorter

pleurocystidia (18–35 µm), presence of broom cells in the stipe surface, and absence of cylindrical to bilobed, elongate cells. Marasmius praeandinus differs by the brownish pileus (up to 15 mm diam.), few and distant lamellae (10-12 per cap), larger basidiospores (8.5–11 µm long), shorter pleurocystidia (up to 42 µm), and the absence of caulocystidia (Singer 1976). Marasmius pseudocorrugatus also has a robust basidiomata (pileus up to 62 mm diam. and stipe 40-75 \times 2.2–2.7 mm) but is still smaller than *M. magnus*. It also differs in having a smaller number of lamellae (14 per cap), intermixed with lamellulae, which are sometimes intervenose; microscopically it has longer basidiospores (8-11.7 μm), shorter pleurocystidia (35–50 μm), and lacks caulocystidia. Marasmius spegazzinii has longer basidiospores $(7.3-11 \,\mu\text{m})$, shorter pleurocystidia (up to 52 μm) and no caulocystidia (Singer 1976). Marasmius spissus is somewhat similar to *M. magnus* because of its large basidiomata (pileus up to 50 mm diam, and stipe ranging from $20-75 \times$ 3–4 mm) and basidiospores size $(5.2-8.5 \times 2.6-3.8 \,\mu\text{m})$, but *M. spissus* has a light yellowish brown (cinnamon) to yellowish pink pileus, crowded lamellae (50 per cap) that are forked to anastomosed, shorter pleurocystidia (19–36 μm), and hymenial *Siccus*-type broom cells, which are also found in the stipitipellis (Gilliam 1976, Desjardin 1989). Marasmius sullivantii differs from M. magnus by the shorter pleurocystidia (16–50 µm), the presence of hymenial Siccus-type broom cells, and by having two types of cells in the stipitipellis (irregular broom cells, often with branched projections, and cylindrical to clavate or lobed cells) (Gilliam 1976, Desjardin 1989). Finally, M. varizae differs by the distant lamellae (10–12 per cap), longer basidiospores (9.5–13.2 μ m), shorter pleurocystidia (25–50 μ m), and the presence of Siccus-type broom cells in the stipitipellis (Singer 1976).

Molecular ITS sequences obtained from the holotype and four paratypes confirm that *M. magnus* is different from other *Marasmius* species for which ITS data are available. The uniqueness of the new species can be detected during the BLAST searches where only sequences assigned to samples of *M. maximus* (sect. *Globulares sensu* Singer) are related with *e*-value equal to 0, but with similarity of 85 %. All others related via BLAST are far different (*e*-value $\neq 0$ and similarities under 90 %).

Within the tree (Figure 3), *M. magnus* (sect. *Sicci* ser. *Haematocephali sensu* Singer) is a very distinct taxon and seems to be closer to *M. wynneae*, *M. maximus*, *M. nivicola*, *M. albimyceliosus* Corner, *M. nigrodiscus* (Peck) Halling (sect. *Globulares sensu* Singer), *M. hypochroides* Berk. & Broome (sect. *Sicci* ser. *Leonini sensu* Singer), *M. cohaerens* (Pers.) Cooke & Quél (sect. *Sicci* ser. *Spinulosi sensu* Singer). All these species form the subclade depicted in the tree with a grey rectangle. Still not entirely defined and out of the scope of this paper, the groups of Singer within sect. *Globulares sensu* Antonín & Noorderloos (2010) have been shown as non-natural in previous (Tan *et al.* 2009, Wannathes *et al.* 2009, Antonín *et al.* 2012) and present papers. The species mentioned above share some similar morphological features to *M. magnus*, but do represent striking distinct species (Singer 1976, Desjardin 1989, Manimohan & Leelavathy 1989, Corner 1996, Wannathes *et al.* 2009, Antonín *et al.* 2010, Antonín *et al.* 2010. All mentioned taxa produce robust basidiomata, but are much smaller than the new species.

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