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ORIGINAL ARTICLE

The genus *Agaricus* in the Caribbean II. Refined phylogeny of *Agaricus* subg. *Spissicaules* with description of two new sections and eight new species

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Abstract

The genus *Agaricus* currently includes about 500 species in seven subgenera. Continuing an inventory of species in the Caribbean, this manuscript's objective was to focus on those in *Agaricus* subg. *Spissicaules*. This subgenus has been recently proposed and divided into four sections by applying a new taxonomic system in which supraspecific taxa are ranked according to their divergence times. We similarly applied this system using Bayesian evolutionary analyses (BEAST). Phylogenetic analyses were performed using maximum likelihood and Bayesian inference methods, based either on multi-gene sequence data (ITS, ncLSU, and *TEF*1) or only on ITS, and including new collections from the Caribbean and one from Argentina. The analyses revealed seven major clades, all with divergence time estimated around 24 Ma. Among those, four were known sections of the subgenus, one remains a putative section, and we introduce the remaining two as new sections, *A.* sect. *Fulventes* and *A.* sect. *Globoterminales*. New samples were distributed among ten species-level clades. For eight of them, new species were proposed. They are distributed as follows: *A. nanofulvens* and *A. basicingulatus* are in the two new sections cited above, respectively; *A. ciferrianus* and *A. omphalodiscus* are in *A.* sect. *Subrutilescentes*; *A. baronii*, *A. furfuripes*, *A. microincrustatus*, and *A. parvisporus* are in *A.* sect. *Rarolentes*. The eight new species and the four sections to which they belong are described in detail. High levels of both species richness and phylogenetic diversity in the Caribbean region contributed to the refinement of the classification of *A.* subg. *Spissicaules*.

Keywords Agaricaceae · Divergence times · ITS · Multigene phylogeny · Neotropics · Taxonomy

Introduction

In the last two decades, taxonomy of the genus *Agaricus* L., which includes about 500 species worldwide, has received considerable attention generally using both traditional and molecular approaches. About 200 new species were described over that time in about 50 publications as summarized in Callac and Chen (2018), followed by an additional seven (Tarafder et al. 2018; Chen et al. 2019; Hussain and Sher 2019; Parra et al. 2019; Phookamsak et al. 2019; Zheng et al. 2019; Liu et al. 2020). In the new taxonomic system of classification proposed by Zhao et al. (2016), higher taxa are restricted to well-supported clades and ranked as subgenera or sections according to their divergence times estimated using a fossil-calibrated molecular clock approach. From recent studies applying this new system, the genus has been split into six subgenera and 24 sections (Callac and Chen 2018). There is a seventh subgenus, *A.* subg. *Conioagaricus* Heinem,

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containing two sections, *A.* sect. *Pulverotecti* Heinem. and *A.* sect. *Striati* Heinem. (Heinemann 1956), which have not yet been studied molecularly in the framework of the new system of classification of Zhao et al. (2016).

In the first study in a series aimed at improving the knowledge of the genus *Agaricus* in the Caribbean region, Parra et al. (2018) proposed three new sections and six new species belonging to *A.* subg. *Minores* (Fr.) R.L. Zhao & Moncalvo, *A.* subg. *Minoriopsis* Linda J. Chen, L.A. Parra, Callac, Angelini & Raspé, and *A.* subg. *Pseudochitonia* Konrad & Maubl. Most Caribbean collections were from the Dominican Republic, but samples from Martinique, Puerto Rico, and the United States Virgin Islands were also included. In this second study, we focus on *A.* subg. *Spissicaules* (Heinem.) R.L. Zhao & Moncalvo. Most Caribbean collections are from the Dominican Republic and Puerto Rico, but samples from Martinique are also included.

Parra et al. (2018) presented a summary of the mycological literature in the Caribbean region with emphasis on *Agaricus* from the Dominican Republic, bringing the total number of species known from the Dominican Republic to eleven. With respect to Puerto Rico, only the following three taxa have previously been reported: *A. johnstonii* Murrill (Murrill 1918), *A. endoxanthus* Berk & Broome (Parra 2013), and *A. lodgeae* L.A. Parra, Angelini & B. Ortiz (Parra et al. 2018). From Martinique, at least 19 species were reported by Pegler (1983), of which eight were described as new. Other species of *Agaricus* in the Caribbean are known from Cuba, Jamaica, and Trinidad (Berkeley and Curtis 1869; Murrill 1918; Baker and Dale 1951; Heinemann 1961, 1962; Pegler 1983) or from an undetermined island (Fries 1851). None of these named species proved to be members of *A.* subg. *Spissicaules*. However, recent phylogenetic studies have revealed the presence of species from the neotropics in all subgenera of *Agaricus* except *A.* subg. *Agaricus* and *A.* subg. *Conioagaricus* (Zhao et al. 2011, 2016; Chen et al. 2017; Parra et al. 2018). Nevertheless, only molecular data were reported for the four unique specimens placed in *A.* subg. *Spissicaules* which, therefore, remained unnamed (Zhao et al. 2011, 2016). Three of them (F2255, F2301, and F2467) were from the Caribbean island Martinique, and the remaining sample (RCGUY07019) was from French Guiana.

Our initial objective which was to inventory the species of *A* subg. *Spissicaule*s in the Caribbean was quickly extended to a refinement of *A.* subg. *Spissicaules* including samples from temperate, subtropical, and tropical climatic areas. Initially, *Agaricus* subsect. *Spissicaules* Heinem. was a subsection of *A* sect. *Agaricus* (Heinemann 1978), which included seven species. Kerrigan (1985) proposed *A.* sect. *Spissicaules* (Heinem.) Kerrigan. Applying the new taxonomic system of classification, in which higher taxa are ranked according to their divergence times, Zhao et al. (2016) proposed *A.* subg. *Spissicaules*. This subgenus was divided into four sections, *A.* sect. *Amoeni* Callac & R.L. Zhao, *A.* sect *Spissicaules*, *A.* sect. *Rarolentes* Kerrigan, and *A.* sect. *Subrutilescentes* Kerrigan, the latter two described the same year by Kerrigan (2016). These four taxa were monophyletic and well supported in multigene-phylogenetic trees. Their divergence times estimated by the means of stem ages in mega-annums (millions of years) were comprised between 19 and 22 Ma and, therefore, earlier than 18 Ma which, in the new system, was the threshold value chosen by Zhao et al. (2016) to delineate sections.

Some taxa in *A.* subg. *Spissicaules* remain unclassified or statistically unsupported. Indeed, in previous phylogenetic analyses only based on ITS sequence data (Zhao et al. 2011, 2016; Kerrigan 2016; Dai et al. 2016), *A.* sect. *Spissicaules*, as emended by Zhao et al. (2016), never appeared as a well-supported monophyletic section. In addition, a clade formed by samples of both or one of the two species, *A. bellanniae* Guinb., Kerrigan & M. Kuo and *A.* sp. (ADK2171), remained unclassified (Zhao et al. 2011, 2016; Dai et al. 2016) or was included in *A.* sect. *Spissicaules* (Kerrigan 2016) without strong support. Therefore, the placement of these species either in an existing section or in a new section remained to be resolved.

Using 25 samples from the Caribbean and one from Argentina, BEAST with the calibration of Zhao et al. (2016) and the same outgroup, we found seven monotypic or well supported monophyletic taxa with divergence times around 24 Ma. New sections are proposed for two of them. Eight new species are proposed and described. The four sections to which they belong are also described. Most of the pending issues cited above were resolved. Species richness and a high level of phylogenetic diversity are demonstrated in the Caribbean.

Materials and methods

Studied collections

Thirty two specimens were studied morphologically and/or molecularly (Table 1) of which 26 are reported on for the first time. Among those, 23 are from the Caribbean region, one from Argentina (AH47621), one from Spain (LAPAG585), and one from the USA (BOS801).

The 23 new collections from the Caribbean region include 13 collections from the Dominican Republic on the island of Hispaniola, and ten collections from the island of Puerto Rico. Most of the collections from the Dominican Republic were made by C. Angelini in the Provinces of Puerto Plata, Santiago, and La Vega, one by B. Ortiz-Santana in Prov. La Vega, and one in Santiago by L. Lacey and D.J Lodge. A description of the vegetation in the areas of Puerto Plata and La Vega is reported in Parra et al. (2018). Most of the

Table 1 List of 142 samples of *Agaricus* with GenBank accession numbers of sequences used in the phylogenetic analyses. New taxa, new samples, and new sequences are in bold. Samples of *A.* subg. *Spissicaules* are ordered as in the ITS ML tree and the remaining samples as in the MCC tree

Taxon	Sample number	GenBank accession number			Country: state, province, or region	Reference
		ITS	LSU	*TEF*1		
A. subg. *Spissicaules*						
A. sect. *Subrutilescentes*						
A. sp.	isolate 119	JN182898	–	–	China: Sichuan Basin	Non-referenced[f]
A. linzhiensis	ZRL2012618 T	KT951378	KT951503	KT951582	China: Tibet	Zhao et al. 2016
A. ciferrianus	**PR6822 T**	**MT436327**	**MT436353**	**MT424828**	Puerto Rico	This study
A. ciferrianus	**PR6585**	**MT436328**	**MT436354**	**MT424829**	Puerto Rico	This study
A. subrutilescens	RWK2122	KJ877775	**MT436355**	**MT424830**	USA: California	Kerrigan 2016; this study
A. subrutilescens	ZRLWXH3276	KT951392	KT951522	KT951585	USA: California[a]	Zhao et al. 2016
A. thiersii	UBC F16985	MF954624	–	–	Canada: British Columbia	Non-referenced[f]
A. thiersii	RWK1940	AY943973	–	–	USA: California	Kerrigan et al. 2005
A. sp.	SHY2012070604	KT951341	KT951475	KT951581	China: Yunnan	Zhao et al. 2016
A. sp.	ZRL2014289	KT951418	–	–	China: Yunnan	Zhao et al. 2016
A. parasubrutilescens	ZRL2014076 T	KT951410	–	–	China: Yunnan	Zhao et al. 2016
A. parasubrutilescens	ZRL2011027	KT951350	KT951521	KT951583	China: Yunnan	Zhao et al. 2016
A. sp.	TL2321	JF495040	–	–	New Zealand	Lebel and Syme 2012
A. inthanonensis	ZRL160	KT951345	KT951486	KT951586	Thailand	Zhao et al. 2016
A. inthanonensis	ZRLLD014 T	KT951422	–	–	Thailand	Zhao et al. 2016
A. inthanonensis	LDMS22	KT951423	–	–	China: Yunnan	Zhao et al. 2016
A. inthanonensis	isolate 121	JN182899	–	–	China: Sichuan Basin	Non-referenced[f]
A. brunneopileatus	ZRL2012115 T	KT951404	KT951489	KT951587	China: Yunnan	Zhao et al. 2016
A. brunneopileatus	ZRL2014144	KT951414	–	–	China: Yunnan	Zhao et al. 2016
A. aff. *inthanonensis*	ZRL20151566	KX186720	–	–	China: Zhejiang	Dai et al. 2016
A. aff. *inthanonensis*	ZRL2015166	KX186722	–	–	China: Fujian	Dai et al. 2016
A. vinosobrunneofumidus	RWK2012	KJ877781	**MT436356**	**MT424831**	USA: Tennessee	Kerrigan 2016; this study
A. vinosobrunneofumidus	NC-1-8341	AY456333	–	–	USA: North Carolina	Edwards et al. 2004
A. vinosobrunneofumidus	MGW1363	KY777365	**MT436357**	–	USA: Tennessee	This study
A. aff. *subrutilescens*	CB08378	KT874933	–	–	Mexico: Mexico State	Non-referenced[f]
A. aff. *subrutilescens*	HC-PNNT-049	KT874934	–	–	Mexico: Mexico State	Non-referenced[f]
A. sp.	CA583	JF797192	–	–	France	Zhao et al. 2011
A. catenariocystidiosus	ZRL2015038 T	KX186706	–	–	China: Zhejiang	Dai et al. 2016
A. catenariocystidiosus	ZRL20151210	KX186713	–	–	China: Zhejiang	Dai et al. 2016
A. sp. 1	**PR6226**	**MT436329**	**MT436358**	**MT424832**	Puerto Rico	This study
A. sp. 1	**PR6202**	**MT436330**	**MT436359**	–	Puerto Rico	This study
A. sp. 1	**PR6214**	**MT436331**	**MT436360**	**MT424833**	Puerto Rico	This study
A. omphalodiscus	F2255 T	JF797198	**MT436361**	**MT424834**	France: Martinique	Zhao et al. 2011; this study
A. sect. A						
A. sp. 2	**JBSD130759**	**MT436332**	**MT436362**	**MT424835**	Dominican Republic	This study
A. sect. *Spissicaules*						
A. lanipedisimilis	ZRL2012193 T	KT951399	–	–	China: Yunnan	Zhao et al. 2016
A. lanipedisimilis	ZRL2012151	KT951366	KT951484	KT951578	China: Yunnan	Zhao et al. 2016
A. planipileus	ZRL20151241	KX186717	–	–	China: Zhejiang	Dai et al. 2016
A. planipileus	ZRL2011250 T	KT951398	–	–	China: Yunnan	Zhao et al. 2016
A. litoraloides	SHY2011073026 T	KT951408	–	–	China: Yunnan	Zhao et al. 2016
A. litoraloides	ZRL2011249	KT951353	KT951523	KT951580	China: Yunnan	Zhao et al. 2016

Table 1 (continued)

Taxon	Sample number	GenBank accession number			Country: state, province, or region	Reference
		ITS	LSU	*TEF*1		
A. sp.	RCGUY07019	JF797199	–	–	French Guiana	Zhao et al. 2011
A. bresadolanus	CA177	DQ185570	–	–	France	Kerrigan et al. 2005
A. bresadolanus	CA10	DQ185569	–	–	France	Kerrigan et al. 2005
A. bresadolanus	CA575	JQ824134	–	–	France	Foulongne-Oriol et al. 2012
A. sp.	ZRLQHY43	KT951386	KT951485	KT951579	China: Shandong	Zhao et al. 2016
A. litoralis	LAPAG420	KT951327	KT951483	KT951572	Spain	Zhao et al. 2016
A. litoralis	CA120	JN204436	–	–	France	Zhao et al. 2012
A. litoralis	strain 816[b]	AJ884642	–	–	Israel	Didukh et al. 2005
A. lanipes	**LAPAG585**	**MT436333**	**MT436363**	**MT424836**	Spain	This study
A. lanipes	CA406	JF797190	–	–	France	Zhao et al. 2011
A. sp.	ZRL2012657	KT951406	–	–	China: Tibet	Zhao et al. 2016
A.* sect. *Fulventes						
A. bellanniae	CA486[c]	JF797189	**MT436364**	**MT424837**	France	Zhao et al. 2011; this study
A. bellanniae	RWK2008 T	KJ877782	–	–	USA: Pennsylvania	Kerrigan 2016
A. bellanniae	MKuoCon5	KJ877783	–	–	USA: Illinois	Kerrigan 2016
A. sp.	ADK2171	JF514517	–	–	Benin	Zhao et al. 2011
A. sp.	CBS 623.89[d]	MH862192	–	–	India	Vu et al. 2019
A. nanofulvens	**AH47621 T**	**MT436334**	**MT436365**	**MT424838**	Argentina	This study
A.* sect. *Globoterminales						
A. basicingulatus	**JBSD130767**	**MT436335**	–	–	Dominican Republic	This study
A. basicingulatus	**JBSD130768**	**MT436336**	–	–	Dominican Republic	This study
A. basicingulatus	**JBSD130761**	**MT436337**	**MT436366**	**MT424839**	Dominican Republic	This study
A. basicingulatus	**JBSD130754 T**	**MT436338**	**MT436367**	**MT424840**	Dominican Republic	This study
A. basicingulatus	**JBSD130760**	**MT436339**	**MT436368**	**MT424841**	Dominican Republic	This study
A. basicingulatus	**JBSD130762**	**MT436340**	**MT436369**	**MT424842**	Dominican Republic	This study
A. basicingulatus	**JBSD130766**	**MT436341**	–	–	Dominican Republic	This study
A. sect. *Amoeni*						
A. kivuensis	Rammeloo5756	JF514541	–	–	Burundi	Zhao et al. 2011
A. sp.	F2467	JF797200	–	–	France: Martinique	Zhao et al. 2011
A. gratolens	ZRL3093 T	JF691548	KT951488	–	Thailand	Zhao et al. 2011; 2016
A. gratolens	NB72	EU118124	–	–	India: Nilgiri Biosphere Reserve	Non-referenced[f]
A. sp.	LAPAF3	JF797184	–	–	Togo	Zhao et al. 2011
A. suthepensis	NTT42	JF514538	–	–	Thailand	Zhao et al. 2011
A. suthepensis	LD2012100 T	KT951409	–	–	Thailand	Zhao et al. 2016
A. amoenomyces	ZRL2010072 T	KT951348	KT951524	KT951638	China: Yunnan	Zhao et al. 2016
A. sp.	NYS122	JF514540	–	–	Benin	Zhao et al. 2011
A. hanthanaensis	SK2012 T	JQ246405	–	–	Sri Lanka	Liu et al. 2015
A. hanthanaensis	GKVK-10[e]	MF403064	–	–	India: Karnataka	Mariselvi and Earanna 2018
A. sect. *Rarolentes*						
A. furfuripes	**DR3106**	**MT436342**	**MT436370**	**MT424843**	Dominican Republic	This study
A. furfuripes	**JBSD127435 T**	**MT436343**	**MT436371**	**MT424844**	Dominican Republic	This study
A. furfuripes	**DR2674**	**MT436344**	**MT436372**	**MT424845**	Dominican Republic	This study

Table 1 (continued)

Taxon	Sample number	GenBank accession number			Country: state, province, or region	Reference
		ITS	LSU	*TEF*1		
A. furfuripes	**JBSD130765**	**MT436345**	**MT436373**	**MT424846**	Dominican Republic	This study
A. furfuripes	F2301	JF727854	–	**MT424847**	France: Martinique	Zhao et al. 2011; this study
A. microincrustatus	**PR6809**	**MT436346**	**MT436374**	**MT424848**	Puerto Rico	This study
A. microincrustatus	**PR6810**	**MT436347**	**MT436375**	**MT424849**	Puerto Rico	This study
A. microincrustatus	**OKM27279**	**MT436348**	**MT436376**	**MT424850**	Puerto Rico	This study
A. microincrustatus	**PR6877 T**	**MT436349**	**MT436377**	**MT424851**	Puerto Rico	This study
A. butyreburneus	AW145	KT951308	–	–	Canada: Ontario	Zhao et al. 2016
A. butyreburneus	RWK2015	KC135887	–	–	USA: Tennessee	Kerrigan 2016
A. butyreburneus	JH105	KT951324	–	–	Canada: Ontario	Zhao et al. 2016
A. butyreburneus	**BOS801**	**MT436350**	**MT436378**	**MT424852**	USA: New York	This study
A. butyreburneus	CA279	JF797191	–	–	France	Zhao et al. 2011
A. parvisporus	**PR6173 T**	**MT436351**	**MT436379**	**MT424853**	Puerto Rico	This study
A. baronii	**DR2523 T**	**MT436352**	**MT436380**	**MT424854**	Dominican Republic	This study
A. albosquamosus	LD2012192 T	KT951394	KT951520	KT951636	Thailand	Zhao et al. 2016
A. albosquamosus	LD201235	KT951395	–	–	Thailand	Zhao et al. 2016
A. leucolepidotus	LD201214 T	KT951336	KT951519	KT951635	Thailand	Zhao et al. 2016
A. subg. *Agaricus*						
A. sect. *Agaricus*						
A. campestris	LAPAG370 T	JQ903618	KP739803	KR006636	Spain	Kerrigan 2016; Parra et al. 2016; Zhao et al. 2016
A. sp.	ZRL2010010	KT951347	KT951511	KT951639	Thailand	Zhao et al. 2016
Outgroup						
Heinemannomyces sp.	ZRL185	KT951346	KT951527	KT951657	Thailand	Zhao et al. 2016
A. subg. *Minoriopsis*						
A. sect. *Minoriopsis*						
A. martinicensis	F2815	JF727855	KX084032	KX198038	France: Martinique	Zhao et al. 2011; Chen et al. 2017
A. sect. *Kerrigania*						
A. rufoaurantiacus	LAPAM15	KT951313	KX671708	KT951641	Dominican Republic	Zhao et al. 2016; Chen et al. 2017
A. subg. *Flavoagaricus*						
A. sect. *Arvenses*						
A. abruptibulbus	ZRL2012005	KT951356	KT951460	KT951626	China: Yunnan	Zhao et al. 2016
A. subrufescens	ZRL2012722	KT951383	KT951451	KT951632	China: Yunnan	Zhao et al. 2016
A. subg. *Minores*						
A. sect. *Minores*						
A. comtulus	LAPAG724	KT951332	KT951448	KT951593	Spain	Zhao et al. 2016
A. huijsmanii	LAPAG639	KF447889	KT951444	KT951571	Spain	Parra 2013; Zhao et al. 2016
A. sect. *Leucocarpi*						
A. leucocarpus	LD201215 T	KU975101	KX083981	KX198048	Thailand	Chen et al. 2017
A. sect. *Pantropicales*						
A. candidolutescens	LD2012129 T	KT951335	KT951525	KT951616	Thailand	Zhao et al. 2016
A. sp.	ZRLWXH3161	KT951391	KT951526	KT951615	China: Guangdong	Zhao et al. 2016
A. subg. *Pseudochitonia*						

Table 1 (continued)

Taxon	Sample number	GenBank accession number			Country: state, province, or region	Reference
		ITS	LSU	*TEF*1		
A. sect. *Nigrobrunnescentes*						
A. desjardinii	WZR2012907	KM657901	KT951474	KT951644	China: Xingjiang	Wang et al. 2015; Zhao et al. 2016
A. padanus	WZR2012903	KM657903	KR006616	KR006644	China: Xingjiang	Wang et al. 2015; Zhao et al. 2016
A. biberi	LAPAG687	KM657919	KR006614	KR006642	Hungary	Wang et al. 2015; Zhao et al. 2016
A. sect. *Rubricosi*						
A. dolichopus	ZRL2012715	KT951382	KT951502	KT951573	China: Tibet	Zhao et al. 2016
A. sp.	ZRLWXH3078	KT951389	KT951464	KT951643	China: Fujian	Zhao et al. 2016
A. kunmingensis	ZRL2012015	KT951361	KT951506	KT951642	China: Yunnan	Zhao et al. 2016
A. sect. *Bohusia*						Zhao et al. 2016
A. bohusii	LAPAG562	KM657928	KR006613	KR006641	Spain	Zhao et al. 2016
A. crassisquamosus	ZRL2012607 T	KT951376	KT951510	KT951645	China: Tibet	Zhao et al. 2016
A. sect. *Flocculenti*						
A. erectosquamosus	LD2012165 T	KT951338	KT951509	KT951565	Thailand	Zhao et al. 2016
A. pallidobrunneus	ZRL2012358 T	KT951370	KT951471	KT951566	China: Yunnan	Zhao et al. 2016
A. sect. *Sanguinolenti*						
A. sylvaticus	ZRL2012013	KT951360	KT951500	KT951570	China	Zhao et al. 2016
A. sylvaticus	ZRL2012568	KT951371	KT951501	KT951568	China: Tibet	Zhao et al. 2016
A. sect. *Brunneopicti*						
A. sp.	ZRL133	KT951344	KT951505	KT951656	Thailand	Zhao et al. 2016
A. duplocingulatus	ZRL2012267	KT951368	KT951504	KT951655	China: Yunnan	Zhao et al. 2016
A. sp.	SWK014	KT951342	KT951482	KT951654	Malaysia: Borneo	Zhao et al. 2016
A. sect. *Cymbiformes*						
A. angusticystidiatus	ZRL2085	KT951434	MG835413	–	Thailand	Zhao et al. 2016; He et al. 2018
A. angusticystidiatus	ZRL2043 T	JF691553	MG835412	–	Thailand	Zhao et al. 2011; He et al. 2018
A. sect. *Crassispori*						
A. variicystis	LD201234 T	KT951339	KT951517	KT951562	Thailand	Zhao et al. 2016
A. sect. *Trisulphurati*						
A. trisulphuratus complex	LAPAF7	KM657924	KR006605	KR006634	Togo	Zhao et al. 2016
A. trisulphuratus complex	SWK079	KT951343	KT951472	KT951561	Malaysia: Borneo	Zhao et al. 2016
A. sect. *Bivelares*						
A. cupressicola	LAPAG889	KT951334	KT951465	KT951649	Italy	Zhao et al. 2016
A. bisporus	LAPAG446	KM657920	KR006611	KR006640	Spain	Wang et al. 2015; Zhao et al. 2016
A. sinodeliciosus	WZR2012822	KM657907	KT951518	KT951648	China: Xingjiang	Wang et al. 2015; Zhao et al. 2016
A. bitorquis	CA427/LAPAG55	KT951320	KT951491	KT951646	Spain	Zhao et al. 2016
A. sect. *Chitonioides*						
A. nevoi	LAPAG257	KM657922	KR006606	KR006635	Spain	Wang et al. 2015; Zhao et al. 2016
A. gennadii	CA339/LAPAG8	KT951318	–	KT951575	Spain	Zhao et al. 2016
A. bernardi	CA383	KT951319	KT951469	KT951576	France	Zhao et al. 2016
A. sect. *Hondenses*						

Table 1 (continued)

Taxon	Sample number	GenBank accession number			Country: state, province, or region	Reference
		ITS	LSU	*TEF*1		
A. bernardiformis	CA433/AVM917	KT951321	KT951467	KT951577	Spain	Zhao et al. 2016
A. grandiomyces	ZRL2012611 T	KM657879	KR006624	KR006652	China: Tibet	Zhao et al. 2016
A. sect. *Xanthodermatei*						
A. pusillobulbosus	ZRL2012627	KM657888	–	KR006654	China: Tibet	Zhou et al. 2016
A. bisporiticus	LD2012111	KJ575611	KT951507	KT951650	Thailand	Thongklang et al. 2014; Zhao et al. 2016
A. microvolvatulus	LD201271	KJ575614	KT951508	KT951651	Thailand	Thongklang et al. 2014; Zhao et al. 2016
A. atrodiscus	MFLU121010 T	KT284912	KT951473	KT951653	Thailand	Ariyawansa et al. 2015; Zhao et al. 2016
A. sp.	ZRL2012629	KM657890	KR006627	KR006656	China: Tibet	Zhao et al. 2016
A. tibetensis	ZRL2012585	KM657895	KR006633	KR006658	China: Tibet	Zhao et al. 2016
A. xanthodermus	LAPAG387	KM657923	KR006609	KR006638	Spain	Zhao et al. 2016

[a] Location according to the collector Wang, Xian-Hua (location wrongly reported in GenBank)

[b] Named as *A. maskae* (syn. *A. litoralis*) in Didukh et al. 2005

[c] Isoparatype of *A. bellanniae* (duplicate of 060923JG cited in the protologue)

[d] Misidentified as *A. romagnesii* (syn. *A. bresadolanus*) in GenBank

[e] Listed as *A.* sp. with photo (Fig. 3j) in Mariselvi and Earanna (2018)

[f] Six non-referenced sequences are listed in the table according to their phylogenetic relationships in the ITS tree. Three of those extend the distribution range of recognized species (*A. thiersii*, *A. gratolens*, and *A. inthanonensis*), while the three remaining sequences represent putative new species in *A.* sect. *Subrutilescentes*

collections from Puerto Rico were made by D.J. Lodge, two by B. Ortiz-Santana, and one by T.J. Baroni in El Yunque National Forest; several collections were also made by researchers of the NSF-funded Basidiomycetes of the Greater Antilles Project (1996–2000). Six collections were made in subtropical wet forest from 250 to 350 m elevation (Bisley, El Verde, Catalina) and four were in lower montane wet and rain forests with an elevation range above 600 m (El Toro, Guzmán, La Mina). Vegetation types in Puerto Rico are according to the Holdridge Life Zone System (Holdridge 1967) as described in Harris et al. (2012). All these collections have been deposited by the authors at the Dr. Rafael M. Moscoso National Botanical Garden (JBSD) with duplicates in the private herbarium of L. A. Parra (LAPAM), and the Center for Forest Mycology Research herbarium (CFMR).

The new specimen AH47621 from Argentina collected by J. C. Zamora and the duplicate AH47624 of the new specimen LAPAG585 of *A. lanipes* from Spain collected by L.A. Parra were deposited at the University of Alcalá de Henares Herbarium (AH). The new specimen BOS801 of *A. butyreburneus* from New York, USA, is deposited at CFMR.

The six remaining specimens are not new. Three (F2255, F2301, CA486) were previously published in Zhao et al. (2011, 2016), two (RWK2122, RWK2012) were published in Kerrigan (2016), and one (MGW1363) was loaned by the University of Tennessee Herbarium (TENN).

These specimens were used to obtain more sequence data except both samples F2255 and F2301 which were also reexamined morphologically. These two specimens were collected in Martinique by J.–P. Fiard and are conserved at the Université de Lille (LIP). All the new sequences are indicated in boldface in Table 1.

Morphological descriptions

Macroscopic descriptions are based on color photographs and field notes from fresh material. Macrochemical reactions if not stated otherwise were determined from herbarium material using Schäffer's reaction, 5% KOH on pileus and stipe surfaces, and in one case Syringaldazine (a more specific reagent than alpha napthol to detect laccases) on context surface of pileus and stipe when cut. Microscopic descriptions are based on photos and data obtained after examining the basidiomata with a Nikon SE compound microscope. Spore measurements followed the statistical method proposed by Heinemann and Rammeloo (1985), where the underlined values are the average of measurements. Herbarium acronyms are from Holmgren and Holmgren (1998). The morphological description of taxa follows the taxonomic treatments published by Parra (2008, 2013).

DNA extraction, PCR, and sequencing

Small tissue samples from specimens were processed following a standard CTAB protocol (Palmer et al. 2008). The internal transcribed spacer region (ITS) of the nuclear ribosomal DNA (nuc-rDNA) including ITS1, 5.8S, and ITS2 was amplified using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) at CFMR and ALVALAB (http://www.alvalab.es/), or the primers ITS5 and ITS4 (White et al. 1990) at INRAE (https://www.inrae.fr/); the 5' end of the large subunit 28S of the nuc-rDNA (LSU) was amplified with primers LR0R (Cubeta et al. 1991) and LR5 (Vilgalys and Hester 1990); and a segment of the translation elongation factor 1-α (*TEF*1) was amplified using primer pair EF1-983/EF1-1567R (Rehner and Buckley 2005). Sequencing was performed at the University of Wisconsin Biotechnology Center (UWBC) for the materials processed at CFMR, by Applied Biosystems at Beckman Coulter Genomics (France) for the materials processed at INRAE, and at StabVida (Caparica, Portugal) for the materials processed at ALVALAB.

Sampling for phylogenetic analyses

The phylogenetic analyses were performed using three datasets. The ITS dataset included 95 sequences representing 48 taxa of *A.* subg. *Spissicaules* and the outgroup *A. campestris*. Of these, 26 were newly generated while 69 were retrieved from GenBank and were used in previous studies (Zhao et al. 2011, 2016; Kerrigan 2016; Dai et al. 2016). The multigene dataset included 43 ITS, 42 LSU, and 40 *TEF*1 sequences representing 28 taxa of *A.* subg. *Spissicaules* and the outgroup *A. campestris*. Of these, 26 ITS, 28 LSU, and 27 *TEF*1 were newly generated and those that remain were retrieved from GenBank following the studies of Zhao et al. (2016). For the maximum clade credibility (MCC) tree, 68 ITS, 66 LSU, and 65 *TEF*1 sequences of 68 specimens representing 63 taxa of six recognized subgenera of *Agaricus* and the outgroup *Heinemanomyces* were used. The new ITS, LSU, and *TEF*1 sequences used for the multigene analyses were included in the MCC dataset and the other sequences used were retrieved from GenBank following the sampling of Zhao et al. (2016) and He et al. (2018). In total, 142 specimens were included for phylogenetic analyses. The sample's origin and GenBank accession numbers are listed in Table 1.

Sequence alignment, divergence time estimation, and phylogenetic analyses

Sequences were aligned by using MAFFT (Katoh and Standley 2013), for each region independently, then manually adjusted in BioEdit v. 7.0.4 (Hall 2007). Alignments were submitted to TreeBase (Submission ID: 26968). Three datasets were prepared for different analyses.

The ITS dataset and combined dataset (ITS, LSU, and *TEF*1) were used for maximum likelihood and Bayesian analyses. The maximum likelihood (ML) analysis was performed in RAxMLHPC2 v. 8.2.4 (Stamatakis 2014) as implemented on the Cipres portal (Miller et al. 2010), under a GTRGAMMA model with one thousand rapid bootstrap (BS) replicates. The combined dataset was partitioned into ITS, LSU, *TEF*1 intron, and *TEF*1 coding sites. The best substitution model for each partition was inferred with the program MrModeltest 2.2 (Nylander 2004): HKY+I+G for ITS, GTR+I+G for LSU, HKY+I for *TEF*1 intron sites, and SYM+I for *TEF*1 coding sites. Bayesian inference (BI) analysis was performed with MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003). Two runs of six Markov chains were run for one million generations and sampled every 100th generations. Burn-in was determined by checking the likelihood trace plots in Tracer v. 1.6 (Rambaut et al. 2014) and subsequently discarded. The outputs were displayed in FigTree v 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

Divergence times were estimated using BEAST v. 1.8 (Drummond et al. 2012). The dataset comprises 68 specimens representing the six subgenera of *Agaricus* and the outgroup. Model selections were performed in jModel Test v. 2 (Darriba et al. 2012) for each gene. An XML file was generated in BEAUTI v. 1.8. Per-gene alignments were imported as separate partitions. Clock and substitution models were set to be unlinked, while the tree prior parameters were set to be linked across partitions. Priors were set according to the previous fossil-calibrated analysis of Zhao et al. (2016). A normal distribution prior was set on the *treeModel.rootHeight* parameter, with a mean of 66 Ma and a standard deviation of 1 Myr. An independent Monte Carlo Markov Chain of 50 million generations was run, logging states every 5000 generations by BEAST v1.8 (Drummond et al. 2012). The log file was viewed in Tracer v. 1.6 (Rambaut et al. 2014) to ensure ESS (effective sample sizes) value greater than 200. An ultrametric maximum-clade-credibility (MCC) tree was summarized using TreeAnnotator 1.8, discarding 10% of states as burn-in and annotating clades with ≥ 0.8 posterior probability.

Specific ITS markers

To find specific markers of new sections or species, their ITS were compared, using BioEdit, to all sequences available in the subgenus or the section to which they belong, respectively. The positions of ITS markers are numbered from the beginning of the ITS1 region following the 3' end (aggatcatta) of the conserved 18S region. This numeration follows the position of section- or species-specific markers in the alignment used for the ITS phylogenetic analysis, or in the sequence of the species, respectively. The exclusive or informative characters are in upper-case and in square brackets in case of insertion or deletion (indel). Flanking characters are indicated in lower-case using IUPAC codes such as "y" to indicate the presence of both characters t and c in the section or the species.

Results

Phylogenetic analyses

For the ITS and combined datasets, we focused only on *A.* subg. *Spissicaules*, and *A. campestris* was used as the outgroup. The aligned ITS dataset consisted of 95 sequences and 723 characters. The combined dataset consisted of sequences (ITS, LSU, and *TEF*1) of 43 specimens and 2044 characters. For each of both ITS and combined datasets, the resulting ML and BI trees had very similar topologies. The bootstrap support values (BS) and Bayesian posterior probabilities (PP) are noted in the ML phylograms of Fig. 1 for ITS analyses, and Fig. 2 for multi-gene analyses.

In both trees (Figs. 1, 2), six of the seven sections revealed in the MCC tree are observed and are monophyletic, except *A.* sect. *Subrutilescentes*, which is polyphyletic in the ITS tree but without strong support values. The phylogeny of the sections in multi-gene and MCC trees remains poorly resolved. However, both trees suggest closer phylogenetic relationships within the three following groups of sections: (i) *Amoeni* and *Rarolentes*, (ii) *Fulventes* and *Spissicaules*, and (iii) *Globoterminales*, *Subrutilescentes* and "*A.* sect. A."

In the ITS tree (Fig. 1), two previously used samples from Martinique and 24 new samples introduced in this study form ten well-supported clades ($BS \geq 80$, $PP \geq 0.95$), of which eight are confirmed as new species in our taxonomic treatment, while the two remaining lineages represent species provisionally named as *A.* sp. 1 and *A.* sp. 2. The ten species-level clades are distributed in sections of *A.* subg. *Spissicaules* as follows: three clades corresponding to the species *A. ciferrianus*, *A. omphalodiscus* and *A.* sp. 1 in *A.* sect. *Subrutilescentes*; a single specimen of *A.* sp. 2 in '*A.* sect. A'; a single specimen of *A. nanofulvens* in *A.* sect. *Fulventes*; a single clade comprising seven specimens of *A. basicingulatus* in *A.* sect *Globoterminales*; and four clades corresponding to species *A. baronii*, *A. furfuripes*, *A. microincrustatus*, and *A. parvisporus* in *A.* sect. *Rarolentes*.

New sections based on divergence times

In the maximum clade credibility (MCC) tree, which is shown in Fig. 3, all six subgenera are well-supported ($PP \geq 0.99$). *Agaricus* subg. *Spissicaules* was subdivided into six major clades and one isolated branch, which are fully supported ($PP = 1$, Fig. 3). In agreement with one of the rules of the classification system proposed by Zhao et al. (2016), these seven clades represent seven taxa of *A.* subg. *Spissicaules*, which can be recognized at section rank since they diverged earlier than 20–18 Ma ago (Table 2), comparable to other sections of the genus. In addition, the species of the new sections (including section A) cannot be included in any recognized section to form clades with significant PP values as this is strongly required according to another rule of the classification system. Therefore, if new sections would not be proposed, these species would remain unclassified. The new taxa *A.* sect. *Globoterminales* and *A.* sect. *Fulventes* are described below in the Taxonomy section, and thus, as much as possible characterized according to the third and last rule of the classification system, while the clade we provisionally named "*A.* sect. A" remains undescribed because it contains a single basidioma without sufficient information for a formal description. In the MCC tree of Fig. 3, we note that the seven sections diverged from four different nodes about 24 Ma ago. Not only does this agree with the basic principles of the system employed that taxa diverging during the same period should be similarly ranked, but also this suggests that a major diversification of species occurred about 24 Ma ago (late Oligocene).

Comparison of the distribution of tropical/subtropical species in the sections of *A.* subg. *Spissicaules*

Based on our sample and climate information reported in Fig. 1, the rates of tropical or subtropical species in the sections can be compared as follows: it is high in five sections: *Amoeni* (8/8), *Rarolentes* (6/7), *Globoterminales* (1/1), *Fulventes* (3/4), and "*A.* sect. A" (1/1). Abundance of tropical species is intermediate in the two remaining sections: *Subrutilescentes* (11/18) and *Spissicaules* (4/9). In the latter, tropical species are grouped into a single clade. In total, 34/48 (71%) of the species or putative species included in our analyses are tropical or subtropical.

Taxonomy

Agaricus sect. ***Fulventes*** Callac, L.A. Parra, B. Ortiz & Linda J. Chen, **sect. nov**.

MycoBank: MB 837353

Type: *Agaricus nanofulvens* L.A. Parra, J.C. Zamora & B. Ortiz.

Etymology: Refers to the fulvous brown color from tawny to buff (*fulvus* in Latin) of the pileus in all the known species of this section and the Latin ending *–entes*, plural participle of *sum* (to be), meaning "those being fulvous."

Morphological description: Gracile, small to medium-sized basidiomata. Pileus 1.8–6(8) cm diam. with light brown color. Stipe with an annulus on its upper third and rhizomorphs at the base. Annulus superous, membranous,

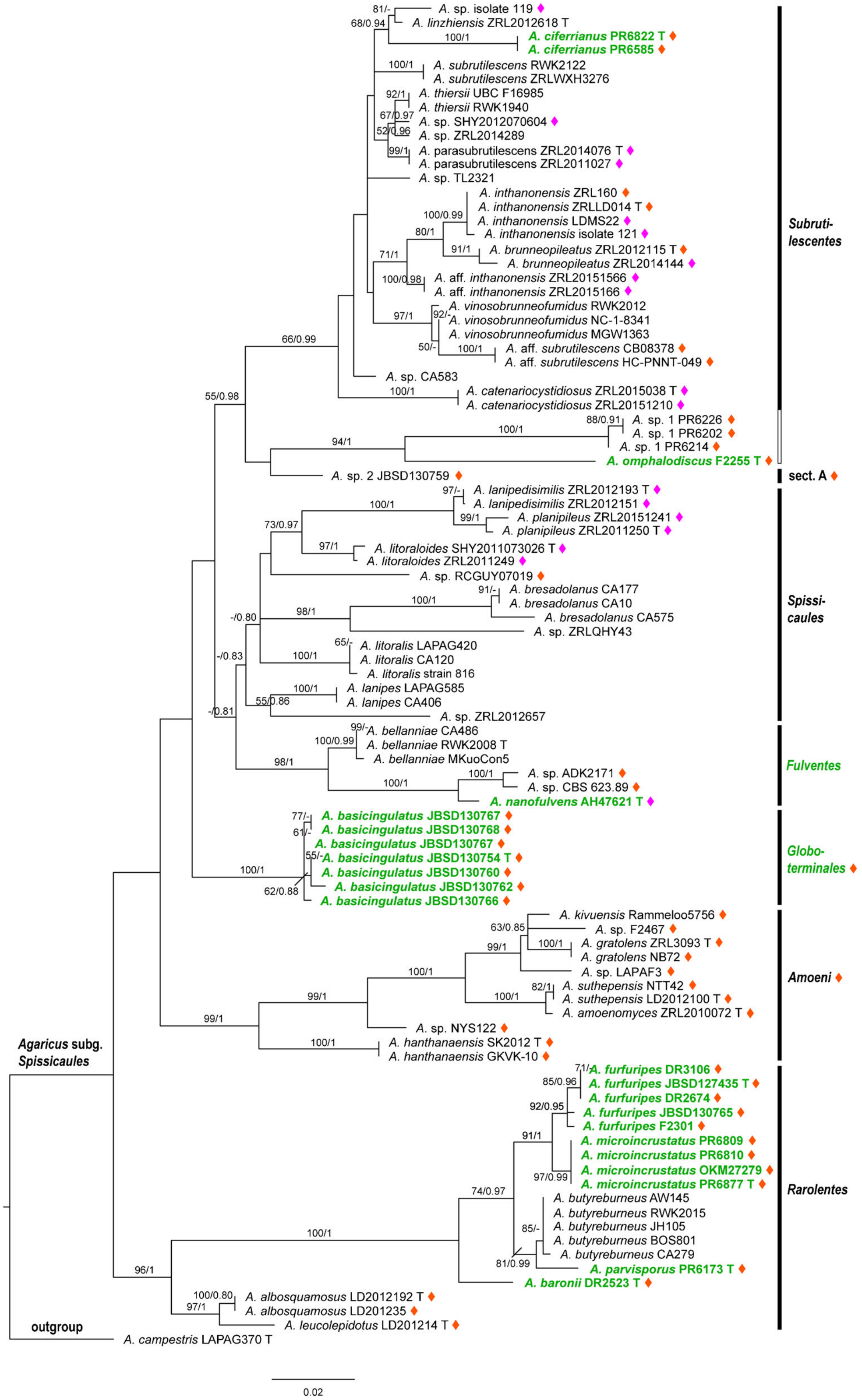
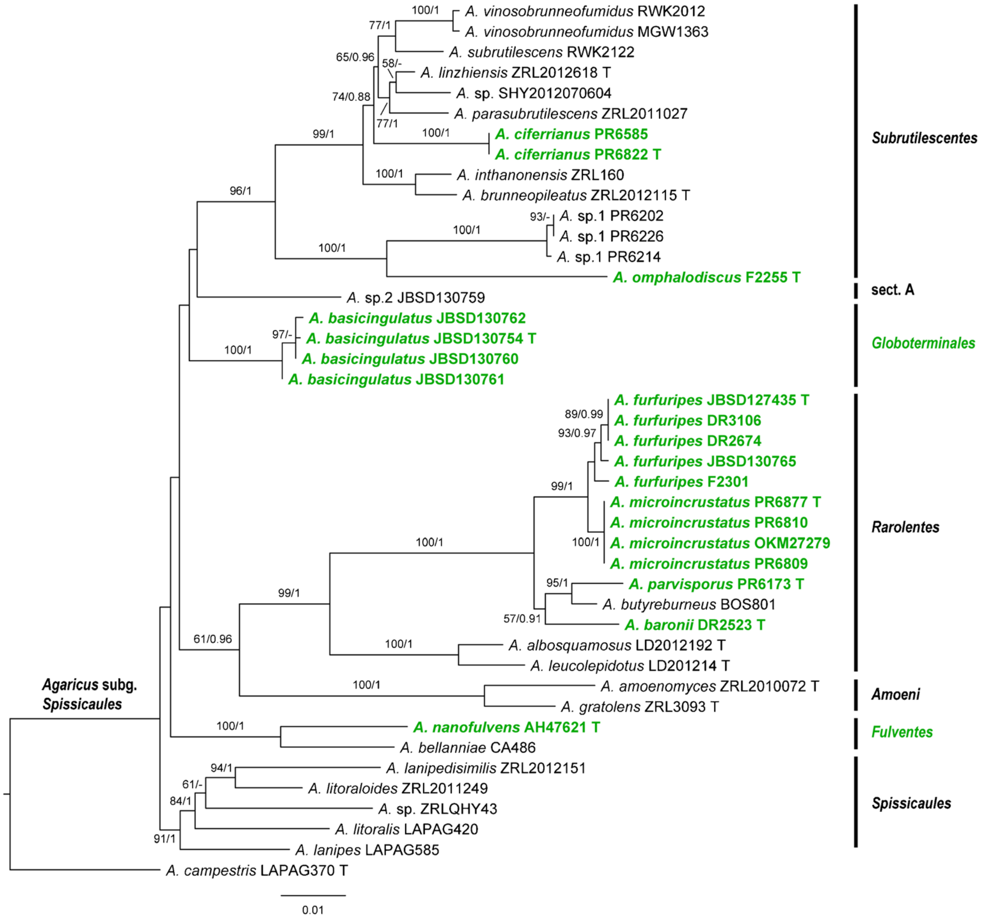


Fig. 1 Phylogeny of *Agaricus* subg. *Spissicaules* inferred from analyses of ITS sequences, rooted with *A. campestris*. Support values along branches are from ML Bootstrap (≥50%), and Bayesian posterior probabilities (PP ≥0.80), respectively. New taxa are highlighted in green. T, type specimen. Red diamond, tropical distribution. Violet diamond, subtropical distribution. Climatic information is based on the Köppen-Geiger climate classification (Peel et al. 2007) with climates of group A considered as "tropical" and those of types Cwa and Cfa as "subtropical." Black and white segments in the bar of *A.* sect. *Subrutilescens* indicate the two clades forming this section, which is polyphyletic in this tree

Fig. 2 Phylogeny of *Agaricus* subg. *Spissicaules* generated from analyses of ITS, LSU, and *TEF*1 sequences, rooted with *A. campestris*. Support values along branches are from ML Bootstrap (≥50%), and Bayesian posterior probabilities (PP ≥0.80), respectively. New taxa are highlighted in green



simple, fibrillose, or somewhat squamulose in its lower surface. Context unchanging or with an obscure yellowing on cutting and with a faint odor of almonds (see note below). Schäffer's and KOH reactions positive but weak on the pileipellis and the rhizomorphs both in fresh and dried basidiomata. Cheilocystidia absent or simple and hardly distinguishable from basidioles, simple. Spores 6.2–7 × 3.9–4.5 µm on average, ellipsoid to elongate-ellipsoid, lacking a rudimentary apical pore. Hyphae of the lower surface of the annulus with inflated elements. Pileipellis composed of hyphae containing diffuse or granular yellowish pigment.

Table 2 Mean stem ages of sections in *A.* subg. *Spissicaules*

Section	Mean of stem age (Ma)
Subrutilescentes	23.96
"section A"	23.96
Globoterminales	24.06
Fulventes	24.06
Spissicaules	24.06
Rarolentes	24.11
Amoeni	24.11

Known distribution: This section is distributed in North America and Europe (*A. bellanniae*), South America (*A. nanofulvens*), Africa (ADK2171), and Asia (CBS523.89).

Edibility: Generally unknown but ADK2171 belongs to a species used for food in Benin. According to the ethnomycological work of Yorou and De Kesel (2001; as *A. goossensiae*), it is called osusu-kpaké in Nagot language, meaning mushroom-cassava, because this species of *Agaricus* always grows around water wells, few meters away from rivulets, where cassava (*Manihot esculenta*) is traditionally processed by soaking, in order to reduce the cyanide content of tubers and/or peels, and lots of organic debris of cassava are left close to the water wells.

Taxonomic comments: The odor was only recorded in French and North American specimens of *A. bellanniae*. The lower surface of the annulus has been examined microscopically only in *A. nanofulvens* because the annulus was missing in dried specimens of *A. bellanniae* and *A.* sp. (ADK2171). The description of the section is based on the examination of available specimens of three species (*A. bellanniae*, *A. nanofulvens*, and *A.* sp./ADK2171).

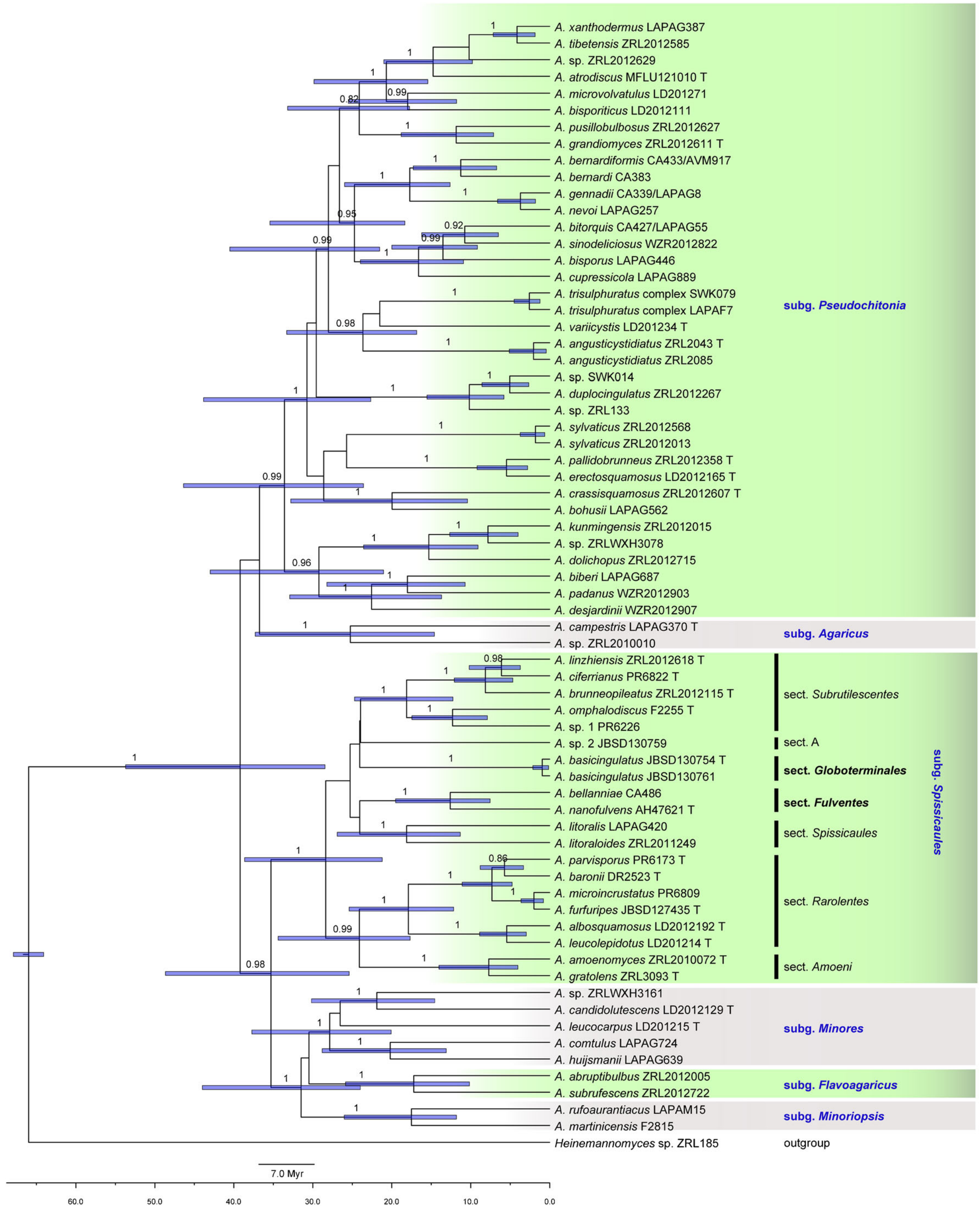


Fig. 3 Maximum clade credibility tree of *Agaricus* resulting from BEAST analysis based on ITS, LSU, and *TEF*1 sequence data with the outgroup *Heinemannomyces* sp. Bayesian posterior probabilities > 80% are indicated. The 95% highest posterior density of divergence time estimations are marked by horizontal bars. New sections are in bold

Information about the remaining specimen MACS125 (Maharashtra Association for the Cultivation of Science) collected in India, from which the culture CBS623.89 was obtained and sequenced, was unsuccessfully requested from the AMH herbarium. Therefore, this sample could not be morphologically compared to the other specimens of the section and we cannot exclude that it could belong to the same species as ADK2171, from which it differs at three positions in the ITS alignment.

Stem age and phylogenetic support: Stem age 24.06 Ma and PP = 1 in MCC tree (Fig. 3); strong support MS/PP = 100/1 in multi-gene tree (Fig. 2), and 98/1 in ITS tree (Fig. 1).

Section-specific ITS markers in A. subg. Spissicaules: ggCAattgctct@653+654. Interestingly, in our alignment, the DNA motif ggggattgctct is fully conserved in the 88 samples of all other sections of *A.* subg. *Spissicaules*.

Agaricus nanofulvens L.A. Parra, J.C. Zamora & B. Ortiz, **sp. nov**. Fig. 4

MycoBank: MB 837355

Etymology: Refers to its small size and the buff brown color of the pileus. From the Latin *nanus* (dwarf) and *fulvens* deriving from the root of *fulvus* (buff brown) and the ending *–ens* singular participle of sum (to be), meaning "the dwarf being fulvous."

Macroscopic description: **Pileus** 1.8–2.2 cm diam., hemispherical, or truncate-conical when young, then convex, finally completely plane, entirely covered with subtle radially arranged warm buff brown fibrils, darker at center. Surface smooth, fibrillose, dull, and dry. Margin thin, initially with a white narrow rim slightly exceeding the lamellae, soon concolorous with the pileus surface and not exceeding the lamellae. **Lamellae** free, up to 0.25 cm broad, straight, or

Fig. 4 Macroscopic characters of *Agaricus nanofulvens* (AH47621, Holotype). **a**–**e** Basidiomata. **d**–**e** Syringaldazine reaction on fresh material. **d** Stipe apex. **e** Stipe base. Microscopic characters of *Agaricus nanofulvens* (AH47621, Holotype). **f**–**l** (**f**–**k** in ammoniacal Congo red; **l** in water). **f** Spores. **g** Typical basidia. **h**–**i** Cupuliform basidia. **j** Hyphae of the lower surface of the annulus. **k**–**l** Pileipellis hyphae. Photos by J.C. Zamora (**a**–**e**) and L.A. Parra (**f**–**l**)

slightly ventricose, intercalated with numerous lamellulae, first pale pink, then bright pink, later pinkish brown, and finally dark brown to almost black with concolorous edge. **Stipe** 2.5–3.5 × 0.3–0.4 cm, clavate or slightly bulbous (0.6–0.8 cm) at base, usually flexuose, stuffed to fistulose, with an annulus on its upper third, above annulus smooth and pale pinkish, below annulus whitish to pale beige, fibrillose, usually with thin transverse brown bands more conspicuous especially at the base which is covered by white felted mycelium and a single thin white rhizomorph. **Annulus** superous, up to 0.2 cm broad, thick and narrow, fragile, white, upper surface smooth, lower surface fibrillose, or sometimes with small floccose pale brown scales near the margin. **Context** white on cutting, becoming grayish brown at stipe base with time. Odor not recorded.

Macrochemical reactions: In fresh specimens syringaldazine negative at the context except for a subtle pink discoloration in the upper part of the stipe and an inconspicuous violet positive tinge at the extreme stipe base. On dried specimens Schäffer's reaction positive, reddish brown on pileus surface, faint reddish orange at the white mycelium of stipe base.

Microscopic description: **Spores** 5.7–6.2–6.8 × 3.7–4–4.3 µm, Q=1.38–1.55–1.76, ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, without apical pore. **Basidia** in young basidiomata 13–23(26) × 7–9.5 µm, usually tetrasporic, some bisporic, clavate, or slightly truncated at the apex, also very abundant on the lamellae edge, sterigmata up to 2 µm long, in mature basidiomata basidia usually collapsed inwards becoming cupuliform with sterigmata and immature spores inside the cup. **Cheilocystidia** absent in young and mature basidiomata. **Pleurocystidia** absent. **Lower surface of the annulus** composed of three types of hyaline hyphae, cylindrical, not or slightly narrowed at septa, 3–10 µm wide; cylindrical with much shorter elements and constricted at the septa, elements easily disaggregating, and inflated elements with round ends up to 35 × 24 µm. Inflated elements not observed. **Pileipellis** a cutis, composed of cylindrical hyphae 3–7 µm, not or slightly constricted at the septa with abundant terminal elements cylindrical or progressively attenuated with rounded apex. In water, hyphae contain abundant granular or diffuse yellowish pigment. **Clamp connections** not observed.

Habit, habitat, occurrence, and distribution: Only collected once in a small group of eight basidiomata in a garden under *Eucalyptus* sp. in Argentina.

Species-specific ITS markers in A. sect. Fulventes: tgcacAcctgt@52, tggttTgccta@124, aagcaTtgctg@170, cttttttt[T]gtaGaagag@477+481.

Material examined: Argentina, Buenos Aires city, Ciudad Universitaria, between the Ciencias Exactas y Naturales Faculty (Pabellón 2) and Pabellón 1, 14 Mar 2012, J.C. Zamora, **AH47621**, **Holotype** (**AH**), LAPAM21, Isotype (LAPAM).

Taxonomic comments: Morphologically, *Agaricus nanofulvens* is similar to other taxa in *A.* sect. *Fulventes* (*A. bellanniae* and ADK2171) in having a warm brown pileus surface, stipe with rhizomorphs at the base, and absence of cheilocystidia at least in some collections. However, it differs from *A. bellanniae* (Kerrigan 2016; Guinberteau 2017) and ADK2171 by having a much smaller fibrillose pileus, stipe with brown bands at the stipe base, and slightly shorter spores. *Agaricus bellanniae* and ADK2171 have a bigger squamose pileus (2.5–8 cm diam. in *A. bellanniae* and 5–6 cm diam. in ADK2171), stipe glabrous at the base, and larger spores (Table 3).

Molecularly, *A. nanofulvens* can be easily identified because, in addition to its five species-specific markers; it differs from *A. bellanniae*, ADK2127, and CBS623.89, at 22, six, and seven additional positions respectively in the ITS alignment. Phylogenetically, *A. nanofulvens*, described from South America, is sister to the clade comprising the samples ADK2171 from Africa (Benin) and CBS623.89 from Asia (India).

Agaricus sect. ***Globoterminales*** Callac, L.A. Parra, Angelini, B. Ortiz & Linda J. Chen, **sect. nov**.

MycoBank: MB 837354

Type: *Agaricus basicingulatus* L.A. Parra, Angelini & B. Ortiz.

Table 3 Pileus and spore size comparison between species of *Agaricus* sect. *Fulventes*

Character	*Agaricus nanofulvens*	*Agaricus bellanniae*			*Agaricus* sp.
	Parra, this study (AH47621)	Kerrigan 2016 (from 5 collections)	Guinberteau 2017 (CA486)	Parra, this study (CA486)	Parra, this study (ADK2171)
Pileus diameter	1.8–2.2 cm	2.5–8 cm	5.1–6 cm	–	5–6 cm
Spore size (µm)	5.7–6.2–6.8 × 3.7–4–4.3	(5.4)6.3–6.5–6.5(7.7) × (4)4.3–4.5–4.6(4.9)	(6.2)7–7.2(8.2) × (3.8)4.2–4.6(5.3)	6.4–6.9–7.2 × 3.8–4–4.2	6–6.4–6.9 × 3.6–3.9–4.1

Etymology: Refers to the globose shape (usually similar to the head of a match) of the terminal elements of the hyphae of the lower surface of the annulus.

Morphological description: Robust, small-sized basidiomata. Pileus (2)2.5–4(4.7) cm diam. with brownish ochraceous to dark brown color. Stipe with an annulus on its upper third and rhizomorphs at the base. Annulus superous, membranous, simple, smooth in its lower surface. Context rufescent, pinkish red on cutting, and without distinctive odor. Schäffer's and KOH reactions not performed on fresh basidiomata, but positive on the stipe base and the rhizomorphs on dried basidiomata. Cheilocystidia very abundant, simple, easily distinguishable from basidioles. Spores 5.6–5.9 × 3.7–3.8 µm on average, ellipsoid, lacking a rudimentary apical pore. Hyphae of the lower surface of the annulus without inflated elements but with short globose terminal elements only slightly wider than subterminal elements. Pileipellis composed of hyphae containing diffuse or granular yellowish pigment.

Known distribution: This section is, so far, only distributed in the Dominican Republic.

Edibility: Unknown.

Stem age and phylogenetic support: Stem age 24\.06 Ma and PP = 1 in MCC tree (Fig. 3); strong support MS/PP = 100/1 in multi-gene tree (Fig. 2), and 100/1 in ITS tree (Fig. 1).

Section-specific ITS markers in A. subg. Spissicaules: gtttcCagatg@14, ctcttGggagc@39, tcttt[T]ggttg@106, tctca[C]ccaga@238, gctgtCttgta@513, yygat[GGT]gtcag@571–573.

Agaricus basicingulatus L.A. Parra, Angelini & B. Ortiz, **sp. nov**. Figs. 5, 6

MycoBank: MB 837356

Etymology: Refers to its annulated brown band near the base of the stipe. From the Latin and Greek *basis* (base) and *cingulatus* (annulate).

Macroscopic description: **Pileus** (2)2.5–4(4.7) cm diam., truncate-conical when young, then completely plane and broadly umbonate at center, in mature specimens with umbo depressed at center (umbilicate), first entirely covered with ochraceous brown to reddish brown fibrils and dark brown entire center, then away from the center radially fibrillose or dissociated in concentrically arranged triangular scales smaller towards the margin, on a lighter, cream-whitish background. Surface smooth, fibrillose to squamulose, dull, and dry. Margin thin, initially with a white narrow rim slightly exceeding the lamellae, soon concolorous with the pileus surface and not exceeding the lamellae. **Lamellae** free, up to 0.3 cm broad, slightly ventricose, intercalated with numerous lamellulae, first white, then bright pink, later reddish pink, finally dark brown-purple with whitish edge. **Stipe** (2.5)3.5–4(5.5) × (0.4)0.5–0.7(0.8) cm, rather robust, clavate to bulbous (0.9–1.5 cm), often curved towards the base, stuffed to fistulose, with an annulus on its upper third, above and below the annulus white, smooth or subtly fibrillose, near the base with one (rarely two or three) thin transverse adpressed brown collar. Base covered by white felted mycelium and provided with a single thick white rhizomorph. **Annulus** superous, up to 0.4 cm broad, thin and narrow, fragile, evanescent, white with light brown edge, upper surface smooth, lower surface smooth to subtly fibrillose. **Context** white on cutting, becoming reddish-pink both in pileus and stipe, without distinctive odor.

Macrochemical reactions: Schäffer's and KOH reactions not performed on fresh basidiomata but positive on the stipe base and the rhizomorphs on dried basidiomata. Schäffer's reaction orange to reddish at stipe base and rhizomorphs, KOH negative to pale yellow at the stipe base.

Microscopic description: **Spores** 5–5.6–6.2(6.4) × 3.5–3.8–4.1 µm, Q=(1.28)1.35–1.47–1.71, ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, without apical pore. **Basidia** 15–24 × 7.5–9 µm, usually tetrasporic, some bisporic, clavate or slightly truncated at the apex, hyaline, some with internal black pigment, sterigmata up to 2 µm long. **Cheilocystidia** 10–27 × 7–15(20) µm, abundant, usually simple, clavate, pyriform, globose, sphaeropedunculate, rarely narrowly lageniform or fusiform, hyaline, some with internal black pigment. **Pleurocystidia** absent. **Lower surface of the annulus** composed of hyaline hyphae, cylindrical, not or slightly narrowed at septa, 2–7 µm wide, without inflated elements but with short globose terminal elements only slightly wider than subterminal elements, spherical, like the head of a match or shortly clavate, 10–18 × 9–12 µm. **Pileipellis** a cutis, composed by cylindrical hyphae 3–10 µm, not or slightly constricted at the septa with abundant terminal elements cylindrical or progressively attenuated with rounded apex. In water, hyphae contain abundant granular or diffuse yellowish pigment. **Clamp connections** not observed.

Habit, habitat, occurrence, and distribution: Solitary or in small groups of 2–5 basidiomata, in lowland broadleaf forests. Uncommon. Recorded only from the Dominican Republic.

Note: The original description is based on seven collections, but all of them came from two contiguous anthropized small broadleaf forests of about 1.4 km^2 in Puerto Chiquito near Sosúa.

Material examined: Dominican Republic, Puerto Plata, Sosúa, Puerto Chiquito, 1 Dec 2016, C. Angelini, JBSD130760 (LAPAM72), 2 Dec 2017, C. Angelini, JBSD130761 (LAPAM97), 17 Dec 2017, C. Angelini, JBSD130762 (LAPAM102), 16 Nov 2018, C. Angelini, **JBSD130754**, **Holotype** (**JBSD**), LAPAM119, Isotype (LAPAM), 16 Nov 2018, C. Angelini, JBSD130766 (LAPAM121), 22 Nov 2018, C. Angelini, JBSD130767 (LAPAM122), 10 Dec 2018, C. Angelini, JBSD130768 (LAPAM123).

Taxonomic comments: This species can be distinguished from any other of the genus *Agaricus* by having small-sized

Fig. 5 Macroscopic characters of *Agaricus basicingulatus*. **a**–**e** Basidiomata. **a**–**b** JBSD130754, Holotype. **c** JBSD130766. **d** JBSD130767. **e** JBSD130768. Photos by C. Angelini

basidiomata, umbilicate brown pileus, darker at center, smooth or subtly fibrillose lower surface of the annulus, a stipe usually with a thin transverse appressed brown collar, rufescent odorless context, spores 5.56 × 3.8 μm on average, abundant cheilocystidia, and hyphae of the lower surface of the annulus with short globose terminal elements only slightly wider than subterminal elements, spherical, like the head of a match or shortly clavate. The most similar species to *A. basicingulatus* is *A. dicystis* Heinem., but the latter differs in having a much larger size (pileus diam. 9 cm) stipe without transverse colored bands near the base, fragile annulus, context reddening only on the cortex of the stipe, longer spores (5.5–7.3 × 3.3–4.2 μm), and hyphae of the scales of the pileus consisting of easily detachable elements (Heinemann 1962).

Agaricus sect. ***Rarolentes*** Kerrigan, Mem. New York Bot. Garden 114: 343. 2016.

MycoBank: MB 802558

Type: *Agaricus butyreburneus* Kerrigan, Guinb. & Callac, Mem. New York Bot. Garden 114: 346. 2016.

Etymology: Refers to the, rare, unusual, and/or variable odors noted in *A. butyreburneus* the type, and the only species described in the original diagnosis of *A.* sect. *Rarolentes*.

Morphological description: Gracile, small to medium-sized basidiomata. Pileus 2.5–9 cm diam. white or covered by pale brown appressed scales with a darker entire center. Stipe with an annulus on its upper third and rhizomorphs or mycelial strands at the base, always distinctively furfuraceous above the basal bulb. Annulus superous, broad, pendant, thin, fragile, squamulose in its lower surface. Context unchanging or with an obscure yellowing or ochraceous tinge on cutting and often with a faint odor of almonds but sometimes fungoid or unpleasant (see note below). Schäffer's and KOH reactions

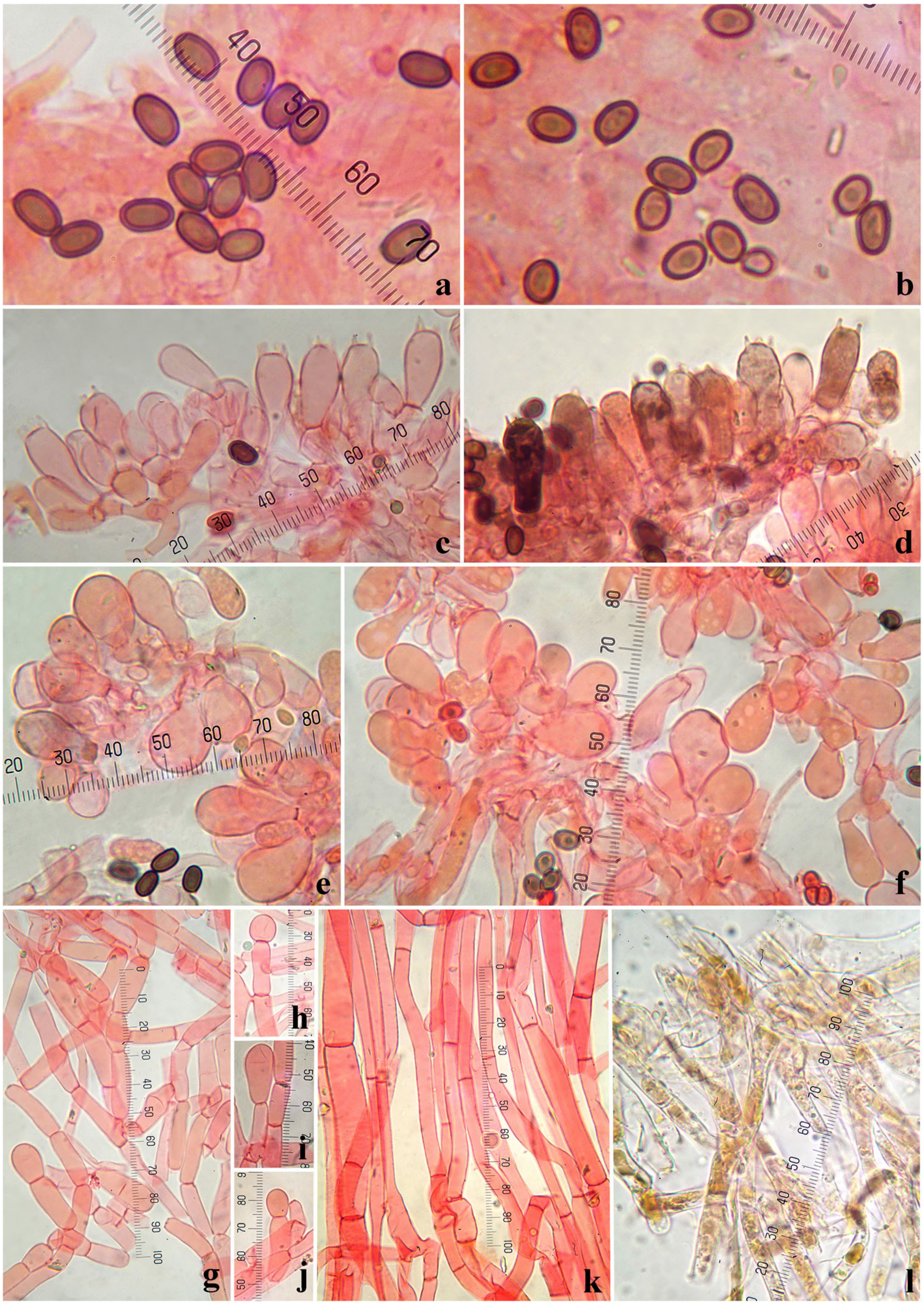


Fig. 6 Microscopic characters of *Agaricus basicingulatus*. **a**–**k** in ammoniacal Congo red; **l** in water. **a**, **c**–**e**, **h**, **k**–**l** JBSD130754, Holotype. **b**, **f** JBSD130767. **g**, **i**, **j** JBSD13076. **a**–**b** Spores. **c**–**d** Basidia. **e**–**f** Cheilocystidia. **g**–**j** Hyphae of the lower surface of the annulus (**h**–**j** globose terminal elements). **k**–**l** Pileipellis hyphae. Photos by L.A. Parra

negative on the pileipellis in dried basidiomata. Cheilocystidia present, claviform, pyriform, globose, or sphaeropedunculate. Spores 4.9–6.8 × 2.7–3.8 μm on average, ellipsoid to elongate-ellipsoid, lacking a rudimentary apical pore. Hyphae of the lower surface of the annulus with abundant globose to inflated elements. Pileipellis composed of hyphae containing diffuse or vacuolar yellowish or pale brown pigment.

Known distribution: This section is distributed in Asia (Thailand), the Caribbean (Dominican Republic, Martinique, and Puerto Rico), Europe (France, Italy, Spain, Czech Republic, and Hungary), and North America (the USA).

Edibility: Unknown.

Taxonomic comments: All species belonging to *A.* sect. *Rarolentes* can have an odor of almonds except *A. parvisporus* with fungoid or unpleasant odor. However, some species exhibit also other odors depending on the collections, in *A. furfuripes* fungoid, and pleasant odors have also been recorded, in *A. microincrustatus* fungoid, or an unpleasant odor like rubber tires has also been noted, and in *A. butyreburneus* fungoid or faintly of shoe or furniture polish odors have also been described (Sánchez and Parra 2019; Kerrigan 2016). So, *A. butyreburneus* is not the only species with a variable odor in this section.

Stem age and phylogenetic support: Stem age 24.11 Ma and PP = 1 in MCC tree (Fig. 3); strong support MS/PP = 99/1 in multi-gene tree (Fig. 2), and 96/1 in ITS tree (Fig. 1).

Section-specific ITS markers in A. subg. Spissicaules: my[Ttt]trycrg@145(146–147). gyyyyyGvmhygr@208.

The T insertion at position 145 is specific to all members of *A.* sect. *Rarolentes* in the subgenus. Interestingly, a supplementary T insertion (position146) is specific of *A. microincrustatus* and *A. furfuripes*, and a third T insertion (position147) is specific to *A. furfuripes*. Because one T was initially present in all samples and since, in addition to the successive T insertions (tandem repeats), a transition C>T occurred at a flanking position, the resulting poly T (positions 144 to 148) appears to have progressively increased from 1T in the other sections of the subgenus to 2T–5T in the taxa of *A.* sect. *Rarolentes*: 2T in *A. albosquamosus* and *A. leucolepidotus*, 3T in *A. butyreburneus*, *A. baronii* and *A. parvisporus*, 4T in *A. microincrustatus*, and 5T in *A. furfuripes*.

Agaricus baronii L.A. Parra, B. Ortiz & Kerrigan, **sp. nov**. Fig. 7

MycoBank: MB 837357

Etymology: The specific epithet honors Timothy J. Baroni for his contribution to the mycological knowledge of the Caribbean.

Macroscopic description: **Pileus** 2.5–8.8 cm diam., at first truncate-conical with plane or broadly umbonate center, then convex with truncate, plane or depressed at center, umbo, completely covered by reddish brown or brown appressed squamules or fibrils on a white, grayish brown, or pale reddish brown background and a darker brown entire center. Surface fibrillose, dull, and dry, not discoloring when rubbed. Margin smooth, nor slightly exceeding the lamellae, sometimes appendiculate by annulus remnants. **Lamellae** free, up to 0.5 cm broad, crowded, straight, intercalated with lamellulae, at first pale pink, then pale grayish vinaceous, dark brown at maturity with entire edge. **Stipe** 4.5–8.5 × 0.6–1 cm, cylindrical, bulbous at the base (1.2–2.4 cm), often curved towards the base, stuffed, with an annulus on its upper third; above annulus white and smooth, below annulus white or pinkish white, becoming slowly brown in some areas, smooth, furfuraceous, or squamulose towards the base. Base sometimes provided with a short rhizomorph or some mycelial strands at the base. **Annulus** superous, up to 1.9 cm broad, white, membranous, thin, fragile, double, upper surface smooth, lower surface with brown squamules towards the margin. **Context** when cut white, unchanging. Odor of almonds.

Macrochemical reactions: Schäffer's reaction negative on fresh and dried basidiomata. KOH negative on dried basidiomata.

Microscopic description: **Spores** 5.2–5.8–6.3 × 3.2–3.5–3.8 µm, Q=1.5–1.68–1.88, ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, without apical pore. **Basidia** 11–20 × 7–9 µm, tetrasporic, clavate or slightly truncated at the apex, hyaline, sterigmata up to 3 µm long. **Cheilocystidia** 11–25 × 6–15 µm, abundant, usually simple, hyaline, clavate, pyriform, globose, or sphaeropedunculate, some septate at the base. **Pleurocystidia** absent. **Lower surface of the annulus** consisting of two types of hyaline hyphae, some cylindrical not or slightly constricted at the septa 2–11 µm wide, and others, abundant, generally consisting of globose, spherical, ovoid, or peanut-shaped inflated elements readily disarticulating, 5–20 × 5–16 µm. **Pileipellis** a cutis, composed by cylindrical hyphae 2–12(16) µm, not or slightly constricted at the septa, hyaline or with diffuse or vacuolar pale brown pigment. **Clamp connections** not observed.

Habit, habitat, occurrence, and distribution: Gregarious, in groups of few basidiomata, on soil. Rare. Recorded only once from the Dominican Republic.

Species-specific ITS markers in A. sect. Rarolentes: tctagGyygggt@18; tctyy[CG]ngagc@39-40; aaagcyCtgctg@178; ttrtrcCgtttt@477; tgkg[t]yArttc@627; ytyraCgaaac@655.

Material examined: Dominican Republic. La Vega, Jarabacoa, road to Salto de Jimenoa, 1 Jan 2003, B. Ortiz-Santana, **DR2523**, **Holotype** (**CFMR**).

Taxonomic comments: Within *A.* sect. *Rarolentes*, *A. butyreburneus*, *A. albosquamosus* L.J. Chen, K.D. Hyde & R.L. Zhao and *A. leucolepidotus* L.J. Chen & R.L. Zhao differ in having a white pileus without colored scales, *A. microincrustatus* differs by its slightly longer spores and mainly by its incrusted cheilocystidia. *Agaricus parvisporus* possesses much smaller spores, and *A. furfuripes*, the closest species, can be morphologically distinguished by having a paler pileus consisting of hyphae without internal granular or diffuse brown pigment, an ochre salmon context, and molecularly by differing at 26 or more positions in its ITS1+2 sequence.

Another morphologically similar species whose current circumscription based on phylogenetic analysis is not yet known is *A. subochraceosquamulosus* Heinem with its pileus surface darker brown colored, but it differs in having an umbonate, pinkish orange, more or less brownish pileus, much smaller spores (3.7–4.7 × 3.1–3.5 µm) and clavate basidioliform cheilocystidia of 14–20 × 5.5–10 µm.

Agaricus furfuripes L.A. Parra, Angelini, Fiard & B. Ortiz, **sp. nov**. Fig. 8

MycoBank: MB 837358

Fig. 7 Macroscopic characters of *Agaricus baronii* (DR2523, Holotype). **a**–**b** Basidiomata. Microscopic characters of *Agaricus baronii* (DR2523, Holotype). **c**–**h** (**c**–**f** in ammoniacal Congo red; **g** in water; **h** in ammonia). **c** Spores. **d** Basidia. **e** Cheilocystidia. **f** Hyphae of the lower surface of the annulus. **g**–**h** Pileipellis hyphae. Photos by B. Ortiz-Santana (**a**–**b**) and L.A. Parra (**c**–**h**)

Etymology: Refers to the furfuraceous ornamentation of the stipe below the annulus. From the Latin *furfureus* (scurfy) and *pes* (stipe).

Macroscopic description: **Pileus** 3–7.5 cm diam., convex subtruncate at center, then completely plane with a depressed or slightly umbonate disc, covered by minute, appressed, pale brown squamules more scattered towards the margin on a whitish background and a darker brown entire center. Surface fibrillose, dull and dry, not discoloring when rubbed. Margin smooth or finely fibrillose, not or slightly exceeding the lamellae. **Lamellae** free, up to 0.4 cm broad, crowded, straight, intercalated with lamellulae, at first pinkish white, finally dark brown in mature basidiomata, with paler and entire or inconspicuously denticulate edge. **Stipe** 4–9 × 0.6–0.8 cm, cylindrical, abruptly bulbous at the base (1.2–2 cm), often curved towards the base, stuffed to fistulose, with an annulus on its upper third; above annulus at first white then pink, smooth, below annulus white, minutely squamulose, more conspicuously furfuraceous towards the base. Base sometimes provided with a single thick rhizomorph or with abundant thin mycelial strands. **Annulus** superous, up to 3.5 cm broad, white, membranous, double, upper surface smooth, lower surface radially fibrillose near the insertion and floccose-squamulose towards the margin. **Context** when cut white, discoloring ochre, salmon with time, with strong odor of bitter almonds in collections JBSD127435 and JBSD130765, fungoid in DR2674 and pleasant in DR3106 and F2301.

Macrochemical reactions: Schäffer's reaction not performed on fresh basidiomata, negative or weakly positive orange on pileus surface in JBSD127435 on dried material. KOH unreadable due to the ochraceous color of the pileus surface on dried material.

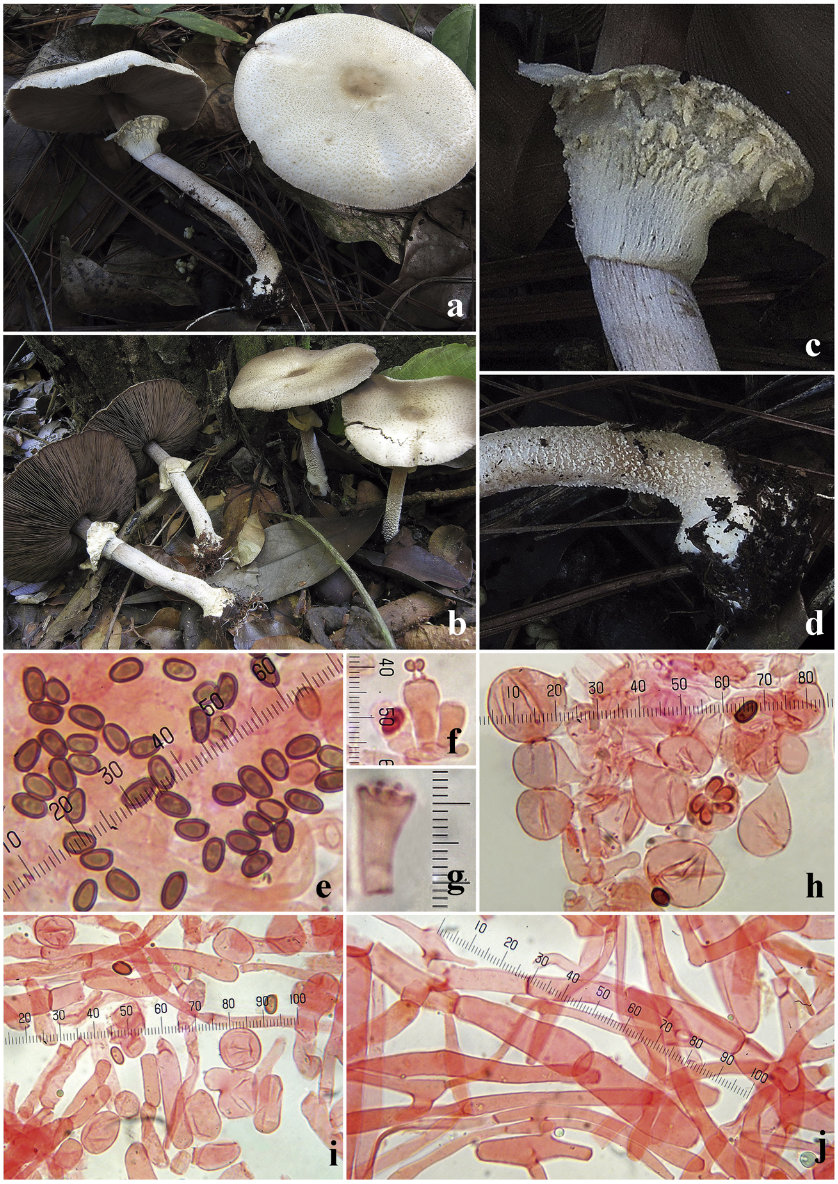


Fig. 8 Macroscopic characters of *Agaricus furfuripes*. **a**–**d** Basidiomata. **a**, **c**–**d** JBSD127435, Holotype. **b** JBSD130765. Microscopic characters of *Agaricus furfuripes*. **e**–**j** in ammoniacal Congo red. **e**, **h**–**j** JBSD127435, Holotype. **f**–**g** F2301. **e** Spores. **f** Typical basidia. **g** Cupuliform basidia. **h** Cheilocystidia. **i** Hyphae of the lower surface of the annulus. **j** Pileipellis hyphae. Photos by C. Angelini (**a**–**d**) and L.A. Parra (**e**–**j**)

Microscopic description: **Spores** 5–5.6–6.4 × 2.9–3.3–3.6(3.8) µm, Q=(1.39)1.5–1.7–1.87(1.97), ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, without apical pore. **Basidia** 13–18 × 5.4–9 µm, tetrasporic, clavate or slightly truncated at the apex, hyaline, some collapsed inwards becoming cupuliform with sterigmata inside the cup, sterigmata up to 2 µm long. **Cheilocystidia** 10–23 × 8–18 µm, abundant, usually simple, broadly clavate, pyriform, ovoid, globose or sphaeropedunculate, hyaline. **Pleurocystidia** absent. **Lower surface of the annulus** consisting of two types of hyaline hyphae, some cylindrical not or slightly constricted at the septa 2–7 µm wide, and others, abundant, generally consisting of globose, spherical, ovoid or peanut-shaped inflated elements easily detachable, 8–21 × 7–16 µm. **Pileipellis** a cutis, composed by cylindrical hyphae 2–16 µm, not or slightly constricted at the septa. **Clamp connections** not observed.

Habit, habitat, occurrence, and distribution: Gregarious, on leaf litter of *Pinus occidentalis* forests in the mountains in the Dominican Republic and mesophilic tropical broad-leaved forests in Martinique. Uncommon. Recorded only from the Dominican Republic and Martinique.

Species-specific ITS markers in A. sect. Rarolentes: ggtca[A]g[g]myt[tT]taycag@128+135, ctggcTrctcg@526, tgag[AG]gtcag@538–539. The insertions A@128), T@135 (and the resulting poly-5T), and G (539) are specific to *A. furfuripes* among the whole subgenus.

Material examined: Dominican Republic, La Vega, Jarabacoa, Jarabacoa golf course area, 11 Nov 2003, O. Perdomo and A. Monegro, DR3106 (CFMR); La Vega, Mun. Jarabacoa, Loc. Jarabacoa, 14 Nov 2017, C. Angelini, **JBSD127435**, **Holotype** (**JBSD**), LAPAM107, Isotype (LAPAM), 23 Nov 2017, C. Angelini, JBSD130765

(LAPAM109); Santiago, Las Placetas, 13 Jan 2003, L. Lacey and D.J. Lodge, DR2674 (CFMR). FRANCE, Martinique, Saint Pierre, Case Pilote, mount Morne Rose, 10 Oct 2000, J.–P. Fiard, F2301 (LIP).

Taxonomic comments: Within *A.* sect. *Rarolentes*, *A. butyreburneus*, *A. albosquamosus* and *A. leucolepidotus* differ in having a white pileus without colored scales and an unchanging context, *A. microincrustatus* differs by its immutable context, its slightly larger spores and mainly by its incrusted cheilocystidia and *A. parvisporus* possesses a darker pileus surface and much smaller spores.

Other morphologically similar species whose current circumscription based on phylogenetic analysis is not yet known are *A. ochraceodiscus* Murrill, *A. ochraceosquamulosus* Heinem. and *A. subochraceosquamulosus* Heinem. The type of *A. ochraceodiscus* has been studied by Smith (1940), Freeman (1979) and Pegler (1987). Spores of the type of *A. ochraceodiscus* were described or iconographied as ovoid or broadly ellipsoidal by these authors. Pegler gave a spore Q coefficient of 1.4 for the type *A. ochraceodiscus* which coincides with the spores drawn by Smith and with Murrill's original description of the spores as "quite broadly ellipsoidal" (Murrill 1918), very different from those of *A. furfuripes*. In addition, the cheilocystidia described from the type study by Smith (26–35 × 7–12 μm) or by Pegler (20–30 × 7–10 μm) are claviform and much narrower than in *A. furfuripes*. *Agaricus ochraceosquamulosus* (Heinemann 1961) differs in having a pileus surface dotted with minute rufous innate squamules, wider spores (5.1–5.7 × 3.6–4 μm) and clavate inconspicuous cheilocystidia 12–20 × 8–9 μm, and *A. subochraceosquamulosus* in having a umbonate, pink orange, more or less brownish, pileus, narrower spores (3.7–4.7 × 3.1–3.5 μm) and clavate basidioliform cheilocystidia 14–20 × 5.5–10 μm.

Agaricus microincrustatus L.A. Parra, B. Ortiz, Lodge & T.J. Baroni, **sp. nov**. Fig. 9

MycoBank: MB 837359

Etymology: Refers to the small incrustations observed on the surface of its microscopic structures. From the Greek and Latin prefix micro- (little, small) and the Latin *incrustatus* (incrusted).

Macroscopic description: **Pileus** 2.5–9 cm diam., conic when immature, then hemispherical to broadly convex, plano-convex with age, covered by minute, appressed, pale brown to dark brown squamules more scattered towards the margin on a whitish, pale grayish tan or yellowish gray background, and a darker brown entire center. Surface fibrillose, dull and dry, not discoloring when rubbed. Margin smooth, not or slightly exceeding the lamellae. **Lamellae** free, up to 0.4 cm broad, crowded, straight, intercalated with lamellulae, at first whitish, soon with a pinkish hue, then brown, dark chocolate brown at maturity, with paler and entire or inconspicuously denticulate edge. **Stipe** 3.3–10 × 0.4–0.65 cm, cylindrical, abruptly bulbous at the base (1–1.5 cm), often curved towards the base, stuffed, with an annulus on its upper third; above annulus white and smooth, below annulus white to grayish pink, smooth, furfuraceous or squamulose towards the base. Base sometimes provided with some mycelial strands. **Annulus** superous, up to 2.5 cm broad, white, membranous, thin, fragile, double, upper surface smooth, lower surface radially fibrillose near the insertion and floccose-squamulose towards the margin with white to pale tan squamules. **Context** when cut white, unchanging, with faint odor of bitter almonds in collection PR6877, fungoid in PR6810 and unpleasant like rubber tires or inner tubes in OKM27279.

Macrochemical reactions: Schäffer's reaction negative on fresh and dried basidiomata. KOH negative on the pileus surface on dried material.

Microscopic description: **Spores** 5.2–5.9–6.8 × 3.1–3.5–4 μm, Q=1.49–1.71–1.94, ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, without apical pore. **Basidia** 14–21 × 5–9 μm, tetrasporic, sometimes monosporic or bisporic near the lamellae edge, clavate or slightly truncated at the apex, hyaline sterigmata up to 2 μm long. **Cheilocystidia** 6–27 × 6–15 μm, abundant, usually simple, broadly clavate, pyriform, ovoid, globose or sphaeropedunculate, hyaline or with minute, widespread epiparietal incrustations of an unknown amorphous refractive substance in all collections examined. The latter more abundant in PR6877. **Pleurocystidia** absent. **Lower surface of the annulus** consisting of two types of hyaline hyphae, some cylindrical, not or slightly constricted at the septa, 2–8(11) μm wide and others, abundant, generally consisting of globose, spherical, ovoid or peanut shaped inflated elements readily disarticulating, 7–20 × 6–13 μm. In PR6877 some hyphae with abundant dense refractive drops of exudate are observed. **Pileipellis** a cutis, composed by cylindrical hyphae 2.7–12 μm, not or slightly constricted at the septa, hyaline or with diffuse pale brown pigment. In PR6877 some hyphae with abundant dense refractive drops of exudate were observed. **Clamp connections** not observed. In PR6809 epipariteal incrustations have been also observed in the basidioles and the lamellar trama hyphae and in PR6810 the lamellar trama hyphae contains numerous small vacuoles of a refractive and dense substance.

Habit, habitat, occurrence and distribution: Gregarious, on humus soil, leaf litter of needles of *Pinus caribaea* in a 30 year old plantation or under *Prestoea montana* (mountain palm). Uncommon. Recorded only from Puerto Rico.

Species-specific ITS markers in A. sect. Rarolentes: gtcttCggty@100, acttgAcyrc@196.

Material examined: Puerto Rico, Luquillo, El Yunque National Forest, near Guzmán, TSBF program plots, under *Pinus caribaea*, 17 Jan 1998, D.J. Lodge, O.K. Miller and

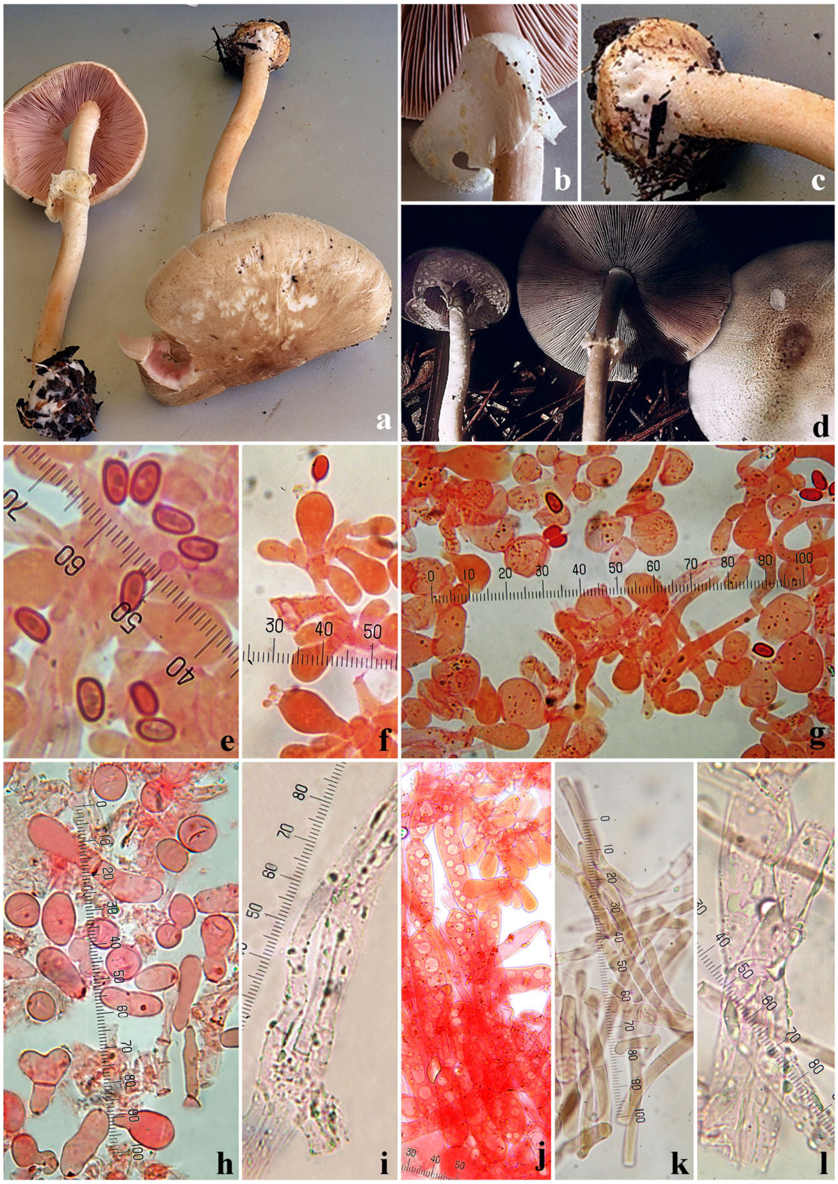


Fig. 9 Macroscopic characters of *Agaricus microincrustatus*. **a**–**d** Basidiomata. **a**–**c** PR6877, Holotype. **d** OKM27279. Microscopic characters of *Agaricus microincrustatus*. **e**–**l**. **e**–**g**, **i**, **k**–**l** PR6877, Holotype. **h** PR6809. **j** PR6820 (**e**–**j**, **j** in ammoniacal Congo red; **i**, **l** in water; **k** in ammonia). **e** Spores. **f** Basidia. **g** Cheilocystidia. **h**–**i** Hyphae of the lower surface of the annulus. **j** Lamellar trama. **k**–**l** Pileipellis hyphae. Photos by T.J. Baroni (**a**–**c**), O.K. Miller, Jr. (**d**), L.A. Parra (**e**–i, **k**–**l**), and B. Ortiz-Santana (**j**)

H. Miller, OKM27279 (NY1758153); Río Grande, El Yunque National Forest, El Verde Field Station, Canopy trimming experiment, block A, upper buffer zone, on humus soil, 18 Sep 2015, D.J. Lodge, PR6809 (CFMR), 18 Sep 2015, V.N. Colón-Fernández, PR6810 (CFMR); Río Grande, El Yunque National Forest, El Toro trail, under *Prestoea montana*, 8 Nov 2016, T.J. Baroni, **PR6877**, **Holotype** (**CFMR**).

Taxonomic comments: Morphologically *A. microincrustatus* can be easily distinguished from the remaining species of the genus *Agaricus* by its cheilocystidia with minute widespread epiparietal incrustations and dense refractive drops of exudate in the hyphae of numerous structures (pileipellis, annulus). In PR6810 these drops are also observed inside of the lamellar trama hyphae.

Agaricus parvisporus L.A. Parra & B. Ortiz, **sp. nov.** Fig. 10

MycoBank: MB 837360

Etymology: Refers to the small spores of the species compared with other members of the genus. From the Latin *parvus* (small) and the latinized Greek *sporus* (spore).

Macroscopic description: **Pileus** 6.7–7.5 cm diam., broadly convex to plane, obtusely umbonate, covered by pale grayish brown appressed squamules or fibrils on a white background and a brown or cinnamon brown entire center. Surface fibrillose, dull and dry. Margin smooth, not or slightly exceeding the lamellae, recurved with age. **Lamellae** free, up to 0.6 cm broad, crowded, straight, intercalated with lamellulae, grayish vinaceous to light brown at maturity with darker entire edge. **Stipe** 6.7–9 × 5.5–6 cm, cylindrical, bulbous at the base (up to 1.5 cm), sometimes curved towards the base, stuffed, with an annulus on its upper third; above annulus white to light brown and smooth, below annulus white,

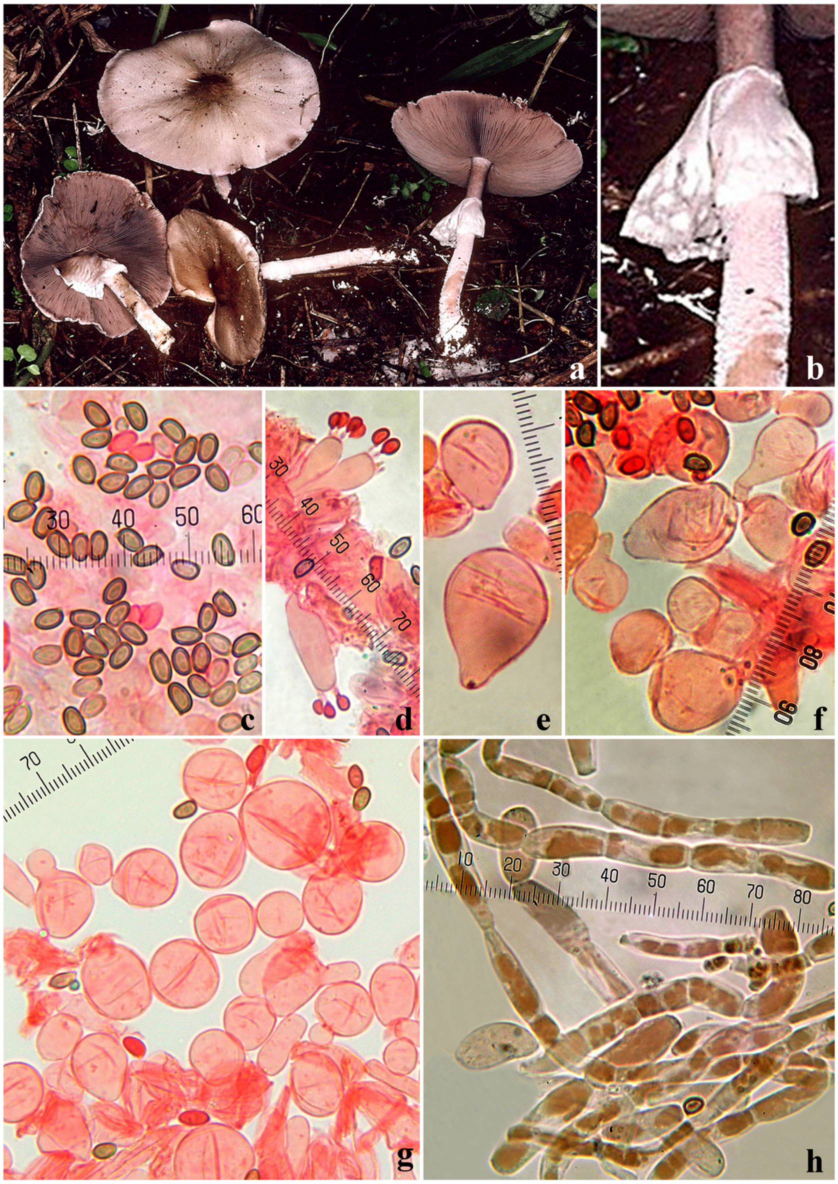


Fig. 10 Macroscopic characters of *Agaricus parvisporus* (PR6173, Holotype). **a–b** Basidiomata. Microscopic characters of *Agaricus parvisporus* (PR6173, Holotype). **c–h** (**c–g** in ammoniacal Congo red; **h** in water). **c** Spores. **d** Basidia. **e–f** Cheilocystidia. **g** Hyphae of the lower surface of the annulus. **h** Pileipellis hyphae. Photos by S.A. Cantrell (**a–b**), L.A. Parra (**c–e**, **h**), and B. Ortiz-Santana (**g**)

becoming yellowish or orangish brown when bruised, furfuraceous or squamulose towards the base. Base sometimes provided with some mycelial strands. **Annulus** superous, up to 1.3 cm broad, white, membranous, double, upper surface smooth, lower surface with white, more or less rounded floccose patches towards the margin. **Context** when cut whitish, unchanging. Odor fungoid or unpleasant.

Macrochemical reactions: Schäffer's reaction negative on fresh and dried basidiomata. KOH negative on dried basidiomata.

Microscopic description: **Spores** 4–4.9–5.8 × 2.4–2.7–3.1 µm, Q=1.54–1.78–2, ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, without apical pore. **Basidia** 15–22 × 5–7.5 µm, tetrasporic, clavate or slightly truncated at the apex, fusiform, hyaline, sterigmata up to 2 µm long. **Cheilocystidia** 12–28(33) × 7–15(18) µm, abundant, simple, hyaline, clavate, pyriform, globose or sphaeropedunculate. **Pleurocystidia** absent. **Lower surface of the annulus** consisting of two types of hyaline hyphae, some cylindrical not or slightly constricted at the septa 2.7–10 µm wide and others, abundant, generally consisting of globose, spherical, ovoid or peanut shaped inflated elements readily disarticulating, 6.3–20.8 × 6.3–18 µm. **Pileipellis** a cutis, composed by cylindrical hyphae 3–10 µm, the broader the more constricted at the septa, hyaline or with diffuse or vacuolar pale brown pigment. **Clamp connections** not observed.

Habit, habitat, occurrence, and distribution: Gregarious, in groups of few basidiomata, on leaf litter. Rare. Recorded only once from Puerto Rico.

Species-specific ITS markers in A. sect. Rarolentes: ttatgAAtctag@11+12; rcctg[T]tytrga@57; ctgga[t]Cgtgag@151; cyyrcTrgagt@215.

Material examined: Puerto Rico, Río Grande, El Yunque National Forest, La Mina Recreation area, Mt. Britton trail, 23 May 2000, S.A. Cantrell and M. Salgado, **PR6173**, **Holotype** (**CFMR**).

Taxonomic comments: Morphologically *A. parvisporus* can be easily distinguished from the remaining species of *A.* sect. *Rarolentes* by its much smaller spores, 4.87 × 2.74 μm on average. The spore size of *A. parvisporus* is a crucial character to distinguish it from other species of the genus *Agaricus*. We have only found 10 taxa originally described with spores less than 3 μm wide on average in the world mycological literature. From them, the reexamination of spores of the types of *A. angustifolius* Murrill, *A. microvolvatulus* Heinem., *A. phaeocyclus* Pat., and *A. placomyces* var. *microsporus* A.H. Smith, by Pegler (1983: 443), Thongklang et al. (2014: 1229), Yang (2000: 435) and Freeman (1979) respectively, has shown that the spores of the first two were 3.3 μm wide on average, those of *A. phaeocyclus* measured (4.5)5–6.5(7.5) × 3–4(4.5) μm, and those of *A. placomyces* var. *microsporus* 4.5–6 × 3–3.8 μm. *Agaricus daliensis* H.Y. His & R.L. Zhao and *A. horakianus* Callac & L.A. Parra (a new name for *Agaricus magnivelaris* Pegler) have been molecularly characterized by Zhou et al. (2016: 111) and Mahdizadeh et al. (2018: 40) respectively, belonging to *A.* subg. *Pseudochitonia*. "*Agaricus cupreobrunneus* var. *microsporus*" M. Kaur & B. Kaur (an invalidly published name, as no type was indicated according Art. 40.6), *A. dennisii* Heinem., *A. lateriticolor* Heinem. and *A. pulverotectus* Heinem. also have spores measuring less than 3 μm wide on average, but morphologically all four are very different from *A. parvisporus* and their spores are either shorter, not reaching the mean length of *A. parvisporus* in the first three, or wider, exceeding the mean width of *A. parvisporus* in the latter. Finally, *A. omphalodiscus* described in this paper also has spores less than 3 μm wide on average but it belongs to *A.* sect. *Subrutilescentes*.

Agaricus sect. ***Subrutilescentes*** Kerrigan, Mem. New York Bot. Garden 114: 333. 2016.

MycoBank: MB 802555

Type: *Agaricus subrutilescens* (Kauffman) Hobson & Stuntz, Mycologia 30: 219. 1938.

Etymology: Refers to the type species *Agaricus subrutilescens*.

Morphological description: Robust to gracile, medium to large-sized basidiomata. Pileus (1.5)3.5–20 cm diam., covered by reddish brown or brown appressed scales with a darker entire center. Lamellae discoloring rose to red when bruised. Stipe with an annulus on its upper third and sometimes with rhizomorphs or mycelial strands at the base, fibrillose to scaly below the annulus, seldom glabrous. Annulus superous, broad, pendant, thick (sometimes thin), squamulose in its lower surface. Context unchanging or weakly reddening mainly at the upper stipe cortex. Odor usually strong, variable (described as fungoid, woody, fruity spicy, unpleasant or similar to *Scleroderma* sp. or *Lepiota cristata*), rarely pleasant, but neither of anise or almonds nor of phenol or iodine (see note below). Schäffer's reaction negative and KOH reaction negative or positive weakly yellow or pale olive green on pileus surface. Cheilocystidia present, usually pyriform, globose or sphaeropedunculate. Spores 4.9–6 × 2.9–4.2 μm on average, ellipsoid to elongate-ellipsoid, lacking a rudimentary apical pore. Hyphae of the lower surface of the annulus devoid of inflated elements. Pileipellis composed of hyphae containing diffuse brown pigment.

Known distribution: This section is distributed in Asia (China and Thailand), the Caribbean (Martinique and Puerto Rico), Europe (widespread all over Europe), and North America (the USA).

Edibility: According to Kerrigan (2016) no species are known to be poisonous. He also comments that Smith (1949) reported that "the broiled buttons [of *A. subrutilescens*] are a delicacy" but later this author (Smith 1975) also cautioned that "some people (myself included) cannot tolerate it". Bon (2005) reported *A. variegans* (considered as synonymous with *A. impudicus* by some authors) as mediocre edible.

Taxonomic comments: Catenulate cheilocystidia and odor of phenol were described in *A. catenariocystidiosus* R.C. Dai & R.L. Zhao (Dai et al. 2016, invalidly published name) but in a recent microscopic examination of the type material by Ling Z.L. (pers. comm., 22-March-2020) of this species, only simple cheilocystidia characteristic of this section were observed, and the unusual odor of phenol must be confirmed in further fresh collections.

Stem age and phylogenetic support: Stem age 23.96 Ma and PP = 1 in MCC tree (Fig. 3); strong support MS/PP = 96/1 in multi-gene tree (Fig. 2), and not monophyletic in ITS tree (Fig. 1).

Section-specific ITS markers in A. subg. Spissicaules: None. However, there is one clade-specific marker for the clade including both *A.* sect. *Subrutilescentes* and the single sample of '*A.* sect. A' in ITS tree (Fig. 1): gggyaRygrkkra@121.

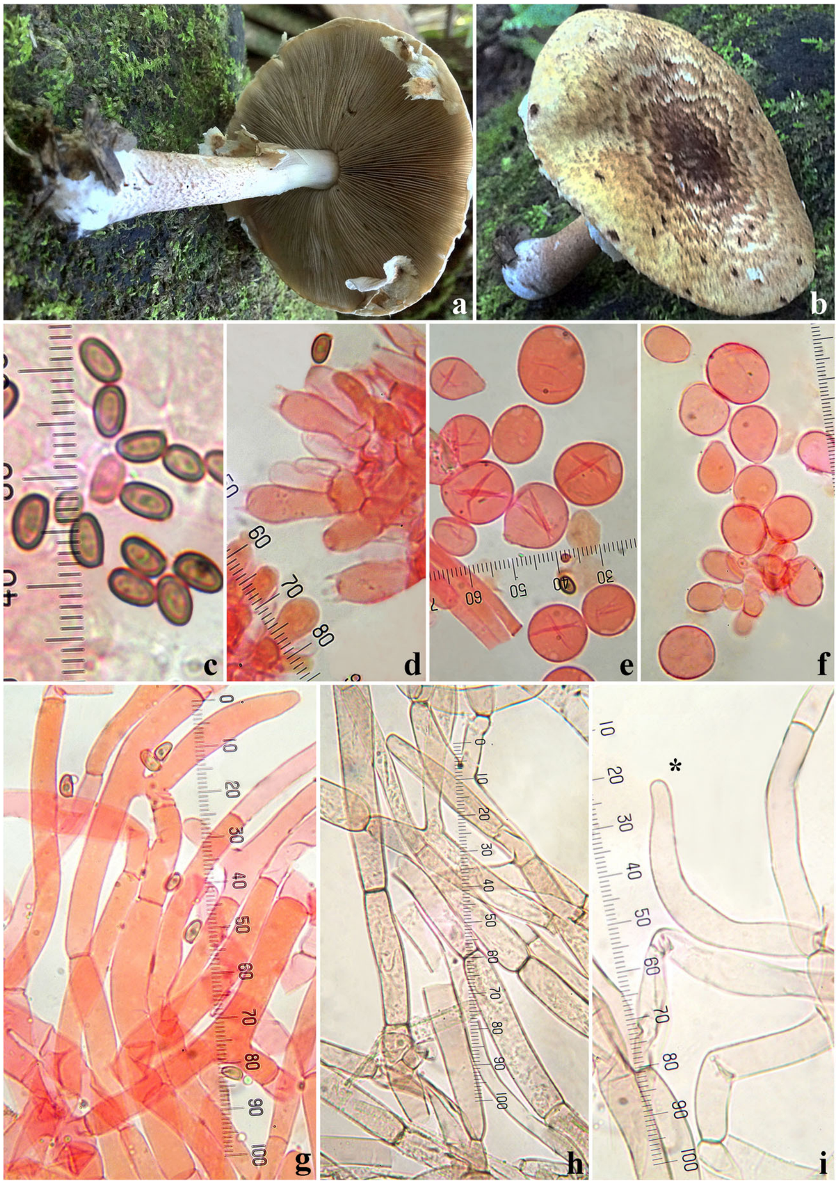
Agaricus ciferrianus L.A. Parra, B. Ortiz & Lodge, **sp. nov.** Fig. 11

MycoBank: MB 837361

Etymology: The specific epithet honors Raffaele Ciferri for his contribution to the mycological knowledge of the Caribbean.

Macroscopic description: **Pileus** 4.5–7 cm diam., at first parabolic then plane or moderately depressed at the center, covered by brown or grayish brown appressed squamules on a grayish yellow to grayish orange background, and a reddish brown to dark brown entire center. Surface fibrillose, dull and dry. Margin smooth, not or slightly exceeding the lamellae,

Fig. 11 Macroscopic characters of *Agaricus ciferrianus* (PR6822, Holotype). **a**–**b** Basidiomata. Microscopic characters of *Agaricus ciferrianus* (PR6822, Holotype). **c**–**i** (**c**–**g** in ammoniacal Congo red; **h**–**i** in ammonia). **c** Spores. **d** Basidia. **e**–**f** Cheilocystidia. **g** Hyphae of the lower surface of the annulus. **h**–**i** Pileipellis hyphae (terminal element marked with an asterisk). Photos by D.J. Lodge (**a**–**b**) and L.A. Parra (**c**–**i**)



appendiculate with large pieces of the annulus. **Lamellae** free, up to 0.4 cm broad, crowded, straight, intercalated with lamellulae, at first white, then grayish pink, later grayish brown, finally dark brown with entire concolorous edge. **Stipe** 6.5–6.8 × 0.5–1.5 cm, cylindrical, bulbous at the base (up to 2 cm), fistulose above, stuffed below, with an annulus on its upper third and a white mycelial pad with a thin rhizomorph at the base; above annulus white or grayish orange and smooth, below annulus appressed squamulose pale reddish brown to grayish brown, unchanging on handling. **Annulus** superous, about 1 cm broad, membranous, double, upper surface white to cream, smooth, lower surface with large brown scales towards the margin. The annulus is mostly appendiculate on pileus margin. **Context** unchanging, brown around the worm holes. Odor fungoid or unpleasant.

Macrochemical reactions: Schäffer's and KOH reactions negative on dried basidiomata.

Microscopic description: **Spores** 4.3–5–5.5(5.7) × 2.9–3.1–3.4 µm, Q=1.41–1.61–1.83, ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, without apical pore. **Basidia** 12–21 × 7–9 µm, tetrasporic, clavate or slightly truncated at the apex, hyaline, sterigmata up to 3 µm long. **Cheilocystidia** 9–22 × 8–20 µm, abundant, usually simple, hyaline, ovoid, pyriform, globose or sphaeropedunculate, some septate at the base. **Pleurocystidia** absent. **Lower surface of the annulus** composed of hyaline hyphae, cylindrical, not or slightly narrowed at septa, 3–12 µm wide, without inflated elements. **Pileipellis** a cutis, composed by cylindrical hyphae 3–13 µm wide, not or slightly constricted at the septa with abundant terminal elements cylindrical or progressively attenuated with rounded apex. In water, hyphae contain abundant granular pale grayish brown pigment. **Clamp connections** not observed.

Habit, habitat, occurrence, and distribution: Solitary, on humus from rotten trees or on soil under bamboo. Rare. Recorded only twice from Puerto Rico.

Species-specific ITS markers in A. sect. Subrutilescentes: ttgaaACatgtt@6-7; rygrkCragyg@126; trtcGg[ca]camyyg@152; tatg[t]tCwAytcat@263-255; tgcyggTy[rctcg]cyyr@550; ytgttTrgcty@674; ysGray[a]aac[tttt]ttyAtgaaygc@701-716. The latter is the 3' end of the alignment.

Material examined: Puerto Rico, Río Grande, El Yunque National Forest, La Mina Recreation Area, Big Tree Trail, under bamboo, 19 Jan 2007, D.J. Lodge, PR6585 (CFMR); El Yunque National Forest, El Verde Field Station, on humus from rotten tree, 15 Jan 2016, D.J. Lodge, **PR6822**, **Holotype** (**CFMR**).

Taxonomic comments: Within *A.* sect. *Subrutilescentes*, *A. ciferrianus* is characterized by its medium-sized basidiomata, and especially the scaly pileus surface on a yellow to orange background, which is white in the remaining scaly species in the section. The most similar species are *A. subrutilescens* (Kauffman) Hotson & D.E. Stuntz, *A. parasubrutilescens* Callac & R.L. Zhao, *A. brunneopileatus* Callac & R.L. Zhao and "*A. catenariocystidiosus*", but all of them differ in having a slender habit and much longer stipes (from 6.8–9.9 cm in "*A. catenariocystidiosus*" to 11–15 cm in *A. brunneopileatus*). In addition, *A. brunneopileatus* differs in having longer spores (4.4–5.8 μm) and narrower cheilocystidia (7–13 μm), "*A. catenariocystidiosus*" has larger spores, especially in width (5–6 × 3–4 μm), *A. parasubrutilescens* possesses a thinner annulus and a context discoloring slightly orange, and *A. subrutilescens* is distinguished by its slightly larger spores (4.5–6 × 3–4.1 μm) and sparse, smaller cheilocystidia (10–17 × 8–12 μm).

Among the taxa from which no molecular characters are available, *A. pseudoargentinus* Albertó & J.E. Wright, according to its original description (Albertó and Wright 1994), is most likely a member of *A.* sect. *Subrutilescentes*, but it differs from *A. ciferrianus* in having a fibrillose to fibrillose-squamulose pileus that is brown without reddish tones, as well as having spores that are larger, 4.7–5.7(6.5) × (3.2)3.7–4.2 μm, with a much lower (1.25–1.45) Q coefficient.

Agaricus omphalodiscus L.A. Parra, Fiard, Callac & B. Ortiz, **sp. nov**. Fig. 12

MycoBank: MB 837362

Etymology: Refers to the pileus with a broad central umbo with an internal depression looking like a navel. From the latinized Greek *omphalos* (navel) and the latinized *discus* from the Greek *discos* (disk, referred to the central part of the pileus).

Macroscopic description: **Pileus** 4–5 cm diam., at first subhemispherical more or less truncated at the apex then plane and broadly umbonate with umbo depressed at center (umbilicate), initially covered by buff to hazelnut appressed fibrils darkening towards the entire center and less dense towards the margin, then obscurely squamulose on a concolorous radially fibrillose background. Surface dull and dry. Margin thin, smooth, not or slightly exceeding the lamellae. **Lamellae** free, up to 0.3 cm broad, crowded, straight, intercalated with lamellulae, at first white, turning remarkably pink when crushed, then dark brown with entire concolorous edge. **Stipe** 4–7 × 0.3–0.35 cm, cylindrical or clavate, fistulose, white becoming grayish with time, with an annulus on its upper third; above annulus smooth, below annulus slightly squamulose when young then glabrous and slightly shiny, unchanging when scratched. **Annulus** superous, about 0.3 cm broad, fibrillose, simple, white, extremely thin, cortiniform, often reduced to some fibrillose remnants at maturity. **Context** unchanging when cut, turning only pink above the insertion of the lamellae. Odor extremely pleasant.

Macrochemical reactions: Schäffer's and KOH reactions negative on dried basidiomata.

Microscopic description: **Spores** (4.5)4.6–5–5.3 × 2.7–2.9–3.1 μm, Q=1.61–1.72–1.85, elongate-ellipsoid, smooth, brown, thick-walled, without apical pore. **Basidia** 13–16 × 6–7 μm, tetrasporic, clavate or slightly truncated at the apex, hyaline or with internal dark brown pigment; some cupuliform (collapsed inwards), sterigmata up to 3 μm long. **Cheilocystidia** 8–25 × 7–15 μm, abundant, usually simple, hyaline, ovoid, pyriform, globose or sphaeropedunculate, some septate at the base. **Pleurocystidia** absent. **Lower surface of the annulus** composed of hyaline hyphae, 3–12 μm wide, cylindrical, constituted by short elements, very constricted at septa and easily disarticulated, without inflated elements. **Pileipellis** a cutis, composed by cylindrical hyphae 2–11 μm, not or slightly constricted at the septa with abundant terminal elements cylindrical or progressively attenuated with rounded apex. In water, hyphae contain abundant granular yellowish brown pigment. **Clamp connections** not observed.

Habit, habitat occurrence, and distribution: In groups, on ground with decomposed branches in degraded xero-mesophilic forest. Rare. Recorded only once from Martinique.

Species-specific ITS markers in A. sect. Subrutilescentes: ttcat[C]cttca@69; rtcrg[CA]camyy@135-136; srray[A]aac[TTTT]ttAytgaaCgc@647+651-654+657+663 (3' end). There are many other markers, only DNA segments with indel are listed.

Material examined: France, Martinique, Pointe Banane forest, 2 Apr 2000, J.-P. Fiard, **F2255**, **Holotype** (**LIP**).

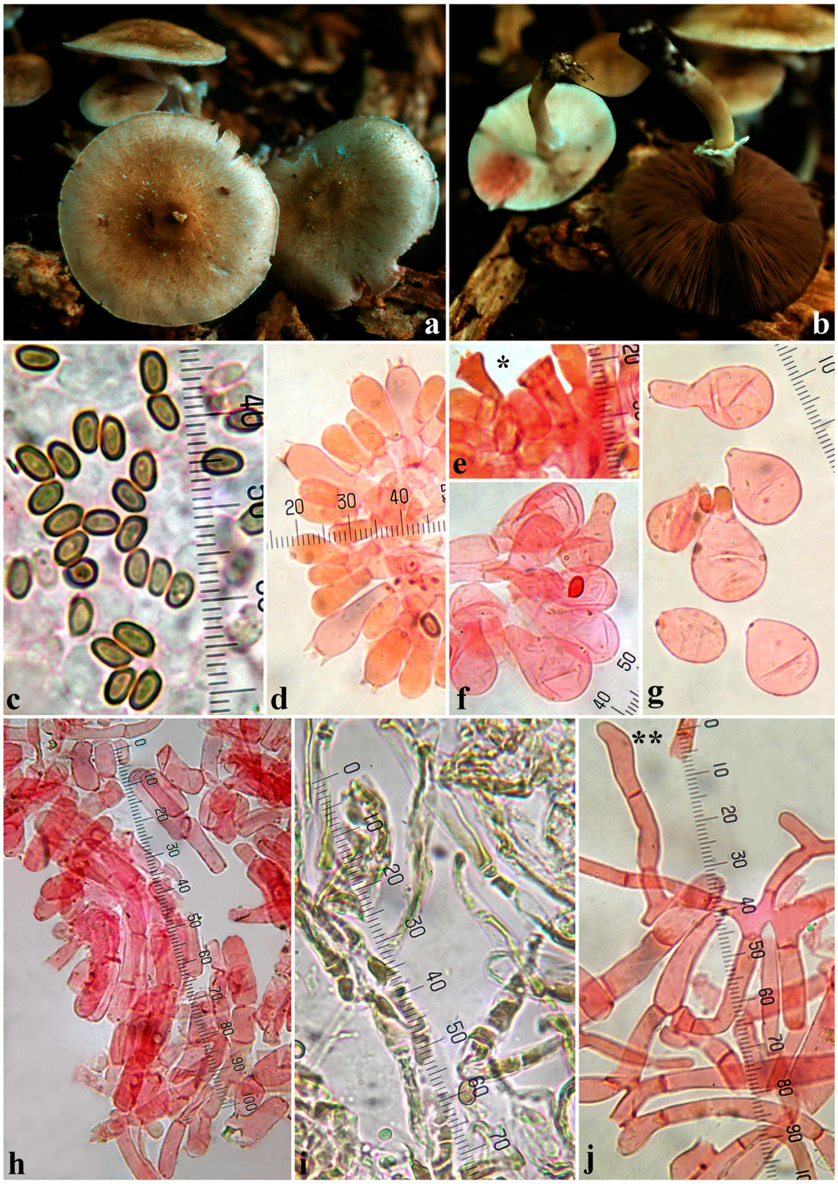


Fig. 12 Macroscopic characters of *Agaricus omphalodiscus* (F2255, Holotype). **a**–**b** Basidiomata. Microscopic characters of *Agaricus omphalodiscus* (F2255, Holotype). **c**–**j** (**c**–**h**, **j** in ammoniacal Congo red; **i** in water). **c** Spores. **d** Typical basidia. **e** Cupuliform basidia (two marked with an asterisk). **f**–**g** Cheilocystidia. **h** Hyphae of the lower surface of the annulus. **i**–**j** Pileipellis hyphae (terminal element marked with two asterisk). Photos by J.-P. Fiard (**a**–**b**) and L.A. Parra (**c**–**j**)

Taxonomic comments: *Agaricus omphalodiscus* is characterized within *A.* sect. *Subrutilescentes* by its small-sized basidiomata, slender habit, umbilicate covered by buff to hazelnut appressed fibrils pileus surface, and the smallest spore.

Among the taxa from which no molecular characters are available, *A. diamantanus* Pegler, *A. silvipluvialis* Pegler and *A. trinitatensis* Baker & Dale are the most similar taxa. *Agaricus diamantanus* differs in having a context discoloring reddish brown, clavate cheilocystidia and wider spores (3.5–4.2 μm) with a much lower (1.39) Q coefficient. *Agaricus silvipluvialis* possesses a grayish brown pileus and lacks cheilocystidia. *Agaricus trinitatensis* has a more robust habit, slightly yellowing context and much bigger spores (5.5–7.5 × 3.5–4 μm).

Discussion

Phylogenetic refinement of *A.* subg. *Spissicaules*

Using BEAST with the same calibration and outgroup as in Zhao et al. (2016) but with a wider and more phylogenetically diversified sample, we found seven major clades with stem ages around 24 Ma, while Zhao et al. (2016) found only four of these clades with stem ages between 19 and 22 Ma. The stem age of *A.* subg. *Spissicaules* is also earlier in our analysis (35 vs. 33 Ma). These earlier divergences times could be due to some differences in the sampling outside *A.* subg. *Spissicaules* such as the addition of representatives of four sections described since 2016, or the deletion of the sample of *A. dilutibrunneus* after noting that its data set included incompatible sequence data. In agreement with the new system of classification, the three new clades were ranked as sections but only two were named and described (*A.* sect. *Fulventes* and *A.* sect. *Globoterminales*), while the third section remains provisionally named "*A.* sect. A" because it includes a single specimen that is not in good condition. In both recent and previous studies, *A.* subg. *Spissicaules* and all its sections except "*A.* sect. A," which includes a single sample, were statistically well supported in both MCC and multigene trees. However, the phylogenetic relationships between the sections were generally poorly supported due to their relatively rapid radiation. There is a notable exception for the clade "Amoeni" which appears closely related to "Rarolentes" (PP=0.99) in our tree, while it was closely related to "Subrutilescentes" (PP=1) in the previous tree. Prudently, we do not emphasize any relationships between the sections, and since the Oligocene-Miocene transition occurred about 23 Ma ago, we conclude that, according to present and previous analyses, a relatively rapid radiation of the sections within *A.* subg. *Spissicaules* occurred in the late Oligocene or in the early Miocene.

Resolving pending issues

The new section *A.* sect. *Fulventes* resolved a pending issue since 2011 (Zhao et al. 2011, 2016). Indeed, both samples CA486 of *A. bellanniae* and ADK2171 (*A.* sp.) which previously formed an unclassified clade are now in *A.* sect. *Fulventes* thanks to new sequence data of CA486 and the new specimen AH47621 from Argentina, which is the type of the new species *A. nanofulvens* being itself the type of *A.* sect. *Fulventes*.

The only samples previously known from the Caribbean region and placed in *A.* subg. *Spissicaules* (Zhao et al. 2011, 2016) were three specimens from Martinique, but they belonged to unknown species. This pending issue is resolved for two of them, thanks to new sequence data, specimen reexamination, and notes of their collector J.-P. Fiard. Specimens F2301 and F2255 now belong to the two newly described species *A. furfuripes* with type specimen from Dominican Republic and *A. omphalodiscus* as type specimen, respectively. Interestingly, *A. furfuripes* and *A. omphalodiscus* are sister to the new species from Puerto Rico *A. microincrustatus* in *A.* sect. *Rarolentes* and *A.* sp. 1 in *A.* sect. *Subrutilescentes*, respectively. This suggests that species diversification has occurred in the Caribbean region but at different periods for these two pairs of species according to the MCC tree. The third specimen F2467 remains unnamed in *A.* sect. *Amoeni*.

Species richness and endemism in the Caribbean region

In this study, twenty five samples from the Caribbean were studied molecularly and another (F2467) was included in the analyses. In total, 26 samples from the Caribbean were included in the analyses, 13 from Dominican Republic, ten from Puerto Rico, and three from Martinique. These 26 samples belong to ten new species of which seven were named and described in detail in the present study, while three remained unnamed (*A.* sp. 1, *A.* sp. 2, and *A.* sp./F2467). Presently, all 26 specimens of *A.* subg. *Spissicaules* found in the Caribbean belong to ten species only known from the Caribbean. Only one species was found on two islands (*A. furfuripes* on both Martinique and Hispaniola - Dominican Republic). The number of different species found in the Dominican Republic, Martinique, and Puerto Rico is four, three, and four, respectively. These data indicate a high endemism in the Caribbean region and possibly at the island level. This also reveals a high species richness that was previously ignored. It is likely that many additional unknown species are distributed among the Caribbean islands. The ten species of *A.* subg. *Spissicaules* found in the Caribbean to date represent 21% (10/48) of all species of the subgenus with available sequence data, of which all were included in our analyses.

A subgenus difficult to circumscribe

A. subg. *Spissicaules* remained ignored in the Caribbean until recently. This can be due to the rarity of certain species, the lack of mycologists and of knowledge on this subgenus, and the difficulty to recognize it. Unfortunately, as phylogenetic knowledge progresses by the inclusion of new sections and species, it becomes more difficult to morphologically circumscribe this subgenus. Indeed, the odor can be pleasant or unpleasant even among collections of the same species. The stipe can exhibit yellow, reddish, or both types of discoloration by bruising and it can be smooth or not below the annulus, which can be simple or double. Microscopically, cheilocystidia are very variable from basidioliform to globose and, in one species, with microincrustations. Inflated elements on the lower side of the annulus can be absent or present like in *A.* subg. *Arvenses*. The only character shared by the species of *A.* subg. *Spissicaules* are the presence of rhizomorphs or mycelial strands at the stipe base and the usually negative or weak Schäffer reaction on the pileus surface. Phylogenetically, in the MCC tree, *A.* subg. *Spissicaules* is a well-supported clade, which is sister to a clade including subgenera *Minoriopsis*, *Minores*, and *Flavoagaricus*.

Phylogenetic diversity in the Caribbean region

The ten species of *A.* sect. *Spissicaules* from the Caribbean are distributed in five different sections as follows. The species A. sp./F2467 that was not studied in detail belongs to the pantropical *A.* sect. *Amoeni*. *Agaricus basicingulatus* and *A.* sp.2 are the single species of the monotypic *A.* sect. *globoterminales* and "*A.* sect. A," respectively. Five species are in *A.* sect. *Rarolentes* and the remaining three are in *A.* sect. *Subrutilescentes*. Interestingly, in the MCC tree (Fig. 3), both sections have similar stem ages around 24 Ma and similar crown ages around 18 Ma (estimations of 17.89 Ma and 18.13 Ma, respectively). In addition, in both sections, one of the two basal clades exclusively or almost exclusively includes species from the Caribbean. Indeed, in *A.* sect. *Rarolentes*, one basal clade includes five species of which four are from the Caribbean (*A. baronii*, *A. furfuripes*, *A. microincrustatus*, and *A. parvisporus*), while the other basal clade includes two species from Thailand. In *A.* sect. *Subrutilescentes*, one basal clade includes two species from the Caribbean (*A. omphalodiscus* and *A.* sp. 1), while the other basal clade includes 18 species of which only one is from the Caribbean (*A. ciferrianus*). The presence of a major Caribbean clade in these two sections suggest that they could have a Caribbean origin, while their relatively similar evolutionary history might reflect an adaptive convergence. The distribution of the ten species from the Caribbean in five sections represents a high level of phylogenetic diversity. It is noteworthy that the two remaining sections *A.* sect. *Fulventes* and *A.* sect. *Spissicaules* do not include species from the Caribbean but include two neotropical species from Argentina (*A. nanofulvens*) and French Guiana (*A.* sp./RCGUY07019), respectively. Therefore, neotropical species are present in all sections.

Climate as a major factor of diversification, and future prospects

Among species with available sequence data, the percentage of tropical or subtropical species now reaches 71% (34/48) in *A.* subg. *Spissicaules*. Most of temperate species (12/14) in *A.* subg. *Spissicaules* belong to *A.* sect. *Subrutilescentes* or *A.* sect. *Spissicaules*. In the latter, tropical and subtropical species form a distinct "tropical" clade, while the temperate species form a paraphyletic group. These data show that the investigation in tropical areas has deeply modified the concept of *A.* sect. *Spissicaules* since the initial proposal of *A.* subsect. *Spissicaules*, 52 years ago, for a group of seven mostly temperate species (Heinemann 1978). The Caribbean data contributed greatly to the knowledge of the subgenus, but they also suggest that the diversity of the subgenus should be investigated in other tropical areas of Africa and South America from which sequence data are available from only a few specimens. Otherwise, *A.* subg. *Spissicaules* could be a good model to compare the evolutionary history of several sections having similar stem ages. For this purpose, collections from more Caribbean islands will be required.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-021-01686-9.

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Availability of data and materials The datasets generated during and/or analyzed during the current study were submitted to TreeBase (Submission ID: 26968). Sequence data that support the findings of this study have been deposited in GenBank. All data generated or analyzed during this study are included in this published article. All specimens described in this study were deposited in institutional herbaria.

Author contribution All authors contributed to the study conception and design. Collecting of specimens was mainly done by Claudio Angelini, Beatriz Ortiz-Santana, and D. Jean Lodge. DNA sequences were generated by Beatriz Ortiz-Santana, Luis A. Parra, and Philippe Callac. Phylogenetic analyses were performed by Beatriz Ortiz-Santana, Jie Chen, and Philippe Callac. Morphological taxonomy was performed by Luis A. Parra and Beatriz Ortiz-Santana. The first draft of the manuscript was written by Beatriz Ortiz-Santana, Jie Chen, Luis A Parra, and Philippe Callac. The review and editing of the manuscript were performed by all the authors. All authors read and approved the final manuscript.

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Declarations

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References

Albertó E, Wright JE (1994) *Agaricus pseudoargentinus* n. sp. from Argentina. Mycotaxon 5:271–278

Ariyawansa HA, Hyde KD, Jayasiri SC et al (2015) Fungal diversity notes 111–252—taxonomic and phylogenetic contributions to fungal taxa. Fungal Divers 75:27–274

Baker RED, Dale WT (1951) Fungi of Trinidad and Tobago. Myc Pap 33:1–123

Berkeley MJ, Curtis MA (1869) Fungi cubenses (Hymenomycetes). J Linn Soc Bot 10:280–341

Bon M (2005) Guía de campo de los hongos de España y Europa. Ed. Omega, Barcelona

Callac P, Chen J (2018) Tropical species of *Agaricus*. In: Sánchez JE, Mata G, Royse DJ (eds) Updates on tropical mushrooms. Basic and applied research. San Cristobal de Las Casas, Chiapas, pp 25–38

Chen J, Callac P, Parra LA, Karunarathna SC, He M-Q, Moinard M, De Kesel A, Raspé O, Wisitrassameewong K, Hyde KD, Zhao R-L (2017) Study in *Agaricus* subgenus *Minores* and allied clades reveals a new American subgenus and contrasting phylogenetic patterns in Europe and Greater Mekong Subregion. Persoonia 38:170–196

Chen J, Callac P, Llarena RC, Mata G (2019) Two species of *Agaricus* subg. *Minoriopsis* from Mexico. Phytotaxa 404:091–101

Cubeta MA, Echandi E, Abernethy T, Vilgalys R (1991) Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. Mol Plant Pathol 81:1395–1400

Dai RC, Li GJ, He MQ, Liu RL, Ling ZL, Wu JR, Zhao R-L (2016) Characterization of four species including one new species of *Agaricus* subgenus *Spissicaules* from Eastern China. Mycosphere 7:405–416

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772

Didukh M, Vilgalys R, Wasser SP, Isikhuemhen OS, Nevo E (2005) Notes on *Agaricus* section *Duploannulati* using molecular and morphological data. Mycol Res 109:729–740

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and BEAST 1.7. Mol Biol Evol 29:1969–1973

Edwards IP, Cripliver JL, Gillespie AR, Johnsen KH, Scholler JM, Turco RF (2004) Nitrogen availability alters macrofungal basidiomycete community structure in optimally fertilized loblolly pine forests. New Phytol 162:755–770

Foulongne-Oriol M, Spataro C, Moinard M, Cabannes D, Callac P, Savoie JM (2012) Development of polymorphic microsatellite markers issued from pyrosequencing technology for the medicinal mushroom *Agaricus subrufescens*. FEMS Microbiol Lett 334:119–126

Freeman AEH (1979) *Agaricus* in North America: type studies. Mycotaxon 8:1–49

Fries EM (1851) Novae symbolae mycologicae, in peregrinis terreis a botanicis danicis collectae. Nova Acta Regiae Soc Sci Upsal, seriei 3(1):17–126

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Guinberteau J (2017) Sur la découverte d'un nouvel agaric amphi-atlantique de la section *Spissicaules* (Heinem.) Kerrigan: *Agaricus bellanniae* Guinb., Kerrigan & M. Kuo

Hall T (2007) BioEdit v7. Available from: http://www.mbio.ncsu.edu/BioEdit/BioEdit.html. Accessed on 19 Aug. 2014

Harris NL, Lugo AE, Brown S, Heartsill-Scalley T (2012) Luquillo experimental forest: research history and opportunities. Experimental Forest and Range EFR-1. US Department of Agriculture, Washington, DC

He MQ, Chuankid B, Hyde KD, Cheewangkoon R, Zhao R-L (2018) A new section and species of *Agaricus* subgenus *Pseudochitonia* from Thailand. MycoKeys 40:53–67

Heinemann P (1956) Champignons récoltés au Congo Belge par Mme M Goossens-Fontana, II *Agaricus* Fr ss. Bull Jard Bot État Bruxelles 26:1–127

Heinemann P (1961) Agarici Austro-Americani: I. *Agaricus* of Trinidad. Kew Bull 15:231–249

Heinemann P (1962) Agarici Austro-Americani: III. Trois *Agaricus* de la Jamaique. Bull Jard Bot État Bruxelles 32:23–28

Heinemann P (1978) ["1977"] Essai d'une clé de détermination des genres *Agaricus* et *Micropsalliota*. Sydowia 30:6–37

Heinemann P, Rammeloo J (1985) De la mesure des spores et son expression. Agarica 6:366–380

Holdridge LR (1967) Life zone ecology. Tropical Science Center, San José

Holmgren PK, Holmgren NH (1998) [continuously updated] Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available from: http://sweetgum.nybg.org/ih/

Hussain S, Sher H (2019) Study in *Agaricus* section *Minores* in Pakistan with the description of two new species. Mycol Prog 18:795–204

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780

Kerrigan RW (1985) Studies in *Agaricus* II. *Agaricus lilaceps* re-evaluated. Mycologia 77:137–141

Kerrigan RW (2016) *Agaricus* of North America. Mem N Bot Gard 39:1–574

Kerrigan RW, Callac P, Guinberteau J, Challen MP, Parra LA (2005) *Agaricus* section *Xanthodermatei:* a phylogenetic reconstruction with commentary on taxa. Mycologia 97:1292–1315

Lebel T, Syme A (2012) Sequestrate species of *Agaricus* and *Macrolepiota* from Australia: new species and combinations and their position in a calibrated phylogeny. Mycologia 104:496–520

Liu JK, Hyde KD, Jones EBG, Ariyawansa HA et al (2015) Fungal Diversity notes 1–110: taxonomic and phylogenetic contributions to fungal species. Fungal Divers 72:1–197

Liu A-Q, Dai R-C, Zhang M-Z, Cao B, Xi Y-L, Wei S-L, Zhao R-L (2020) Species of *Agaricus* section *Agaricus* from China. Phytotaxa 452:1–18

Mahdizadeh V, Parra LA, Safaie N, Mohammadi-Goltapeh E, Chen J, Guinberteau J, Callac P (2018) A phylogenetic and morphological overview of sections *Bohusia*, *Sanguinolenti*, and allied sections within *Agaricus* subg. *Pseudochitonia* with three new species from France, Iran, and Portugal. Fungal Biol 122:34–51

Mariselvi M, Earanna N (2018) Molecular identification and screening of mushrooms for antibacterial property against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. J Appl Nat Sci 10:791–796

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Gateway Computing Environments Workshop (GCE), 2010. IEEE:1–8

Murrill WA (1918) The Agaricaceae of tropical North America VIII. Mycologia 10:62–85

Nylander JAA (2004) MrModeltest 2.2 Program distributed by the author. Evolutionary Biology Centre, Uppsala University

Palmer JM, Lindner DL, Volk TJ (2008) Ectomycorrhizal characterization of an American chestnut (*Castanea* dentate)-dominated community in western Wisconsin. Mycorrhiza 19:27–36

Parra LA (2008) *Agaricus* L., *Allopsalliota* Nauta & Bas. Fungi Europaei 1. Edizioni Candusso, Alassio, Italy

Parra LA (2013) *Agaricus* L., *Allopsalliota* Nauta & Bas. Fungi Europaei 1A. Candusso Edizioni s.a.s., Alassio

Parra LA, Tan Y, Xu ML, Zhou JL, Wang B, Zhao R-L (2016) A reexamination of *Allopsalliota* indicates synonymy with *Micropsalliota* (Agariceae, Agaricaceae, Agaricales, Basidiomycota). Mycoscience 57:303–310

Parra LA, Angelini C, Ortiz-Santana B, Mata G, Billette C, Rojo C, Chen J, Callac P (2018) The genus *Agaricus* in the Caribbean. Nine new taxa mostly based on collections from the Dominican Republic. Phytotaxa 345:219–271

Parra LA, Cappelli A, Kerrigan RW, Bizio E (2019) [2018] *Agaricus porphyrocephalus* subsp. *alpinus* a new subspecies collected in the Italian Alps. Micol Veg Medit 33:67–88

Peel MC, Finlayson BL, McMahon TA (2007) Updated world map of the Köppen-Geiger climate classification. Hydrol Earth Syst Sci Discuss 11:1633–1644

Pegler DN (1983) Agaric flora of the Lesser Antilles. Kew Bull Addit Ser 9:1–667

Pegler DN (1987) A revision of the Agaricales of Cuba 2. Species described by Earle and Murrill. Kew Bull 42:855–888

Phookamsak R, Hyde KD, Jeewon R et al (2019) Fungal diversity notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungi. Fungal Divers 95:1–273

Rambaut A, Suchard MA, Xie D, Drummond A (2014) Tracer v1.6. Available from http:// beast.bio.ed.ac.uk/Tracer. Accessed on 24 July 2015

Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97:84–98

Ronquist F, Huelsenbeck JP (2003) MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Sánchez L, Parra LA (2019) Làmina 1851. In: Societat Catalana de Micologia Eds. Bolets de Catalunya de la Península Ibèrica i de les Illes Balears 38 Colleció. Barcelona

Smith AH (1940) Studies in the genus *Agaricus*. Pap Mich Acad Sci 25: 107–138

Smith AH (1949) Mushrooms in their natural habitat. Sawyer, Portland

Smith AH (1975) A field guide to western mushrooms. University of Michigan Press, Ann Arbor

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313

Tarafder E, Dutta AK, Sarkar J, Acharya K (2018) A new species of *Agaricus* sect. *Brunneopicti* from Eastern India. Phytotaxa 374: 139–146

Thongklang N, Nawaz R, Khalid AN, Chen J, Hyde KD, Ruilin Z, Parra LA, Hanif M, Moinard M, Callac P (2014) Morphological and molecular characterization of three *Agaricus* species from tropical Asia (Pakistan, Thailand) reveals a new group in section *Xanthodermatei*. Mycologia 106:1220–1232

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246

Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol 92:135–154

Wang ZR, Parra LA, Callac P, Zhou JL, Fu WJ, Dui SH, Hyde KD, Zhao R-L (2015) Edible species of *Agaricus* (*Agaricaceae*) from Xinjiang Province (Western China). Phytotaxa 202:185–197

White TJ, Bruns T, Lee SS, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322 p. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York

Yang Z-L (2000) Type studies on agarics described by N. Patouillard (and his coauthors) from Vietnam. Mycotaxon 75:431–476

Yorou SN, De Kesel A (2001) Connaissances ethnomycologiques des peuples Nagot du centre du Bénin (Afrique de l'Ouest). Syst Geogr Plants 71:627–637

Zhao R-L, Karunarathna SC, Raspé O, Parra LA, Guinberteau J, Moinard M, De Kesel A, Barroso G, Courtecuisse R, Hyde KD, Guelly AK, Desjardin ED, Callac P (2011) Major clades in tropical *Agaricus*. Fungal Divers 51:279–296

Zhao R-L, Hyde KD, Desjardin DE, Raspé O, Soytong K, Guinberteau J, Karunarathna SC, Callac P (2012) *Agaricus flocculosipes* sp. nov., a new potentially cultivatable species from the tropics. Mycoscience 53:300–311

Zhao R-L, Zhou J-L, Chen J, Margaritescu S, Sánchez-Ramírez S, Hyde KD, Callac P, Parra LA, Li G-J, Moncalvo JM (2016) Towards standardizing taxonomic ranks using divergence times – a case study for reconstruction of the *Agaricus* taxonomic system. Fungal Divers 78:239–292

Zheng J, Li J, Song Y, Wang G, Qiu L (2019) *Agaricus rubripes* sp. nov., a new species from Southern China. Nova Hedwigia 109:233–246

Zhou J-L, Su A-Y, Su H-Y, Wang B, Callac P, Guinberteau J, Hyde KD, Zhao R-L (2016) A description of eleven new species of *Agaricus* sections *Xanthodermatei* and *Hondenses* collected from Tibet and the surrounding areas. Phytotaxa 257:99–121