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Jie Chen, Philippe Callac, L.A. Parra, S.C. Karunarathna, M.-Q. He, et al.. Study in *Agaricus* subgenus *Minores* and allied clades reveals a new American subgenus and contrasting phylogenetic patterns in Europe and Greater Mekong Subregion. *Persoonia*, 2017, 38, pp.170-196. 10.3767/003158517X695521 . hal-01605877

HAL Id: hal-01605877

<https://hal.science/hal-01605877>

Submitted on 26 May 2020

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Study in *Agaricus* subgenus *Minores* and allied clades reveals a new American subgenus and contrasting phylogenetic patterns in Europe and Greater Mekong Subregion

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Key words

Agaricaceae
Agaricus sect. *Minores*
divergence times
multi-gene phylogeny
taxonomy

Abstract Within *Agaricus* subg. *Minores*, A. sect. *Minores* remains a little-studied section due generally to its delicate sporocarps often lacking taxonomically relevant morphological characters. To reconstruct the section, using the recent taxonomic system based on divergence times, and to evaluate the species diversity of A. sect. *Minores* in the Greater Mekong Subregion, 165 specimens were incorporated in phylogenetic analyses. A dated tree based on nuclear ITS, LSU and *tef1-α* sequence data allowed us to better circumscribe A. subg. *Minores* and to propose a new subgenus, A. subg. *Minoropsis*, which is only known from tropical and subtropical regions of the Americas. A larger tree based on ITS sequences indicated that, with 81 phylogenetic species, the reconstructed section *Minores* is now one of the largest sections in the genus. Within A. subg. *Minores*, a new section, A. sect. *Leucocarpi*, and eleven new species are described from the Greater Mekong Subregion. Thirty-eight species of A. sect. *Minores* from this region of Asia were distributed in multiple clades that successively diverged over the past 24 million years. In contrast, species reported from Europe mostly grouped in a single non-tropical clade, suggesting a major species diversification following the middle Miocene climatic optimum.

Article info Received: 13 September 2016; Accepted: 12 January 2017; Published: 28 April 2017.

INTRODUCTION

Agaricus (*Agaricaceae*, *Basidiomycota*) is a large genus comprising more than 400 species worldwide that are common in forests or grasslands (Zhao et al. 2011, Karunarathna et al. 2016). In the field, *Agaricus* species are easily distinguishable by having a fleshy pileus with free lamellae which produce brown spores, and an annulate stipe. The taxonomy of the genus has been well developed during the last two decades by using molecular phylogenetic tools (Challen et al. 2003, Kerrigan et al. 2005, 2008, Zhao et al. 2011, Parra 2013, Thongklang et al. 2014, Chen et al. 2015, Gui et al. 2015), which essentially reshaped our understanding of some of the morphologically recognized sections. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA has been largely used for establishing the phylogenetic relationships among the members of the genus, for example, in the phylogenetic study of Zhao et al. (2011), an eight-section taxonomic system was well supported, including seven additional strongly supported clades (TRI to TRVII). The fact that these clades were

exclusively from subtropical or tropical regions suggested that geographical and climatic factors had played a major role in the evolutionary history of the genus. Nevertheless, the ITS region alone is generally insufficient to delimit taxa or to resolve their relationships especially for those of higher rank than species (Matheny et al. 2007, Zhao et al. 2016). Zhao et al. (2016) proposed standardization of the taxonomic ranks based on divergence times. And using multi-gene phylogenetic analyses and molecular clock methods, a revised taxonomic system was proposed in which the genus *Agaricus* was divided into five subgenera and 20 sections (Zhao et al. 2016).

Among the five subgenera of *Agaricus* considered in Zhao et al. (2016), the present study focuses on A. subg. *Minores*, which accommodates species with positive reaction to KOH, usually positive, seldom negative Schäffer's cross-reactions (aniline × nitric acid) at the pileus surface or stipe base, yellowish staining when rubbed or cut and an anise-like or almond odour (Parra 2008, 2013, Zhao et al. 2016). The above traits are also shared by species of A. subg. *Flavoagaricus*, but taxa of A. subg. *Minores* could be further recognized by simple annulus (vs bilayered) and microscopically, generally by simple cheilocystidia (vs catenulate) and absence of inflated elements at the lower surface of the annulus.

For the delimitation of the subgenus, we followed the revised system of Zhao et al. (2016) in which clades that diverged 30–33 or 18–26 million years ago (Ma) were ranked as subgenera or sections, respectively. In this recent study A. subg. *Minores* consisted of three sections: A. sect. *Laeticolores*, A. sect. *Minores*, and one unnamed section (Zhao et al. 2016). *Agaricus* sect. *Laeticolores* was represented by a single species identified as *A. rufoaurantiacus*, while A. sect. *Minores* included not only A. sect. *Minores* as reported in Zhao et al. (2011), but also the closely related tropical clades TRV to TRVII reported

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in the same study, because they diverged too recently to be ranked as sections (Zhao et al. 2016). Although they belong in *A. sect. Minores* and this has been phylogenetically established in the new taxonomic system, a comprehensive morphological study of certain specimens is still needed to better circumscribe the section and its species. Species of *A. sect. Minores* are commonly distributed in temperate and tropical regions (Zhao et al. 2011, Parra 2013), and can also be found in harsh environments especially the sequestrate (secotiid) species adapted to xeric conditions (Thiers 1984, Lebel 2013).

Agaricus sect. Minores is well documented in Europe with 21 recognized species (Parra 2013). However, the species diversity in other areas is poorly explored. It must be noted that before the application of molecular techniques, few species were described in this group (Peterson et al. 2000). Some species seem to be widespread and have been recorded in several continents, for example, *A. brunneolus*, *A. comtulus*, and *A. purpurellus* (Heinemann 1961, 1962, 1980, 1990, Pegler 1977). Since species of *A. sect. Minores* usually produce small sporocarps, and lack morphological characters useful for species recognition, the question arises as to whether these species are widely distributed or whether there are any cryptic species, that are morphologically indistinguishable, but genetically distant (Bickford et al. 2007).

The Greater Mekong Subregion (GMS) is a region around the Mekong River basin in Southeast Asia, which includes Cambodia, Laos, Myanmar, Thailand, Vietnam, and Yunnan Province of China. It is also located within the so-called Indo-Burma hotspot, one of the 34 global biodiversity hotspots identified by Conservation International (Fisher & Christopher 2007). A project entitled 'Inventory and taxonomy of *Agaricus* species in Thailand, Laos, Malaysia and Yunnan (China); domestication and evaluation of species of nutritional or medicinal interest' has been carried out since 2010 and has already revealed a high species diversity in this region (Zhao et al. 2011, 2012, 2013, 2016, Chen et al. 2012, 2015, Karunarathna et al. 2016, Thongklang et al. 2014). Most of our samples have been collected in the framework of this project. In other respects, we have also contributed to the study of *A. sect. Minores* in the monograph of the genus *Agaricus* in Europe (Parra 2013), and therefore, the European diversity is also widely represented in our phylogenetic analyses. The present study aims to:

- i phylogenetically reconstruct *A. subg. Minores* following the new taxonomic system proposed by Zhao et al. (2016) and taking into account allied clades; and
- ii compare the species diversity in GMS and Europe.

MATERIALS AND METHODS

Materials examined and morphological observations

Fresh samples were mostly collected from 2006 to 2015 from Thailand and Yunnan Province, Southwest China, and seven were from Africa, Brazil, Dominican Republic and Malaysia. Specimens are deposited in MFLU (Mae Fah Luang University Herbarium) with duplicates at HMAS (Herbarium Mycologium, Chinese Academy of Sciences, Beijing, China), the African specimens are deposited in BR (the herbarium of the Botanic Garden Meise in Belgium). The Brazilian specimen is deposited in the Eliseo Battistin private herbarium, the Dominican specimens are deposited in JBSD (National Herbarium of Santo Domingo, Dr Rafael M. Moscoso National Botanical Garden), and the Malaysia specimens are deposited in KLU (Herbarium of Kuala Lumpur). In addition, two specimens of *A. laeticolor* (holotype *Goossens5272* and paratype *Goossens5371*) from Africa were loaned from BR herbarium and three specimens from Martinique and Guadeloupe are from LIP herbarium (Uni-

versité de Lille, France). Facesoffungi numbers (Jayasiri et al. 2015) are provided for new taxa.

Samples were photographed *in situ* or in laboratory, and odour and colour change (when rubbed or cut) were recorded in the field. The macroscopic characters were recorded according to the methodology described by Largent (1986). KOH and Schaffer's reactions were performed as described by Chen et al. (2015). Micromorphological features were examined from dried specimens following the protocols of Largent et al. (1977) including anatomy of lamellae, pileipellis and partial veil, and features of basidiospores, basidia and cystidia. Measurements of anatomical features (basidiospores, basidia and cheilocystidia) were presented based on at least 20 measurements, and include \bar{x} = the mean of length by width \pm SD; Q = the quotient of basidiospore length to width, and Q_m = the mean of Q -values \pm SD.

DNA extraction, PCR and sequencing

At the Institut National de la Recherche Agronomique (INRA), DNA was isolated from dried specimens following a CTAB protocol as described by Zhao et al. (2011). At the Southwest Forestry University, a commercial DNA extraction kit (E.Z.N.A. Forensic Kit, D3591-01, Omega Bio-Tek) was used for DNA extraction. DNA sequences were obtained from three loci: the internal transcribed spacer (ITS), nuclear large ribosomal sub-unit (nrLSU) and translation elongation factor 1- α (*tef-1 α*). Protocols for amplification of ITS and nrLSU regions followed those of White et al. (1990) with some modifications (Zhao et al. 2010), by using primers ITS4 and ITS5, LR0R and LR5, respectively. Amplification of the *tef-1 α* region using primers EF1-983F and EF1-1567R (Morehouse et al. 2003) followed the procedure described as below:

- 1 initial denaturation at 94 °C for 3 min;
- 2 denaturation at 94 °C for 30 s;
- 3 annealing at 56 °C for 40 s;
- 4 extension at 72 °C for 50 s;
- 5 repeat for 40 cycles starting at step 2;
- 6 leave at 72 °C for 10 min.

Sequencing was performed on ABI Prism Genetic analyzer (Applied Biosystems) at Beckman Coulter Genomics, England or on ABI 3730 XL DNA analyzer (Applied Biosystems) at Shanghai Majorbio Bio-Pharm Technology Co., Ltd, China. Consensus sequences were assembled by using SeqMan package of Lasergene software v. 7.1 (DNASTar, Madison, WI, USA). All sequences have been deposited in GenBank and their accession numbers are given in Table 1.

Sequence alignment, divergence time estimation and phylogenetic analyses

A total of 165 specimens were incorporated in phylogenetic analyses. In addition to the sequences generated from this study, 109 ITS sequences, 43 nrLSU sequences and 39 *tef-1 α* sequences were retrieved from GenBank (Geml et al. 2008, Zhao et al. 2011, 2016, Lebel & Syme 2012, Lebel 2013, He & Zhao 2015, Liu et al. 2015, Bates et al. 2016, Li et al. 2016) and their accession numbers are given in Table 1. Sequences were aligned, for each region independently using MAFFT (Katoh & Standley 2013), then manually adjusted in BioEdit v. 7.0.4 (Hall 2007). The ITS alignment was treated with Gblocks 0.91b (Castresana 2000), eliminating poorly or ambiguously aligned positions or DNA segments. Alignments have been submitted to TreeBase (submission ID 19813).

Divergence times were estimated using BEAST v. 1.8 (Drummond et al. 2012) based on 111 sequences. We first constructed an XML file with BEAUTI v. 1.8. Per-gene alignments were imported as separate partitions. Clock and substitution models were set to be unlinked (independently estimated for each

Table 1 GenBank accession numbers and samples used in the molecular analyses

Subgenus/section	Species	Taxa no.	Collection	Public database accession number			Geographic origin
				ITS	LSU	TEF	
Outgroup	<i>Heinemannomyces</i> sp.		ZRL185	KT951346	KT951527	KT951657	Thailand
<i>Agaricus</i>	<i>A. campestris</i> T		LAPAG370	JQ903618	KP739803	KR006636	Spain
	<i>A. sp.</i>		CA637	KT951322	KT951468	KT951633	France
	<i>A. sp.</i>		ZRL2012006	KT951357	KT951466	KT951634	Yunnan, China
<i>Flavoagaricus/Arvenses</i>	<i>A. arvensis</i> T		LAPAG450	KF114474	KP739801	KX198047	Spain
	<i>A. fissuratus</i>		WC777	AY484683	–	–	Denmark
	<i>A. flocculosipes</i>		ZRL2012105	KT951365	KT951463	KT951618	Yunnan, China
	<i>A. inapertus</i>		ECVel2339	AF482834	AF482878	–	USA
	<i>A. sp.</i>		ZRL2012630	KT951379	KT951495	KT951621	Tibet, China
<i>Minores/Leucocarpus</i>	<i>A. subrufescens</i>		ZRL2012722	KT951383	KT951451	KT951632	Yunnan, China
	<i>A. leucocarpus</i>		LD201226	KU975102	KX083982	KX198049	Thailand
	<i>A. leucocarpus</i>		SCK089	KU975090	–	–	Thailand
	<i>A. leucocarpus</i> T		LD201215	KU975101	KX083981	KX198048	Thailand
<i>Minores/Minores</i>	<i>A. sp.</i>	1	ZRL2012012	KT951359	KT951494	KT951597	Yunnan, China
	<i>A. columellatus</i>	2	MIN 938394	KJ912899	–	–	USA
	<i>A. colpetei</i> T	3	TL2424	JX984565	–	–	Australia
	<i>A. aridicola</i>	4	CA101	JF797195	AF261478	–	France
	<i>A. aridicola</i>	4	LAPAG589	KT951331	KX084027	KX198081	Spain
	<i>A. sp.</i>	5	CA848	JF727864	KT951445	KT951605	Thailand
	<i>A. sp.</i>	6	PS036	KU975087	KX084035	KX198036	Thailand
	<i>A. laeticulus</i> T	7	Goossens5272	KX671705	–	–	DR Congo
	<i>A. laeticulus</i>	7	Goossens5371	KX671704	–	–	DR Congo
	<i>A. sp.</i>	8	NTS73	KU975099	–	–	Thailand
	<i>A. sp.</i>	9	NTT33	JF514535	–	–	Thailand
	<i>A. sp.</i>	10	ZRL2011156	KT951352	KT951480	KT951603	Yunnan, China
	<i>A. flavopileatus</i> T	11	MS596	KU975121	KX084022	KX198078	Yunnan, China
	<i>A. flavopileatus</i>	11	MS603	KU975122	KX084023	KX198045	Yunnan, China
	<i>A. sp.</i>	12	ZRLD013	KT951384	KT951516	KT951604	Thailand
	<i>A. luteopallidus</i>	13	SCK121	KU975092	–	–	Thailand
	<i>A. luteopallidus</i>	13	LD2012113	KU975124	KX084026	KX198080	Thailand
	<i>A. luteopallidus</i>	13	SCK099	KU975095	–	–	Thailand
	<i>A. luteopallidus</i>	13	LD2012120	KU975123	KX084024	KX198079	Thailand
	<i>A. luteopallidus</i>	13	NTF26	JF514526	–	–	Thailand
	<i>A. luteopallidus</i>	13	SCK120	KU975093	–	–	Thailand
	<i>A. luteopallidus</i>	13	SCK138	KU975094	–	–	Thailand
	<i>A. luteopallidus</i> T	13	ZRL3088	JF691543	KX084025	–	Thailand
	<i>A. luteopallidus</i>	13	NTSCR1	KU975100	–	–	Thailand
	<i>A. callacii</i> T	14	AH42929	KF447899	KX083984	KX198051	Canary Islands (Spain)
	<i>A. chartaceus</i> T	15	H6271	JF495048	–	–	Australia
	<i>A. lamellipeditus</i> T	16	MDBF61/96	JX984559	–	–	Australia
	<i>A. cf. wariatodes</i>	17	MEL2058664	JF495050	–	–	Australia
	<i>A. wariatodes</i>	18	TWM1589	JF495052	JF495030	–	Australia
	<i>A. parvibicolor</i> T	19	LD2012116	KP715162	KX084016	KX198075	Thailand
	<i>A. parvibicolor</i>	19	ZRL3091	JF691546	KX084015	–	Thailand
	<i>A. purpureofibrillosus</i>	20	NTF63	KU975098	–	–	Thailand
	<i>A. purpureofibrillosus</i> T	20	ZRL3080	JF691542	KX084021	–	Thailand
	<i>A. sp.</i>	21	CA843	JF727866	KX084029	KX198040	Thailand
	<i>A. sp.</i>	22	ZRL2012004	KT951355	KT951457	KT951608	Yunnan, China
	<i>A. sp.</i>	23	ZRL2012714	KT951381	KT951476	KT951607	Tibet, China
	<i>A. sp.</i>	24	ZRL2011039	KT951351	KT951449	KT951606	Yunnan, China
	<i>A. sp.</i>	25	LD201252	KU975103	KX083983	KX198050	Thailand
	<i>A. sp.</i>	26	ADK2751	JF514519	–	–	Bénin
	<i>A. sp. (A. semotus)</i>	27	PDD68575	AF059224	AF059224	–	New Zealand
	<i>A. campbellensis</i> T	28	GAL9420	DQ232644	DQ232657	–	New Zealand
	<i>A. sp.</i>	29	GAL5812	EF460364	EF460389	–	USA
	<i>A. sp.</i>	30	ZRL3056	JF691541	KX084020	–	Thailand
	<i>A. megalosporus</i>	31	LD2012142	KU975120	KX084019	KX198077	Thailand
	<i>A. megalosporus</i>	31	ZRL2012199	KT951367	KT951470	KT951595	Yunnan, China
	<i>A. megalosporus</i> T	31	LD030	JF514521	–	–	Thailand
	<i>A. sp.</i>	32	CA846	JF727865	KT951452	KT951601	Thailand
	<i>A. fimbrimarginatus</i> T	33	LD201250	KU975119	KX084017	KX198076	Thailand
	<i>A. sp.</i>	34	ZRL2044	JF691540	KX084018	–	Thailand
	<i>A. robustulus</i>	35	ADK2905	JF514520	–	–	Bénin
	<i>A. robustulus</i> T	35	CA847	KU975086	KX084034	KX198039	Thailand
	<i>A. robustulus</i>	35	AK075	KU975088	–	–	Malaysia
	<i>A. robustulus</i>	35	MAR145	KU975089	–	–	Malaysia
	<i>A. robustulus</i>	35	ZRL2012357	KT951369	KT951496	KT951610	Yunnan, China
	<i>A. robustulus</i>	35	NT055	JF727846	–	–	Thailand
	<i>A. purpurellus</i>	36	LAPAG682	KF447903	KX083993	KX198059	Italy
	<i>A. purpurellus</i>	36	LAPAG944	KU975076	KX083994	KX198060	Czech Republic
	<i>A. jacobii</i>	37	LAPAG942	KU975081	KX083995	–	Spain
	<i>A. jacobii</i> T	37	AH44505	KF447895	KX083996	KX198061	Spain
	<i>A. marisae</i>	38	LAPAG138	KU975083	KX083998	KX198065	Spain
	<i>A. marisae</i> T	38	LAPAG111	JF797182	–	–	Spain
	<i>A. edmondai</i> T	39	LAPAG80	KF447902	–	–	Spain
	<i>A. edmondai</i>	39	LAPAG412	KT951326	KT951481	KT951590	Spain
	<i>A. kerriganii</i>	40	LAPAG808	KT951306	KT951442	KT951589	Spain
	<i>A. kerriganii</i> T	40	AH44509	KF447893	KX083999	KX198066	Spain
	<i>A. cf. kerriganii (A. diminutivus)</i>	41	WC912	AY484681	–	–	USA
	<i>A. dulcidulus</i>	42	PRM909627	KF447894	–	–	Czech Republic
	<i>A. iesu-et-marthae</i>	43	LAPAG41	KF447904	–	–	Spain
	<i>A. brunneolus</i>	44	LAPAG654	KU975077	–	KX198063	Czech Republic
	<i>A. brunneolus</i>	44	LAPAG938	KU975082	KX083997	KX198062	Spain
	<i>A. sp.</i>	45	GAL3083	EF460374	EF460399	–	USA
	<i>A. friesianus</i> T	46	F156208	KF447907	–	–	Sweden

Table 1 (cont.)

Subgenus/section	Species	Taxa no.	Collection	Public database accession number			Geographic origin
				ITS	LSU	TEF	
<i>Minores/Minores</i> (cont.)	<i>A. friesianus</i>	46	LAPAG592	KT951316	KX083992	KT951594	France
	<i>A. matrum</i>	47	LAPAG916	KU975080	KX083990	KX198057	Spain
	<i>A. matrum</i> T	47	AH44506	KF447896	KX083991	KX198058	Spain
	<i>A. heinemannianus</i>	48	LAPAG302	KF447906	—	KX198056	Spain
	<i>A. heinemannianus</i> T	48	AH19381	KF447905	—	—	Spain
	<i>A. pallens</i>	49	LAPAG441	KF447898	—	KX198067	Spain
	<i>A. pallens</i>	49	LAPAG580	KF447897	—	—	Spain
	<i>A. arrillagarum</i>	50	LAPAG810	KF447900	KX083985	KT951592	Spain
	<i>A. arrillagarum</i> T	50	AH44508	KF447908	—	—	France
	<i>A. gemlii</i> T	51	AH44510	KF447891	KX083989	—	Spain
	<i>A. gemlii</i>	51	LAPAG286	KU975079	KX083988	KX198055	Spain
	<i>A. comtulus</i>	52	LAPAG724	KT951332	KT951448	KT951593	Spain
	<i>A. comtulus</i>	52	LAPAG303	KU975078	KX083986	KT951592	Spain
	<i>A. luteomaculatus</i>	53	CA331	KF447901	—	KX198053	France
	<i>A. sp.</i>	54	ZD1528	KU975104	KX083987	KX198054	Yunnan, China
	<i>A. gemloides</i> T	55	ZRL2014084	KT633271	—	—	Yunnan, China
	<i>A. gemloides</i>	55	ZRL2014009	KT633272	—	—	Yunnan, China
	<i>A. sp.</i>	56	ZRLWXH3067	KT951387	KT951497	KT951611	Jiangxi, China
	<i>A. sp.</i>	57	ZRL3102	JF691545	KX084028	—	Thailand
	<i>A. coccyginus</i> T	58	ZRL2012485	KU245979	—	—	Tibet, China
	<i>A. coccyginus</i>	58	ZRL2012576	KT951372	KT951499	KT951596	Tibet, China
	<i>A. coccyginus</i>	58	ZRL2014430	KU245980	—	—	Yunnan, China
	<i>A. huijsmanii</i>	59	LAPAG639	KF447889	KT951444	KT951571	Spain
	<i>A. sp.</i>	60	PYP014	KU975091	—	—	Thailand
	<i>A. sp.</i>	61	ADK3580	KU975097	—	—	Bénin
	<i>A. sp.</i>	62	NT62	JF727845	—	—	Thailand
	<i>A. patris</i>	63	ZRL3101	JF691544	KX084013	—	Thailand
	<i>A. patris</i> T	63	LD201224	KU975118	KX084012	KX198073	Thailand
	<i>A. sodalis</i> T	64	LD2012159	KP715161	KX084014	KX198074	Thailand
	<i>A. sodalis</i>	64	LD2011029	KP715160	—	—	Thailand
	<i>A. pseudolutosus</i> T	65	AH11488	KF447890	—	—	Spain
	<i>A. pseudolutosus</i>	65	LAPAG454	KT951329	KT951453	KT951602	Spain
	<i>A. sp.</i>	66	MATA774	JF727871	—	—	Mexico
	<i>A. sp.</i>	67	ZRLWXH3076	KT951388	KT951458	KT951612	Fujian, China
	<i>A. sp.</i>	68	ZRLWXH3150	KT951390	KT951447	KT951609	Guangdong, China
	<i>A. sp. (A. diminutivus)</i>	69	Vellinga2360	AF482831	AF482877	—	USA
	<i>A. viridopurpurascens</i> T	70	Horak68/79	JF514525	—	—	New Zealand
	<i>A. sp.</i>	71	TL2154	JF495059	—	—	Australia
	<i>A. sp.</i>	72	TL2307	JF495058	—	—	Australia
	<i>A. brunneolutosus</i>	73	MS541	KU975112	KX084007	—	Yunnan, China
	<i>A. brunneolutosus</i> T	73	MS514	KU975111	KX084006	—	Yunnan, China
	<i>A. sp.</i>	74	MS386	KU975113	KX084008	KX198044	Yunnan, China
	<i>A. sp.</i>	75	CA935	KU975085	KX084036	KX198034	Thailand
	<i>A. badioniveus</i> T	76	LD2012131	KU975117	—	KX198072	Thailand
	<i>A. flammicolor</i>	77	ZRL2012270	KU975116	KX084011	KX198071	Yunnan, China
	<i>A. flammicolor</i>	77	LD201225	KU975115	KX084010	KX198070	Thailand
	<i>A. flammicolor</i> T	77	LD201502	KU975114	KX084009	KX198042	Thailand
	<i>A. sp.</i>	78	CA845	KU975084	KX084033	KX198035	Thailand
	<i>A. sp.</i>	79	NTT72	JF514539	—	—	Thailand
	<i>A. fulvoaurantiacus</i>	80	MS316	KU975106	KX084001	KX198043	Yunnan, China
	<i>A. fulvoaurantiacus</i> T	80	LD201404	KU975107	KX084002	KX198069	Yunnan, China
	<i>A. fulvoaurantiacus</i>	80	MS549	KU975105	KX084000	KX198068	Yunnan, China
	<i>A. luteofibrillosus</i>	81	ZRL2014136	KU245974	—	—	Yunnan, China
	<i>A. luteofibrillosus</i>	81	LD201501	KU975108	KX084003	KX198041	Thailand
	<i>A. luteofibrillosus</i> T	81	ZRL2013484	KU245972	—	—	Yunnan, China
	<i>A. luteofibrillosus</i>	81	ZRL2110	KU975109	KX084004	—	Thailand
	<i>A. luteofibrillosus</i>	81	ZRL3039	KU975110	KX084005	—	Thailand
	<i>A. luteofibrillosus</i>	81	NTT37	JF514537	—	—	Thailand
<i>Minores</i> /sect. 1	<i>A. candidolutescens</i> T		LD2012129	KT951335	KT951525	KT951616	Thailand
	<i>A. sp.</i>		LAPAM14	KT951312	—	KT951613	Dominican Republic
<i>Minoriopsis</i>	<i>A. sp.</i>		LAPAM45	KX671701	—	—	Dominican Republic
	<i>A. sp.</i>		ZRLWXH3161	KT951391	KT951526	KT951615	Guangdong, China
	<i>A. martinicensis</i>		F2815	JF727855	KX084032	KX198038	Martinique (France)
	<i>A. martinicensis</i>		LAPAM16	KX671699	KX671709	KX671706	Dominican Republic
	<i>A. rufoaurantiacus</i>		LAPAM15	KT951313	KX671708	KT951641	Dominican Republic
	<i>A. aff. rufoaurantiacus</i>		CL/GNAD05090	JF727857	KX084031	—	Guadeloupe (France)
	<i>A. sp.</i>		LAPAM28	KX671700	KX671710	KX671707	Dominican Republic
	<i>A. sp.</i>		LAPAM34	KX671703	—	—	Dominican Republic
	<i>A. sp.</i>		LAPAM66	KX671702	—	—	Brazil
	<i>A. sp. (A. comtulus)</i>		HAI0386	AJ884624	—	—	USA
<i>Pseudochitonina</i>	<i>A. sp. (A. johnstonii)</i>		F1779	JF727853	KX084030	KX198037	Martinique (France)
	<i>A. bisporus</i>		LAPAG446	KM657920	KR006611	KR006640	Spain
<i>Spissicaules</i>	<i>A. bitorquis</i>		WZR2012827	KM657916	KT951492	KT951647	Xinjiang, China
	<i>A. sinodeliciosus</i>		WZR2012822	KM657907	KT951518	KT951648	Xinjiang, China
	<i>A. albosquamosus</i> T		LD2012192	KT951394	KT951520	KT951636	Thailand
	<i>A. gratolens</i> T		ZRL3093	JF691548	KT951488	—	Thailand
	<i>A. leucolepidotus</i> T		LD201214	KT951336	KT951519	KT951635	Thailand
	<i>A. litoralis</i>		LAPAG475	KT951393	KX083980	KX198046	Spain
	<i>A. litoraloides</i>		ZRL2011249	KT951353	KT951523	KT951580	Yunnan, China
	<i>A. sp.</i>		AW145	KT951308	—	KT951637	Canada

Note New taxa are in **bold**. 'T' refers to type specimen. Species numbering in *A. sect. Minores* follows the order observed in the ITS ML tree of Fig. 3. Species name of the sequences deposited in GenBank is included in parenthetical citation after the identification adopted in this work whether when both differ due to a clear misidentification (*A. comtulus*), the same identification is indicated in GenBank for divergent sequences of obviously unrelated samples (*A. diminutivus*), or identification is based on a dubious and confused name (*A. semotus*) or based on material not matching the original description (*A. johnstonii*).

gene partition), while the tree prior parameters were set to be linked across partitions (concatenation). Substitution models were chosen based on jModelTest v. 2 (Darriba et al. 2012). In this case, we used normal distribution prior on the *treeModel.rootHeight* parameter, which has an initial value of 66 Myr for the genus *Agaricus* and a standard deviation of 1 Myr. The initial value is according to the previous fossil-calibrated analysis of Zhao et al. (2016). We ran an independent Monte Carlo Markov Chains of 50 million generations, logging states every 5 000 generations. The log file was opened in Tracer v. 1.6 (Rambaut et al. 2014) to evaluate convergence and mixing, and to ensure that Effective Sample Sizes were at least 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.

Maximum Likelihood analysis was performed using RAxML-HPC2 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 for each gene. A reciprocal 70 % bootstrap support approach was used to compare the tree topologies from individual genes. There was no significant incongruence between the datasets, so the ITS, nrLSU, and *tef-1 α* sequences were concatenated in BioEdit v. 7.0.4 (Hall 2007) for subsequent phylogenetic analyses.

The combined dataset was partitioned into ITS1, 5.8S, ITS2, nrLSU, *tef-1 α* intron and *tef-1 α* coding sites. The best substitution model for each partition was inferred with the program MrModeltest 2.2 (Nylander 2004): GTR+I+G for ITS1, 5.8S, ITS2, nrLSU, and *tef-1 α* intron sites, and SYM+I+G for *tef-1 α* coding sites. Bayesian Inference (BI) analysis was performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Six Markov chains were run for five million generations and sampled every 100th generation. Burn-in was determined by checking the likelihood trace plots in Tracer v. 1.6 (Rambaut et al. 2014) and subsequently discarded. Maximum parsimony (MP) analysis was performed in PAUP* 4.0b10 (Swofford 2004), by heuristic searches with unordered characters, random addition of sequences, gaps treated as missing data, and the tree bisection reconnection (TBR) branch swapping. Bootstrap values (BS) were obtained from 1 000 replicates. A node is considered to be strongly supported if at least two of the three analyses show a bootstrap support value (BS) ≥ 70 % and/or a posterior probability (PP) ≥ 0.95 .

Phylogenetic species recognition criterion

Because the taxa belonging to *A. sect. Minores* often lack distinctive morphological characters, we applied the multilocus genealogical phylogenetic species recognition approach (Taylor et al. 2000, Dettman et al. 2003) to delimit the species boundaries. A phylogenetic species is recognized when it matches either of the two criteria:

- 1 a genealogical concordant group that is present in the majority of the single-locus genealogies; or
- 2 a clade that is strongly supported by at least one single-locus genealogy and is not contradicted by any other locus (Ge et al. 2014).

Automatic Barcode Gap Discovery (ABGD) method was used for primary species delimitation (Puillandre et al. 2012). In certain cases, species circumscription was improved by examining polymorphisms in ITS alignment, taking into account insertions/deletions and heteromorphisms which are relatively frequent in species of *Agaricus* and reflect allelic polymorphisms and heterozygosity.

RESULTS

Phylogenetic analyses

We generated 166 new sequences for this study, including 56 of ITS, 60 of nrLSU, and 50 of *tef-1 α* . For dating analysis, a maximum clade credibility (MCC) tree is represented in Fig. 1. For this analysis 111 samples were used. Representatives of each five subgenera recognized by Zhao et al. (2016) were included and a specimen from the genus *Heinemannomyces* was used as an outgroup taxon. Eighty-six of these samples belong to *A. subg. Minores*.

In the multi-gene analyses as in the ITS analysis below, some redundant sequences were deleted, only subgenera related to *A. subg. Minores* were represented and *A. campestris* was used as outgroup. For multi-gene analyses, the final alignment contained 99 samples and was 2004 characters in length. The likelihood value of the final ML tree was -16287.144026. The topologies of the trees generated by the Bayesian and the maximum parsimony analyses were very similar to the ML tree (Fig. 2) except for few ungrouped samples such as *A. aridicola*/LAPAG589, *A. callacii*/AH42929, *A. sp.*/CA843, *A. sp.*/ZRL3080, *A. sp.*/ZRLWXH3150, *A. sp.*/ZRL2012004, and *A. sp.*/ZRL2012012.

For the ITS ML analysis 45 specimens belonging to 20 species were added. The aligned ITS dataset consisted of 150 sequences and was 751 nucleotides in length. The final alignment contained 676 characters after excluding ambiguous regions. Maximum likelihood analysis resulted in one ML tree with optimization likelihood value -8012.130834. To facilitate comparison between the trees and more specifically in *A. sect. Minores*, the major clades and the species of this section were numbered following the order in which they appeared in the multi-gene tree (from I to XI in Fig. 2) and in the ITS tree (from 1 to 81 in Fig. 3), respectively. Despite the different number of sequences used in multi-gene and ITS trees the same 11 major clades of *A. sect. Minores* were represented in both trees.

New subgenus and sections based on divergence times

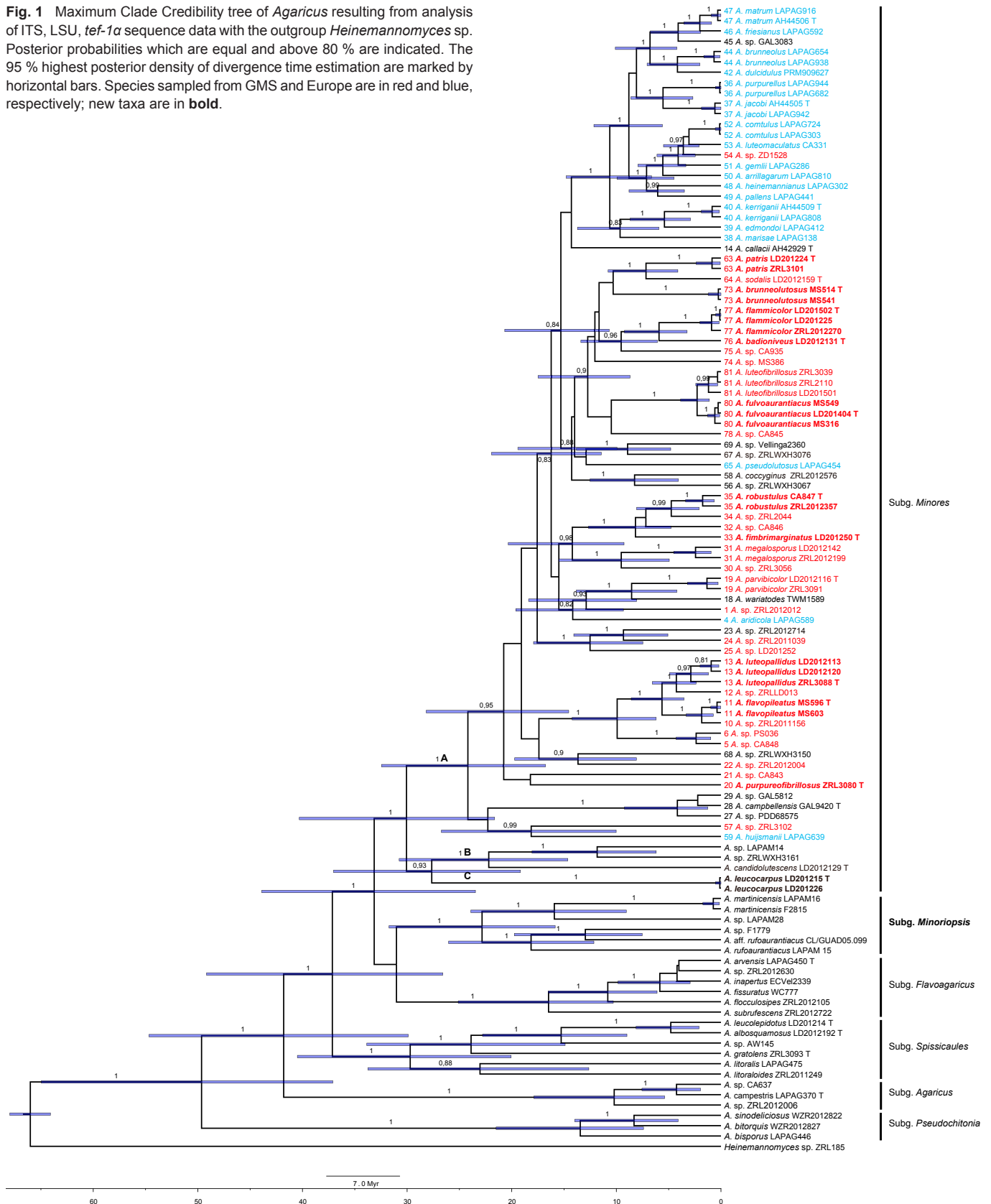
We used the taxonomic system of classification introduced by Zhao et al. (2016) with the following criteria to recognize subgenera and sections in the genus *Agaricus*:

- i they must be monophyletic and statistically well-supported in the multi-gene analyses;
- ii estimated stem ages for subgenera and sections are c. 30 Ma and c. 20 Ma, respectively; and
- iii they should be identifiable phenotypically, whenever possible.

Table 2 Mean stem ages of well-supported clades ($P \geq 0.99$) within or related to *A. subg. Minores*.

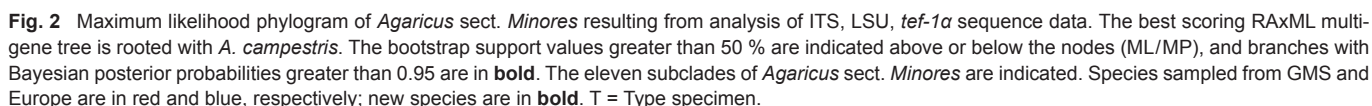
Subgenera	Section/major clades	Mean of stem age (Ma)
<i>Flavoagaricus</i>		31.02
	<i>Arvenses</i>	31.02
<i>Minores</i>		33.15
	<i>Leucocarpi</i>	27.64
	<i>Minores</i>	30.06
	A-I	17.56
	A-II	12.88
	A-III	17.40
	A-IV	14.21
	A-V	14.21
	A-VI	22.28
	A-VII	14.30
	A-VIII	14.26
	A-IX	12.89
<i>Minoriopsis</i>		31.02

Fig. 1 Maximum Clade Credibility tree of *Agaricus* resulting from analysis of ITS, LSU, *tef-1 α* sequence data with the outgroup *Heinemannomyces* sp. Posterior probabilities which are equal and above 80 % are indicated. The 95 % highest posterior density of divergence time estimation are marked by horizontal bars. Species sampled from GMS and Europe are in red and blue, respectively; new taxa are in **bold**.



In the genus *Agaricus*, MCC tree revealed that six well-supported clades had a stem age over 30 Ma (Fig. 1). Five of them correspond to subgenera that were previously recognized as *A. subg. Agaricus*, *A. subg. Flavoagaricus*, *A. subg. Minores*, *A. subg. Pseudochitonina*, and *A. subg. Spissicaules* by Zhao et al. (2016). The remaining clade was well supported in the MCC tree (PP ≥ 0.99 ; Fig. 1), in the multi-gene ML tree (ML: BS = 98, MP: BS = 90, PP > 0.95 ; Fig. 2) and in the ITS ML tree (BS = 80; Fig. 3). Its stem age was estimated to 31.02 Ma (Table 2). Therefore, this clade represents a new subgenus and

is named below as *A. subg. Minoropsis*. It includes five species in the MCC tree distributed in two sister clades which are well supported (PP ≥ 0.99 ; Fig. 1) and represent two sections since they diverged 22.83 Ma ago (Table 2). Using a broader sampling, the new subgenus includes eight species in the ITS ML tree and the two new sections remain well supported with BS values of 99 and 91, respectively. The new subgenus corresponds to the clade TRII in Zhao et al. (2011) and was represented by a single specimen (LAPAM15) in Zhao et al. (2016), which was included in *A. [subg. Minores] sect. Laeticolores*.



Using more samples, our analysis indicates that this previous placement was incorrect as well as the name of the section. Indeed, we obtained the ITS sequence of a type specimen of *A. laeticulus* (a *nom. nov.* for the illegitimate name *A. laeticolor*, the type of the section) and this sample was placed in *A. sect. Minores* in the ITS tree (Fig. 3; see also the taxonomic treatment of *A. sect. Minores*).

The clade corresponding to *A. subg. Minores* was subdivided in three clades A, B, and C, which are well supported in the MCC tree (PP \geq 0.99; Fig. 1) and in the multi-gene ML tree (ML: 95, 97, and 100; MP: 84, 75, and 100; PP $>$ 0.95; Fig. 2). In the system of classification adopted here, they represent three sections, respectively, since their stem ages are over 20 Ma (Fig. 1, Table 2). The clade A corresponds to *A. sect. Minores* since it includes the type (*A. comtulus*) of this section. The clade B was previously reported as clade A2 by Zhao et al. (2016) and now corresponds to an unnamed section, while clade C represents a new section of the subgenus. Clades B and C are sister and thus have the same stem age which was estimated to 27.64 Ma (Table 2). In the ITS ML tree, clades corresponding to the different subgenera and sections were recovered except for *A. subg. Minores*. Therefore, using only ITS sequence data, it was not possible to circumscribe *A. subg. Minores* and the phylogenetic relationships between the three sections of this subgenus remained unresolved. In the ITS ML tree (Fig. 3), only clades B and C are well supported (BS of 80 and 100, respectively), while clade A, corresponding to *A. sect. Minores* is poorly supported (BS $<$ 50). However, the placement of the species in the three sections/clades of the ITS tree does not differ from their placement in the multi-gene MCC or ML trees.

Major clades in *A. sect. Minores*

In the multi-gene ML tree, 11 subclades were revealed within *A. sect. Minores* and numbered from A-I to A-XI (Fig. 2). Except clade A-XI, all subclades received moderate to strong support. The clade A-VII is a core clade of *A. sect. Minores* and includes the type *A. comtulus*. It contains 20 species: 16 of the 19 species from Europe included in this study, two from China (*A. gemloides* and a putative sister taxon represented by ZD1528), and two unnamed species represented by samples from North America GAL3083 and WC912, respectively. The latter, originally identified as *A. diminutivus* is closely related or could belong to *A. kerriganii*. The tropical clades TRV, TRVI, and TRVII previously revealed in the phylogenetic analysis of Zhao et al. (2011) were distributed in clades A-III, A-IV, and A-X, respectively.

In the MCC tree, clades A-I to A-X were well supported (PP \geq 0.99; Fig. 1) but the samples of clade XI were distributed in several clades forming a group paraphyletic to clade A-X. Species are distributed in the 11 clades as in the multi-gene and ITS ML trees except one sample [57] ZRL3102 which ap-

pears to group with *A. huijsmanii*. The estimated mean stem and crown ages of *A. sect. Minores* were 30.06 and 24.19 Ma. In this section the broadest clade TRVII diverged relatively late, since its estimated stem and crown ages were 14.30 and 10.63 Ma, respectively.

In the ITS ML tree (Fig. 3), *A. sect. Minores* and the same 11 major clades were recovered, but were phylogenetically poorly supported, except the four clades A-III, -IV, -VI, and -X. *Agaricus laeticulus* clustered in *A. sect. Minores*, showing close affinities with two undescribed species in clade A-III.

Phylogenetic species recognition

In total, 60 species-level groups were recognized belonging to *A. sect. Minores* based on the combined dataset using the ABGD method. In addition, 22 species-level groups were recognized among 45 specimens for which only ITS sequence data are available. A more accurate species circumscription was performed in some groups of closely related samples exhibiting highly polymorphic ITS sequences including insertions, deletions and heteromorphisms, which are not taken in consideration in phylogenetic analyses. The distribution of putative alleles at such variable positions of the ITS alignment was examined in detail in the three following groups:

- 1 Collections NTF63 and ZRL3080 which were recognized as two entities in the ITS dataset, appear to belong to the same putative species. The two sequences differed at six positions, of which four were heteromorphic in ITS sequence of NTF63 but in each case one of the two nucleotides was also present in ZRL3080; indeed, these two samples which differ at only two positions and share putative alleles at four other positions are likely to be the same species.
- 2 In a group of nine samples (SCK121, NTF26, LD2012113, SCK099, SCK120, LD2012120, NTSCR1, SCK138, and ZRL3088) of which seven having non-redundant sequences were included in the ITS analysis, NTSCR1 and ZRL3088 formed a clade which might represent a distinct entity in the phylogenetic tree of Fig. 3. Polymorphisms were detected at 13 positions among the nine samples (Table 3). However, at 12 of the 13 polymorphic positions, heteromorphisms were found in one to four samples. Taking into account the heteromorphic positions, NTSCR1 and ZRL3088 do not have any characteristic alleles. Only the sample SCK121 differs from all the other samples by a characteristic allele at position 489. Therefore, we consider that the polymorphism among this group of samples likely reflects allelic diversity within a single species.
- 3 *Agaricus luteofibrillosus* is represented by six samples (species number 81) within clade A-XI of Fig. 3. They formed a polytomy with a clade containing three samples of the entity numbered 80. The phylogenetic relationships between the two entities remained poorly resolved likely

Table 3 Polymorphisms at 13 positions within ITS rDNA sequences of nine samples of *Agaricus luteopallidus*.

Sample	Positions in the ITS alignment (657 nts)												
	109	145	181	198	201	207	231	489	498	511	545	625	630
NTF26	C	T	A	G	T	C	C	T	T	T	G	T	C
LD2012113	C	T	A	G	T	C	C	T	C	C	A	T	C
SCK099	C	T	R	G	K	C	C	T	Y	Y	R	T	C
SCK120	C	Y	R	K	K	Y	M	T	Y	Y	R	Y	Y
LD2012120	C	Y	R	K	K	Y	M	T	Y	Y	R	Y	Y
NTSCR1	M	C	R	K	K	T	M	T	Y	Y	R	Y	Y
SCK138	C	C	G	T	G	T	A	T	T	T	G	C	T
ZRL3088	C	C	G	T	G	T	A	T	T	T	G	C	T
SCK121	A	C	A	G	T	T	C	C	T	C	G	T	C

Note Heteromorphisms: M: A and C; K: G and T; R: A and G; Y: C and T. Characters are in **bold** types when a nucleotide is shared with the specimen SCK121.

Fig. 3 Maximum likelihood phylogram of *Agaricus* sect. *Minores* resulting from analysis of ITS sequence data. The best scoring RAxML ITS tree is rooted with *A. campestris*. The bootstrap support values greater than 50 % are indicated. The eleven subclades of *Agaricus* sect. *Minores* are indicated. Species sampled from GMS and Europe are in red and blue, respectively; new species are in **bold**. T = Type specimen. * an ungrouped sample in the multi-gene trees of Fig. 1 and 2, and which therefore, probably, does not belong to the clade A-XI. Circle symbol indicates secotioid species.

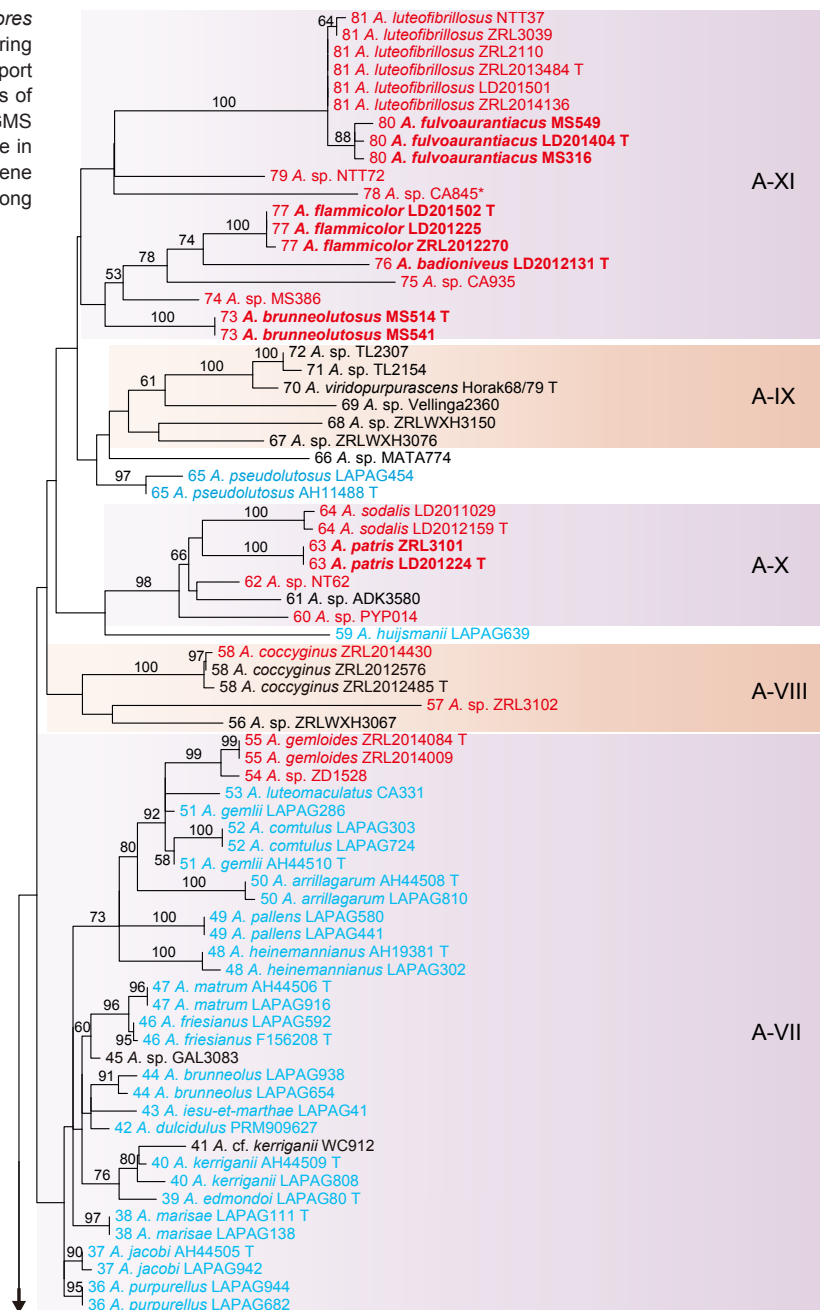
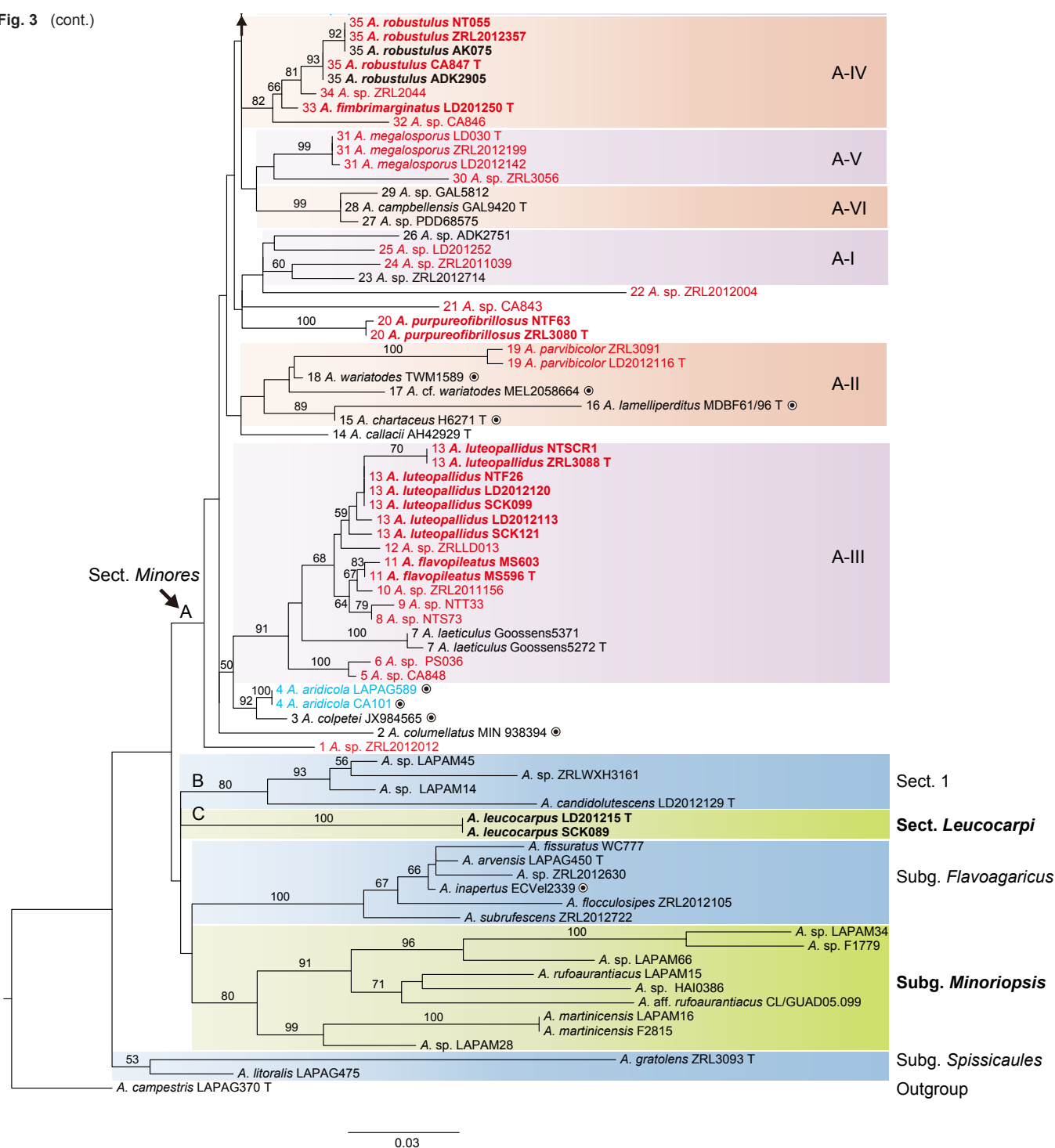


Table 4 Characters at 16 polymorphic positions within ITS rDNA sequences of 14 samples of *A. fulvoaurantiacus* and *A. luteofibrillosus*.

Sample	Positions in the ITS alignment (667 nts) reflecting variability															
	Within <i>A. fulvoaurantiacus</i>								Within <i>A. luteo</i>				Interspecific			
	38	87	138	202	235	257	259	481	23	26	204	468	603	632	634	646
<i>A. fulvoaurantiacus</i>																
MS549	T	C	C	T	C	A	A	A	T	T	–	A	–	C	C	A
MS316	C	C	Y	T	C	K	A	G	T	T	–	A	–	C	C	A
LD201404	T	T	C	K	Y	K	W	R	T	T	–	A	–	C	C	A
<i>A. luteofibrillosus</i>																
LD201501	T	C	T	T	C	T	A	G	T	T	T	A	G	T	–	G
ZRL2110	T	C	T	T	C	T	A	G	K	K	–	N	G	T	–	G
ZRL3039	T	C	T	T	C	T	A	G	T	T	T	G	G	T	–	G
NTT37	T	C	T	T	C	T	A	G	T	T	–	G	G	T	–	G
ZRL2013484	T	C	T	T	C	T	A	G	T	T	–	R	G	T	–	G
ZRL2012200	T	C	T	T	C	T	A	G	T	T	–	R	G	T	–	G
ZRL2014136	T	C	T	T	C	T	A	G	T	T	–	A	G	T	–	G
ZRLWXH3112	T	C	T	T	C	T	A	G	T	T	–	G	G	T	–	G
ZRLWXH3183	T	C	T	T	C	T	A	G	T	T	–	G	G	T	–	G
ZRL2012121	T	C	T	T	C	T	A	G	T	T	–	G	G	T	–	G
ZRL2012359	T	C	T	T	C	T	A	G	T	T	–	G	G	T	–	G

Note Heteromorphisms: K: G and T; R: A and G; W: A and T; Y: C and T. N: unidentified nucleotide (A, T, G or C). –: absent nucleotide.

Fig. 3 (cont.)



due to insertions, deletions and heteromorphisms. The variability observed at 16 polymorphic positions among the two entities is reported in Table 4. Three samples of *A. luteofibrillosus* were not included in the phylogenetic analysis because their sequences were redundant. The three samples of entity 80 clearly differ from the 11 samples of *A. luteofibrillosus* at the four positions 511, 545, 625, and 630. Moreover, the polymorphism at the eight positions 38, 87, 138, 202, 235, 257, 259, and 481 was observed only among the samples of the putative new species 80, while the polymorphism at the four remaining positions 23, 26, 204, and 468 was found only among the samples of *A. luteofibrillosus*. Knowing that samples differing at more than two positions generally belong to different species in genus *Agaricus* (Zhao et al. 2011), the clade containing the three samples MS549, MS316, and LD201404 is regarded as a distinct species from *A. luteofibrillosus*.

After these adjustments, 81 phylogenetic species were ultimately recognized and numbered in *A. sect. Minores* (Fig. 3). Among them, 44 are named species including ten newly described in this study and 37 taxa remain unnamed either because their sequences were retrieved from GenBank or because our material and/or morphological/macrochemical information were insufficient.

Geographic distribution of species of *A. subg. Minores* and *A. subg. Minoriopsis*

Geographic distribution of the species ordered by subgenera, sections and sectional subclades are summarized in Table 5. We first note that all species of *A. subg. Minoriopsis* are from the Americas, mostly from tropical or subtropical areas.

In *A. sect. Minores* most of the species included in this study are from Europe (19) or GMS (38) and among the 24 remaining species, 10 are from Australasia. Among the 19 European

Table 5 Geographic distribution of 94 species of *Agaricus* subg. *Minores* and *A.* subg. *Minoriopsis*.

Subgenus	Section	Subclade	Number of species						
			Total	EUR ^a	GMS ^a	ASI ^a	AFR ^a	AME ^a	AUS ^a
<i>Minores</i>	<i>Minores</i>	A-I	4	0	2	1	1	0	0
		A-II	5	0	1	0	0	0	4
		A-III	9	0	8	0	1	0	0
		A-IV	4	0	4 ^b	0	0	0	0
		A-V	2	0	2	0	0	0	0
		A-VI	3	0	0	0	0	1	2
		A-VII	20	16	2	0	0	2	0
		A-VIII	3	0	2	1	0	0	0
		A-IX	6	0	0	2	0	1	3
		A-X	5	0	4	0	1	0	0
		A-XI	7	0	7	0	0	0	0
		Ungrouped	13	3	6	0	1	2	1
		Total. (<i>Min.</i>)	81	19	38	4	4	6	10
<i>Minoriopsis</i>	A. sect. 1		4	0	1	1	0	2	0
	<i>Leucocarpi</i>		1	0	1	0	0	0	0
			8	0	0	0	0	8	0

Notes ^a EUR = Europe; GMS = the Greater Mekong Subregion; ASI = Asia (China); AFR = Africa; AME = Americas; AUS = Australasia.

^b One of the four is also found in Malaysia and in Africa.

species, three are ungrouped and 16 (84 %) are in the same clade (A-VII). Clade A-VII also includes two non-tropical sister species from GMS (Yunnan) [54, 55] and two others from North America [41, 45]. Clade A-VII is a core clade of the section: it contains most of the European species including the type *A. comtulus* and it does not contain any tropical species. In contrast, 32 of the 38 GMS species of *A.* sect. *Minores*, mostly from tropical area in Thailand, are distributed in nine of the 11 subclades, while the six remaining species are ungrouped.

Nine samples belonging to eight secotioid species were included in our studies. One is *A. inapertus*, a species of *A.* sect. *Arvenses*, while the seven remaining species were considered or suspected to belong to *A.* sect. *Minores* (Zhao et al. 2011, Lebel & Syme 2012, Lebel 2013, Bates et al. 2016). Our ITS ML tree not only confirms that the seven species belong to *A.* sect. *Minores*, but also, as in Lebel (2013), that four Australian species (*A. chartaceus*, *A. lamelliperditus*, *A. variatodes* and *A.* cf. *variatoles*) are closely related to each other, while the fifth Australian species *A. colpetei* ('*colpeteii*') is related to the European species *A. aridicola*. Representatives of these two groups (*A. aridicola* and *A. variatodes*) were included in the MCC tree and appear in Fig. 1 to be in the same moderately supported clade (PP = 0.82). However, the multi-gene ML tree does not confirm this result and the position of the seventh species, *A. columellatus* from the USA remains uncertain in the ITS ML tree. Therefore, we cannot conclude that the seven secotioid species are closely related, but also this hypothesis cannot be excluded.

TAXONOMY

Here we present descriptions of 13 new taxa (one subgenus, one section, and 11 species from the Greater Mekong Subregion). In addition we propose one new combination and one new species record from Thailand. Generally speaking, within *A.* sect. *Minores*, the number of morphological characters which are available for species distinction is scarce, usually with a large level of overlap between closely related species. In some cases, unequivocal identification of individual collections would not be possible without molecular data.

Agaricus* subgenus *Minoriopsis Linda J. Chen, L.A. Parra, Callac, Angelini & Raspé, *subg. nov.* — MycoBank MB818040

Facesoffungi number. FoF 02280.

Type. *Agaricus martinicensis* Pegler, Kew Bull., Addit. Ser. 6: 446. 1983.

Etymology. Referring to the similarities to *A.* subg. *Minores*.

Original description and delimitation of *Agaricus* subg. *Minoriopsis* — Schäffer's reaction immediately and strongly positive dark reddish purple, rarely reddish brown and KOH difficult to observe but positive yellow when observable, on the pileus surface of dried specimens. Odour of anise or of bitter almonds when rubbed or cut. Annulus superous, thick at the margin, double, fibrillose squamose or, sometimes with squames radially arranged as a cogwheel near the margin in its lower surface, which, under the microscope is composed only by thin cylindrical hyphae. Cheilocystidia generally simple or with a septum at the base, clavate, pyriform, more or less globose, fusiform, sometimes rostrate or absent in some specimens. Spores lacking a rudimentary apical pore.

Stem age and phylogenetic support — In the MCC tree (Fig. 1 and Table 2), the clade corresponding to *A.* subg. *Minoriopsis* has a stem age of 31.02 Ma and is well supported (PP ≥ 0.99). It has 98/90 bootstrap support (ML/MP) in multi-gene phylogenetic analysis (Fig. 2). In the present analyses, *A.* subg. *Minoriopsis* includes five to eight species distributed in two sister subclades, which potentially represent respectively two sections since they diverged 22.83 Ma ago.

Agaricus* subgenus *Minores (Fr.) R.L. Zhao & Moncalvo, Fung. Diversity 78: 257. 2016

Type. *Agaricus comtulus* Fr. designated by Heinemann, Bull. Jard. Bot. État Bruxelles 26: 42. 1956.

Agaricus [subg. *Minores*] section 1

Clade B (Clade A2 in Zhao et al. 2016). Four specimens (LD2012129/*A. candidolutescens*, LAPAM14, LAPAM45 and ZRLWXH3161) cluster together (Fig. 3) in clade B, sister to *A.* sect. *Leucocarpi*. Since some important morphological data are lacking, we refrain from describing this section here.

Agaricus* [subg. *Minores*] section *Leucocarpi Linda J. Chen & Callac, *sect. nov.* — MycoBank MB818041

Facesoffungi number. FoF 02281.

Type. *Agaricus leucocarpus* Linda J. Chen, Callac, R.L. Zhao & K.D. Hyde.

Etymology. The epithet '*Leucocarpi*' is following the name of the type *A. leucocarpus*.

Original description and delimitation of *Agaricus* sect. *Leucocarpi* — Schäffer's reaction negative, KOH reaction positive. Surface of basidiomes often flavescent when rubbed. Odour of almonds. Annulus superous, membranous, smooth on both sides. Cheilocystidia present, simple, pyriform or broadly clavate.

Stem age and phylogenetic support — In the MCC tree (Fig. 1 and Table 2), *A. sect. Leucocarpi* has a stem age of 27.64 Ma and is well supported (PP \geq 0.99). It has strong bootstrap support (ML/MP) in multi-gene phylogenetic analyses (Fig. 2).

Agaricus leucocarpus Linda J. Chen, Callac, R.L. Zhao & K.D. Hyde, *sp. nov.* — MycoBank MB818042; Fig. 4, 5

Facesoffungi number. FoF 02282.

Etymology. The epithet '*leucocarpus*' refers to the white sporocarp of this species.

Pileus 2.5–4 cm diam, 1–3 mm thick at disc; at first parabolic, becoming hemispherical to plano-convex, finally applanate; surface dry, smooth, completely white with light brownish or ochre tinges at the disc. Margin straight, not exceeding the lamellae, often with appendiculate remains of the annulus. *Lamellae* free, crowded, 3 mm broad, ventricose, with intercalated lamellulae, at first white, turning pinkish when touched, then pink to greyish brown, finally brown. *Stipe* 40–65 \times 2–5 mm (5–9 mm at base), cylindrical with a subbulbous base, fistulose, surface smooth both above and below the annulus, white, flavescent when rubbed or by handling. *Annulus* simple, superous, membranous, white, fragile. *Context* firm, white, unchanging when cut. *Odour* of almonds.



Fig. 4 *Agaricus leucocarpus*. a. Overall morphology *in situ* (SCK089); b. appendiculate margin (holotype LD201215); c. lamellae when mature (LD201507); d. overall morphology (LD201226); e. lamellae when young (LD201226).

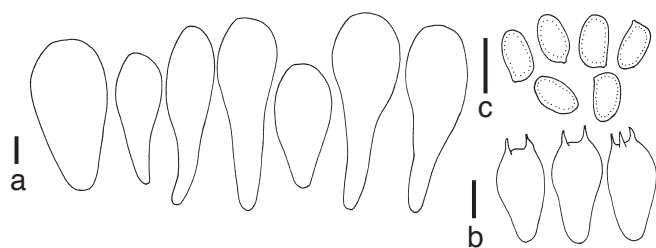


Fig. 5 Microscopic characters of *Agaricus leucocarpus*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars = 5 µm.

Spores (4.3–)4.5–5 × 3–3.5 µm, ($x = 4.7 \pm 0.17 \times 3.2 \pm 0.12$ µm, $Q = 1.32–1.61$, $Q_m = 1.48 \pm 0.02$, $n = 20$), ellipsoid, smooth, brown, thick-walled. **Basidia** 11–14 × 6.5–8 µm, broadly clavate, hyaline, smooth, 4-spored. **Cheilocystidia** (11–)18.5–26 × 6–15 µm, simple, pyriform or broadly clavate, hyaline, smooth. **Pleurocystidia** absent. **Pileipellis** a cutis composed of hyphae of 4–8 µm wide, cylindrical, not or slightly constricted at the septa, hyaline.

Macrochemical reactions — KOH reaction positive, yellow. Schäffer's reaction negative on dry specimen.

Habitat — Solitary on soil, in grassland of roadside; or scattered on leaf litter mixed with compost.

Material examined. THAILAND, Chiang Rai Prov., Mae Fah Luang University gate, 27 July 2015, J. Chen, LD201507; Chiang Mai Prov., Tharnthong Lodges, 31 May 2012, J. Chen, LD201215 (holotype MFLU12-0859); Chiang Mai Prov., Tharnthong Lodges, 3 June 2012, J. Chen, LD201226 (MFLU12-0870); Chiang Rai Prov., Bandu, 31 July 2011, S.C. Karunaratna, SCK089 (MFLU11-1283).

Notes — *Agaricus leucocarpus* is a species morphologically well characterized by its slender, pure white sporocarps, with a brownish tinge at disc, small spores and simple cheilocystidia. Considering its morphology, discoloration when rubbed and the almond smell, it is very likely to be a member of *A. sect. Minores*. However, it shows negative Schäffer's reaction, which is in disagreement with *A. sect. Minores*. Among the other known sections, possibly related to *A. sect. Minores*, *A. sect. Lanosi* is characterized by negative Schäffer's reaction, and *A. haemosarcus*, is the only species showing pure white sporocarps. But it can easily be distinguished from *A. leucocarpus* by its woolly pileus and stipe surface, and strong reddening when cut (Heinemann 1956, Parra 2013). Since the attempts

at sequencing the type of *A. sect. Lanosi* failed, in the absence of sequence data from any species of the section, and because the new species does not exhibit any woolly veil, which is a main character of this section, we have no reason to place *A. leucocarpus* in *A. sect. Lanosi*. According to the phylogenetic analyses, *A. leucocarpus* corresponds to clade C, which constitutes *A. subg. Minores* (Fig. 2) with the two clades A (*A. sect. Minores*) and B.

***Agaricus* [subg. *Minores*] section *Minores* (Fr.) Henn. in Engler & Prantl, Nat. Pflanzenfam. 1(1**): 238. 1898**

≡ *Agaricus* [unranked] *Minores* Fr., Hymenomyc. Eur.: 281. 1874.

Type. *Agaricus comtulus* Fr., designated by Heinemann (1956) 42.

= *Agaricus sect. Laeticolors* Heinem., Kew Bull. 15(2): 144. 1961.

Type. *Agaricus laeticulus* Callac, L.A. Parra, Linda J. Chen & Raspé, nom. nov. — MycoBank MB818070.

Etymology. A composite word from the Latin *laetus* meaning cheerful, pleasant, bright and the suffix *-culus* denoting diminutive. Thus, *laeticulus* is 'the little bright'.

Agaricus laeticulus Callac, L.A. Parra, Linda J. Chen & Raspé, is a replacement name for *Agaricus laeticolor* Heinem. & Gooss.-Font., Bull. Jard. Bot. État 26: 42. 1956, an illegitimate name because of the existence of the earlier homonym *Agaricus laeticolor* Lév., Icon. Champ. Paulet: 36. 1855.

***Agaricus badioniveus* Linda J. Chen, R.L. Zhao & K.D. Hyde, sp. nov. — MycoBank MB818047; Fig. 6, 7**

Facesoffungi number. FoF 02283.

Etymology. Refers to the pileus with tawny fibrils on a white background.

Pileus 3.5 cm diam, 3 mm thick at disc; convex and truncated at disc; surface dry, with yellowish brown fibrils, densely at disc, and progressively sparse towards the margin, on a white background. Margin straight, not exceeding the lamellae, with appendiculate remains of the annulus. **Lamellae** free, crowded, 3 mm broad, with intercalated lamellulae, ventricose, pinkish to brown with time. **Stipe** 45 × 7 mm (12 mm at base), cylindrical with a bulbous base, surface above the ring smooth, below the ring fibrillose, white, strongly flavescent when bruised. **Annulus** simple, membranous, superous, white, fragile. **Context** firm, white, flavescent when cut. **Odour** of strong almonds.

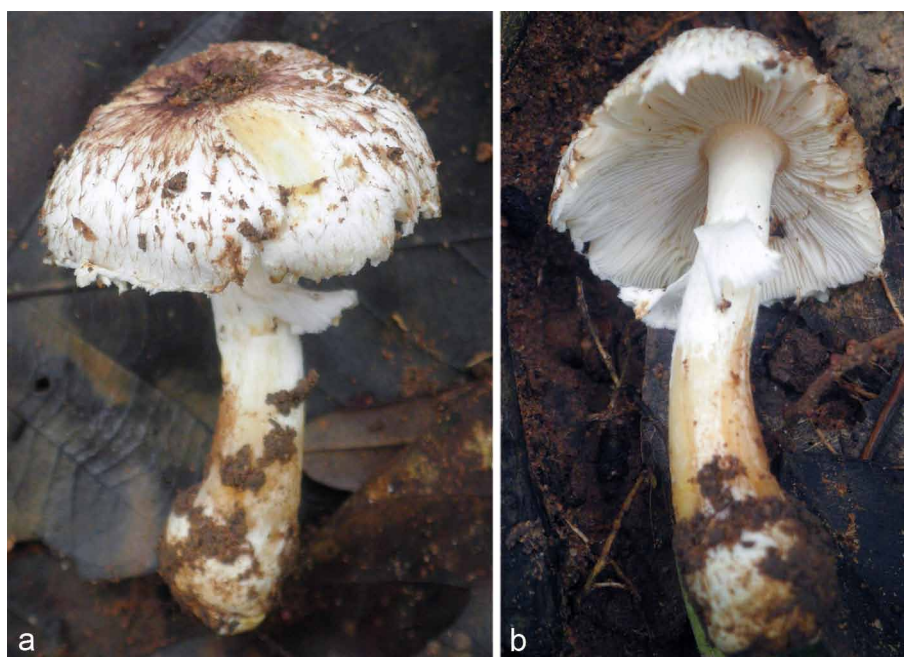


Fig. 6 *Agaricus badioniveus* (holotype LD2012131). a. Pileus surface; b. lamellae and stipe.

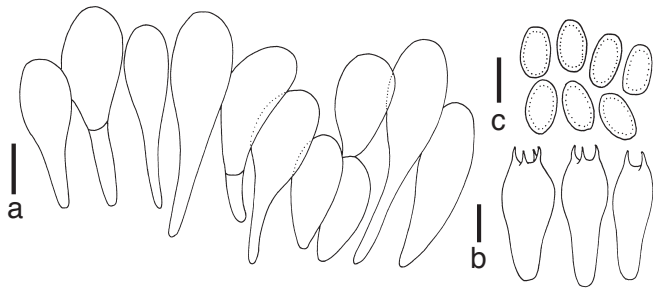


Fig. 7 *Agaricus badioniveus* (holotype LD2012131). a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars: c = 10 μ m, d–e = 5 μ m.

Spores (5–)5.4–5.8(–6.2) \times 3.1–3.5(–3.8) μ m, ($x = 5.6 \pm 0.12 \times 3.3 \pm 0.11$ μ m, $Q = 1.54$ – 1.86 , $Q_m = 1.67 \pm 0.01$, $n = 20$), ellipsoid, smooth, brown, thick-walled. **Basidia** 15–19 \times 6.5–7 μ m, clavate to broadly clavate, hyaline, smooth, 4-spored. **Cheilocystidia** 23–35(–40) \times 9–12(–16) μ m, abundant, simple, or septate at base, pyriform, clavate or narrowly clavate, with yellowish pigments, smooth. **Pleurocystidia** absent. **Pileipellis** a cutis constituted of cylindrical hyphae of 6–9 μ m wide, not or slightly constricted at the septa, with brownish pigment.

Macrochemical reactions — KOH reaction positive, yellow. Schäffer's reaction positive, reddish orange on dry specimen.

Habitat — Solitary on soil, in forest.

Material examined. THAILAND, Chiang Rai Prov., Doi Pui site1, 25 July 2012, J. Chen, LD2012131 (holotype MFLU12-0964).

Notes — *Agaricus badioniveus* is characterized by a pileus surface covered with yellowish brown fibrils, simple cheilocystidia and spores on average size of 5.6 \times 3.3 μ m.

In gross morphology, *A. badioniveus* is highly similar to *A. megalosporus*. However, the latter species has larger sporocarps (the pileus diameter can reach 10 cm) and spores (6 \times 3.5 μ m on average, Chen et al. 2012). According to phylogenetic results, *A. badioniveus* is closely related to *A. flammicolor*, a species easily distinguished by its bright orange colour.

Agaricus brunneolutosus Linda J. Chen, Karun. & K.D. Hyde, *sp. nov.* — MycoBank MB818048; Fig. 8, 9

Facesoffungi number. FoF 02284.

Etymology. Refers to the brown yellow colour of the pileus.

Pileus 5.5–8.5 cm diam, 3–5 mm thick at disc; convex to applanate, or uplifted; surface dry, covered with brown fibrils, densely at disc and radially arranged elsewhere, somewhat sparse towards the margin, on a white to yellowish white background. Margin straight, shortly exceeding the lamellae, often with appendiculate remains of the annulus. **Lamellae** free, crowded, 3–5 mm broad, with intercalated lamellulae, at first white, then pinkish brown, finally dark brown. **Stipe** 70–110 \times 7–10 (10–12 at base) mm, clavate or tapering upwards,

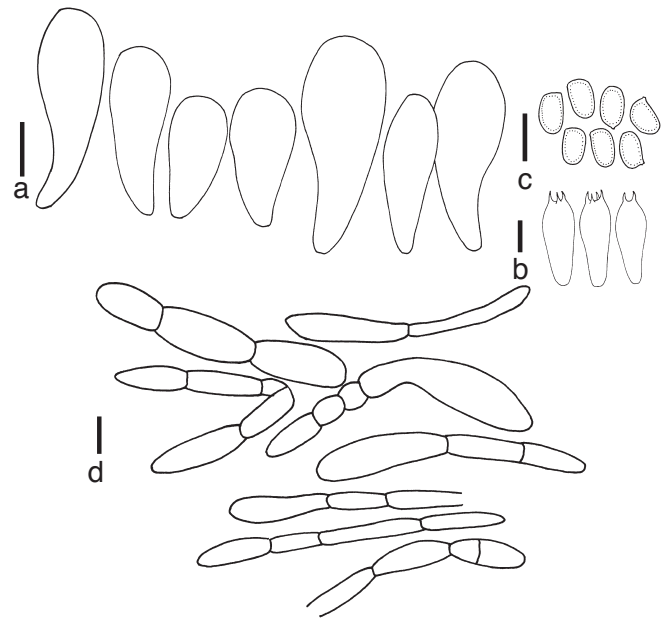


Fig. 8 Microscopic characters of *Agaricus brunneolutosus*. a. Cheilocystidia; b. basidia; c. basidiospores; d. pileipellis. — Scale bars: a = 10 μ m, b–c = 5 μ m, d = 20 μ m.

fistulose, surface above the ring smooth, below fibrillose, white, flavescent when bruised. **Annulus** simple, superous, membranous, upper surface smooth, lower surface fibrillose, white, changing to yellowish with time or when rubbed. **Odour** of almonds. **Context** firm, discoloration when cut not recorded. **Spores** 3.9–4.7(–5.2) \times 2.7–3.3 μ m, ($x = 4.3 \pm 0.22 \times 2.9 \pm 0.14$ μ m, $Q = 1.32$ – 1.65 , $Q_m = 1.48 \pm 0.03$, $n = 20$), ellipsoid, smooth, brown, thick-walled. **Basidia** 13–18 \times 5.5–8 μ m, clavate to broadly clavate, hyaline, smooth, 4-spored. **Cheilocystidia** 17–42 \times 9–15 μ m, abundant, simple, pyriform to broadly clavate, hyaline or with yellowish pigments, smooth. **Pleurocystidia** absent. **Pileipellis** a cutis composed of hyphae of 5–13 μ m wide, cylindrical, hyaline or with light yellow pigments, smooth, sometimes constricted at the septa; terminal elements observed cylindrical, 13–25 μ m wide, with rounded or attenuate apex.

Macrochemical reactions — KOH reaction positive, yellow. Schäffer's reaction positive, reddish orange on dry specimen.

Habitat — Solitary on soil, in forest dominated by *Casternopsis* and *Lithocarpus*.

Material examined. CHINA, Yunnan Prov., Mengsong, 7 July 2012, S.C. Karunarathna, MS514 (holotype MFLU16-0976; isotype HMAS279153); Yunnan Prov., Mengsong, 10 July 2012, S.C. Karunarathna, MS541 (MFLU16-0977, HMAS279154).

Notes — *Agaricus brunneolutosus* is distinguished by its yellowish white pileus, entirely covered with brown fibrils, small spores on average size of 4.3 \times 2.9 μ m, large cheilocystidia and the pileipellis hyphae with terminal elements 13–25 μ m wide.



Fig. 9 *Agaricus brunneolutosus*. a–b. Overall morphology *in situ* (holotype MS514).

Among the members of *A.* sect. *Minores*, few species have spores on average shorter than 5 μm : *A. comtulus*, *A. dulcidulus*, *A. edmondoi*, *A. entibigae*, *A. friesianus*, *A. matrum*, and *A. pallens*. However, *A. comtulus* (Parra 2013) and *A. entibigae* (Peterson et al. 2000) have wider spores. The remaining taxa usually have pinkish, reddish pink or reddish purple fibrils on the pileus disc, and are white elsewhere. Additionally, they are well separated by molecular data (Fig. 2). *Agaricus brunneolutosus* forms a sister clade with *A. fulvoaurantiacus* and *A. luteofibrillosus*, however, the two latter species have larger spores (larger than 5 \times 3 μm on average).

Agaricus fimbrimarginatus Linda J. Chen, Callac & K.D. Hyde, *sp. nov.* — MycoBank MB818049; Fig. 10, 11

Facesoffungi number. FoF 02285.

Etymology. Refers to the appendiculate remains on the pileus margin.

Pileus 4 cm diam, 3 mm thick at disc; applanate and slightly depressed at disc; surface dry, with purplish fibrils, densely at disc, radially arranged elsewhere, and sparse towards the margin, on a dirty white background. Margin straight, shortly exceeding the lamellae, with appendiculate remains of the annulus. *Lamellae* free, crowded, 3 mm broad, with intercalated lamellulae, ventricose, pinkish to brown with time. *Stipe* 47 \times 7–8 mm, cylindrical with a slightly bulbous base, surface above the ring smooth, below the ring fibrillose, white, strongly flavescent when bruised. *Annulus* simple, membranous, superous, white, fragile. *Context* firm, white, flavescent when cut. *Odour* strong of almonds.

Spores (4.4–)4.5–4.9 \times (2.9–)3–3.3 μm , ($x = 4.7 \pm 0.11 \times 3.2 \pm 0.09 \mu\text{m}$, $Q = 1.36\text{--}1.59$, $Q_m = 1.46 \pm 0.01$, $n = 20$), ellipsoid, smooth, brown, thick-walled. *Basidia* 12–17 \times 5–6 μm , clavate to broadly clavate, hyaline, smooth, 4-spored, rarely 2-spored. *Cheilocystidia* 15–26 \times 8–12 μm , simple, pyriform or broadly clavate, with yellowish pigments, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis, composed of cylindrical hyphae of 4–9 μm wide, not or slightly constricted at the septa, with brownish pigment.

Macrochemical reactions — KOH reaction positive, yellow. Schäffer's reaction positive, reddish on dry specimen.

Habitat — Solitary on soil, in grassland along roadside.

Material examined. THAILAND, Chiang Mai Prov., Mae Sa, 25 June 2012, P. Callac & J. Chen, LD201250 (holotype MFLU12-0891).

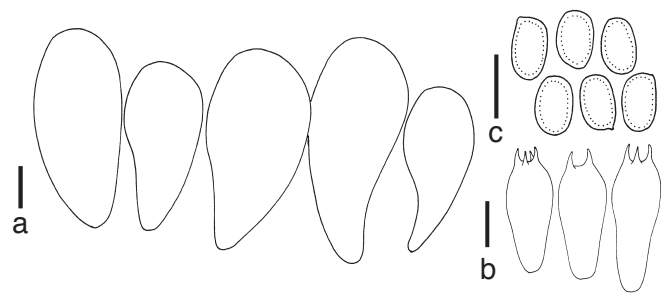


Fig. 10 Microscopic characters of *Agaricus fimbrimarginatus*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars = 5 μm .

Notes — *Agaricus fimbrimarginatus* is characterized by a pileus surface covered with purplish fibrils, simple cheilocystidia and small spores less than 3.5 μm wide.

Several members of *A.* sect. *Minores* resemble *A. fimbrimarginatus* by exhibiting a reddish brown to purplish brown, fibrillose pileus surface, such as, *A. brunneolus*, *A. dulcidulus*, *A. gemlii*, *A. megalosporus* and *A. patris*. However, *A. brunneolus*, *A. gemlii*, *A. megalosporus* and *A. patris* are easily distinguished by their larger spores (wider than 3.5 μm on average, Chen et al. 2012, Parra 2013). *Agaricus dulcidulus* differs in its smaller spores (4.3 \times 3 μm on average, Parra 2013).

According to the phylogenetic analyses, *A. fimbrimarginatus* shows close affinities to *A. robustulus*. However, the latter species differs in its robust sporocarps and larger spores and molecularly has four nucleotides in difference in the ITS sequences, two differences in LSU and with more than 20 differences in *tef-1 α* sequences.

Agaricus flammicolor Linda J. Chen, Callac, R.L. Zhao & K.D. Hyde, *sp. nov.* — MycoBank MB818050; Fig. 12, 13

Facesoffungi number. FoF 02286.

Etymology. The epithet '*flammicolor*' refers to the orange colour like a flame.

Pileus 4–7 cm diam, 2–4 mm thick at disc, at first parabolic, sometimes truncated at disc, then becoming hemispherical to convex, finally applanate; surface dry, with bright orange fibrils, densely at disc and radially arranged elsewhere, sometimes with fibrils bunching together into finely squamules, on a white background; strongly flavescent when bruised. Margin



Fig. 11 *Agaricus fimbrimarginatus* (holotype LD201250). a. Pileus surface; b. lamellae and stipe.

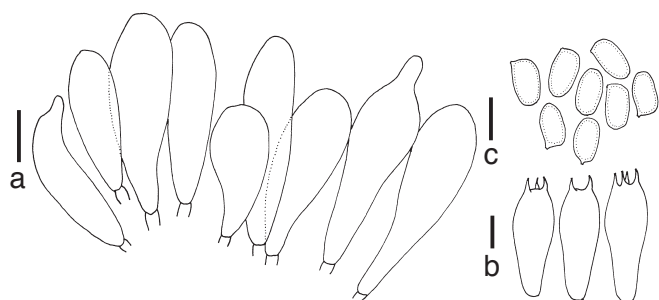


Fig. 12 Microscopic characters of *Agaricus flammicolor*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars: a = 10 μ m; b–c = 5 μ m.

incurved, shortly exceeding the lamellae, often with appendiculate remains of the annulus. *Lamellae* free, crowded, 3–4 mm broad, with intercalated lamellulae, first white, then pinkish brown, finally dark brown. *Stipe* 50–87 \times 4–6 mm (8–12 mm at base), clavate or cylindrical with slightly bulbous base, fistulose, surface above the ring smooth, below the ring heavily fibrillose, white, strongly flavescens when rubbed. *Annulus* simple, superous, membranous, upper surface smooth, lower surface fibrillose, white, except sometimes with orange tinge close to the margin at the lower surface. *Odour* of almonds. *Context* firm, white, slightly yellowish at stipe when cut. *Spores* 4.4–5.3(–6.2) \times 2.5–3.2 μ m, (\bar{x} = 4.9 \pm 0.25 \times 2.9 \pm 0.15 μ m, Q = 1.53–1.91, Q_m = 1.69 \pm 0.04, n = 20), ellipsoid to oblong, smooth, brown, thick-walled. *Basidia* 12–16 \times 5–6 μ m, broadly clavate, hyaline, smooth, 4-spored. *Cheilocystidia*

21–45 \times 10–25 μ m, abundant, simple, pyriform, broadly clavate or spheeropedunculate, rarely rostrate or mucronated, with yellowish pigment, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 5–7 μ m diam, cylindrical, hyaline, smooth, not constricted at the septa.

Macrochemical reactions — KOH reaction positive, bright yellow. Schäffer's reaction positive, bright orange.

Habitat — Solitary or scattered, on soil, under an *Albizia* tree.

Material examined. CHINA, Yunnan Prov., Cangyuan county, 11 July 2012, P. Callac & J. Guinbertau, ZRL2012270 (HMAS279148). — THAILAND, Chiang Rai Prov., Mae Fah Luang University, 28 July 2015, J. Chen, LD201502 (holotype MFLU16-0982); Chiang Mai Prov., Thang Thong village, 31 June 2012, J. Chen, LD201225 (MFLU12-0869).

Notes — *Agaricus flammicolor* is well characterized by a pileus surface covered with bright orange fibrils or fine squamules, spores on average less than 3 μ m in width, and with simple and large cheilocystidia containing yellowish pigments. Among the known taxa of *A.* sect. *Minores*, species with a pileus surface showing orange tinges are very rare. *Agaricus entibigae*, originally described from Hawaii, also has a pale orange to brownish orange pileus, but it differs in having its stipe surface base covered with reddish squamules, wider spores (3.8 μ m on average) and smaller cheilocystidia (Peterson et al. 2000). According to the phylogenetic results (Fig. 2), *A. flammicolor* is closely related to *A. badioniveus*/LD2012131, another new species treated in this study.



Fig. 13 *Agaricus flammicolor*. a. Overall morphology *in situ* (holotype LD201502), coin = 24 mm diam; b. pileus surface (holotype LD201502); c. lamellae and stipe surface (ZRL2012270).

Agaricus flavopileatus Linda J. Chen, Karun. & Callac, *sp. nov.*
— MycoBank MB818051; Fig. 14, 15

Facesoffungi number. FoF 02287.

Etymology. The epithet '*flavopileatus*' refers to the yellow pileus.

Pileus 4–6 cm diam, 3–4 mm thick at disc; at first parabolic, then hemispherical to plano-convex, truncate or slightly depressed at disc, finally applanate; surface dry, covered with greyish yellow to yellow ochre fibrils or squamules, densely at disc and radially or concentrically arranged elsewhere, on a white to yellowish white background; sometimes squamules are not uniformly distributed on pileus surface. Margin straight, shortly exceeding the lamellae, often with appendiculate remains of the annulus. *Lamellae* free, crowded, 3–4 mm broad, with intercalated lamellulae, pink to brown, finally chocolate brown. *Stipe* 30–75 × 4–12 mm, clavate or tapering upwards, with rhizomorphs, fistulose, surface above the ring smooth, below the ring fibrillose, white, strongly flavescent when bruised. *Annulus* single, membranous, superous, white, upper surface

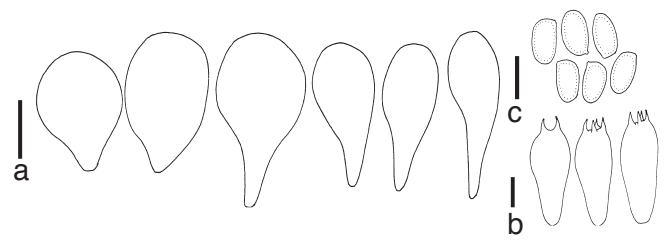


Fig. 14 Microscopic characters of *Agaricus flavopileatus*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars = 5 µm.

smooth, lower surface fibrillose. *Context* firm, white. *Odour* of almonds.

Spores 4.6–5.2(–5.3) × (2.6–)2.7–3.3(–3.4) µm, ($x = 4.8 \pm 0.13 \times 2.9 \pm 0.15$ µm, $Q = 1.42–1.87$, $Q_m = 1.65 \pm 0.1$, $n = 20$), ellipsoid, smooth, brown, thick-walled. *Basidia* 11–17 × 4–6 µm, clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 14–28 × 5–18 µm, abundant, simple, pyriform to broadly clavate, or sphaeropedunculate, with yellowish pigments, smooth. *Pleuro-*



Fig. 15 *Agaricus flavopileatus*. a. Overall morphology *in situ* (holotype MS596); b. lamellae and strong yellowing when bruised on stipe surface (holotype MS596); c. pileus surface (holotype MS596); d. overall morphology *in situ* (MS603); e. pileus surface (MS603).

cystidia absent. *Pileipellis* a cutis composed of hyphae of 4–14 μm wide, cylindrical, hyaline or with yellowish brown pigments, smooth, sometimes constricted at the septa.

Macrochemical reactions — KOH reaction positive, yellow. Schäffer's reaction positive, reddish orange on dry specimen.

Habitat — Solitary or scattered on soil, in forest.

Material examined. CHINA, Yunnan Prov., Mengsong, 21 July 2012, S.C. Karunaratna, MS596 (holotype MFLU16-0984); Yunnan Prov., Mengsong, 22 July 2012, S.C. Karunaratna, MS603 (MFLU16-0983, HMAS279150).

Notes — *Agaricus flavopileatus* is morphologically well characterized by the white to yellowish white pileus, radially or concentrically covered with greyish yellow to yellowish brown fibrils or squamules, small spores and the simple, pyriform, broadly clavate, or sphaeropedunculate cheilocystidia.

Comparing with other members of *A. sect. Minores*, which sometimes also have a yellowish to ochre pileus surface, *A. flavopileatus* can be distinguished as follows: *A. azoetes* and *A. pseudolutosus* have larger spores, with an average of $6.37 \times 4.78 \mu\text{m}$ and $5.7 \times 4.3 \mu\text{m}$, respectively (Peterson et al. 2000, Parra 2013); *A. comtus* has wider spores, on average $4.87 \times 3.55 \mu\text{m}$ (Parra 2013), and phylogenetically, it is quite distant from *A. flavopileatus* (Fig. 2); *A. luteoflocculosus* differs in having larger spores ($5.95 \times 4.1 \mu\text{m}$ on average), the lower side of the annulus is floccose and stipe surface has fibrillose woolly scales (Parra 2013).

Agaricus fulvoaurantiacus Linda J. Chen & Karun., *sp. nov.* — MycoBank MB818052; Fig. 16, 17

Facesoffungi number. FoF 02288.

Etymology. Refers to the tawny orange colour of the pileus.

Pileus 3.7–7 cm diam, 3–5 mm thick at disc, at first parabolic, then convex or plano-convex, finally applanate; surface dry, with light brownish yellow to brownish orange fibrils, densely at disc and radially arranged elsewhere, or sometimes squamose with appressed squamules or thick scales, against a white background. Margin incurved, shortly exceeding the

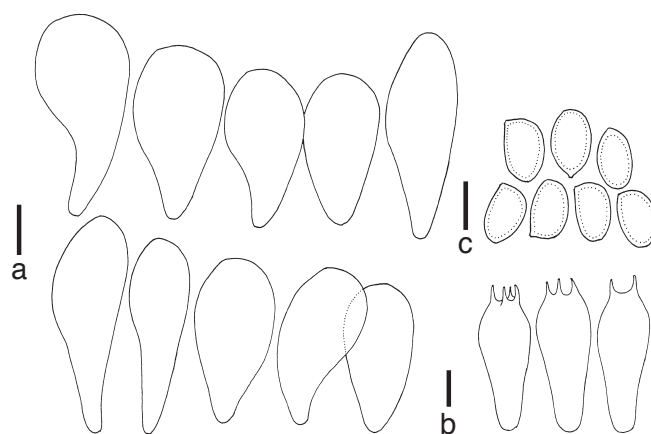


Fig. 16 Microscopic characters of *Agaricus fulvoaurantiacus*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars = 5 μm .

lamellae, often with appendiculate remains of the annulus. **Lamellae** free, crowded, 2–5 mm broad, with intercalated lamellulae, first white, then pinkish brown, finally dark brown. **Stipe** 50–70 \times 6–8 mm (11 mm at base), clavate, with numerous rhizomorphs, fistulose, surface above the ring smooth, below the ring with light yellowish brown appressed fibrillose scales, white, strongly flavescent when bruised. **Annulus** simple, superous, membranous, white, upper surface smooth, lower surface decorated with tiny yellowish flakes, connected with the stipe by cortinate fibrils. **Odour** of almonds. **Context** firm, white, flavescent when cut.

Spores (5.2–)5.6–6.1 \times 3.5–4.1 μm , ($x = 5.8 \pm 0.22 \times 3.8 \pm 0.18 \mu\text{m}$, $Q = 1.26\text{--}1.73$, $Q_m = 1.51 \pm 0.01$, $n = 20$), ellipsoid, smooth, brown, thick-walled. **Basidia** 16–18 \times 6–8 μm , clavate to broadly clavate, hyaline, smooth, 4-spored, rarely 2-spored. **Cheilocystidia** (12–)17–30 \times 9–13 μm , abundant, simple, pyriform, broadly clavate or sphaeropedunculate, hyaline or with yellowish pigments, smooth. **Pleurocystidia** absent. **Pileipellis** a cutis composed of hyphae of 6–12 μm diam, cylindrical, hyaline or with light yellow pigments, smooth, rarely constricted at the septa.



Fig. 17 *Agaricus fulvoaurantiacus*. a–b. Overall morphology *in situ* (holotype LD201404); c. scales on pileus surface (MS316); d. fibrils on pileus surface (MS549); e. annulus (MS549).

Macrochemical reactions — KOH reaction positive, bright yellow. Schäffer's reaction positive, bright orange.

Habitat — Solitary or gregarious on soil, in forest or tea plantations.

Material examined. CHINA, Yunnan Prov., Mengsong, 23 June 2012, S.C. Karunaratna, MS316 (MFLU16-0974, HMAS279151); Yunnan Prov., Mengsong, 11 July 2012, S.C. Karunaratna, MS549 (MFLU16-0978, HMAS279152); Yunnan Prov., Mengsong, 3 July 2014, J. Chen, LD201404 (holotype MFLU16-0980; isotype HMAS279149).

Notes — *Agaricus fulvoaurantiacus* is well characterized by a pileus surface covered with light brownish yellow to brownish orange fibrils or fibrillose squamules, concolorous fibrillose scales on the lower stipe surface, an annulus with tiny yellowish flakes on the lower surface, spores on average $5.8 \times 3.8 \mu\text{m}$, and the simple cheilocystidia, hyaline or containing yellowish pigments.

Generally speaking, *A. fulvoaurantiacus* is very similar to *A. luteofibrillosus* by having the same appearance of pileus and stipe. However, *A. luteofibrillosus* has narrower spores ($5.8 \times 3.2 \mu\text{m}$ on average) and different cheilocystidia which are sometimes in short chains (see *A. luteofibrillosus* below) or sep-

tate at the base (Li et al. 2016). According to the phylogenetic analyses (Fig. 2), they are closely related. Indeed, *A. fulvoaurantiacus* differs at four positions in ITS sequences, one position in LSU (except MS316 which is heteromorphic (C and T) at this position), and six positions in *tef-1 α* sequences.

Macromorphologically, *A. luteoflocculosus* roughly resembles *A. fulvoaurantiacus* by having the bright yellow fibrillose scales on both pileus and stipe surface. However, it differs by its smaller spores ($5.1 \times 3.7 \mu\text{m}$ on average) and the habitat on rotting seaweed of the species *Fucus vesiculosus* on the sea shore (Parra 2013).

Agaricus luteofibrillosus M.Q. He, Linda J. Chen & R.L. Zhao, Fung. Diversity 78: 126. 2016 — Fig. 18, 19

Pileus 3–10 cm diam, 4–6 mm thick at disc, at first parabolic, then hemispherical or plano-convex, finally applanate or plano-concave, occasionally with a slightly depressed centre; surface dry, initially and uniformly covered with appressed fibrils of a brownish orange tone and more densely at disc, with pileus expansion, the disc remains unbroken, disrupting into subtle squamules or triangular scales appressed or upturned else-



Fig. 18 *Agaricus luteofibrillosus*. a–c. Overall morphology, coin = 24 mm diam; d. annulus and stipe surface; e. flavescent when bruised.

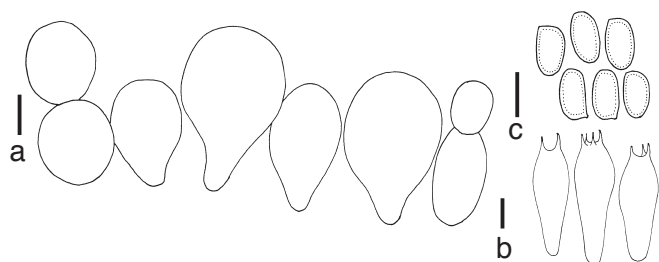


Fig. 19 Microscopic characters of *Agaricus luteofibrillosus*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars = 5 µm.

where, on a yellowish white background, flavescent when rubbed. Margin incurved or straight, not exceeding the lamellae, often with appendiculate remains of the annulus. *Lamellae* free, crowded, 3–7 mm broad, with intercalated lamellulae, first white, then brownish orange, finally dark brown. *Stipe* 40–120 × 3–15 (5–25 at base) mm, abruptly bulbous, or rounded with rhizomorphs, fistulose, surface above the ring smooth, below the ring fibrillose woolly of a brownish orange colour, yellowish discoloration when rubbed. *Annulus* simple, superous, thick when young, with cortinate fibrils connected with stipe, membranous when mature, fragile, smooth on both surfaces, white, sometimes with brownish orange tinge towards the margin. *Odour* of almonds. *Context* firm, white, discolouring slightly yellowish when cut.

Spores (4.7–)5.1–5.9(–6) × 2.8–3.5(–3.8) µm, ($x = 5.4 \pm 0.22 \times 3.2 \pm 0.19$ µm, $Q = 1.44–1.91$, $Q_m = 1.72 \pm 0.01$, $n = 20$), ellipsoid, smooth, brown, thick-walled. *Basidia* 16–20 × 6–8 µm, clavate to broadly clavate, hyaline, smooth, 4-spored, rarely 2-spored. *Cheilocystidia* 16–22(–30) × 8–15 µm, abundant, simple or sometimes in short chains (in this case, elements measuring 8–11 × 5–9 µm), globose, pyriform, or sphaeropedunculate, rarely clavate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 5–12.5 µm diam, cylindrical, hyaline or with light yellow pigment, smooth, occasionally constricted at the septa.

Macrochemical reactions — KOH reaction positive, bright yellow. Schäffer's reaction positive, bright orange.

Habitat — Caespitose or gregarious on soil, in *Fagaceae* and *Pinaceae* mixed forest.

Material examined. THAILAND, Chiang Rai Prov., Doi Mae Salong, 22 June 2015, J.Z. Sun, LD201501 (MFLU16-0981); Chiang Mai Prov., Tong Jown, 3 Aug. 2005, R.L. Zhao, ZRL2110 (BBH19490, HMAS279140); Chiang Mai Prov., Pathumvikaram Temple, 8 June 2006, Tim, ZRL3039 (BBH19545, HMAS279155); Chiang Mai Prov., Doi Suthep, 20 June 2010, K. Wisitrasameewong, NTT037.

Notes — *Agaricus luteofibrillosus* is a species recently described from China. It is morphologically characterized by its yellowish white pileus surface covered with brownish orange squamules or triangular scales and the stipe with concolour fibrils. Our collections match well with the original diagnosis, except for their slightly smaller spores (5.8 × 3.4 µm on average, Li et al. 2016), which can be considered as intraspecific variation. This is the first record of *A. luteofibrillosus* from Thailand.

Agaricus luteofibrillosus is most similar to *A. fulvoaurantiacus* in macro-morphology. The differences between the two species are noted in *A. fulvoaurantiacus*.

Agaricus luteopallidus Linda J. Chen, Karun., R.L. Zhao & K.D. Hyde, *sp. nov.* — MycoBank MB818053; Fig. 20, 21

Facesoffungi number. FoF 02289.

Etymology. Refers to the pallid yellow colour of the pileus.

Pileus 3–6 cm diam, 2.5–3 mm thick at disc, conico-truncate when young, then convex to hemispherical, finally appanate;

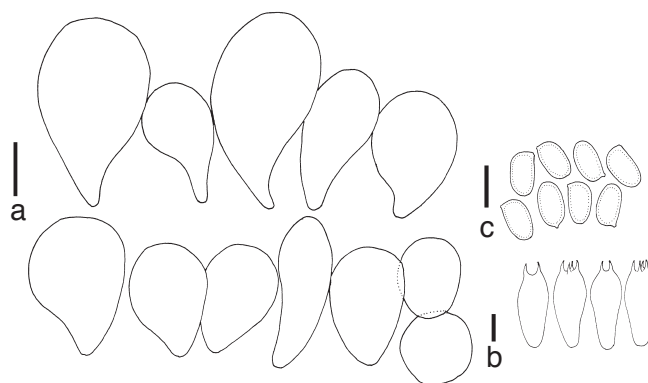


Fig. 20 Microscopic characters of *Agaricus luteopallidus*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars: a = 10 µm; b–c = 5 µm.

surface dry, with pallid yellow to light brownish yellow fibrils, densely at disc, with pileus expansion, outside the unbroken disc, the surface disrupts into finely triangular scales, on a white background; turning yellowish when rubbed. Margin straight, not exceeding the lamellae, often with appendiculate remains of the annulus. *Lamellae* free, crowded, ventricose, 3–4 mm broad, with intercalated lamellulae, first pink, then pinkish brown, finally dark brown. *Stipe* 65–95 × 5–11 mm, cylindrical or with slightly bulbous base, with numerous rhizomorphs, fistulose, surface above the ring smooth, below the ring fibrillose, white, strongly flavescent when bruised or by handling. *Annulus* simple, superous, cortinate when young, membranous when mature, fragile, white. *Context* firm, white, unchanging when cut. *Odour* of almonds.

Spores (4.5–)5–6 × (3–)3.2–4 µm, ($x = 5.4 \pm 0.36 \times 3.6 \pm 0.3$ µm, $Q = 1.38–1.83$, $Q_m = 1.52 \pm 0.02$, $n = 20$), ellipsoid, smooth, brown, thick-walled. *Basidia* 13–20 × 5.5–7 µm, clavate to broadly clavate, hyaline, smooth, 4-spored, rarely 2-spored. *Cheilocystidia* 14–28 × 10–22 µm, abundant, simple, rarely in short chains, globose to pyriform or sphaeropedunculate, rarely clavate, with yellowish pigments, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 4–11 µm diam, cylindrical, with yellowish membranous pigments, smooth, at times slightly constricted at the septa.

Macrochemical reactions — KOH reaction positive, bright yellow. Schäffer's reaction positive, bright reddish orange.

Habitat — Solitary, scattered or gregarious on soil, in grassland or rotted litter.

Material examined. THAILAND, Chiang Rai Prov., Parnae Lao Park, 2 Aug. 2006, R.L. Zhao, ZRL3088 (holotype BBH19604; isotype HMAS279147); Chiang Mai Prov., Doi Suthep, 29 June 2010, P. Sysouphanthong, NTF26; Chiang Rai Prov., Mae Fah Luang University park, 3 Aug. 2010, S.C. Karunarathna, NTS-CR01 (MFLU10-0674); 5 Mar. 2011, S.C. Karunarathna, SCK099 (MFLU11-1285); 5 June 2011, S.C. Karunarathna, SCK120 (MFLU11-1287); 5 July 2011, S.C. Karunarathna, SCK121 (MFLU11-1287); Chiang Mai Prov., MRC, 13 May 2011, S.C. Karunarathna, SCK138 (MFLU11-1296); Chiang Rai Prov., Mae Fah Luang University, 20 July 2012, J. Chen, LD2012113 (MFLU12-0950); 21 July 2012, J. Chen, LD2012120 (MFLU12-0956).

Notes — *Agaricus luteopallidus* is well characterized by having a pileus surface covered with pale yellow to light brownish yellow fibrils or triangular squamules, spores 5.4 × 3.6 µm on average, and the simple cheilocystidia containing yellowish pigments.

In general, several species resemble *A. luteopallidus* by having a pileus surface with yellowish tinge, and later covered with fibrillose scales, such as *A. xantholepis*, *A. azoetes* and *A. luteoflocculosus*. According to the original diagnosis, *A. xantholepis*, which has been considered as a synonym of *A. brunneolus* (Parra 2013), exhibits a distinctively bulbous base up to 15 mm broad and has smaller spores, 4–5.5 × 3 µm (Parra 2013). *Agaricus azoetes* was originally described from Hawaii



Fig. 21 *Agaricus luteopallidus*. a. Overall morphology *in situ* (SCK120); b. overall morphology at laboratory (holotype ZRL3088).

and can be easily distinguished from *A. luteopallidus* by its smaller sporocarps not exceeding 4.5 cm, wider basidiospores ($5.7 \times 4.3 \mu\text{m}$ on average), lacking of cheilocystidia and the arid habitats (Peterson et al. 2000). *Agaricus luteoflocculosus* differs from the new species by the floccose on the lower side of the annulus and fibrillose woolly scales on the stipe surface (Parra 2013).

According to the phylogenetic results, *A. luteopallidus* is closely related to *A. flavopileatus*. The latter differs at 6 positions in ITS sequences, and more than 20 positions in *tef-1a* sequences.

Agaricus patris Linda J. Chen, Callac, K.D. Hyde & R.L. Zhao, *sp. nov.* — MycoBank MB818054; Fig. 22, 23

Facesoffungi number. FoF 02290.

Etymology. This species honours all the fathers in the world but it is written in singular (*patris*: of the father) with a plural sense because the plural *patrum* (of the fathers) is very much alike to *matrum* an epithet already used in *Agaricus*.

Pileus 4.5–5 cm diam, 3–4 mm thick at disc; convex to applanate; surface dry, covered with purplish brown to reddish brown or dark purple fibrillose scales, dense at disc and progressively sparse towards the margin, on a greyish white background; no discoloration when rubbed. Margin incurved, then becoming straight, shortly exceeding the lamellae, often with appendiculate remains of the annulus. **Lamellae** free, crowded, 4 mm broad, with intercalated lamellulae, ventricose, pink to light brown, finally dark brown. **Stipe** 45–68 \times 5–7 mm (8–15 mm at base), cylindrical with a bulbous base, fistulose, surface above the ring smooth, below the ring tomentose, white, flavescent or orange-ochre when rubbed. **Annulus** simple, membranous, superous, white, fragile. **Context** firm, white, somewhat flavescent when cut. **Odour** of almonds.

Spores $(5.5\text{--})5.8\text{--}6.2\text{--}6.5 \times (3.3\text{--})3.5\text{--}4.0\text{--}4.2 \mu\text{m}$, ($x = 6 \pm 0.16 \times 3.7 \pm 0.15 \mu\text{m}$, $Q = 1.49\text{--}1.72$, $Q_m = 1.58 \pm 0.01$, $n = 20$), ellipsoid, rarely oblong, smooth, brown, thick-walled. **Basidia** $14\text{--}22 \times 6\text{--}7 \mu\text{m}$, clavate, hyaline, smooth, 4-spored, rarely 2-spored. **Cheilocystidia** $16\text{--}34 \times 7\text{--}13 \mu\text{m}$, simple,



Fig. 22 *Agaricus patris*. a. Pileus surface (holotype LD201224); b. lamellae and stipe (holotype LD201224); c. pileus surface (ZRL3101); d. section view (ZRL3101).

clavate to broadly clavate or sphaeropedunculate, hyaline or sometimes with yellowish pigments, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 6–13 μm wide, cylindrical, often with brownish pigments, constricted at the septa.

Macrochemical reactions — KOH reaction positive, yellow. Schäffer's reaction positive, reddish on dry specimen.

Habitat — Solitary, on soil of roadside.

Material examined. THAILAND, Chiang Mai Prov., Mae Pong Nature Trail, 3 June 2012, J. Chen, LD201224 (holotype MFLU2012-0868; isotype HMAS279139); Chiang Mai Prov., MRC, 13 Sept. 2006, R.L. Zhao, ZRL3101 (BBH19617, HMAS279143).

Notes — *Agaricus patris* is morphologically characterized by having a pileus surface covered with fibrillose scales, of variable colour ranging from purplish brown to reddish brown or dark purple, spores $6 \times 3.7 \mu\text{m}$ on average, and simple cheilocystidia. Indeed, in view of gross morphology, *A. patris* is hardly distinguished from many members of the section, such as *A. brunneolus*, *A. dulcidulus*, *A. gemlii*, and *A. megalosporus*. From average spore size, *A. patris* can be easily separated from *A. dulcidulus*, which has the smallest size within the section ($4.31 \times 3 \mu\text{m}$, Parra 2013). *Agaricus gemlii* differs in its habitat which is in damp Atlantic environments near the coast (Parra 2013). When the collections consist of robust, fleshy specimens with pilei exceeding 7 cm, *A. brunneolus* and *A. megalosporus*

are easily distinguished from *A. patris*; otherwise, the sequence data is essential for doubtless identification. According to the phylogenetic results, *A. patris* is closely related to *A. sodalis*, a species recently described from Thailand. However, the latter species differs by its pileus surface which is covered with violet brown fibrils, mainly densely arranged at the disc, rare or absent towards the margin and slightly shorter spores ($5.4 \times 3.6 \mu\text{m}$ on average, Liu et al. 2015). Phylogenetically, they differ at more than 15 positions in both ITS and *tef-1 α* sequences.

Agaricus purpureofibrillosus Linda J. Chen, R.L. Zhao & K.D. Hyde, *sp. nov.* — MycoBank MB818055; Fig. 24, 25

Facesoffungi number. FoF 02291.

Etymology. The epithet '*purpureofibrillosus*' refers to purplish fibrils on the pileus of this species.

Pileus 2–3 cm diam, 1 mm thick at disc; at first conical, then convex to plano-convex, finally applanate; surface dry, entirely covered with purplish fibrils, dense at disc and more sparse towards the margin, on a white background; strongly flavescent

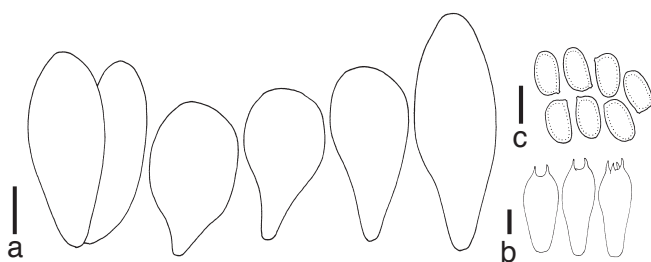


Fig. 23 Microscopic characters of *Agaricus patris*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars = 5 μm .

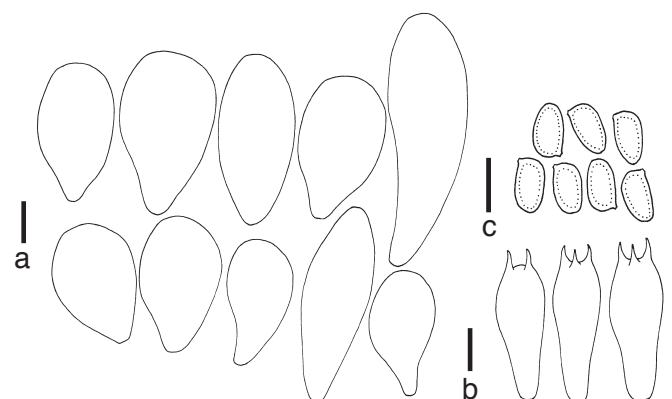


Fig. 24 Microscopic characters of *Agaricus purpureofibrillosus*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars = 5 μm .



Fig. 25 *Agaricus purpureofibrillosus* (holotype ZRL3080). a. Overall morphology in laboratory; b. section view; c. pileus surface; d. lamellae; e. annulus.

when margin is bruised. Margin straight, shortly exceeding the lamellae, often with appendiculate remains of the annulus. *Lamellae* free, crowded, 2 mm broad, with intercalated lamellulae, at first white, then pink, brown when mature. *Stipe* 36–45 × 3–6 mm, cylindrical fistulose, surface both above and below the ring smooth, silky, white, strongly flavescent when rubbed. *Annulus* simple, membranous, superous, white, fragile. *Context* firm, white, flavescent when cut. *Odour* of almonds.

Spores 4.5–5(–5.3) × 2.7–3 µm, ($\bar{x} = 4.9 \pm 0.12 \times 2.9 \pm 0.14$ µm, $Q = 1.25$ – 1.66 , $Q_m = 1.69 \pm 0.02$, $n = 20$), ellipsoid or amygdaliform, smooth, brown, thick-walled. *Basidia* 16–22 × 6–7 µm, clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 9–25 × 7–15 µm, abundant, simple or rarely septate at base, pyriform, sphaeropedunculate, or broadly clavate, with yellowish pigments, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 6–12.5 µm wide, cylindrical, often with crystalline brownish pigment inside, constricted at the septa.

Macrochemical reactions — KOH reaction positive, yellow. Schäffer's reaction positive, reddish orange on dry specimen.

Habitat — Solitary, in soil of roadside.

Material examined. THAILAND, Chiang Mai Prov., MRC, 10 July 2006, T.H. Li, ZRL3080 (holotype BBH19596; isotype HMAS279145); Chiang Rai Prov., Mae Sae, 28 July 2010, N. Tongklang, NTF063 (MFLU).

Notes — *Agaricus purpureofibrillosus* is morphologically well characterized by its slender sporocarps, a pileus surface entirely covered with purplish fibrils, small spores and simple cheilocystidia.

Among the members of *A. sect. Minores*, numerous species morphologically resemble *A. purpureofibrillosus* by sharing a slender sporocarp and purplish fibrillose pileus, such as *A. dulcidulus*, *A. gemlii*, *A. parvibicolor*, and *A. purpurellus*. However, they can be distinguished on account of the following

characters: *A. dulcidulus* has smaller spores (4.3×3 µm on average) and grows under broadleaved trees as *Quercus* or *Carpinus* (Parra 2013); *A. gemlii* differs in its larger spores (5.6×3.8 µm on average) and the habitat in damp Atlantic environments near the coast (Parra 2013); *A. parvibicolor*, a species recently described from Thailand, differs by the finely striate pileus margin and larger spores (5.2×3.3 µm on average; Liu et al. 2015); *A. purpurellus* differs in its wider spores (5.2×4 µm on average) and the distinctive habitat in conifer woods (Parra 2013). Otherwise, the molecular data is essential for unequivocal identification.

Agaricus robustulus Linda J. Chen, Callac, L.A. Parra, K.D. Hyde & De Kesel, *sp. nov.* — MycoBank MB818056; Fig. 26, 27

Facesoffungi number. FoF 02292.

Etymology. The epithet '*robustus*' refers to the small but robust appearance of the sporocarps of this species.

Pileus 2–6(–8.5) cm diam, 2–3 mm thick at disc; at first parabolic, becoming conico-convex to convex, sometimes with truncated centre, finally applanate; surface dry, with reddish brown or dark golden brown fibrils, densely at disc, soon with pileus expansion, outside the unbroken disc the surface disrupts into triangular scales, concentrically arranged on a dirty white background. Margin incurved, becoming straight when mature, not exceeding the lamellae, often with appendiculate remains of the annulus. *Lamellae* free, crowded, 4 mm broad, with intercalated lamellulae, subventricose to ventricose, at first white to pink, then light brown, finally dark brown. *Stipe* 20–40(–95) × 6–10 mm (13–14 mm at base), cylindrical with a bulbous base, fistulose, surface above the ring smooth, below



Fig. 26 *Agaricus robustulus*. a. Overall morphology *in situ* (holotype CA847); b. annulus and stipe (holotype CA847); c. overall morphology *in situ* (ADK2905); d. section view (ADK2905).

the ring fibrillose, sometimes with camel-coloured appressed scales, white, strongly flavescent when rubbed. *Annulus* simple, superous, membranous, occasionally somewhat floccose on the below side, white, fragile. *Context* firm, white, flavescent when cut. *Odour* of almonds.

Spores 5.4–6.2(–6.6) × 3–4 µm, (\bar{x} = 5.8 ± 0.25 × 3.7 ± 0.16 µm, Q = 1.47–1.74, Q_m = 1.56 ± 0.04, n = 20, Asiatic collections), ellipsoid, rarely oblong, smooth, brown, thick-walled; 4.4–6.1 × 3.1–3.6(–3.8) µm, (\bar{x} = 5.2 ± 0.43 × 3.3 ± 0.18 µm, Q = 1.37–1.79, Q_m = 1.56 ± 0.11, n = 30, African collection), ellipsoid, rarely oblong, smooth, brown, thick-walled. *Basidia* 12–22 × 6–9 µm, clavate to broadly clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 16–40(–66) × 14–20(–23) µm, simple, ovoid, pyriform or broadly clavate with a thin base, with yellowish pigments, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of cylindrical hyphae 4–13(–15) µm diam,

not or slightly constricted at the septa, the thicker the more constricted. With greyish brown diffuse internal pigment. One terminal element observed 8 µm wide with progressively attenuated and rounded apex.

Macrochemical reactions — KOH reaction positive, yellow. Schäffer's reaction positive, reddish on dry specimen.

Habitat — Solitary or scattered in sandy soil of secondary forest or in park.

Material examined. BENIN, Borgou Prov., Wari Maro, 19 Sept. 2000, A. De Kesel, ADK2905 (BR). — CHINA, Yunnan Prov., Lincang, Yongde County, 15 July 2012, Q.H. Yu, ZRL2012357 (HMAS273958). — MALAYSIA, Langkawi Island, 21 Apr. 2013, P. Callac, AK075 (KLU); 22 Apr. 2013, J. Ha, K Yun & P. Callac, MAR145 (KLU). — THAILAND, Chiang Mai Prov., Chiang Mai University, 25 July 2010, J. Guinberteau, CA847 (holotype MFLU16-0973); Chiang Mai Prov., Doi Suthep Pui National Park, 15 Aug. 2009, S.C. Karunaratna, NT055 (MFLU).

Notes — *Agaricus robustulus* is morphologically well characterized by its fleshy sporocarps, the reddish brown or dark golden brown, fibrillose or squamose pileus, spores with mean of 5.8 × 3.7 µm and simple cheilocystidia.

The average spore size of the new species is slightly different between Asian and African collections which can be considered as intraspecific variation. Several species morphologically resemble *A. robustulus* by having fleshy sporocarps, fibrillose or squamose pileus and variable colour from reddish brown to purplish brown, such as: *A. brunneolus*, *A. goossensiae*, and *A. megalosporus*. *Agaricus goossensiae* differs by its larger spores (6.3 × 4.4 µm on average, re-examination of the holotype GF929) and inconspicuous cheilocystidia; *A. brunneolus* and *A. megalosporus* are easily separated when their pilei exceed 7 cm diam. Otherwise, the sequence data are crucial for an accurate identification.

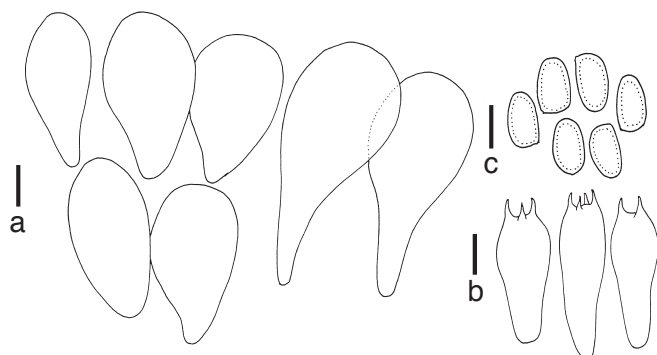


Fig. 27 Microscopic characters of *Agaricus robustulus*. a. Cheilocystidia; b. basidia; c. basidiospores. — Scale bars = 5 µm.

DISCUSSION

Advancements in the classification of the genus *Agaricus*

Zhao et al. (2011) provided evidence for seven strongly supported major tropical clades (TRI to TRVII) in the genus *Agaricus* that were not represented in the traditional classification mainly based on temperate species of the genus. Zhao et al. (2016) later proposed a new system of classification with taxonomic ranks based on the divergence times. Divergence times of between (18–)20–26 Ma or higher than 30 Ma were used to raise well-supported clades to sectional or subgenus taxonomic ranks, respectively, in *Agaricus*. As a result, 20 sections and five subgenera were proposed (Zhao et al. 2016). Among those, the following three sections were included in *A. subg. Minores*: *A. sect. Minores*, an unnamed section and *A. sect. Laeticolores*. The latter was so named because *A. rufoaurantiacus*, which was the single species of the tropical clade TRII that Zhao et al. (2016) included in their multi-gene analysis, had been previously placed in *A. sect. Laeticolores* by Heinemann (1961). In our multi-gene analyses, we included five species of the tropical clade TRII and also the type specimen of *A. laeticulus* that is the type of *A. sect. Laeticolores*. Firstly, our results demonstrate that this clade could not represent *A. sect. Laeticolores* because the type specimen of *A. laeticulus* was placed outside of this clade and nested in *A. sect. Minores*, indicating that Heinemann (1978) erroneously included *A. rufoaurantiacus* in *A. sect. Laeticolores*. Secondly, this clade diverged 31.01 Ma ago in our MCC tree and was therefore raised to the subgenus rank as *A. subg. Minoriopsis*. In addition, the newly described species *A. leucocarpus* not only diverged 27.54 Ma ago from the other members of the *A. subg. Minores*, but also, it is morphologically distinct from the species of *A. sect. Minores*. Therefore, it is excluded from *A. sect. Minores* and is considered as the type in a new section named *A. sect. Leucocarpi*.

In conclusion, *A. subg. Minores* still consists of three sections, but they have changed as follows with respect to the system proposed by Zhao et al. (2016): the new monospecific section *A. sect. Leucocarpi* is introduced, while the erroneously named *A. sect. Laeticolores* is excluded and raised to the subgenus rank as *A. subg. Minoriopsis*. The two remaining sections *A. sect. Minores* and an unnamed section are unchanged. The genus *Agaricus* currently comprises six subgenera (*Agaricus*, *Arvenses*, *Minores*, *Minoriopsis*, *Pseudochitonina*, and *Spissicaules*).

The reaction of Schäffer is among the most pertinent taxonomic characters at the sectional or subgeneric rank in the genus *Agaricus*. It is useful to identify both new section and new subgenus proposed in this study: it is positive dark reddish purple or reddish brown in dried specimens in the new subg. *Minoriopsis*, while the positive reaction is orange or red in the phylogenetically related subgenera *A. subg. Flavagaricus* and *A. subg. Minores* except in the new section *Leucocarpi* for which the reaction is negative.

What are the future prospects? Three putative sections, one in *A. sect. Minores* and two in *A. sect. Minoriopsis*, all corresponding to well-supported clades with stem ages earlier than 20 Ma, require further studies with more samples to be described and circumscribed as new sections. About a dozen of ungrouped species of *A. sect. Minores* also require supplementary studies and more specifically *A. huijsmanii* that may belong to a clade which diverged more than 20 Ma ago. In the genus *Agaricus* some species such as *A. martineziensis*, *A. heterocystis*, or the entire clade TRIV including *A. deserticola*, remain unclassified. We did not include these in the analysis because only ITS sequence data were available and preliminary tests based on

these data suggested they were not closely related to *A. subg. Minores*.

It can be noted that, except the clade TRIV, the six other well-supported tropical clades reported by Zhao et al. (2011) are now included in the new classification. TRI is *A. sect. Brunneopicti* within *A. subg. Pseudochitonina* (Chen et al. 2015, Zhao et al. 2016); TRII is *A. subg. Minoriopsis* (this study); TRIII is *A. sect. Amoeni* within *A. subg. Spissicaules* (Zhao et al. 2016); TRV, TRVI, and TRVII diverged too recently to be raised to sectional rank and thus remain in *A. sect. Minores* (Zhao et al. 2016, this study).

Reconstruction of *Agaricus* section *Minores* and evolutionary considerations

Phylogenetic reconstruction has been made so far in the genus *Agaricus* for five sections (*Arvenses*, *Bivelares*, *Brunneopicti*, *Nigrobrunnescentes*, and *Xanthodermatei*) based on ITS sequence data (Challen et al. 2003, Kerrigan et al. 2005, Thongklang et al. 2014, Chen et al. 2015, Gui et al. 2015, Parra et al. 2015). The successive studies of Zhao et al. (2011), Lebel (2013) and Zhao et al. (2016) included 25–30 species of *A. sect. Minores* roughly distributed in 5–7 major subclades. The present study includes 81 species distributed in 11 major subclades.

Delimitation of *A. sect. Minores* has always been problematic. Here, we present a section with estimated mean stem and crown ages of 30.06 and 24.19 Ma, respectively. This means that clades diverging between 24.19 Ma and 20 Ma could be also ranked at the sectional rank in the system of classification that we adopted with the condition they form a strongly supported clade. However, one early divergent clade is not well-supported in Fig. 1. It includes elements which are not grouped in the other analyses: [59] *A. huijsmanii* (Europe), [57] ZRL3102 (Thailand), and [27–28–29] the clade A-VI which is a curiosity since it includes *A. campbellensis* from a subantarctic island and *A. sp. GAL 5812* from arctic tundra in Alaska (Geml et al. 2008).

Only two regions are relatively well represented in our study: Europe with 19 species and Greater Mekong Subregion with 38 species. Completely different phylogenetic patterns are observed in these two regions. In Europe 16 species belong to the same clade A-VII and the three remaining samples are ungrouped. In Greater Mekong Subregion 32 species are distributed in 9 of the 11 clades. This difference neither results from the larger number of species from Greater Mekong Subregion, nor from the fact that Thailand (tropical) and Yunnan (subtropical) have been regrouped since the 27 species reported from Thailand are distributed in six clades and the 14 species reported from Yunnan (three are both in Yunnan and in Thailand) are also distributed in six clades. Therefore, compared to Europe, Greater Mekong Subregion is remarkable both by its species richness and by its phylogenetic diversity. Fig. 1 shows very well that the Greater Mekong Subregion diversity results from multiple species diversification that have occurred over the past 24 Myr, while most of the species today present in Europe result from a major diversification event that occurred relatively recently. The estimated stem and crown ages of the clade TR-VII were 14.30 and 10.63 Ma, respectively. This diversification might have followed the middle Miocene climatic optimum (15 Ma), likely accompanying the re-installation of the temperate vegetation in Europe (Pound et al. 2012). More investigations are required to establish to which extent, the species of the A-VII clade should be specifically adapted to temperate climates. In Fig. 3, with a broader sampling, two North American species and two species from Yunnan are also found in clade A-VII, but still no typically tropical species. Our sampling is not sufficient in non-European temperate regions to determine where this diversification occurred, but it is likely that climatic changes in Europe were favourable for its extension.

Seven secotioid species were included in the analysis. It was expected to establish whether they are related or how many times the gasteroid morphology, considered an adaption to xeric conditions, appeared independently. We did not found strong evidence for either, but only some indications that most species might be related. Presently, we cannot reject the hypothesis they would have a common ancestor.

Species diversity in *Agaricus* section *Minores*

The present study is far from comprehensive, however, it includes all of the tropical and temperate species of *A. sect. Minores* with ITS sequence data available in GenBank. In total, 81 phylogenetic species are recognized worldwide. Sequence data have not been obtained for the following dozen of species:

- six that we failed to sequence: one only known from Estonia (*A. luteoflocculosus*) and five from Hawaii (*A. azoetes*, *A. cheilotulus*, *A. entibigae*, *A. kiawetes*, and *A. xeretes*).
- two from Japan (Imai 1938) that are not traceable and lack a designated holotype (*A. comptulellus* and *A. semotellus*).
- The four remaining species are *A. johnstonii* from tropical North America (Murrill 1918), *A. nothofagorum* and *A. singeri* from tropical South America (Heinemann 1962, 1986, 1990, 1993) and *A. heinemanniensis* from India (Natarajan & Purushothama 1996).

Because of the rather brief descriptions generally given in the past and our inability to re-examine the type specimens of these species, we conservatively accept these as good species in *A. sect. Minores*. Therefore, *A. sect. Minores* may comprise at least 93 species.

Our results suggest that the species diversity of *A. sect. Minores* is largely underestimated. This is partly due to the fact that many areas, especially tropical regions, are underexplored. Secondly, before the application of molecular techniques (primarily DNA sequencing), species were typically identified and clumped by gross morphology. However, species diversity can be masked by a lack of discriminant morphological differences between cryptic species (Bickford et al. 2007). As a consequence, potentially valuable good species may have been misidentified as known taxa. This occurred for *A. marisae*, which was considered as *A. heinemannianus* until sequence data proved its novelty (Parra 2013). This is also the case in the present study for an unnamed species of *A. subg. Minoriopsis* represented by the samples HAI10186 and HAI10371 from North Carolina (USA), previously misidentified as *A. comtulus* (Didukh et al. 2005). Indeed, according to our phylogenetic analyses of hundreds of collections of the genus *Agaricus*, none of the European taxa except *A. subrufescens* are conspecific with tropical taxa of Southeast Asia. Therefore, the multiple records in literature of species like *A. purpurellus* from various regions including Africa, South Asia and tropical South America (Heinemann 1961, 1962, 1980, 1986, 1993) may appear doubtful and remains to be confirmed by sequencing specimens from these regions.

In *A. sect. Minores*, species richness appears much higher in tropical areas since 21 species are recognized throughout Europe while 27 species or putative species are recorded mainly from northern Thailand in the present study. Three of the 27 species have been also recorded from subtropical areas of Yunnan (China), while 11 other species have been recorded in Yunnan, but not in Thailand. This makes a total of 38 species reported from only two countries of the Greater Mekong Subregion. It is a good indication of the potential high species diversity in this area which also includes Cambodia, Lao, Myanmar, and Vietnam.

The distribution range of these species is unknown but it could be relatively broad for few of them such as *A. robustulus*, which has been reported from Africa, Malaysia, and Thailand. How-

ever, we did not find any conspecific record between samples from Europe, Greater Mekong Subregion, and Australasia which represent 83 % (67/81) of the species included in this study. It can be reasonably expected that at least 200 species of *A. sect. Minores* could occur worldwide.

Edibility of species of *A. sect. Minores* is generally unknown and they are not consumed because they are small-sized in general and hard to identify. However, to our knowledge intoxication has never been reported by any species. They have a pleasant odour and *A. brunneolus*, the largest European species of the section, sometimes abounds and is locally consumed (Cappelli 2011). In Greater Mekong Subregion, some medium-sized or attractive fleshy species such as *A. megalosporus* and *A. robustulus* should be tested for their edibility.

Acknowledgements The authors are grateful to P. Sysouphanthong, N. Thongklang, and J. Guinbertau, and to the team of the Mushroom Research Centre of the University of Malaya who helped with field work or provided us fungal specimens. This research was supported by The French Ministry of Foreign Affairs (project AGASIA of the regional program Bio-Asie); the National Natural Science Foundation of China (Project ID: 31470152 and 31360014), the Innovative Group of Edible Mushrooms Industry of Beijing (Project ID:BAIC05-2017), the Key Research and Development Program from Government of Guangxi Zhuang Autonomous Region (Project ID: 2016AB05317) and the Thailand Research Fund Grant (BRG 5580009) entitled 'Taxonomy, Phylogeny and Biochemistry of Thai Basidiomycetes'.

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