

Towards standardizing taxonomic ranks using divergence times – a case study for reconstruction of the *Agaricus* taxonomic system

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Abstract The recognition of taxonomic ranks in the Linnean classification system is largely arbitrary. Some authors have proposed the use of divergence time as a universally standardized criterion. *Agaricus* (Agaricaceae, Agaricales) is a mushroom genus that contains many species of high commercial value. Recent studies using ITS sequence data discovered 11 new phylogenetic lineages within the genus, however their taxonomic ranks were uncertain due to the lack of criteria to define them within traditional taxonomy. In this study, we analyzed ITS sequence data from 745 collections (nearly 600 being newly generated) including 86 from type specimens of previously recognized subgenera and sections. Many monophyletic groups were recognized, but most basal relationships were unresolved. One hundred and fourteen representatives of the identified ITS clades were selected in order to produce a multi-gene phylogeny based on combined LSU, *tef1 α* , and *rpb2* sequence data. Divergence times within the multi-gene phylogeny were estimated using BEAST v1.8.

Based on phylogenetic relationships and with respect to morphology, we propose a revised taxonomic system for *Agaricus* that considers divergence time as a standardized criterion for establishing taxonomic ranks. We propose to segregate *Agaricus* into five subgenera and 20 sections. Subgenus *Pseudochitonina* is substantially emended; circumscription of the subgenera *Agaricus* and *Flavoagaricus* is restricted to taxa of sections *Agaricus* and *Arvenses*, respectively; and two new subgenera (*Minores* and *Spissicaules*) are introduced. Within *Pseudochitonina*, sections *Bivelares*, *Brunneopicti*, *Chitonioides*, *Nigrobrunnescens*, *Sanguinolenti* and *Xanthodermatei* are maintained, but the latter two are reduced because we raise subsection *Bohusia* to sectional rank and a clade within section *Xanthodermatei* is formally introduced as section *Hondenses*; and sections *Rubricosi*, *Crassispori*, *Flocculenti*, and *Amoeni* are introduced. Section *Laeticolores* is placed in the subgenus *Minores* and sections *Rarolentes* and *Subrutilescentes* are placed in the subgenus

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Spissicaules. Twenty-two new species belonging to various sections are described. This work exemplifies that ITS data, while useful at lower taxonomic levels (i.e., detection of species and species groups), are of limited value for inferring deeper phylogenetic relationships. Finally, we suggest that the establishment of a standardized taxonomic system based on divergence times could result in a more objective, and biologically more meaningful, taxonomic ranking of fungi.

Keywords Agaricaceae · Divergence times · Multi-gene phylogenetics · Systematics · Taxonomy

Introduction

Current systematic and taxonomic research focuses on organizing and classifying species, genera, and higher ranks using a combination of characters, including molecular, morphological, ecological, and biogeographical data (e.g., Hyde et al. 2013, Maharachchikumbura et al. 2015). However, as several biologists have pointed out, the Linnean system lacks universal standardized criteria that can comprehensively recognize taxonomic ranks. Fifty years ago, W. Hennig suggested that a taxonomic rank should reflect its geological age, and that time divergence could be used as a universally standardized criterion in the systematics of all known organisms (Hennig 1966). However, it was not until the advent of DNA sequence data analyses and molecular clocks that this idea was further considered.

One of the first studies that aimed at reconciling taxonomic ranks and time divergence estimates is that of Avise and John (1999). These authors studied the molecular evolution of fish (14 species and 9 genera), anthropoid primates (7 species in several families), and fruit flies (14 species of *Drosophila*). They found a notable disparity in the ages of lineages and their taxonomic rank; for example, all fruit flies, shared common ancestors within a single genus more than 40 million years ago, while some primates formed different families a few million years ago, and cichlid fish were placed in several different genera that formed a few thousand years ago. As a result, they proposed a standardized temporal scheme for biological classification using approximate dates of nodes in an evolutionary tree, as a universal ranking criterion.

Advances in divergence time estimation based on DNA sequence data provide a way to convert molecular change into evolutionary time (Robinson and Robinson 2001; Drummond et al. 2006; Drummond et al. 2012). Fossil isotopic ages aid estimation of molecular divergence time analyses by providing points of calibration (Berbee and Taylor 2010). Two fungal fossil specimens – one within the order Agaricales (Berbee and Taylor 2010) and the other within the order Hymenochaetales (Smith et al. 2004) – provide a means for

divergence time calibrations in the basidiomycetes. Although recent reports have successfully established divergence times in fungi (Hibbett and Matheny 2009), these studies did not use these data for the establishment of a taxonomic system.

The saprotrophic genus *Agaricus* is the type genus of the family *Agaricaceae* (*Agaricales*), and has a worldwide distribution. Three hundred and seventy-five species are currently recognized in the genus (Zhao et al. 2011). However, the count should now exceed 400 due to recent discoveries of new species (Chen et al. 2012; 2015; Gui et al. 2015; Lebel 2013; Lebel and Syme 2012; Parra 2013; Wang et al. 2015; Zhao et al. 2012). *Agaricus* includes many species that have high nutritional and medicinal value, leading to it being a well-studied genus (Largeteau et al. 2011; Wisitrassameewong et al. 2012). Historically, the bulk of morphotaxonomic and systematic foundations in *Agaricus* has stemmed from mycological work in Europe, North America, tropical Asia, and Africa (Bohus 1995; Cappelli 1984; Heinemann 1978; Kerrigan 1986; Konrad and Maublanc 1952; Kühner and Romagnesi 1953; Möller 1950, 1952; Pilát 1951; Moser 1967–1983; Singer 1986; Wasser 1980). In recent years, *Agaricus* has been well-studied in Europe in a combination of molecular (ITS) and morphological analyses (Parra 2008, 2013).

The first molecular phylogenetic studies in *Agaricus* were carried out by Mitchell (1999) from the use of ITS and LSU sequence data from 16 species. Later, Geml et al. (2004) conducted analyses with a larger sample (42 species), revealing six clades. Some sections have been shown to be monophyletic (*Arvenses*, *Bivelares*, *Xanthodermatei*), while others (*Sanguinolenti* and *Spissicaules*) were not (Challen et al. 2003; Geml et al. 2004; Kerrigan et al. 2005, 2008). In a more global analysis based on the ITS sequence data from 128 species, an eight-section taxonomic system was proposed (Parra 2008, 2013), including seven new strongly supported and four moderately supported unnamed clades, mainly from tropical areas (Zhao et al. 2011). Recently, four additional sections, *Brunneopicti*, *Nigrobrunnescetes*, *Rarolentes* and *Subrutilescentes*, have been recognized from tropical and temperate regions (Chen et al. 2015; Parra et al. 2014; Wang et al. 2015; Kerrigan 2016 in press), bringing to twelve the number of currently recognized sections in *Agaricus*. Historically, the genus *Agaricus* has been separated into three subgenera *Agaricus*, *Lanagaricus* and *Conioagaricus* using morphological and macrochemical reactions characters (Heinemann 1956a, b; 1978; Singer 1986). The first two subgenera are well accepted by most mycologists (Parra 2013), however the subgenus *Conioagaricus* is heterogenous; its members have an epithelium pileipellis that is completely different from all *Agaricus* species from the other subgenera, which lead Heinemann (1978) to suggest that members of this section should be relocated to other genera, even though he erected this subgenus (Heinemann 1978). Unfortunately, DNA data

are not available for the subgenus *Conioagaricus*, which would help clarify its taxonomic position.

This study samples members of each of the twelve sections currently recognized and reports a new classification of subgenera and sections within *Agaricus* based on extensive morphological examination of specimens in combination with analysis of multi-gene sequence data. We propose a new classification system of the genus that reflects evolutionary divergence time, while concomitantly redefining taxonomic groups based on phylogenetic relationships.

Materials and methods

Sampling

Our dataset is composed of ITS sequences from 745 specimens (including 64 type specimens of known species and 22 herein newly introduced types), mainly from tropical and temperate areas of Asia (China, Thailand, and Malaysia), Europe (France, Spain) and North America (Canada and the U.S.A.), representing each of the 12 sections currently recognized (Parra 2008, 2013; Chen et al. 2015; Parra et al. 2014; Wang et al. 2015; Kerrigan 2016 in press), as well as seven new clades (Zhao et al. 2011). ITS sequence data for nearly 600 collections are new contributions. Nearly 150 other ITS sequences are mainly from our previous studies (Chen et al. 2015, Wang et al. 2015; Zhao et al. 2011). In the latter case, we only used sequence data attached to voucher specimens, or on whose identification we felt we could confidently rely (Callac and Guinberteau 2005; Chen et al. 2012, 2015; Kerrigan 2016 in press; Kerrigan et al. 2005, 2008; Thongklang et al. 2014; Parra 2008, 2013; Wang et al. 2015; Zhao et al. 2011, 2012, 2013).

Morphological examination

The protocol of morphological study, description of morphological characters and macrochemical reactions follow Largent's methodology (Largent 1986a, b) and our previous studies (Parra 2008; Zhao et al. 2011). Specimens are deposited in the Herbarium Mycologicum Academiae Sinicae (HMAS); CGAB herbarium (INRA); Luis A. Parra private herbarium (LAPAG); Mae Fah Luang University Herbarium (MFLU) and BIOTEC Bangkok Herbarium (BBH). Herbarium acronyms are those of Thiers (<http://sweetgum.nybg.org/ih/>). Faces of fungi numbers (Facesoffungi) and Fungal Names numbers (FN) are as explained in Jayasiri et al. (2015) and Fungal names (2013). Colour terms are according to Kornerp and Wanscher (1978) or the Online Auction Colour Chart™ (<http://OnlineAuctionColorChart.com>). Measurements of basidiospores, basidia and cheilocystidia are calculated from at least 20 measurements. Basidiospore

measurements include the range of all spores length by width, then \bar{x} , the mean of all spores \pm SD (Standard Deviation), Q, the range of the quotient length/width of all basidiospores, and Qm, the mean Q of all spores \pm SD.

PCR amplification and sequencing

Genomic DNA was extracted from dried specimens using E.Z.N.A. Forensic DNA Kit (OMEGA Bio-Tek, Norcross, GA, USA) or using 96-well plates (Dentinger et al. 2010). We used primers ITS4 and ITS5 (White et al. 1990) for the ITS1-5.8S-ITS2 region (ITS) of rDNA region, primers LROR and LR5 (Moncalvo et al. 2000, 2002) for the nuclear LSU-rDNA region, primers EF1-983 F and EF1-1567R (Morehouse et al. 2003) for translation elongation factor alpha (*tef-1 α*), and primers b6F and b7.1R (Matheny et al. 2007) for RNA polymerase II subunit II (RPB2). Genes were amplified by polymerase chain reaction (PCR) using the procedures mentioned in Zhao et al. (2011); Moncalvo et al. (2000); Morehouse et al. (2003); Matheny et al. (2007). The PCR products were sent to Shuoyang Biotech Company (Kunming city, China) for sequencing or were sequenced at the Royal Ontario facilities with an Applied Biosystems 3730 DNA Analyzer (Life Technologies, Carlsbad, CA, USA).

Preliminary ITS tree

The 745 ITS sequences used in this study could not be unambiguously aligned because of numerous insertions/deletions. Nevertheless, in order to obtain a better *a priori* understanding of clade diversity within *Agaricus*, we conducted ITS analyses of these sequences using neighbor-joining bootstrapping (NJ) in PAUP*4.0 from various alignment methods (not shown). This preliminary NJ tree served as a map for sampling strategies, both for sequencing other genes and for the divergence time analysis. One hundred and fourteen samples representing species from each of the recognized sections, main lineages within each section, and 'unclassified' taxa, were subsequently selected for generating LSU, *tef-1 α* , and *rpb2* sequence data.

Divergence time estimation of clades (crown vs. stem ages)

The term "clade" refers to a monophyletic group of organisms, which includes the most recent common ancestor of all of its members and their descendants (UCMP Glossary: Phylogenetics <http://www.ucmp.berkeley.edu/glossary/gloss1phylo.html>). The last common ancestor of a living clade plus all of its descendants is called the crown group. The stem group—which may include extinct lineages—refers to the lineage(s) that originated between the crown age and divergence time between the crown group and the sister clade

(Budd 2001; Budd and Jensen 2000). This means that for any given group of taxa the crown age is always younger than the stem age (Stadler et al. 2014). The length of branch between stem ancestor and crown clade depends on factors such as the timescale, the net diversification rate, and the species richness of the clade (McPeck and Brown 2007; Stadler et al. 2014). Here we use stem ages to reflect divergence times, as crown ages may change with taxon sampling.

Calibration strategies for estimation of the stem age of *Agaricus*

We conducted a fossil-calibrated analysis to estimate the time of divergence between *Agaricus* and its token outgroup *Heinemannomyces* within the Agaricomycetes. We selected 30 *Agaricus* species representative of the main sections and ITS clades within the genus for which we had LSU and *tef-1 α* sequence. We integrated these data into the Agaricomycetes matrix of Sánchez-Ramírez et al. (2015) that used two fossils: *Archaeomarasmius leggetti* Hibbett et al., an agaricoid fruiting body preserved in 90 million years old Dominican amber (Hibbett et al. 1997) as representative of the minimum age of *Agaricales*; and *Quatsinoporites cranhamii* S.Y. Sm. et al., a poroid fruiting body from Apple Bay on Vancouver Island from 113 million years ago (Smith et al. 2004) as representative of the minimum age of *Hymenochaetales*. Members of the *Boletales* + *Atheliales* were set as the sister clade of *Agaricales*. The *Agaricus* + *Heinemannomyces* sequence data were aligned using MUSCLE v3.6 Edgar (2004a, b), and then combined with a previously aligned matrix with these outgroups as in Sánchez-Ramírez et al. (2015) using Mesquite v2.75 (Maddison and Maddison 2007). Outgroups in this matrix included 84 species from Agaricomycetes, members from the orders *Agaricales*, *Boletales*, and *Hymenochaetales*, and the agaricoid clades hygrophoroid, tricholomatoid, and marasmioid. Introns in the *tef-1 α* region and a few poorly aligned sites in LSU were removed from the analysis.

Divergence times were estimated using BEAST v1.8 (Drummond et al. 2012). We first constructed an XML file with BEAUTI v1.8. Single gene alignments were imported as separate partitions. Clock and substitution models were set to be unlinked (independently estimated for each gene partition), while the tree prior parameters were set to be linked across partitions (concatenation). As substitution models, we used the GTR + G + I and SYM + G + I, for LSU and *tef-1 α* , respectively, as suggested by jModelTest v2 (Darriba et al. 2012). We used the uncorrelated lognormal relaxed clock model (Drummond et al. 2006; Lepage et al. 2007), specifying a gamma distribution for the *ulcd.mean* parameter with a shape of 1.0, scale of 1E-3, and offset 0. On the calibrated nodes, we specified a prior gamma distribution with an arbitrarily long

tail (scale of 50) and offset ages of 90 and 113 Ma for *Agaricales* and *Hymenochaetales*, respectively (Sánchez-Ramírez et al. 2015). We ran four independent Monte Carlo Markov Chains of 50 million generations, logging states every 5000 generations. We compared the log files of each run in Tracer v1.6 (Rambaut et al. 2013; <http://tree.bio.ed.ac.uk/software/tracer/>) evaluating convergence and mixing, ensuring that effective sample sizes (ESS) 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.

Divergence time estimation within *Agaricus*

We selected 114 taxa from a total of 745 *Agaricus* samples for dating analyses based on the preliminary ITS NJ tree and per-sample data completeness. We used ITS, LSU, *tef-1 α* , and *rpb2* sequence data, and *Heinemannomyces* as the outgroup taxon (Table 1). Each gene was separately aligned in MUSCLE 3.6 with default settings (Edgar 2004a, b). Ambiguously aligned regions were removed from phylogenetic analyses. Single-gene phylogenies were constructed using Bayesian methods and possible significant conflicts among the single-gene trees were tested (none were detected; data not shown). The dating analysis was performed from the concatenated data set in a manner similar to that described above, using BEAST v1.8; however, we calibrated the nodes by using the highest posterior density (HPD) age values for the *Agaricus* + *Heinemannomyces* divergence. In this case, we used normal distribution prior on the *treeModel.rootHeight* parameter, which had a mean of 66 Ma and a standard deviation of 1 Myr. The substitution models for LSU and *tef-1 α* were the same as above, while for ITS and RPB2 they were TVM + I + G and TrN + I + G, respectively, as indicated by jModelTest v2.

Establishment of subgenera and sections of *Agaricus*

We used the following criteria to recognize subgenera and sections: (i) they must be monophyletic and statistically well-supported in the multi-gene analyses; (ii) their respective stem ages should be roughly equivalent, and subgenera stem ages must be older than section stem ages; and (iii) they should be identifiable phenotypically, whenever possible. With regard to the second criterion, estimated stem ages for subgenera and sections were estimated to be ca. 30 Ma and ca. 20 Ma, respectively (Table 2). However we kept the divergence times of sections *Agaricus* and *Arvenses*, because they are the only sections in subgenera *Agaricus* and *Flavoagaricus*, respectively.

Table 1 Materials used in the analyses

No.	Subgenera	Sections	Species	Collection Number	LSU	ITS	Tef1- α	RPB2	Location
1	Minores (Clade A)	Minores (A1)	<i>A. aridicola</i>	LAPAG589	–	KT951331	–	KT951540	Castellón, Spain
2			<i>A. arrillagarum</i>	LAPAG810	–	KF447900	KT951592	KT951536	Asturias, Spain
3			<i>A. callacii</i>	AH-42929 ^a	–	KF447899	–	–	Canarias, Spain
4			<i>A. comtulus</i>	LAPAG724	KT951448	KT951332	KT951593	KT951532	Burgos, Spain
5			<i>A. edmondoi</i>	LAPAG412	KT951481	KT951326	KT951590	–	Segovia, Spain
6			<i>A. friesianus</i>	LAPAG592	–	KT951316	KT951594	KT951535	Pyrenées Atlantiques, France
7			<i>A. genlii</i>	AH-44510 ^a	–	KF447891	–	–	Canarias, Spain
8			<i>A. huijismanii</i>	LAPAG639	KT951444	KF447889	KT951571	–	Navarra, Spain
9			<i>A. kerriganii</i>	AH-44509 ^a	–	KF447893	–	–	León, Spain
10			<i>A. kerriganii</i>	LAPAG808	KT951442	KT951306	KT951589	KT951528	Madrid, Spain
11			<i>A. matrum</i>	AH-44506 ^a	–	KF447896	–	–	La Rioja, Spain
12			<i>A. matrum</i>	LAPAG855	KT951443	KT951310	KT951598	KT951533	Asturias, Spain
13			<i>A. pallens</i>	LAPAG926	–	KT951315	KT951591	KT951531	Lapland, Sweden
14			<i>A. pseudolutosus</i>	LAPAG454	KT951453	KT951329	KT951602	–	Burgos, Spain
15			<i>A. sp.</i>	ZRL2012012	KT951494	KT951359	KT951597	–	Yunnan, China
16			<i>A. sp.</i>	ZRL2012004	KT951457	KT951355	KT951608	KT951545	Yunnan, China
17			<i>A. sp.</i>	ZRLWXH3076	KT951458	KT951388	KT951612	–	Fujian, China
18			<i>A. sp.</i>	ZRLLD013	KT951516	KT951384	KT951604	–	Thailand
19			<i>A. sp.</i>	CA848	KT951445	JF727864	KT951605	KT951543	Thailand
20			<i>A. sp.</i>	CA921	–	KT951323	–	KT951544	–
21			<i>A. sp.</i>	ZRL2011156	KT951480	KT951352	KT951603	–	Yunnan, China
22			<i>A. sp.</i>	ZRLWXH3150	KT951447	KT951390	KT951609	–	Guangdong, China
23			<i>A. sp.</i>	CA846	KT951452	JF727865	KT951601	KT951538	Thailand
24			<i>A. sp.</i>	ZRL2012357	KT951496	KT951369	KT951610	–	Yunnan, China
25			<i>A. sp.</i>	ZRL2012199	KT951470	KT951367	KT951595	KT951537	Yunnan, China
26			<i>A. sp.</i>	ZRLWXH3067	KT951497	KT951387	KT951611	–	Jiangxi, China
27			<i>A. sp.</i>	ZRL2012576	KT951499	KT951372	KT951596	–	Tibet, China
28			<i>A. sp.</i>	ZRL2012714	KT951476	KT951381	KT951607	KT951542	Tibet, China
29			<i>A. sp.</i>	ZRL2011039	KT951449	KT951351	KT951606	KT951541	Yunnan, China
30		Laeticolores	<i>A. rufoaurantiacus</i>	LAPAM15	–	KT951313	KT951641	KT951558	Sosúa, Dominican Republic
31		Incertae Sedis or Laeticolores	<i>A. candidolutescens</i>	LD2012129 ^a	KT951525	KT951335	KT951616	–	Thailand
32			<i>A. sp.</i>	ZRLWXH3161	KT951526	KT951391	KT951615	–	Guangdong, China
33			<i>A. sp.</i>	LAPAM14	–	KT951312	KT951613	KT951557	Sosúa, Dominican Republic
34	Flavoagaricus (clade B)	Arvenses	<i>A. abruptibulbus</i>	ZRL2012005	KT951460	KT951356	KT951626	–	Yunnan, China
35			<i>A. arvensis</i>	MA-Fungi 80999 ^b	–	KF114474	–	–	Burgos, Spain

Table 1 (continued)

No.	Subgenera	Sections	Species	Collection Number	LSU	ITS	Tef1- α	RPB2	Location
36			<i>A. augustus</i>	ZRL2012598	KT951477	KT951373	KT951629	–	Tibet, China
37			<i>A. cf. essettei</i>	ZRL2012026	KT951450	KT951363	KT951630	–	Yunnan, China
38			<i>A. essettei</i>	ZRL2012599	KT951514	KT951374	KT951625	–	Tibet, China
39			<i>A. flocculosipes</i>	ZRL2012105	KT951463	KT951365	KT951618	–	Yunnan, China
40			<i>A. flocculosipes</i>	JM03-193	–	KT951325	KT951617	KT951555	Thailand
41			<i>A. gemellatus</i>	ZRL2012631	KT951478	KT951380	KT951623	–	Tibet, China
42			<i>A. subrufescens</i>	CA276	KT951461	KT951317	KT951631	–	–
43			<i>A. subrufescens</i>	ZRL2012722	KT951451	KT951383	KT951632	–	Yunnan, China
44			<i>A. sp.</i>	ZRL2012630	KT951495	KT951379	KT951621	–	Tibet, China
45			<i>A. sp.</i>	ZRL2012030	KT951455	KT951364	KT951628	–	Yunnan, China
46			<i>A. sp.</i>	ZRL2012608	KT951498	KT951377	KT951627	–	Tibet, China
47			<i>A. sp.</i>	ZRL2012002	KT951462	KT951354	–	–	Yunnan, China
48			<i>A. sp.</i>	ZRL2012604	KT951515	KT951375	KT951620	–	Tibet, China
49			<i>A. sp.</i>	SHY2011073117	KT951459	KT951407	KT951622	–	Yunnan, China
50	Spissicaules (clade C)	Subrutilescentes (C1)	<i>A. parasubrutilesces</i>	ZRL2011027	KT951521	KT951350	KT951583	–	Yunnan, China
51			<i>A. parasubrutilesces</i>	ZRL2012025	KT951487	KT951362	KT951584	–	Yunnan, China
52			<i>A. parasubrutilesces</i>	SHY2011073115	–	KT951396	–	–	Yunnan, China
53			<i>A. parasubrutilesces</i>	ZRL2011015	–	KT951397	–	–	Yunnan, China
54			<i>A. parasubrutilesces</i>	ZRL3020	–	KT951401	–	–	Thailand
55			<i>A. parasubrutilesces</i>	ZRLWXH3151	–	KT951402	–	–	Guangdong, China
56			<i>A. parasubrutilesces</i>	ZRL2012196	–	KT951405	–	–	Yunnan, China
57			<i>A. parasubrutilesces</i>	ZRL2014076 ^a	–	KT951410	–	–	Yunnan, China
58			<i>A. parasubrutilesces</i>	ZRL2014086	–	KT951411	–	–	Yunnan, China
59			<i>A. parasubrutilesces</i>	ZRL2014090	–	KT951412	–	–	Yunnan, China
60			<i>A. parasubrutilesces</i>	ZRL2014113	–	KT951417	–	–	Yunnan, China
61			<i>A. parasubrutilesces</i>	ZRL2014114	–	KT951413	–	–	Yunnan, China
62			<i>A. parasubrutilesces</i>	ZRL2014116	–	KT951415	–	–	Yunnan, China
63			<i>A. parasubrutilesces</i>	ZRL2014416	–	KT951415	–	–	–
64			<i>A. parasubrutilesces</i>	ZRL2014418	–	KT951419	–	–	–
65			<i>A. parasubrutilesces</i>	ZRL2014419	–	KT951416	–	–	–
66			<i>A. parasubrutilesces</i>	ZRL2014448	–	KT951420	–	–	Yunnan, China
67			<i>A. inthanonensis</i>	LDMS22	–	KT951423	–	–	Yunnan, China
68			<i>A. inthanonensis</i>	ZRLD014 ^a	–	KT951422	–	–	Thailand
69			<i>A. linzhienensis</i>	ZRL2012618 ^a	KT951503	KT951378	KT951582	KT951553	Tibet, China
70			<i>A. brunneopileatus</i>	ZRL2012115 ^a	KT951489	KT951404	KT951587	–	Yunnan, China

Table 1 (continued)

No.	Subgenera	Sections	Species	Collection Number	LSU	ITS	Tef1- α	RPB2	Location
71			<i>A. brunneopileatus</i>	ZRL2014144	–	KT951414	–	–	
72			<i>A. subrutilescens</i>	ZRLWXH3276	KT951522	KT951392	KT951585	KT951554	China
73			<i>A. subrutilescens</i>	ZRLWXH3316	–	KT951403	–	–	China
74			<i>A. subrutilescens</i>	ECV2418	–	KT951421	–	–	
75			<i>A. sp.</i>	ZRL160	KT951486	KT951345	KT951586	–	Thailand
76			<i>A. sp.</i>	SHY2012070604	KT951475	KT951341	KT951581	KT951552	Yunnan, China
77			<i>A. sp.</i>	CA583	–	JF797192	–	–	
78			<i>A. sp.</i>	F2255	–	JF797198	–	–	
79			<i>A. sp.</i>	ZRL2014289	–	KT951418	–	–	
80			<i>A. sp.</i>	RWK1940	–	AY943973	–	–	USA
81		<i>Amoeni</i> (C2)	<i>A. amoenus</i>	ZRL2010072 ^a	KT951524	KT951348	KT951638	–	Yunnan, China
82			<i>A. gratulens</i>	ZRL3093 ^a	KT951488	JF691548	–	KT951560	Thailand
83			<i>A. kivuensis</i>	Rammeloo5756	–	JF514541	–	–	
84			<i>A. stuehpenensis</i>	LD2012100 ^a	–	KT951409	–	–	Thailand
85			<i>A. sp.</i>	F2467	–	JF797200	–	–	
86			<i>A. sp.</i>	LAPAF3	–	JF797184	–	–	Plateaux, Togo
87			<i>A. sp.</i>	NTT42	–	JF514538	–	–	Thailand
88			<i>A. sp.</i>	NYS122	–	JF514540	–	–	Thailand
89		<i>Rarolentes</i> (C3)	<i>A. albosquamosus</i>	LD2012192 ^a	KT951520	KT951394	KT951636	–	Thailand
90			<i>A. albosquamosus</i>	LD201235	–	KT951395	–	–	Thailand
91			<i>A. hanthanaensis</i>	SK2012 ^a	–	KT951424	–	–	
92			<i>A. leucolepidotus</i>	LD201214 ^a	KT951519	KT951336	KT951635	–	Thailand
93			<i>A. sp.</i>	AW145	–	KT951308	KT951637	KT951550	Awenda, Canada
94			<i>A. sp.</i>	JH105	KT951490	KT951324	–	KT951551	Jokers Hill, ON, Canada
95			<i>A. sp.</i>	CA279	–	JF797191	–	–	
96			<i>A. sp.</i>	F2301	–	JF727854	–	–	
97			<i>A. bresadolanus</i>	CA177	–	DQ185570	–	–	
98			<i>A. lanipes</i>	CA406	–	JF797190	–	–	
99			<i>A. litoralis</i>	LAPAG420	KT951483	KT951327	KT951572	–	Burgos, Spain
100			<i>A. litoralis</i>	CA829	–	JF727867	–	–	
101			<i>A. litoralis</i>	LAPAG475	–	KT951393	–	–	Burgos, Spain
102			<i>A. planipileus</i>	ZRL2011248	–	KT951400	–	–	Yunnan, China
103			<i>A. planipileus</i>	ZRL2011250 ^a	–	KT951398	–	–	Yunnan, China
104			<i>A. lanipedisimilis</i>	ZRL2012151	KT951484	KT951366	KT951578	–	Yunnan, China
105			<i>A. lanipedisimilis</i>	ZRL2012193 ^a	–	KT951399	–	–	Yunnan, China

Table 1 (continued)

No.	Subgenera	Sections	Species	Collection Number	LSU	ITS	Tef1- α	RPB2	Location
106			<i>A. litoraloides</i>	ZRL2011249	KT951523	KT951353	KT951580	–	Yunnan, China
107			<i>A. litoraloides</i>	SHY2011073026 ^a	–	KT951408	–	–	
108			<i>A. sp.</i>	ZRLQHY43	KT951485	KT951386	KT951579	–	Shandong, China
109			<i>A. sp.</i>	RC_GUY07019	–	JF797199	–	–	
110			<i>A. sp.</i>	ZRL2012657	–	KT951406	–	–	Tibet, China
111	<i>Pseudochitonina</i> (clade D)	<i>Chitonoides</i> (D1)	<i>A. bernardiformis</i>	CA433/AVM917	KT951467	KT951321	KT951577	–	
112			<i>A. cf. bernardi</i>	CA383	KT951469	KT951319	KT951576	–	
113			<i>A. gennadii</i>	CA339	–	KT951318	KT951575	KT951547	
114			<i>A. pilosporus</i>	LAPAG227	–	KT951425	–	–	Burgos, Spain
115			<i>A. nevoi</i>	LAPAG535	–	KT951330	KT951574	–	Teruel, Spain
116			<i>A. nevoi</i>	LAPAG257	KR006606	KM657922	KR006635	–	Burgos, Spain
117			<i>A. bisporus</i>	LAPAG446	KR006611	KM657920	KR006640	–	Burgos, Spain
118		<i>Bivelares</i> (D2)	<i>A. bitorquis</i>	WZR2012827	KT951492	KM657916	KT951647	–	Xingjiang, China
119			<i>A. bitorquis</i>	CA427	KT951491	KT951320	KT951646	–	
120			<i>A. sinodeliciosus</i>	WZR2012822	KT951518	KM657907	KT951648	–	Xingjiang, China
121			<i>A. cupressicola</i>	LAPAG889	KT951465	KT951334	KT951649	–	Roma, Italy
122		<i>Hondenses</i> (D3)	<i>A. biannulatus</i>	LAPAG611 ^a	–	JF896229	–	–	Sardinia, Italy
123			<i>A. freirei</i>	CA186	–	DQ185553	–	–	
124			<i>A. hondensis</i>	RWK1938	–	DQ182513	–	–	USA
125			<i>A. phaeolepidotus</i>	CA217	–	DQ185552	–	–	
126			<i>A. grandionyceus</i>	ZRL2012611 ^a	KR006624	KM657879	KR006652	–	Tibet, China
127									
128			<i>A. atrodiscus</i>	LD2012185 ^a	KT951473	KT284912	KT951653	–	Thailand
129			<i>A. bisporiticus</i>	LD2012111	KT951507	KJ575611	KT951650	–	Thailand
130			<i>A. bisporiticus</i>	MCR25	–	KJ575608	–	–	Pakistan
131			<i>A. fusopunctatus</i>	LD2012115	–	KJ575612	–	–	Thailand
132			<i>A. lasibarii</i>	LAPAG115 ^a	–	AY943975	–	–	Landes, France
133			<i>A. microvohatulus</i>	LD201271	KT951508	KJ575614	KT951651	–	Thailand
134			<i>A. murinocephalus</i>	ZRL3044	–	JF691555	–	–	Thailand
135			<i>A. parvigrinus</i>	CA158 ^a	–	AY899267	–	–	
136			<i>A. tibetensis</i>	ZRL2012585 ^a	KR006633	KM657895	KR006658	–	Tibet, China
137			<i>A. lollocanensis</i>	MATA688	–	AY703913	–	–	
138			<i>A. lythocarpus</i>	ZRLWXH3077 ^a	KR006618	KM657889	KR006645	–	Fujian, China
139			<i>A. xanthodermulus</i>	CA160 ^a	–	AY899273	–	–	
140			<i>A. xanthodermus</i>	LAPAG387	KR006609	KM657923	KR006638	–	Soria, Spain

Table 1 (continued)

No.	Subgenera	Sections	Species	Collection Number	LSU	ITS	Tef1- α	RPB2	Location
141			<i>A. xanthodermis</i>	CA15	–	AY899271	–	–	
142			<i>A. xanthosarcus</i>	Goossens5415	–	JF514523	–	–	
143			<i>A. sp.</i>	RP32	–	KT951340	KT951652	KT951559	Rogers Park, Canada
144			<i>A. sp.</i>	ZRL2012629	KR006627	KM657890	KR006656	–	Tibet, China
145	<i>Trisulphurati</i> (D5)		<i>A. ignicolor</i>	ZRL2132 ^a	–	JF691558	–	–	Thailand
146			<i>A. trisulphuratus</i> complex	LAPAF7	KR006605	KM657924	KR006634	–	Plateaux, Togo
147			<i>A. trisulphuratus</i> complex	Swk079	KT951472	KT951343	KT951561	–	Lanjak-Entimau, Malaysia
148			<i>A. trisulphuratus</i> complex	LAPAF4	–	JF727843	–	–	Plateaux, Togo
149			<i>A. trisulphuratus</i> complex	ZRL2128	–	JN664955	–	–	Thailand
150			<i>A. trisulphuratus</i> complex	ZRL3014	–	KT951436	–	–	Thailand
151			<i>A. trisulphuratus</i> complex	ZRL3072	–	KT951437	–	–	Thailand
152			<i>A. trisulphuratus</i> complex	ZRL2123	–	JF691557	–	–	Thailand
153			<i>A. trisulphuratus</i> complex	ZRL2014023	–	KT951428	–	–	China
154			<i>A. trisulphuratus</i> complex	ZRL2014024	–	KT951429	–	–	China
155			<i>A. trisulphuratus</i> complex	ZRL2014025	–	KT951430	–	–	China
156			<i>A. trisulphuratus</i> complex	ZRL2014026	–	KT951431	–	–	China
157			<i>A. trisulphuratus</i> complex	ZRL2014030	–	KT951432	–	–	China
158	<i>Crassispori</i> (D6)		<i>A. campestroides</i>	LAPAF2	–	JF727842	–	–	Plateaux, Togo
159			<i>A. lamellicistans</i>	ZRL3099 ^a	–	JF691556	–	–	Thailand
160			<i>A. varicystis</i>	LD201228	–	KT951426	–	–	Thailand
161			<i>A. varicystis</i>	LD201234^a	KT951517	KT951339	KT951562	–	Thailand
162			<i>A. sp.</i>	ZRL2043	–	JF691553	–	–	Thailand
163			<i>A. sp.</i>	ZRL2085	–	KT951434	–	–	Thailand
164	<i>Sanguinolenti</i> (D7)		<i>A. benesii</i>	LAPAG283	–	JF797179	–	–	Burgos, Spain
165			<i>A. dilutibrunneus</i>	ZRL2012010^a	KT951512	KT951358	KT951569	–	Yunnan, China
166			<i>A. sylvaticus</i>	ALG07_213	–	KT951307	KT951567	KT951546	Algonquin, ON, Canada
167			<i>A. sylvaticus</i>	LAPAG382	KR006608	KM657929	KR006637	–	Burgos, Spain
168			<i>A. sylvaticus</i>	ZRL2012013	KT951500	KT951360	KT951570	–	Thailand
169			<i>A. sylvaticus</i>	ZRL2012568	KT951501	KT951371	KT951568	–	Tibet, China
170	<i>Bohusia</i> (D8)		<i>A. bohussii</i>	LAPAG562	KR006613	KM657928	KR006641	–	Madrid, Spain
171			<i>A. crassiquamosus</i>	ZRL2012607^a	KT951510	KT951376	KT951645	–	Tibet, China
172			<i>A. sp.</i>	WC913	–	AY484684	–	–	
173			<i>A. haematinus</i>	ZRL2109	–	KT951435	–	–	Thailand
174			<i>A. haematinus</i>	ZRL2136 ^a	–	JF691552	–	–	Thailand
175			<i>A. pseudolangei</i>	ZRL3012 ^a	–	JF691551	–	–	Thailand

Table 1 (continued)

No.	Subgenera	Sections	Species	Collection Number	LSU	ITS	Tef1- α	RPB2	Location
176		<i>Brunneopicti</i> (D9)	<i>A. bingensis</i>	ADK1992	–	KJ540954	–	–	Atakora, Bénin
178			<i>A. brunneopictus</i>	ADK2564	–	JF514518	–	–	Plateau Atlantique, Bénin
178			<i>A. brunneosquamulosus</i>	ZRL4017	–	JF691549	–	–	Thailand
179			<i>A. brunneosquamulosus</i>	LD2012105	–	KJ540968	–	–	Thailand
180			<i>A. chiangnaiensis</i>	NTS113	–	JF514531	–	–	Thailand
181			<i>A. duplovingulatus</i>	ZRL3064	–	KJ540966	–	–	Thailand
182			<i>A. megacystidiatus</i>	LD2012179	–	KF305946	–	–	Thailand
183			<i>A. niveogramulatus</i>	LD201124	–	KJ540959	–	–	Thailand
184			<i>A. sordidocarpus</i>	LD201237	–	KJ540946	–	–	Thailand
185			<i>A. subsaharianus</i>	ADK4732	–	JF440300	–	–	Ouagadougou, Burkina Faso
186			<i>A. toluenolens</i>	CA911	–	KJ540947	–	–	–
187			<i>A. sp.</i>	Swk014	KT951482	KT951342	KT951654	KT951549	Lanjak-Entimau, Malaysia
188			<i>A. sp.</i>	ZRL2012267	KT951504	KT951368	KT951655	–	Yunnan, China
189			<i>A. sp.</i>	ZRL133	KT951505	KT951344	KT951656	–	Thailand
190		<i>Flocculenti</i> (D10)	<i>A. erectosquamosus</i>	LD2012165^a	KT951509	KT951338	KT951565	–	Thailand
191			<i>A. pallidobrunneus</i>	ZRL2012358^a	KT951471	KT951370	KT951566	–	Yunnan, China
192		<i>Nigrobrunnescens</i> (D11)	<i>A. biberi</i>	LAPAG687	KR006614	KM657919	KR006642	KT951548	Hungary
193			<i>A. boisseletii</i>	CA123	–	DQ182531	–	–	–
194			<i>A. caballeri</i>	AH-44503 ^a	–	KJ575605	–	–	Zaragoza, Spain
195			<i>A. destardini</i>	WZR2012907	KT951474	KM657901	KT951644	–	Xinjiang, China
196			<i>A. erythrosarx</i>	MURU6080 ^a	–	JF495068	–	–	–
197			<i>A.</i>	–	–	KJ577973	–	–	–
198			<i>A. nigrobrunnescens</i>	DEH632	–	JX308267	–	–	Hawaii, USA
199			<i>A. padanus</i>	WZR2012903	KR006616	KM657903	KR006644	–	Xinjiang, China
200			<i>A. pattersoniae</i>	RWK1415	–	AY943974	–	–	–
201			<i>A. dolichopus</i>	ZRL2012715	KT951502	KT951382	KT951573	–	Tibet, China
202		<i>Rubricosi</i> (D12)	<i>A. dolichopus</i>	ZRL2014120^a	–	KT951433	–	–	–
203			<i>A. kunningensis</i>	ZRL2012015	KT951506	KT951361	KT951642	–	Yunnan, China
204			<i>A. kunningensis</i>	ZRL2012007 ^a	–	KT951427	–	–	Yunnan, China
205			<i>A. magnivelaris</i>	F2389	–	JF727851	–	–	–
206			<i>A. variabilicolor</i>	ZRL4002	–	KT951438	–	–	Thailand
207			<i>A. variabilicolor</i>	ZRL4007 ^a	–	KT951439	–	–	Thailand
208			<i>A. variabilicolor</i>	ZRL4012	–	KT951440	–	–	Thailand
209			<i>A. sp.</i>	ZRLWXH3078	KT951464	KT951464	KT951643	–	Fujian, China
210			<i>A. sp.</i>	ZRLWXH3140	–	KT951441	–	–	Guangdong, China

Table 1 (continued)

No.	Subgenera	Sections	Species	Collection Number	LSU	ITS	Tef1- α	RPB2	Location
211			<i>A. sp.</i>	NT020	–	JF797197	–	–	Thailand
212		<i>Incertae Sedis</i>	<i>A. sp.</i>	LD2012162	KT951493	KT951337	KT951563	–	Thailand
213		<i>Incertae Sedis</i>	<i>A. sp.</i>	ZRL2010099	KT951479	KT951349	KT951564	–	Yunnan, China
214		<i>Incertae Sedis</i>	<i>A. nigrogracilis</i>	ZRL2012014 ^a	KR006621	KM657882	KR006647	–	Yunnan, China
215		TRa	<i>A. sp.</i>	CA820	–	JF727861	–	–	–
216	<i>Agaricus</i> (Clade E)	<i>Agaricus</i>	<i>A. campestris</i>	CA637	KT951468	KT951322	KT951633	–	–
217			<i>A. campestris</i>	LAPAG370	KR006607	KM657927	KR006636	KT951556	Madrid, Spain
218			<i>A. sp.</i>	ZRL2010010	KT951511	KT951347	KT951639	–	Thailand
219			<i>A. sp.</i>	ZRLLD026	KT951513	KT951385	KT951640	–	Thailand
220			<i>A. sp.</i>	ZRL2012006	KT951466	KT951357	KT951634	–	Yunnan, China
221		unknown	<i>A. cf. goossensiae</i>	ADK2171	–	JF514517	–	–	Borgou, Bénin
222		unknown	<i>A. sp.</i>	CA486	–	JF797189	–	–	–
223	Outgroup		<i>Heinemannomyces sp.</i>	ZRL185	KT951527	KT951346	KT951657	–	Thailand

^a means Type or Isotype; ^b means epitype or future type; Collecting number in **BOLD** means used in all trees, in regular means only used in ITS trees

Table 2 Divergence times with subgenera and sections

Subgenera	Sections	Mean of stem age (Ma)
<i>Agaricus</i> (clade E)		30.57
	<i>Agaricus</i>	30.57
<i>Flavoagaricus</i> (clade B)		30.06
	<i>Arvenses</i>	30.06
<i>Minores</i> (clade A)		30.06
	<i>Minores</i>	26.07
	<i>Laeticolores</i>	26.07
<i>Pseudochitonina</i> (clade D)		30.57
	<i>Bivelares</i>	18.16
	<i>Bohusia</i>	24.36
	<i>Brunneopicti</i>	17.75
	<i>Chitonioides</i>	18.16
	<i>Crassispori</i>	18.03
	<i>Flocculenti</i>	21.89
	<i>Hondenses</i>	19.35
	<i>Nigrobrunnescetes</i>	23.30
	<i>Rubricosi</i>	23.30
	<i>Sanguinolenti</i>	23.45
	<i>Trisulphurati</i>	18.03
	<i>Xanthodermatei</i>	19.58
	<i>Spissicaules</i> (clade C)	
<i>Amoeni</i>		19.15
<i>Rarolentes</i>		21.98
<i>Spissicaules</i>		21.98
<i>Subrutilescentes</i>		19.15

Additional ITS analysis within some sections

There were a few taxonomically important specimens (type specimens or type species of known sections) for which we obtained ITS sequences, but not LSU, *tef-1 α* and/or *rpb2* sequences. For these we performed independent analyses of ITS sequences in order to identify “proxy” specimens that could represent them in the multigene phylogeny (Table 1). ITS sequence data were initially aligned using MUSCLE 3.6 with default settings (Edgar 2004a, b), then manually adjusted in Mesquite (<http://mesquiteproject.org/mesquite/mesquite.html>). The alignments have been submitted to TreeBase (TreeBase No. 18680 and 18682). Maximum-likelihood analyses were performed using RAxML v7 (Stamatakis 2006) with a GTR+G substitution model. To assess the statistical support of clades we ran 1000 fast-bootstrap (BS) replications under the GTR-CAT approximation. Bayesian analysis was performed with MrBayes-3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Heulsenbeck 2003), with four chains (one cold, three incrementally heated) for 10,000,000 generations and Trees were sampled every 100 generations. Those trees sampled prior to searches reaching a split deviation

frequency value of 0.01 were discarded as the burn-in, and the remaining trees were used to calculate Bayesian posterior probabilities (PPs).

Results and discussion

Hennig (1966) and Avise and John (1999) suggested that time divergence could serve as a more objective and biologically informative criterion for the delimitation of taxonomic ranks. Here we present the first case study that attempts to use this criterion for the systematics revision of a fungal genus while trying to minimally disrupt its taxonomic ranks as they were recognized in the past. The limitations and uncertainties of molecular divergence time estimation have been discussed in van Tuinen and Torres (2015).

Estimation of the age of *Agaricus*

The ultrametric MCC tree of the Agaricomycetes (Supplementary Fig. 1) supports the stem ages of the order *Hymenochaetales*, *Boletales*+*Atheliales*, and *Agaricales* to be (mean values) 158, 141, and 151 Ma, respectively. The mean estimated stem age of *Agaricus* was 66 Ma.

Estimation of the age of subgenera and sections of *Agaricus*

The MCC tree for *Agaricus* (Fig. 1) shows five monophyletic clades with stem ages of 30–33 Ma. These clades are named A, B, C, D, and E, all of them having PP values ≥ 0.99 (Fig. 1). We propose to rank them as subgenera. Within these five clades, 18 subclades that diverged 18–26 Ma ago were strongly supported statistically. We propose to rank them as sections.

Clade A is proposed as subgenus *Minores*. It includes section *Minores* as reported in Zhao et al. (2011); it diverged 30 Ma ago and is separated into two 26 Ma-old clades (A1 represents section *Minores*, A2 are section unknown and section *Laeticoloris*).

Clade B, which is sister to Clade A, is proposed as subgenus *Flavoagaricus*. This clade is equivalent to section *Arvenses* in Geml et al. (2007), Parra (2013) and Zhao et al. (2011). Because this clade diverged 30 Ma ago, we propose to raise it to the subgenus level using the older name “*Flavoagaricus*”, which now includes solely section *Arvenses*.

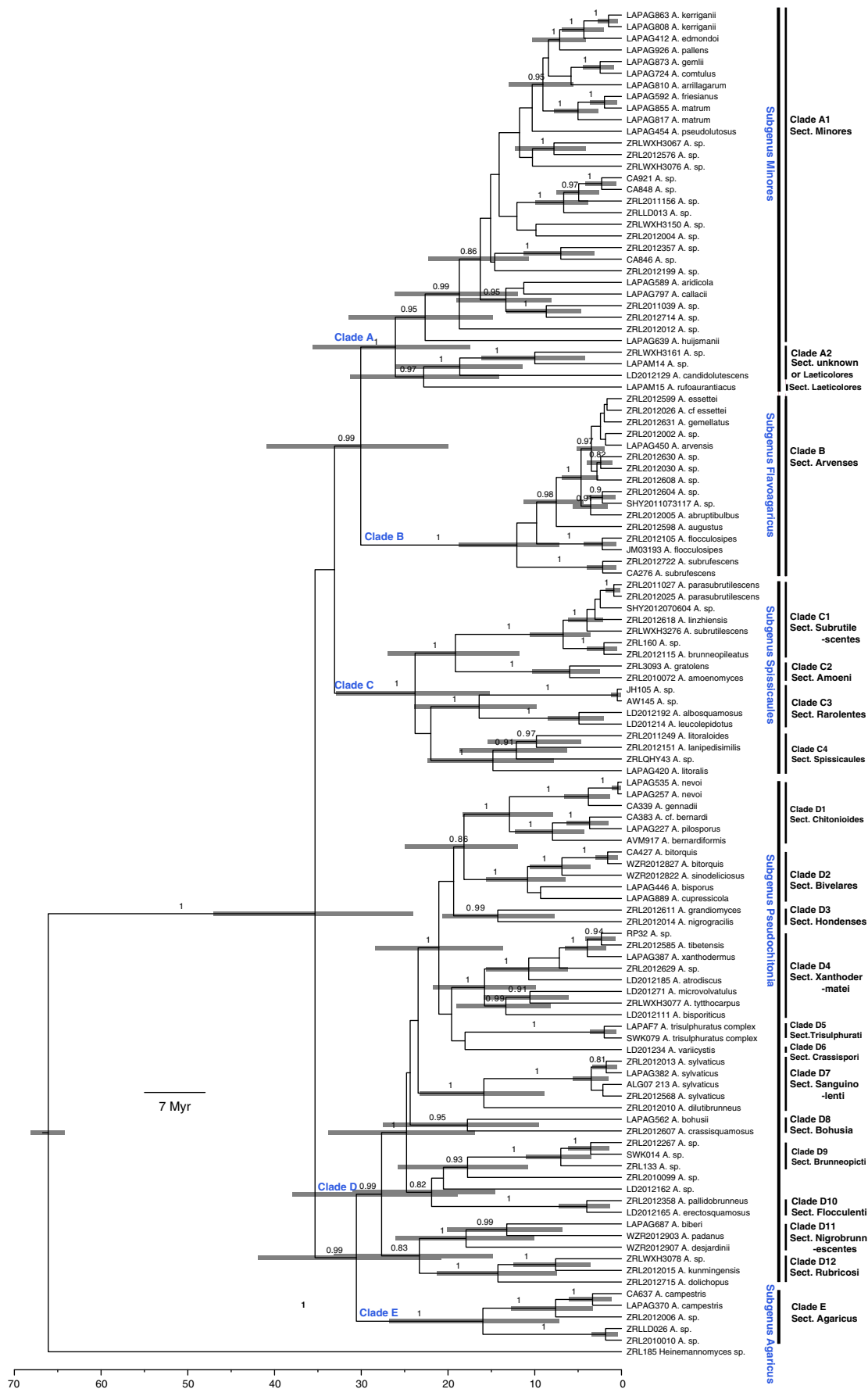
Clade C (33 Ma) includes four clades (C1–4), which range in stem ages from 18 to 23 Ma. Clade C4 is roughly equivalent to the section *Spissicaules* (Heinemann 1978; Parra 2008). Clade C2 is equivalent to clade TRIII in Zhao et al. (2011) and is described as a new section *Amoeni* in the present study. Clades C1 and C3 correspond to sections *Subrutilescentes* and *Rarolentes* (Kerrigan 2016).

Fig. 1 Maximum Clade Credibility tree of *Agaricus* based on ITS, LSU, *tef-1 α* , and *rpb2* genes sequences with the outgroup *Heinemannomyces* sp.. Posterior probability which are equal and above 80 % are annotated at the internodes. The 95 % highest posterior density of divergence time estimation are marked by horizontal bars

Clade D is composed of twelve well-supported clades (clades D1–12, Fig. 1) which diverged 18 to 26 Ma ago. We propose to recognize these clades at section level. Sections *Chitonioides* (clade D1), *Bivelares* (clade D2), *Brunneopicti* (clade D9) and *Nigrobrunnescetes* (clade D11) are supported with high PP values, in agreement with previous studies (Parra et al. 2014; Wang et al. 2015; Chen et al. 2015; Didukh et al. 2005; Challen et al. 2003). Section *Xanthodermatei* was suggested to include three monophyletic groups (represented here by *A. xanthodermus* Genev., *A. bisporiticus* Nawaz, Callac, Thongklang & Khalid, and *A. hondensis* Murrill) based on earlier studies (Geml et al. 2007; Callac and Guinberteau 2005; Kerrigan et al. 2005; Zhao et al. 2011, 2012, Thongklang et al. 2014). However, we found that this section is polyphyletic, and splits into two lineages (clades D3 and D4). Clade D4 contains *A. xanthodermus*, the type species of section *Xanthodermatei*, while clade D3 comprises samples ZRL2012611 and ZRL2012014 and is distant from clade D4. Section *Sanguinolenti* was split into two subsections (*Sanguinolenti* and *Bohusia*) by Parra (2008), which were polyphyletic in Zhao et al. (2011). In the present study, these two lineages (represented here by *A. sylvaticus* Schaeff. and *A. bohusii* Bon, respectively) are represented as clades D7 and D8. Tropical clades TRb was not well-supported in previous studies (Zhao et al. 2011); however, in this study it splits into well-supported clades D5 and D6 in Fig. 1, and based on their stem ages, they are treated as two sections. Clade D10, composed of *A. pallidobrunneus* sp. nov. and *A. erectosquamosus* sp. nov. is also well-supported, and a more detailed ITS analysis (Fig. 2) shows that it may be related to the tropical clade TRa of Zhao et al. (2011). Clade D12 is another well-supported clade in both the multi-gene and ITS trees, and based on the ITS tree it is related to TRc (Zhao et al. 2011). We found two isolated lineages close to clade D9 and D10, LD2012162 and ZRL2010099, but the present data could not clarify their precise location. Clade E is monophyletic with strong support with a stem age of 31 Ma; it represents the section *Agaricus*.

Within-clade relationships based on ITS sequence data

In order to better understand evolutionary relationships within clades in Fig. 1 and for which we have large ITS sampling sequences that align with little ambiguities (and in some cases in order to link taxonomically important samples such as type specimens to a proxy representative in our higher level



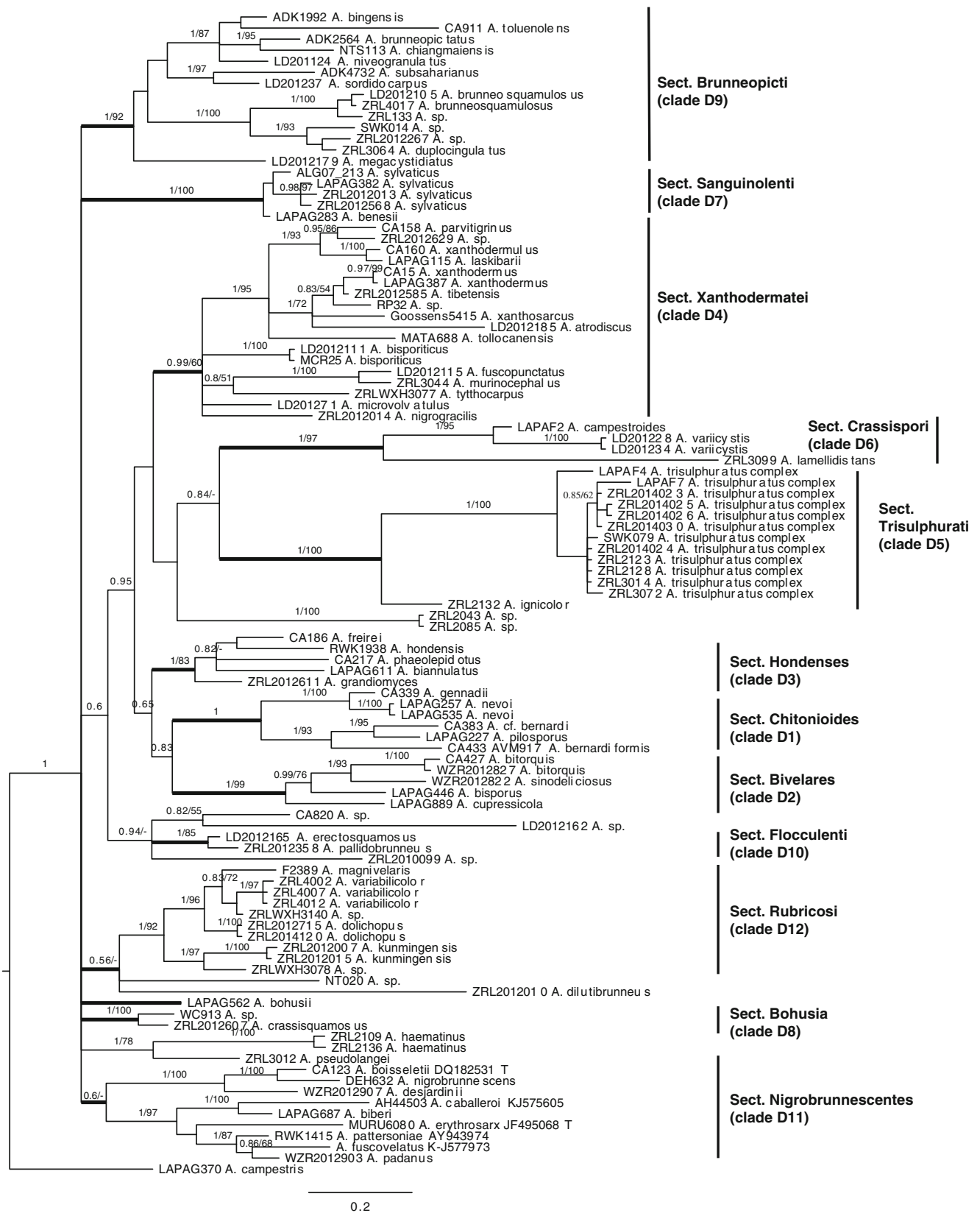


Fig. 3 Phylogeny of *Agaricus* subgenus *Pseudochitonina* generated from Bayesian analysis of ITS sequences, rooted with *A. campestris*. Bootstrap support (BS) values > 50 % and Bayesian posterior probability (PP)

values > 80 % (or >50% in some main clades) are given at the internodes (PP/BS). “T” means the sequence is from type specimen; a clade with thick branch is corresponding a certain section in Fig. 1

Table 3 Main morphological characters of *Agaricus* sections

Subgenera	Sections	KOH reaction	Schäffer's reaction	Odour	Discoloration on touching	Discoloration on exposure	Annulus	Cheilocystidia	Others
<i>Agaricus</i>	<i>Agaricus</i>	–	–	Mushroomy, sometimes of anise	None or slightly yellowish or reddish	Pink, strong red or yellow	Superous or intermediate, simple or double	Absent or indistinct, some many, pyriform, ovoid	
<i>Flavaagaricus</i>	<i>Arvenses</i>	+	+	Anise or almond	Yellow	Yellow, reddish, or none	Superous, double	Generally catenulate	
<i>Minores</i>	<i>Minores</i>	+	+	Anise or almond	Yellow	Yellow	Superous, simple	Simple, clavate, pyriform, contorted or none	
<i>Laeticolores</i>	<i>Laeticolores</i>	+	+	Anise or almond	Indistinct or yellow	None, yellow or ochraceous orange	Superous, simple or double	Variable in shape	
<i>Pseudochitonina</i>	<i>Bivelares</i>	–	–	Indistinct or mushroomy	Reddish or none	Pink to reddish	Intermediate, inferous or sometimes coexisting superous and inferous, simple or double	Clavate, some septate at the base	Innoxious
<i>Bohusia</i>	<i>Bohusia</i>	+	+ and violet-ceous purple	Pleasant	Orange-yellow or red	Slightly yellow or red	Superous, double	Present, most clavate	
<i>Brunneopicti</i>	<i>Brunneopicti</i>	+	– or weakly +	Pleasant, almond, unpleasent, phenol	Faint to strong yellow, orange, brownish rufescent or red	Faint to strong yellow, orange, rufescent	Superous, double	Pyriform to broadly clavate	Known only from palaeotropics
<i>Chitonioides</i>	<i>Chitonioides</i>	–	–	Indistinct or mushroomy	Indistinct or reddish	Pink to reddish or none	Inferous, simple	Clavate, sometimes cylindrical contorted, some septate at the base	spore with apical endosporal thickening, shape variable cymbiform or ellipsoid
<i>Crassispori</i>	<i>Crassispori</i>	+	–	Phenol, ink	None	None	Superous, double	Shape highly variable, often sinuous, capitate or clavate	
<i>Flocculentii</i>	<i>Flocculentii</i>	+	–	Indistinct or variable	None	None	Superous, double	Present and abundant, globose to pyriform or sphaeropedunculate	
<i>Hondenses</i>	<i>Hondenses</i>	+	+	Generally faint, or pleasant rare iodine or phenol	Indistinct	Weakly yellow, then reddish	Superous, double	Present or not, if present clavate, pyriform, globose or sphaeropedunculate	

Table 3 (continued)

Subgenera	Sections	KOH reaction	Schäffer's reaction	Odour	Discoloration on touching	Discoloration on context exposure	Annulus	Cheilocystidia	Others
<i>Nigrobrunnescentes</i>		–	–	Indistinct or mushroomy	Reddish brown, dark brown	brown or none Pink, reddish brown, or vinaceous red	Superous or intermediate, simple or double	Clavate, pyriform, subglobose, catenulate or not, some septate at the base.	
	<i>Rubricosi</i>	indistinct or slightly reddish brown	–	Indistinct, pleasant, or phenol	Reddish brown	Red	Superous, double	Abundant, pyriform	
<i>Sanguinolenti</i>	–	–	–	Indistinct or mushroomy	Reddish brown	Reddish brown to blood red	Superous, simple	Abundant, clavate, some septate at the base	
<i>Trisulphurati</i>	+	–	–	Carbolic acid, ink, or mushroomy	None	None	Superous or intermediate	Clavate, or subcapitate	Universal veil developed
<i>Xanthodermatei</i>	+	–	–	Iodine or phenol mostly	Yellow	Yellow or red	Superous, simple or double	Present or not, if present globose often	Toxic
<i>Spissicaules</i>	–	–	–	Generally faint, or pleasant of anise at the stipe base	Yellow	Pink, reddish or none	Simple, superous	shaeropedunculate Cylindrical to clavate, some septate at base	
<i>Rarolentes</i>	–	–	–	Solvent, rubber, or sometimes of almonds or anise	None	None or pink, reddish	Superous	Various, cylindrical to clavate	
<i>Amoeni</i>	– or +	– or +	– or +	Pleasant or almond	Weakly yellow or red	None or slightly orange	Superous, simple or double	Rare or abundant. Variable, some septa at the base.	
<i>Subrutilescentes</i>	teal to olivaceous green	–	–	Similar to <i>Leptota cristata</i>	None or gill red on bruising	None, weakly pink or yellow	Superous, simple or double	Variable, some septa at the base	

groups in the backbone tree (Fig. 1); thus, more extensive ITS analyses were not performed for these groups.

Taxonomy

The diagnostic characters in the field of the genus *Agaricus* are brown to dark brown, free, never deliquescent lamellae, a pileus easily separable from the stipe and the presence of one or several simple or double annuli. Microscopically, the pileipellis is always a cutis, the hymenophoral trama is mostly slightly interwoven, sometimes regular when young, the hyphae are devoid of clamp connections and the basidiospores lack a true apical pore. Basidiome surface discolouration when touched or rubbed and of the context when bruised or cut, biochemical reactions, and odour are also crucial to the taxonomical characterization at species, section and subgenus level.

The developmental process of annulus formation is often very complex in *Agaricus* species, so that some terms are used specifically in this genus. For example, the terms superous, inferous and intermediate are used when a mechanical test shows if it peels upwards (generally like a skirt), if it peels downwards (generally like a sock), or if it breaks (generally perpendicular or completely appressed to the stipe), respectively, on pulling (Parra 2008). It must be noticed that the annulus can be inferous, but visually pendant (Parra 2008). Also the annulus may be simple (when smooth in its underside or double (two layers), when floccose or squamose on its underside, and if double it is important to know if this ornamentation is arranged radially like a cogwheel. Furthermore, in some species these two layers can be completely separated, such as in *A. duplocingulatus* Heinem. All characters of the annulus are basic requirement in the genus *Agaricus* when characterizing certain subgenera, sections and species.

In order to provide an outline of the *Agaricus* taxonomic system, the main morphological characters of each section are shown in Table 3, and detailed descriptions of all the taxa at any rank are also given.

1. *Agaricus* subgenus *Agaricus*

Facesoffungi number: FoF01165; Fig. see Parra 2008, pp198;540–548

Type species *Agaricus campestris* L., Sp. Pl. 2: 1173 : Fr., Syst. Mycol. 1:281. 1821.

Agaricus subgenus *Agaricus* only contains *A.* section *Agaricus*

Delimitation of *Agaricus* subgenus *Agaricus* and *A.* section *Agaricus*

The diagnosis of this subgenus is the same as that of *A.* section *Agaricus*. KOH and Schäffer's reactions negative on white areas of the surface of the pileus. Pileus surface unchanging or slightly yellowing, rarely rufescent on touching. Context

often turning pink or strongly reddening and then becoming brownish over time near the connection between the stipe and pileus, and sometimes yellow at the base of the stipe on exposure. Odour usually indistinct or mushroomy, but in some species of anise in young basidiomes. Annulus superous or intermediate, simple or double, membranous or fibrillose. Cheilocystidia absent or indistinct, basidia-like, some species abundant, large, globose, piriform or ovoid, such as *A. langei* (F.H. Møller) F.H. Møller and *A. depauperatus* (F.H. Møller) Pilát, never catenulate.

Stem age and phylogenetic support

The subgenus *Agaricus* has long been considered a group containing all sections of this subgenus in temperate areas (Heinemann 1978; Singer 1986; Parra 2008). This study shows that section *Agaricus* as represented by the type species *A. campestris* (clade E in Fig. 1) is a monophyletic group, which is supported with 1 PP value, and this result concurs with previous studies (Geml et al. 2007; Zhao et al. 2011). The present circumscription of the subgenus *Agaricus* is restricted to that of *A.* section *Agaricus* with the remaining temperate sections placed in other subgenera. The stem age of this clade is 30.57 Ma and the isolated phylogenetic placement indicates that this clade should represent a higher rank in this genus.

2. *Agaricus* subgenus *Flavoagaricus* Wasser, Fl. Fung. RSS Ukrainicae: 138. 1980.

Facesoffungi number: FoF01166; Fig. see Parra 2013, pp835-849

Type species *Agaricus arvensis* Schaeff., designated by Wasser, Fl. Fung. RSS Ukrainicae: 138.1980.

Agaricus subgenus *Flavoagaricus* only contains *A.* section *Arvenses* (Konrad & Maubl.) Konrad & Maubl., Encycl. Mycol. 14: 104. 1948.

Delimitation of *Agaricus* subgenus *Flavoagaricus* and *Agaricus* section *Arvenses*

The diagnosis of this subgenus is the same as that of *A.* section *Arvenses*. KOH and Schäffer's reactions positive on all surfaces of basidiome. The surface of pileus often discolours yellow on touching. Context turning yellow, reddish, or not discolouring on exposure. Odour of anise or bitter almonds. Annulus superous, double with lower layer woolly, floccose, breaking in scales often radially arranged as a cogwheel. Cheilocystidia generally catenulate.

Stem age and phylogenetic support

This subgenus/section is reflected in clade B (Fig. 1) which is a monophyletic group with 1 PP support, and the type species of section *Arvenses*, *A. arvensis*, nests well in this clade along with other known species from this section, such as *A. essettei*

Bon, *A. abruptibulbus* Peck, *A. augustus* Fr., and *A. flocculosipes* R.L. Zhao, Desjardin, Guinb. & K.D. Hyde. The analysis also indicates its stem age is 30.06 Ma; thus, it is a natural subgenus, and includes only the section *Arvenses*.

3. *Agaricus* subgenus *Minores* (Fr.) R.L. Zhao & Moncalvo. stat. nov.

Fungal Names: FN570194

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

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

Facesoffungi number: FoF01167; Fig. see Parra 2013, pp 968–975

Etymology: the epithet “*Minores*” is following the name of section *Minores*.

Delimitation of *Agaricus* subgenus *Minores*

KOH reaction positive, Schäffer’s reaction usually positive, seldom negative. Surface of pileus often discolouring yellow on touching. Context often turning yellow on exposure. Odour of anise or bitter almonds. Basidiomes often slender, small-to-medium sized. Annulus superous, if simple, thin, fragile, smooth on both sides; if double upper side smooth and lower surface floccose or squamose. Cheilocystidia simple, clavate, pyriform, sometimes absent, scattered or rare.

Stem age and phylogenetic support

Subgenus *Minores* (clade A) is fully supported and its stem age is 30.06 Ma. The phylogeny also has a closer position to the subgenus *Flavoagaricus* than others.

The subgenus includes sections *Minores* (Fr.) Henn. and *Laeticolores* Heinem.

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

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

Facesoffungi number: FoF01168; Fig. see Parra 2013, pp 968–975

Delimitation of *Agaricus* section *Minores*

The characters of this section are those of subgenus *Minores*, but the Schäffer’s reaction is positive and the lower surface of the annulus is neither floccose nor squamose. The universal veil is poorly developed or absent.

Stem age and phylogenetic support

This section is reflected in clade A1 in Fig. 1 with 0.95 PP support, and its stem age is 26.07 Ma. In this clade, there are 26 species including the type species of this section and three

type specimens. In Zhao et al. (2011), three clades (clades TRV, VI, and VII) were strongly supported based on ITS sequence data, which are composed of 10 unnamed species from tropical areas and *A. aridicola* Geml, Geiser & Royse ex Mateos, J. Morales, J. Muñoz, Rey & Tovar from temperate areas. These three clades are sister to the section *Minores* (Zhao et al. 2011). In this study, samples CA848 and LAPAG589 (*A. aridicola*), which were members of TRV in Zhao et al. (2011), are included. The tree (Fig. 1) shows that CA848 clusters with the new samples CA921, ZRL2011156 and ZRLLD013 in clade A1 (section *Minores*), which is fully supported with a 12 Ma stem age. Sample LAPAG589 (*A. aridicola*) nested with other new samples (LAPAG797) as a clade also within clade A1. Another sample, CA846, represents the clade TRVI (Zhao et al. 2011) and clusters with sample ZRL2012357, which is fully supported with a 14 Ma stem age, and also nests in clade A1. Because the clades related to TRV and VI are younger than those sections that we accept, and in the topology they are distributed in other well-supported clades within clade A1, we conclude that the members of TRV and VI should belong to section *Minores* (clade A1).

3.2 *Agaricus* [subgenus *Minores*] section *Laeticolores* Heinem., Kew Bull. 15(2) : 244. 1961.

Type species: *Agaricus laeticolor* Heinem. & Gooss.-Font., designated by Heinemann, Kew Bull. 15(2): 244. 1961.

Facesoffungi number: FoF01169; Fig. see Heinemann 1956, p99

Delimitation of section *Laeticolores*

The characters of this section are those of subgenus *Minores*, but the Schäffer’s reaction is positive, seldom negative and the lower surface of the annulus is smooth, heavily fibrillose, squamose. The universal veil is developed and is often present on the pileus, while the annulus margin and stipe base forms small squamules.

Stem age and phylogenetic support

This section is represented by only one species, *A. rufoaurantiacus* Heinem. in Fig. 1, and its stem age is 26.07 Ma. Section *Laeticolores* was established by Heinemann (1961) based on samples from Africa and Central America in *Agaricus* subgenus *Lanagaricus* Heinem. Heinemann included seven species in this section in his world key to the genus *Agaricus* (Heinemann 1978). One of them, *A. rufoaurantiacus* has been used in our analyses (LAPAM 15) and this section also is studied in molecular phylogeny by first time.

Clade A2 (section unknown or *Laeticolores*)

Three specimens (LD2012129 introduced as new species *A. candidolutescens* in this paper, LAPAM14 and ZRLWXH3161) cluster together (Fig. 1), and they are sister

to section *Laeticolores* (represented by *A. rufoaurantiacus*). Because of the lack of some important morphological information and the limitation of sampling in section *Laeticolores*, this section is unresolved in this study.

Agaricus candidolutescens L.J. Chen & R.L. Zhao, sp. nov. Figs. 4a, b and 11.

Fungal Names: FN570195

Facesoffungi number: FoF01170

Etymology: “candido” refers to the white surface of basidiome; “lutescens” refers to slightly yellow of context on cutting.

Typus: THAILAND, Chiang Rai Prov., Doi Pui, 25 July 2012, collector Jie Chen, LD2012129 (MFLU12-0962, holotype; HMAS373994, isotype).

Fig. 4 Macrocharacters of *A. candidolutescens* (specimen LD2012129, Type a, b), *A. crassisquamosus* (specimen ZRL2012607, Type, c, d); *A. trisulphuratus* complex (specimen ZRL3073, e, f), and *A. ignicolor* (specimen ZRL2132, Type, g–i)

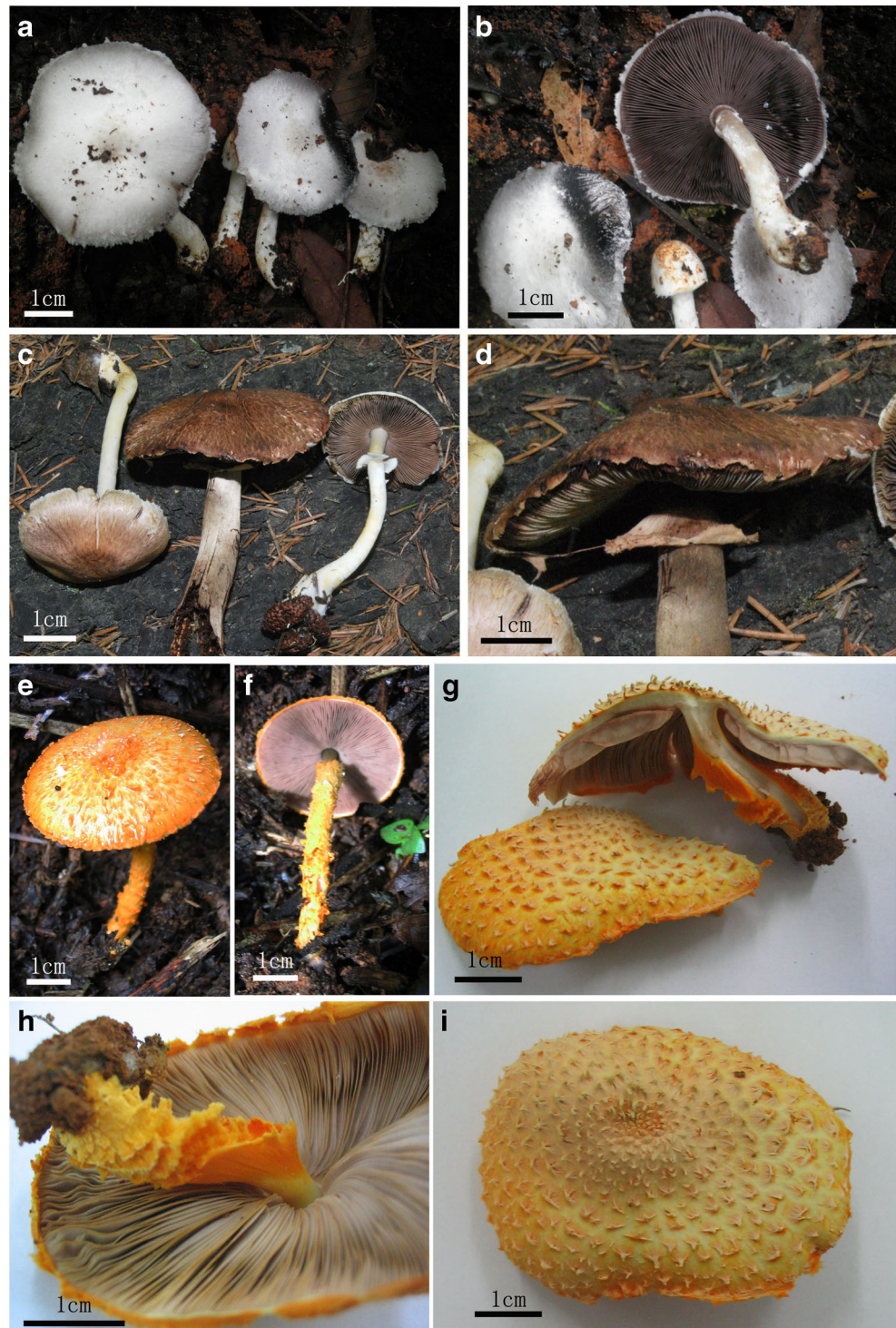


Fig. 5 Macrocharacters of *A. lamellidistans* (specimen ZRL3099, Type, **a**; ZRL3074, **b–d**), *A. variicystis* (specimen LD201234, Type, **e**; LD201228, **f**); *A. dilutibrunneus* (specimen ZRL2012010, Type, **g–i**)



Original description: *Pileus* 27–42 mm diam., 2 mm broad at disc, convex to applanate; surface dry, smooth, silky, with woolly-fibrillose squamules towards the margin, pure white; margin appendiculate. *Lamellae* free, crowded, ventricose, intercalated with lamellulae, with more than 5 series, 2.5–4 mm broad, at first pink, then pinkish brown, later dark brown and finally almost black. *Stipe* 40–55 × 3–8 (10–12 at base) mm, cylindrical with a bulbous base, narrowly hollow, white,

staining yellow when bruised, smooth above the ring, fibrillose below the ring. *Annulus* membranous, simple, white, fragile, upper side smooth, lower side fibrillose. *Context* firm, at first white on cutting, both in pileus and stipe, and quickly turning yellow at the stipe base, finally after 15 min uniformly brown. *Odour* of almonds.

Macrochemical reactions: KOH reaction positive yellow, Schäffer's reaction negative.

Fig. 6 Macrocharacters of *A. erectosquamosus* (specimen LD2012165, Type, **a, b**), *A. pallidobrunneus* (specimen ZRL2012358, Type, **c, d**); *A. litoraloides* (specimen SHY2011073026, **e, f**), and *A. planipileus* (specimen ZRL2011248, Type, **g, h**)



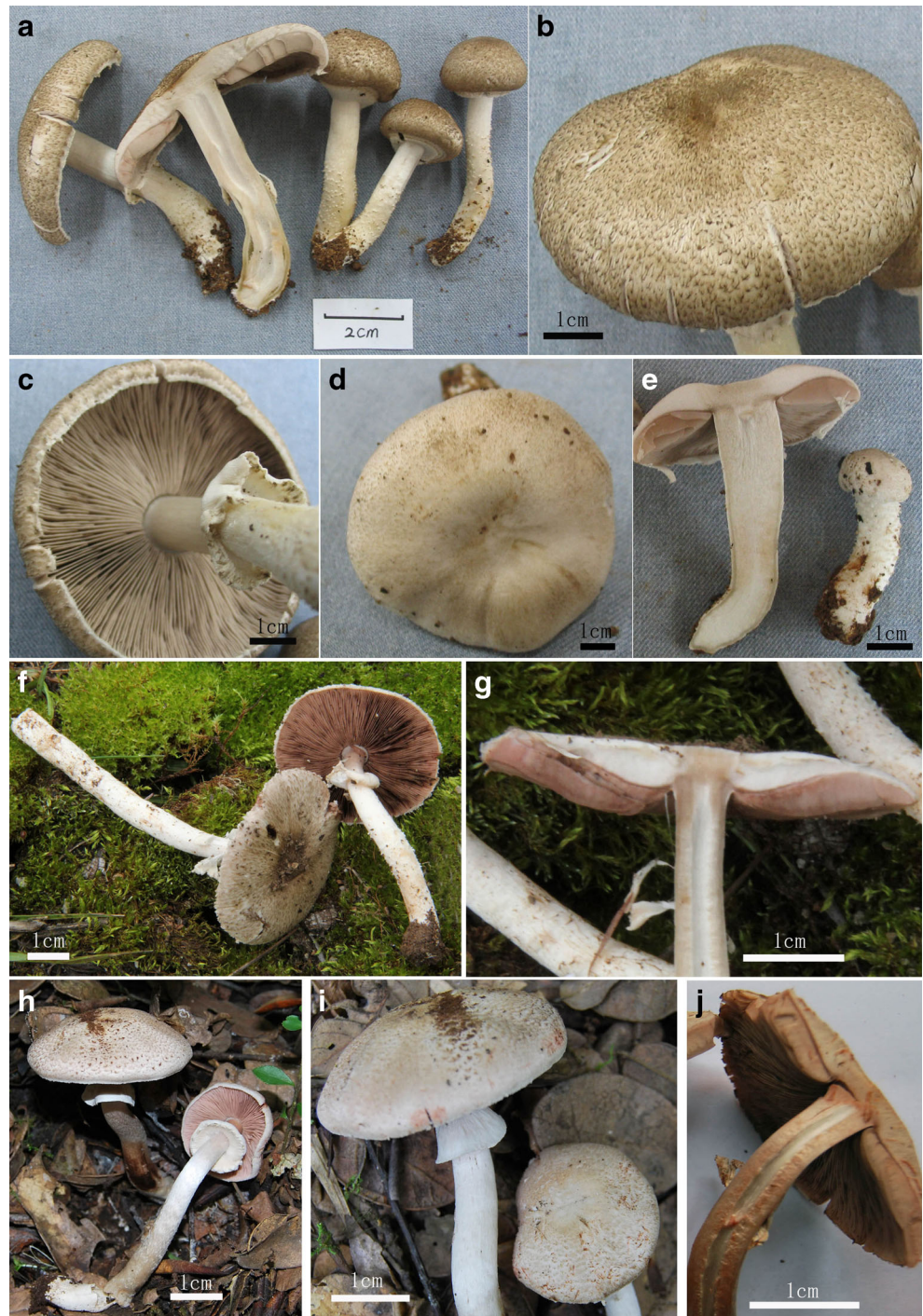
Basidiospores $6.4\text{--}7.3$ (-7.5) \times $3.9\text{--}4.5$ μm , [$\bar{x} = 6.8 \pm 0.32 \times 4.2 \pm 0.2$, $Q = 1.49\text{--}1.77$, $Q_m = 1.6 \pm 0.05$, $n = 20$], ellipsoid, smooth, dark brown, thick-walled, without germ pore. *Basidia* $11\text{--}13 \times 6.5\text{--}8$ μm , clavate, hyaline, smooth, 4-spored. *Cheilocystidia* $18\text{--}38 \times 7.5\text{--}13$ μm , rare, simple, pyriform or sphaeropedunculate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae $5\text{--}9$ μm in diam.,

cylindrical, hyaline, smooth, at times slightly constricted at the septa.

Habitat: in groups, in rich soil of tree stump.

Notes: Compared to all species of section *Laeticolores*, which is the closest section in phylogeny, *A. candidolutescens* differs in having a pure white, whole basidiome, and a woolly-fibrillose, pileus margin and

Fig. 7 Macrocharacters of *A. variabilicolor* (specimen ZRL4012, **a–c**; ZRL4007, Type, **d, e**), *A. dolichopus* (specimen ZRL2014120, Type, **f, g**); *A. kunmingensis* (specimen ZRL2012007, Type, **h–j**)



lower surface of annulus (Heinemann 1978). In the phylogenetic tree (Fig. 1), unnamed sample ZRLWXH3161 from tropical China is sister to *A. candidolutescens*; however, this Chinese collection has a dark brown, squamose pileus. In morphology, *A. candidolutescens* is similar to *A. haematoscarcus* Heinem. et Gooss. (section *Lanosi*) in its pure white and woolly-fibrillose pileus, however, *A. haematoscarcus* has a strong red, blood colour on cutting, wider basidiospores (4.8–5.6 μm wide in Heinemann

1956a; 4.5–5.5 μm wide in Parra 2013), and basidiospores with an acute apex.

4. *Agaricus* subgenus *Pseudochitonina* Konrad & Maubl., Icon. Select. Fung. 6, fasc. 6: 61. 1927

Type species: *Agaricus pequinii* (Boud.) Konrad & Maubl., Icon. Select. Fung. 6, fasc. 6: 61. 1927, here designated by L.A. Parra.

Facesoffungi number: FoF01171; Fig. see Parra 2008, pp641–642

Fig. 8 Macrocharacters of *A. lanipedisimilis* (specimen LD2012193, Type, **a, b**; ZRL2012151, **c, d**), *A. albosquamosus* (specimen LD2012192, Type, **e, f**; LD201235, **g, h**); *A. leucolepidotus* (specimen LD201214, Type, **i, j**), and *A. amoenomyces* (specimen ZRL2010072, Type, **k–m**)



Delimitation of *Agaricus* subgenus *Pseudochitonia*

This subgenus contains the most sections in the genus *Agaricus*. KOH and Schäffer's reactions are negative or positive. Staining yellowish, pink, reddish brown or indistinct on touching the surfaces or on cutting the context.

Odour generally mushroomy and pleasant, sometimes indistinct or also unpleasant, like fish or like ink or phenol, and in this latter case often accompanied with an intense yellow discolouration on cutting and bruising, mainly at pileus margin and stipe base. Annulus variable, superous or inferous, simple or double (typically 2 layers and

Fig. 9 Macrocharacters of *A. suthepensis* (specimen LD2012100, Type, **a, b**), *A. gratolens* (specimen ZRL3093, Type, **c–e**); *A. parasubrutescens* (specimen ZRL2014076 Type, **f–h**; ZRL2014090, **i**), and *A. inthanonensis* (specimen LD014, Type, **j–l**)



separated far away). Cheilocystidia present or absent, generally simple and not catenulate.

Stem age and phylogenetic support

This subgenus (clade D) is well-supported in the multi-gene and ITS trees. Its stem age is 30.57 Ma. The subgenus *Agaricus* is the closest group. There are twelve strongly supported clades (D6 is represented by a single sample in Fig. 1) which diverged 18–23 Ma, and these clades are reflected in

twelve sections: *Agaricus* sect. *Bivelares* (Kauffman) L.A. Parra, *A.* sect. *Bohusia* (L.A. Parra) L.A. Parra & R.L. Zhao stat. nov., *A.* sect. *Brunneopicti* Heinem., *A.* sect. *Chitonioides* Romagn., *A.* sect. *Crassispori* R.L. Zhao, *A.* sect. *Flocculenti* J. Chen, K.D. Hyde & R.L. Zhao, *A.* sect. *Hondenses* R.L. Zhao & L.A. Parra sect. nov., *A.* sect. *Nigrobrunnescentes* K. P. Peterson, Desjardin, & Hemmes, *A.* sect. *Rubricosi* R.L. Zhao, *A.* sect. *Sanguinolenti* Jul. Schäff & F.H. Möller ex L.A. Parra, *A.* sect. *Trisulphurati* Heinem., and *A.* sect. *Xanthodermatei* Singer.

Fig. 10 Macrocharacters of *A. brunneopileatus* (specimen ZRL2012115, Type, **a–c**), *A. linzhiensis* (specimen ZRL2012618, Type, **e–g**)



4.1 *Agaricus* [subgenus *Pseudochitonina*] section *Bivelares* (Kauffman) L.A. Parra, *Fungi Europaei* 1. *Agaricus* L. *Allopsalliota* Nauta & Bas: 265. 2008.

Type species: *Agaricus rodmanii* (Peck) Lloyd, designated by Singer, *The Agaricales in Modern Taxonomy* 3rd ed.: 461. 1975.

Facesoffungi number: FoF01172; Fig. Parra 2008, pp285, 597–602

Delimitation of *Agaricus* section *Bivelares*

KOH and Schäffer's reactions negative. Surface of pileus discolouring reddish or not. Context often turning pink to reddish or unchanging on exposure. Odour indistinct or mushroomy. Basidiomes generally stout, pileus diameter almost equal to stipe length. Annulus intermediate or with one

or more inferous annuli, sometimes coexisting with a superous annulus. Cheilocystidia present, mostly clavate, some septate at the base (such as *A. subperonatus*). No toxic species and easy to reproduce on artificial compost, such as *A. bisporus* (J.E. Lange) Imbach.

Stem age and phylogenetic support

The section (clade D2) is represented by well-known cultivated species, such as *A. bisporus* and *A. bitorquis* (Quél.) Sacc. This section is fully supported as a monophyletic group in analyses of combined multigene sequence data (Fig. 1) and ITS sequence data (Fig. 3). These results are consistent with previous studies (Didukh et al. 2005; Challen et al. 2003; Li et al. 2014). The stem age of this section is 18.16 Ma.

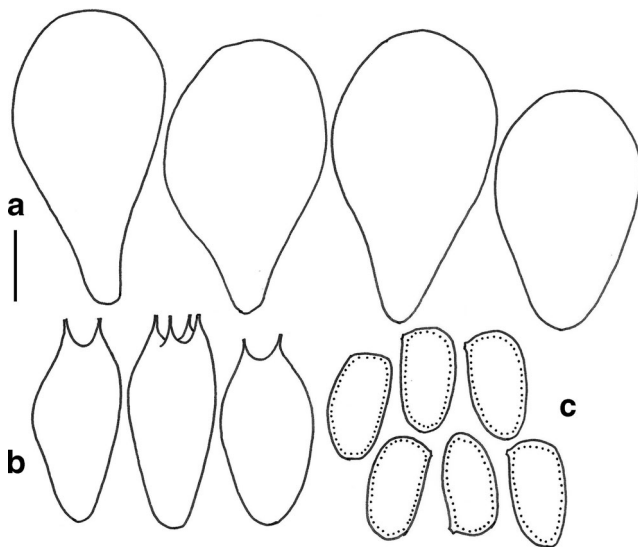


Fig. 11 Microcharacters of *A. candidolutescens* L.J. Chen & R.L. Zhao (LD2012129). **a.** Cheilocystidia **b.** Basidia **c.** Basidiospores, bars a, b, c = 5 μ m

4.2 *Agaricus* [subgenus *Pseudochitonina*] section *Bohusia* (L.A. Parra) L.A. Parra & R.L. Zhao, stat. nov.

Fungal Names: FN570196

\equiv *Agaricus* [subgenus *Agaricus*] subsection *Bohusia* L.A. Parra, *Fungi Europaei* 1. *Agaricus* L. *Allopsalliota* Nauta & Bas: 159. 2008. [basionym]

Type species: *Agaricus bohusii* Bon, designated by L.A. Parra, *Agaricus* L. *Allopsalliota* Nauta & Bas: 159. 2008.

Facesoffungi number: FoF01173; Fig. see Parra 2008, pp 648–655, 706

Delimitation of *Agaricus* section *Bohusia*

KOH reaction positive yellow only at the stipe, Schäffer's reaction positive red or violaceous purple. Orange-yellow discolouration on stipe surface on touching often present. Context slightly yellow, red or unchanging on exposure. Odour mushroomy. Annulus superous, thick, double. Cheilocystidia present, clavate.

Stem age and phylogenetic support

This section is represented by *A. bohusii* (LAPAG562) and *A. crassisquamosus* sp. nov., (ZRL2012607) as a monophyletic clade with strong support in the multi-gene tree (Fig. 1, clade D8). Its stem age is 24.36 Ma. We conclude that subsection *Bohusia* (Parra 2008) should be raised to sectional level. In the ITS tree (Fig. 3), this new species clusters with specimen WC913 (not included in multi-gene analysis), which is fully supported. Presently, these three species are recognized as members of section *Bohusia*, and comprise two main lineages; one is represented by *A. bohusii* (Parra 2008) and the other lineage is *A. crassisquamosus* sp. nov. and specimen WC913.

Zhao et al. (2011) found that specimens ZRL3012 and ZRL2136 were loosely related to *A. bohusii* in a previous ITS analysis, but in the present study they did not cluster with *A. bohusii* and form an isolated clade (Fig. 3). The reliability of their phylogenetic placement needs to be further examined using more gene sequence data.

Agaricus crassisquamosus R.L. Zhao, sp. nov. Figs. 4c, a and 12.

Fungal Names: FN570224

Facesoffungi number: FoF01174

Etymology: epithet “crassisquamosus” refers to the presence of thick squamules on the pileus of this species.

Typus: China, Tibet, Milin, Nanyigou National Forest Park, Alt. 3089 m, E94°21'24", N29°15'71", collected by R.L. Zhao, 29 July, 2012, ZRL2012607 (HMAS273991, holotype).

Original description: *Pileus* 30–60 mm in diam., 5–7 mm thick at the disc, convex, subumbonate; surface dry, completely covered by large, appressed, dark reddish brown (oac523) fibrillose squamules; margin exceeding and crenate.

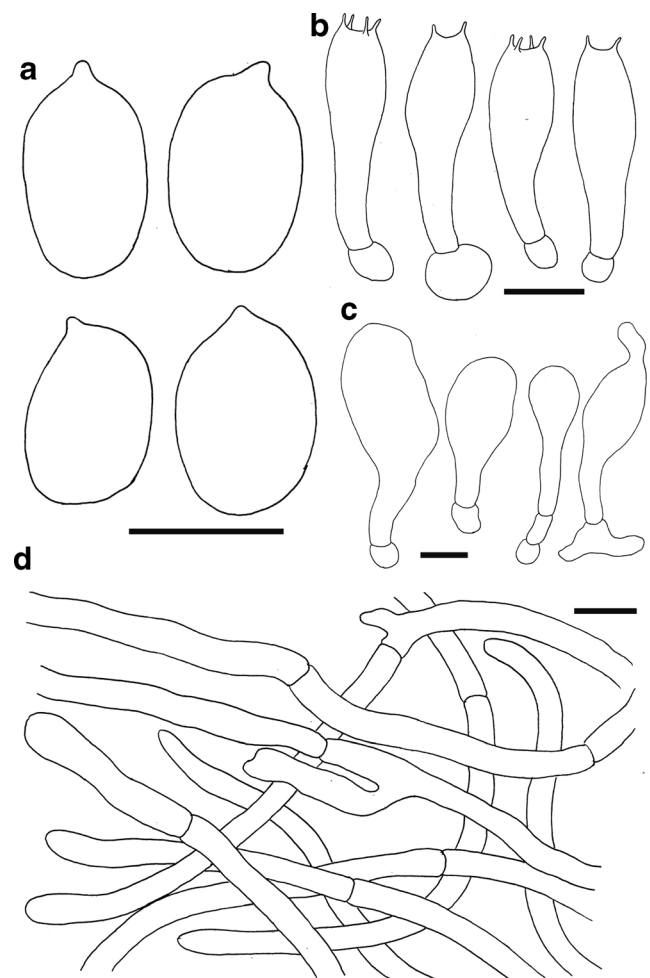


Fig. 12 Microcharacters of *A. crassisquamosus* R.L. Zhao, sp. nov. (ZRL 2012607). **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a = 5 μ m, b, c, d = 10 μ m

Lamellae free, crowded, 5–7 mm width, intercalated with lamellulae, brown, dark brown (oac524) when mature. *Stipe* 60–80 × 6–11 (apex) – 10–20 (base) mm, clavate with a subbulbous base, hollow, surface smooth and white above and below the annulus, becoming yellow on touching. *Annulus* membranous, superous, pendant, double, the upper side striate, lower side densely fibrillose or floccose, white; *Context* fleshy, on cutting white both in pileus and stipe, and colour unchanging on exposure. *Odour* pleasant.

Macrochemical reactions: KOH positive yellow, Schäffer's reaction positive red.

Basidiospores 6.1–7.3 × 4–4.8 μm [\bar{x} = 6.4 ± 0.3 × 4.3 ± 0.2, Q = 1.4–1.6, Q_m = 1.5 ± 0.1, n = 20], ellipsoid, brown, smooth and thick-walled, without germ pore. *Basidia* 19–26 × 6–10 μm, clavate, 4-spored. *Cheilocystidia* 19–48 × 8–18 μm, clavate, pyriform with long stipe, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 4–8 μm in diam., long cylindrical, curved, branched, containing brown pigments.

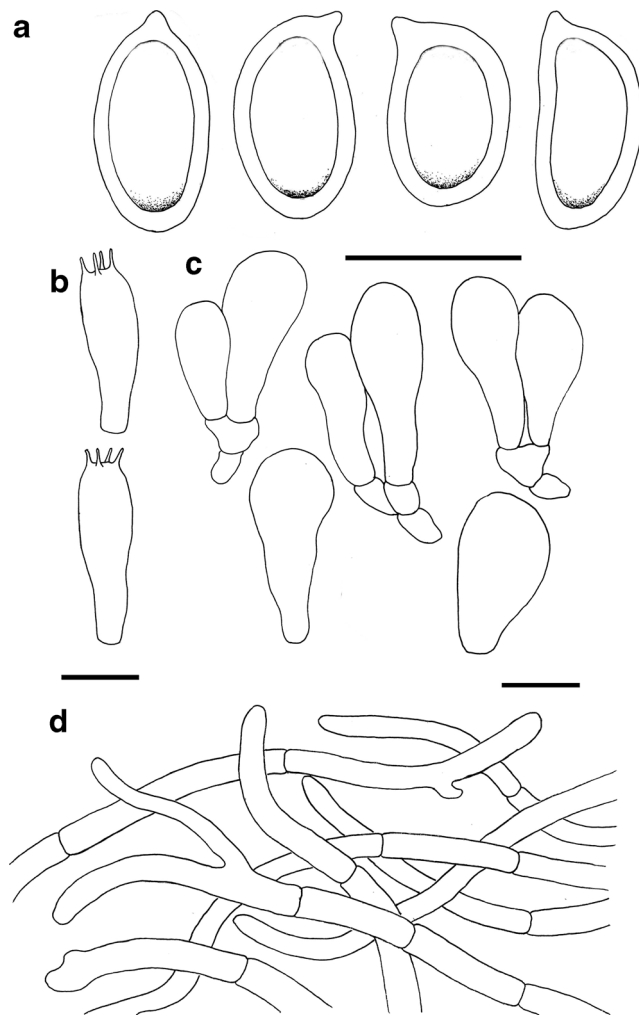


Fig. 13 Microcharacters of *A. lamellidistans* R.L. Zhao, sp. nov. (ZRL3074) **a.** basidiospores, **b.** basidia, **c.** Cheilocystidia, **d.** pileipellis, bars: a = 5 μm, b, c, d = 10 μm

Habitat: scattered in forests dominated by *Picea* spp.

Notes: This new species is characterized in the field by medium to large basidiomes, and a thick and large, completely squamose-covered pileus. It is similar to *A. bohusii* in morphology; however, *A. bohusii* has broadly ellipsoid spores (Q_m = 1.24), and clavate to cylindrical cheilocystidia, 6–10 μm wide (Parra 2008), which are different from *A. crassisquamosus*. The phylogeny also shows that they are different species.

4.3 *Agaricus* [subgenus *Pseudochitonina*] section *Brunneopicti* Heinem., Bull. Jard. Bot. État Bruxelles 26: 71 (1956).

Type species: *Agaricus brunneopictus* Heinem. & Gooss.-Font., by Heinemann, Bull. Jard. Bot. État Bruxelles 26: 74 (1956).

Facesoffungi number: FoF01175; Fig. see Heinemann 1956, p75

Delimitation of section *Brunneopicti*

KOH reaction positive or faint, Schäffer's reaction negative, rarely weakly positive. Context discolouration when bruised

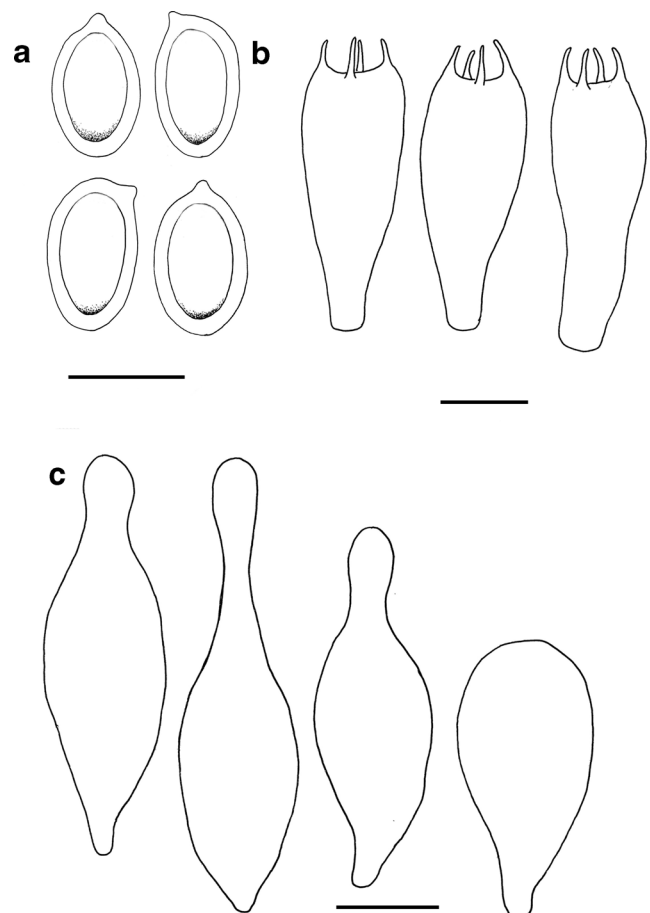


Fig. 14 Microcharacters of *A. variocystis* L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov. (LD201234) **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, bars: a, b = 5 μm, c = 10 μm

faint to strong yellow, orange, rufescent, brownish rufescent or red. Odour from pleasant bitter almond to unpleasant, like phenol or solvent used in marker pens. Pileus roughly covered with punctiform squamules, or brownish, larger squamules. Annulus superous, double or complex with scales or cortinate fibrils on the lower surface. Cheilocystidia usually present, pyriform to broadly clavate. Known only from palaeotropics.

Stem age and phylogenetic support

This section is fully supported as a monophyletic group by multi-gene and ITS analysis (clade D9 in Figs. 1 and 2), and the stem age is 17.75 Ma. This section was introduced by

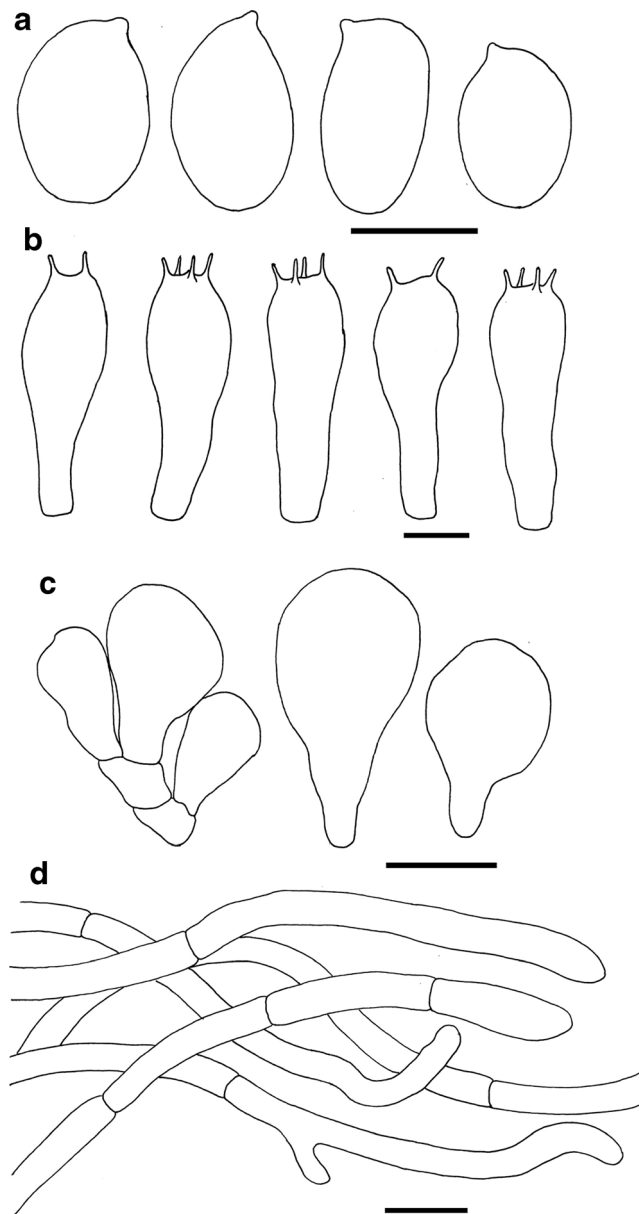


Fig. 15 Microcharacters of *A. erectosquamosus* L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov. (LD2012165) **a.** basidiospores, **b.** basidia, **c.** Cheilocystidia, **d.** pileipellis, bars: a, b = 5 μm , c, d = 10 μm

Heinemann (1956a), but a reconstruction has recently been made by Chen et al. (2015) along with several new species (Chen et al. 2015; Karunarathna et al. 2014). Our study is consistent with the previous work (Chen et al. 2015; Zhao et al. 2011).

4.4 *Agaricus* [subgenus *Pseudochitonina*] section *Chitonioides* Romagn., Bull. Soc. Mycol. Fr. 102(1): 118. 1986.

Type species: *Agaricus pequinii* Boud., designated by Romagnesi, Bull. Soc. Mycol. France 102(1): 118. 1986.

Facesoffungi number: FoF01176; Fig. see Parra 2008, pp. 641–642

Delimitation of section *Chitonioides*

KOH and Schäffer's reactions negative. Surface of pileus discolouring indistinct or reddish. Context often turning pink to reddish or unchanging on exposure. Odour indistinct or mushroomy. Basidiomes generally stout, pileus diameter almost equal to stipe length. Annulus inferous, peronate, simple. Cheilocystidia present, clavate, sometimes cylindrical contorted, some septate at the base.

Stem age and phylogenetic support

This section also is fully supported and sister to section *Bivelares* in multi-gene and ITS analyses (clade D1, Figs. 1 and 2), which concurs with previous studies (Parra 2008; Zhao et al. 2011; Wang et al. 2015). This section was formed 18.16 Ma, which is also in the time range as other sections.

4.5 *Agaricus* [subgenus *Pseudochitonina*] section *Crassispori* R.L. Zhao, sect. nov.

Mycobank: MB814599

Type species: *Agaricus lamellidistans* R.L. Zhao.

Facesoffungi number: FoF01177; Figs. 5a–d and 13

Etymology: from *crassus*, *crassi-* means thick, so “*crassispori*” referring to the thick walled basidiospores.

Original description and delimitation of section

Crassispori

KOH reaction positive, Schäffer's reaction negative. Not staining on touching and cutting. Smell phenol, ink. Annulus superous, membranous, double and lower side always wooly or cortinate. Cheilocystidia present, shape highly variable, often sinuous, capitate or clavate. Basidiospore with an apical endospore thickening, shape variable cymbiform or ellipsoid.

Stem age and phylogenetic support

This section is represented by *A. variicystis* (LD201234) in multi-gene analysis with a stem age of 18.03 Ma. It is fully

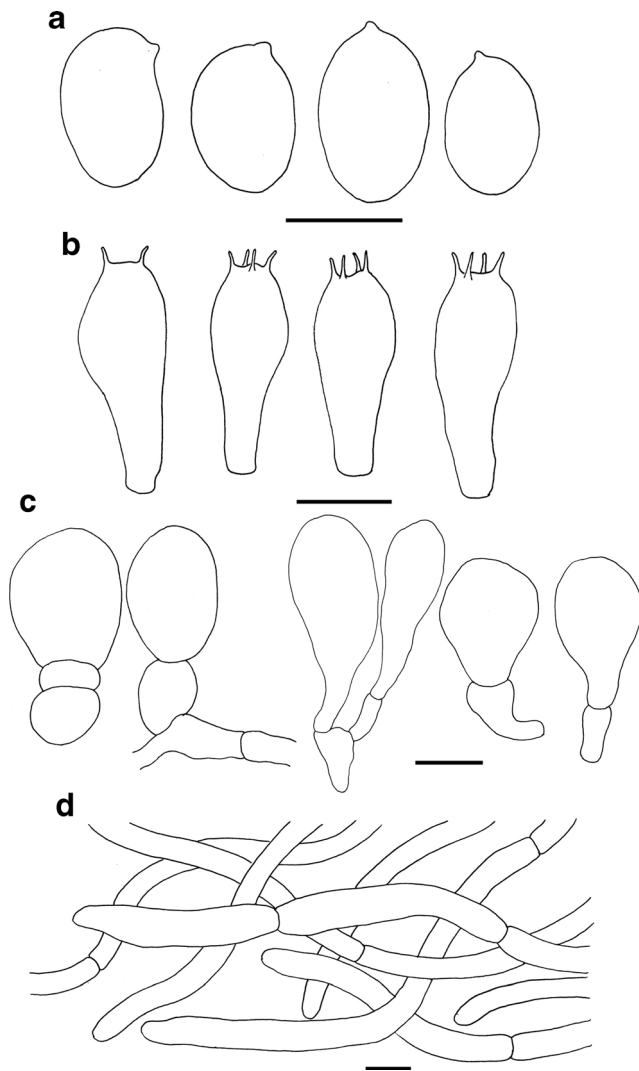


Fig. 16 Microcharacters of *A. pallidobrunneus* R.L. Zhao, sp. nov. (ZRL2012358). **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis; bars: a = 5 μ m, b, c, d = 10 μ m

supported in ITS analysis and comprises *A. variicystis* sp. nov., *A. lamellidistans* sp. nov., and *A. campestroides* Heinem. & Gooss.-Font. This section is sister to section *Trisulphurati*. Morphologically, the species of this section are distinct in having basidiospores with an apical endospore thickening, which is rare in the genus *Agaricus*, but common in the genus *Micropsalliota*. *Agaricus campestroides* was introduced from Congo. At that time it was reported as a member of *A.* section *Campestres* (Heinemann 1956a), which is a homotypic synonym of *A.* section *Agaricus* (Cappelli 1984).

Agaricus lamellidistans R.L. Zhao, sp. nov. Figs. 5a–d and 13

Fungal Names: FN570197

Facesoffungi number: FoF01178

Etymology: epithet “lamellidistans” refers to the distant lamellae of this species, which is not common in most *Agaricus* species.

Typus: Thailand, Chiang Mai Prov., Mae Taeng Dist., Tung Joaw village, forest trail, N19°08.07' E98°38.90', elev. 1300 m., 5 September 2006, collected by Ruilin Zhao, ZRL3099 (BBH 19615, **holotype**; HMAS 274007, **isotype**).

Original description: *Pileus* 12–36 mm in diam., 2 mm thick at disc, hemisphaerical, extending broad conic, convex with broad umbo and deflexed margin in most cases, some without umbo; surface dry, glabrous to fibrillose, lacking squamules, generally white or cream, disc light orange, brownish orange (6C5), fading to margin, margin exceeding. *Lamellae* free, less crowded than usual for the genus or slightly distant, intercalated with lamellulae, 4–5 mm broad, ventricose, at first greyish brown (7D2, 7D3), then brownish grey, finally dark brown. *Stipe* 18–80 \times 2–4 (apex)–4–5 (base) mm, cylindrical to subclavate, hollow or stuffed with rhizomorphs at the base, white to slightly grey, smooth above the annulus, tomentose below the annulus and almost strigose towards the base, not staining on touching. *Annulus* membranaceous, superous, pendent, simple, fugacious, white, with radiate striations, up to 4 mm broad. *Context* firm, white both in pileus and stipe of young basidiomes, and grey to yellowish brown in stipe for mature basidiomes, colour unchanging on exposure. *Odour* of carbolic acid or ink.

Macrochemical reaction: KOH reaction positive brightly yellow, Schäffer’s reaction negative.

Basidiospores 5.5–6.5 \times 3.2–4 μ m [\bar{x} = 6 \pm 0.2 \times 3.8 \pm 0.3, Q = 1.4–2, Q_m = 1.61 \pm 0.39, n = 20], cymbiform to ellipsoid, smooth, thick-walled, with a slightly apical, endospore thickening, without a germ pore, reddish brown. **Basidia** 13–18 (–22) \times 4–7 μ m, hyaline, smooth, 4-spored. **Cheilocystidia** 12–32 \times 7–16 μ m, pyriform, broad-clavate, smooth, hyaline. **Pleurocystidia** absent. **Pileipellis** a cutis consisted of hyphae 4–9 μ m in diam., light yellow or hyaline, smooth, branched, not constricted at the septa. **Annulus** consisting of the same hyphae as pileipellis.

Habitat: solitary or scattered in small groups in red soil.

Other material examined: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma village, N18°48.62' E98°54.60', elev. 1145 m., 2 July 2005, collected by Thanh Huyen Le, ZRL2081 (BBH 19464; HMAS 274013); same location, 7 June 2006, collected by Thanh Huyen Le, ZRL3034 (BBH 19550); same location, 13 June 2006, collected by Todd Osmundson, ZRL3063 (BBH 19579; HMAS 274004); Chiang Mai Prov., Mae Taeng Dist., Tung Joaw village, forest trail, N19°08.07' E98°38.90', elev. 1300 m., 30 June 2006, collected by Ruilin Zhao, ZRL3074 (BBH 19590; HMAS 274012); same location, 5 September 2006, collected by Ruilin Zhao, ZRL3098 (BBH 19614; HMAS 274020).

Notes: This species is often found in the field on the sides of trails in forests of Thailand. It is distinct from most *Agaricus* species in the distance between lamellae being

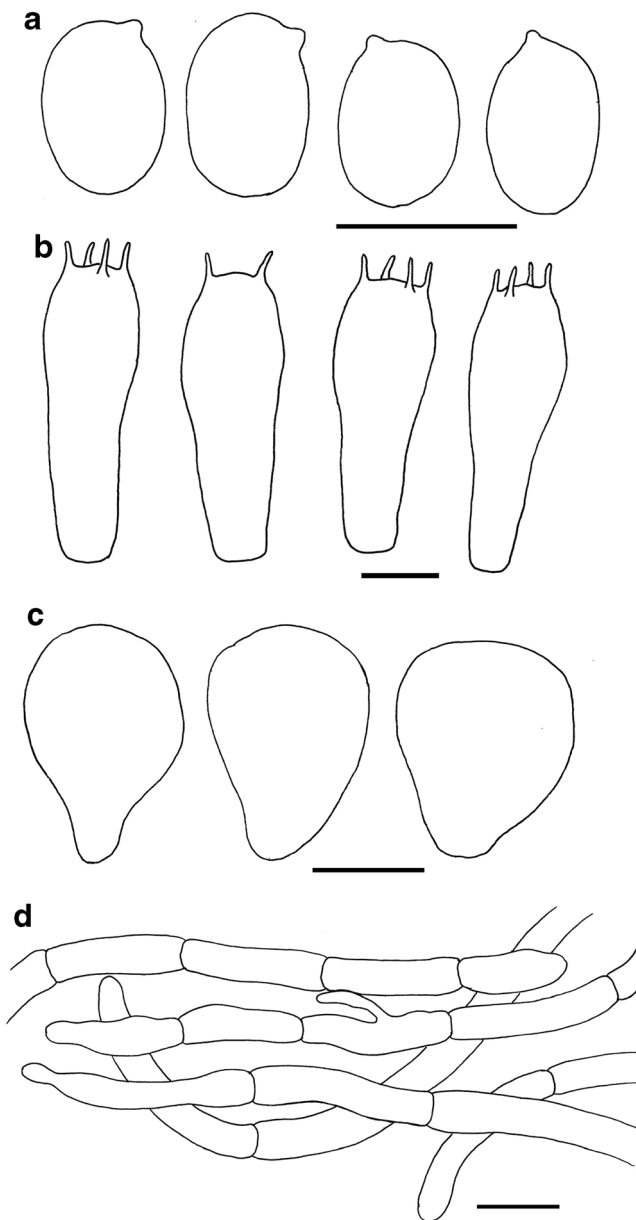


Fig. 17 Microcharacters of *A. variabilicolor* R.L. Zhao, sp. nov. (ZRL4012) **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a, b = 5 μ m, c, d = 10 μ m

further than most *Agaricus* species, which are often crowded. Under the microscope, it is similar to species of section *Crassispori* in having cymbiform spores with an apical endosporal thickening. The known species *A. campestroides* belongs to this section, and it can be distinguished from *A. lamellidistans* as it has a pleasant smell and lacks cheilocystidia, while, *A. lamellidistans* has an ink smell and abundance of cheilocystidia (Heinemann 1956a).

Agaricus variicystis L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov. Figs. 5e, f and 14.

Fungal Names: FN570198

Facesoffungi number: FoF01179

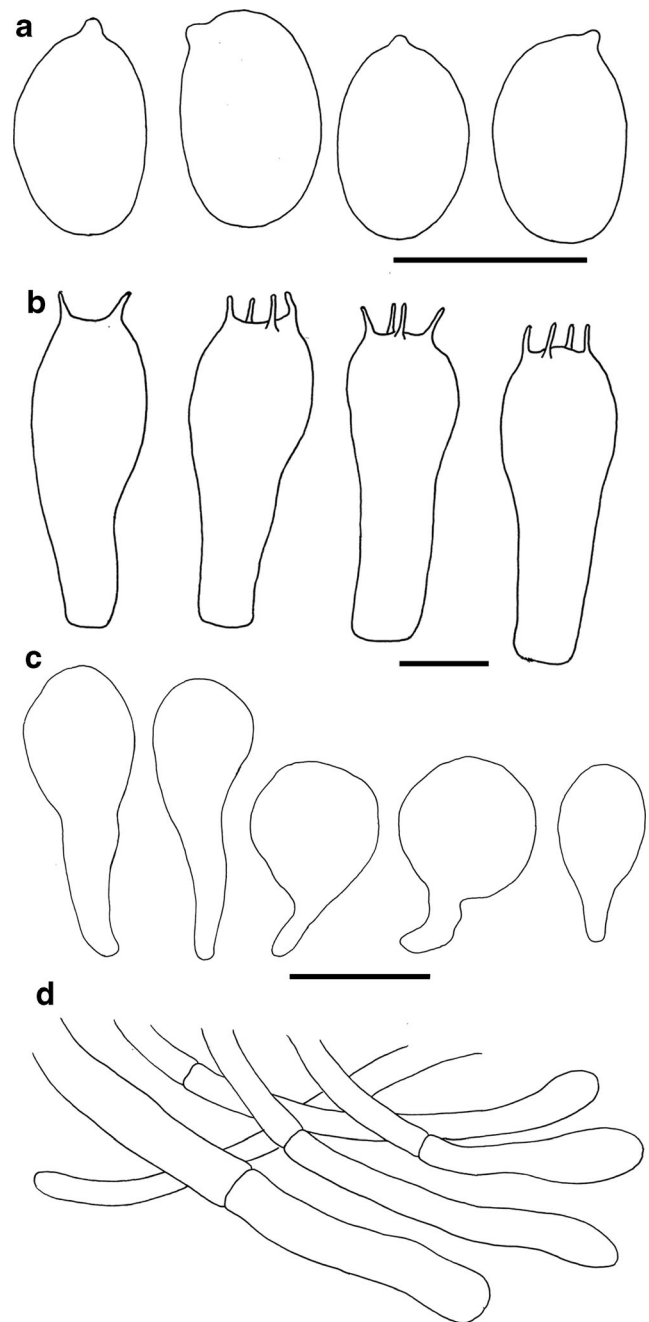


Fig. 18 Microcharacters of *A. dolichopus* R.L. Zhao, sp. nov. (ZRL2014120) **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a, b = 5 μ m, c, d = 10 μ m

Etymology: epithet “variicystis” refers to variable shapes of cheilocystidia in this species.

Typus: THAILAND, Chiang Mai Prov., 3 km down the road from Tharnthong Lodges, 4 June 2012, collector Jie Chen, LD201234 (MFLU12-0878, **holotype**; HMAS273996, **isotype**).

Original description: *Pileus* 53–75 mm in diam., 1–2 mm thick at disc, convex to hemispherical, more or less truncate

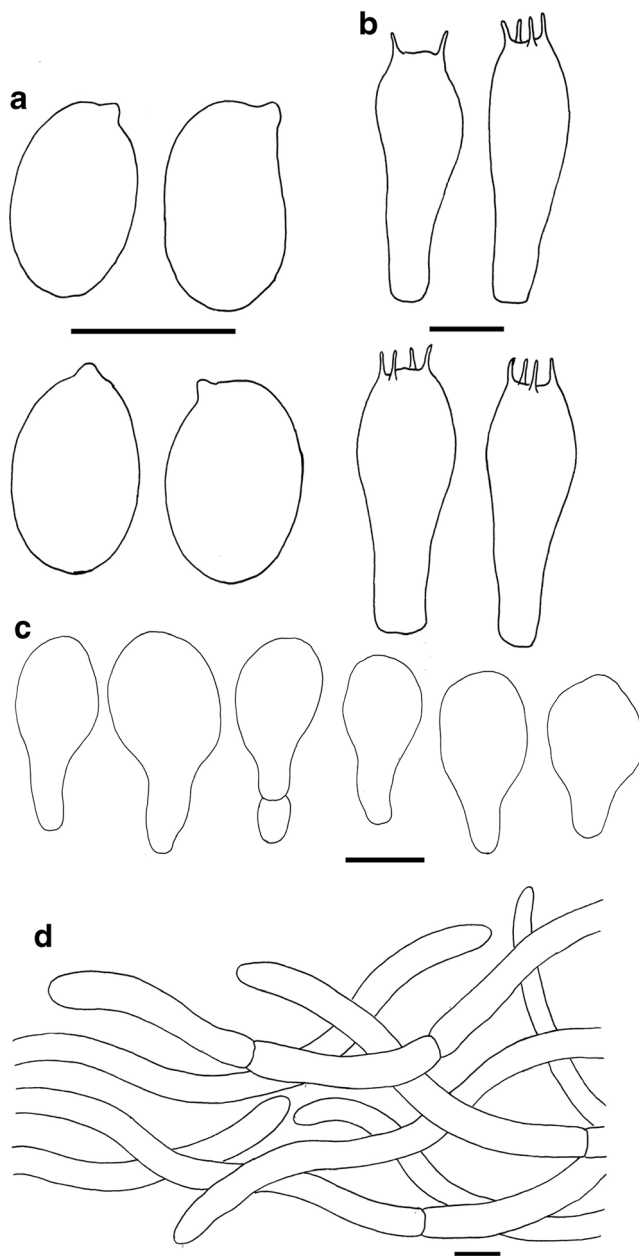


Fig. 19 Microcharacters of *A. kunmingensis* R.L. Zhao, sp. nov. (ZRL2012007) **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a, b = 5 μ m, c, d = 10 μ m

at the disc; surface dry, smooth, occasionally exhibiting very few squamules, on a brownish grey (7C2) background, close to the margin; margin straight. *Lamellae* free, very crowded, with intercalated lamellulae, 5–7 mm broad, greyish brown to dark brown. *Stipe* 64–94 \times 4–7 (apex) – 8–11 (base) mm, cylindrical to slightly enlarged towards the base, hollow, white, surface smooth, tomentose close to the base (composed of mycelium), staining greyish orange when bruised. *Annulus* membranous, simple, fragile. *Context* firm, on cutting pale white both in pileus and stipe, without discolouring on exposure. *Odour* of phenol.

Macrochemical reactions: KOH reaction positive yellow, Schäffer's reaction negative.

Basidiospores 5.6–6.4 (–6.8) \times 3.4–3.8 (–4.1) μ m, [\bar{x} = 6.1 \pm 0.26 \times 3.6 \pm 0.17, Q = 1.47–1.78, Q_m = 1.66 \pm 0.04, n = 20], ellipsoid to fusiform, brown, smooth, thick-walled, endospore thickening, without a germ pore. *Basidia* 12–15.5 \times 6–6.5 μ m, clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 20–55 \times 10–23 μ m, abundant, variable in shape, rarely pyriform or broadly clavate, often lageniform to utriform, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 4–10 μ m in diam., cylindrical, hyaline, smooth, sometimes slightly constricted at the septa.

Habitat: scattered or in groups, in soil under the bamboo woods or in forest clearings.

Other material examined: THAILAND, Chiang Mai Prov., 3 km down the road from Tharnthong Lodges, 4 June 2012, collector Jie Chen, LD201228 (MFLU 12-0872; HMAS273951).

Notes: This species is distinguished by its uniquely-shaped cheilocystidia, which is very rare in *Agaricus*. Phylogenetic analyses also support it as a distinct species.

4.6 *Agaricus* [subgenus *Pseudochitonina*] section *Flocculentii* L.J. Chen, K.D. Hyde & R.L. Zhao sect. nov.

Mycobank: MB814600

Facesoffungi number: FoF01180; Figs. 6a, b and 15

Type species: *Agaricus erectosquamosus* L.J. Chen, K.D. Hyde & R.L. Zhao

Etymology: epithet of “*Flocculentii*” referring to the pileus and stipe surfaces floccose.

Original description and delimitation of *Agaricus* section *Flocculentii*

KOH reaction yellow to orange, Schäffer's reaction negative. Surface of pileus and stipe not discolouring on bruising. Context lacking discolouration on exposure. Odour indistinct or variable, phenol or pleasant. Universal veil generally developed, such that the surfaces of pileus and stipe are covered in heavily fibrillose squamules. Annulus superous, double, upper layer smooth and lower layer thick, floccose or cogwheel-like. Cheilocystidia present and abundant.

Stem age and phylogenetic support

This section is represented by *A. pallidobrunneus* and *A. erectosquamosus* in Fig. 1 as clade D10, which is fully supported and the stem age is 21.89 Ma. In the ITS tree, this clade is also well-supported (1/85 PP/BS) and thus clade D10 is recognized as a new section. The ITS phylogenetic tree indicates that this new section is associated with unnamed sample CA820, which was shown to belong to clade TRa in Zhao et al. (2011) and unnamed samples ZRL2010099 and LD2012162 (Fig. 3) with strong support (0.94/- PP/BS).

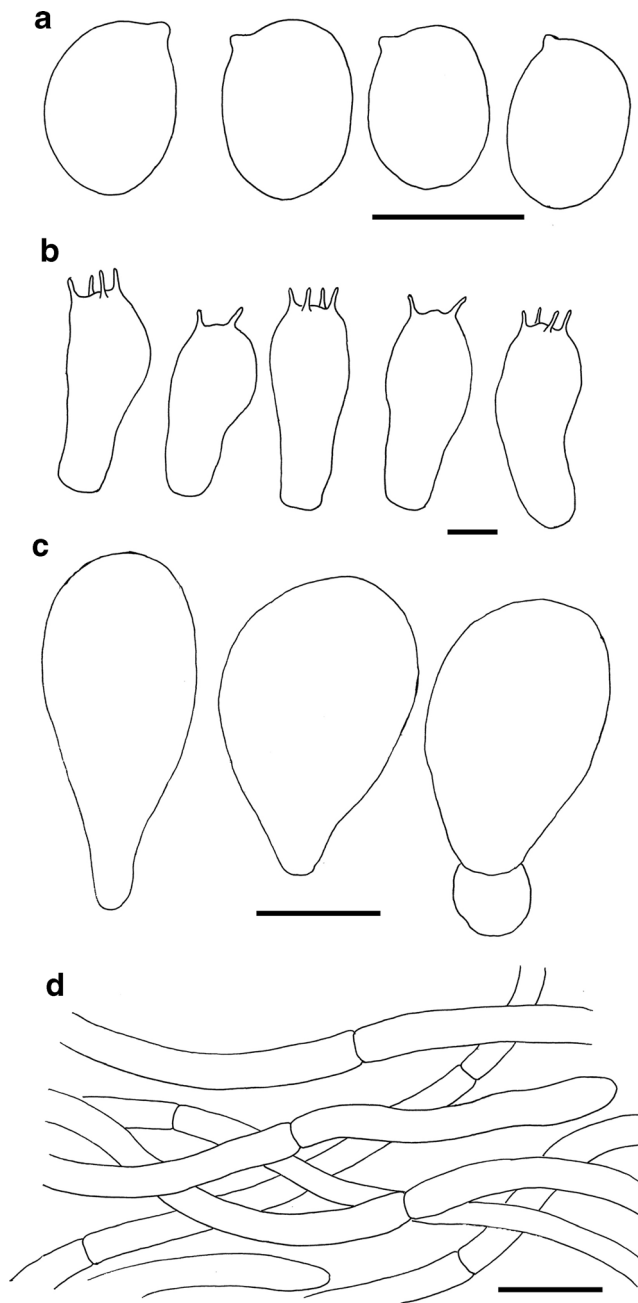


Fig. 20 Microcharacters of *A. dilutibrunneus* R.L. Zhao, sp. nov. (ZRL2012010) **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a, b = 5 μ m, c, d = 10 μ m

Because of the lack of other gene sequences from those three unnamed samples, we could not confirm their phylogenetic relationship. Presently we conclude that the strongly supported clade D10 is a new section, and presently contains two new species: *A. erectosquamosus* and *A. pallidobrunneus*.

Agaricus erectosquamosus L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov. Figs. 6a, b and 15

Fungal Names: FN570199

Facesoffungi number: FoF01181

Etymology: the epithet “erectosquamosus” refers to the squamose pileus covered by upturned squames.

Typus: THAILAND, Chiang Mai Prov., Chiang Mai Zoo, 4 August 2012, collector Jie Chen, LD2012165 (MFLU12-0993, **holotype**; HMAS273992, **isotype**).

Original description: *Pileus* 40–50 mm in diam., 5 mm thick at the disc, convex to plane; surface dry, whole cap covered by squamules, erect, dense at the disc, brown against a dirty white background; margin decurved and exceeding. *Lamellae* free, 5 mm broad, ventricose, crowded, intercalated with lamellulae, reddish brown, brown to dark brown with age. *Stipe* 45 \times 5 mm, cylindrical to slightly clavate upon maturing, hollow, smooth and white above the annulus, and squamose, white to light brown below the annulus, with indistinctive staining on touching. *Annulus* membranous, double, large, entire, pendant or subperonate, upper surface smooth, white and lower surface heavy floccose, and often cogwheel-like, white and light brown at the margin. *Context* fleshy, on cutting pale white both in pileus and stipe, and without discoloration on exposure. *Odour* indistinct or slightly phenol.

Macrochemical reactions: KOH reaction orange, Schäffer’s reaction negative.

Basidiospores (6.6–) 6.9–7.6 \times 4.1–4.6 (–5) μ m, [\bar{x} = 7.2 \pm 0.31 \times 4.4 \pm 0.2, Q = 1.51–1.73, Qm = 1.63 \pm 0.05, n = 20], ellipsoid to oblong, smooth, brown, thick-walled, without a germ pore. *Basidia* 15–22 \times 6.5–7.5 μ m, clavate to broadly clavate, hyaline, smooth, 4-(2-) spored. *Cheilocystidia* 18–30 \times 9–16.5 μ m, abundant, simple, or occasionally in short chains, globose to pyriform or sphaeropedunculate, rarely clavate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 4–12.5 μ m in diam., shortly cylindrical, with brownish membranous pigments, smooth, distinctively constricted at the septa. *Annulus* composed of hyphae 7–10 μ m in diam., which are hyaline and smooth.

Habitat: scattered in soil of grasslands.

Notes: see the notes of *A. pallidobrunneus* for the details.

Agaricus pallidobrunneus R.L. Zhao, sp. nov. Figs. 6c, d and 16

Fungal Names: FN570225

Facesoffungi number: FoF01182

Etymology: the epithet “pallidobrunneus” refers to light (pallido) brown (brunneus) coloured pileus.

Typus: China, Yunnan Prov., Yongde county, Daxueshan Xiang, Pintian Village, 17 July 2012, collected by R.L. Zhao ZRL2012358 (HMAS273999, **holotype**).

Original description: *Pileus* 105 mm in diam., 7 mm thick at disc, convex to plane; surface dry, whole cap covered by fibrils, and broken into small squamules, appressed or upturned, dense at the disc and brown, light brown towards the margin; margin decurved and exceeding. *Lamellae* free, 11 mm broad, ventricose, crowded, intercalated with lamellulae, reddish brown, brown with age. *Stipe* 110 \times 13–20 mm, cylindrical and widening towards base, slightly

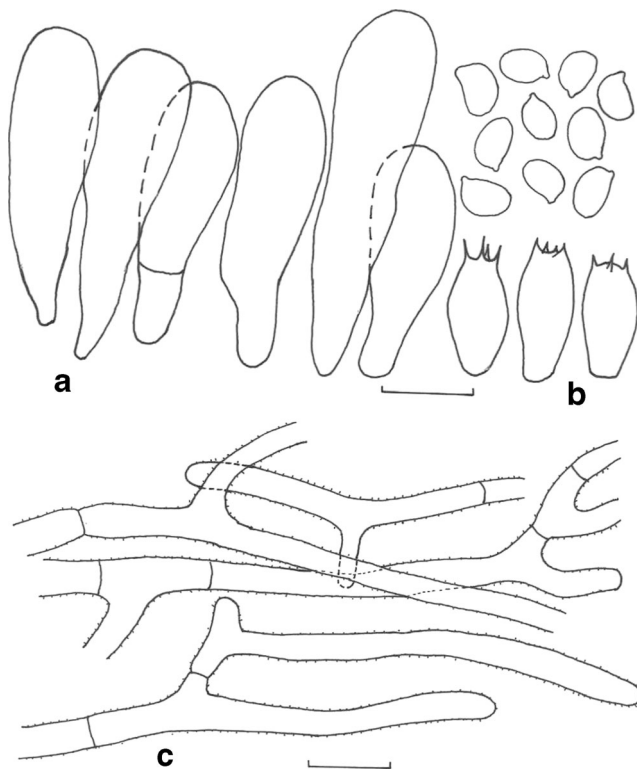


Fig. 21 Microcharacters of *A. trisulphuratus* complex (ZRL2111). **a.** cheilocystidia, **b.** basidia and basidiospores, **c.** pileipellis, bars =10 μm

clavate, hollow, surface white, smooth and white above the annulus, covered by small squamules which are the same as those of the pileus at the lower of annulus, not staining on touching. *Annulus* membranous, double, large, entire, with crenate edge, pendant, upper surface (upper layer) smooth, white and lower surface (lower layer) floccose, white and light brown towards the margin. *Context* firm, on cutting greyish white both in pileus and stipe, not discolouring on exposure. *Odour* pleasant.

Macrochemical reactions: KOH reaction yellow, Schäffer's reaction negative.

Basidiospores 6.1–7.8 \times 3.5–4.5 (–5.2) μm , [\bar{x} = 6.7 \pm 0.4 \times 4.3 \pm 0.4, Q = 1.4–1.8, Qm = 1.6 \pm 0.1, n = 20], ellipsoid to oblong, brown, smooth, thick-walled, without germ pore. **Basidia** 16–3 \times 6–9 μm , clavate to broadly clavate, hyaline, smooth, 4-(2-) spored. **Cheilocystidia** 17–35 \times 11–21 μm , abundant, simple, pyriform or sometimes in short chains, globose to ellipsoid, 11–25 μm in diam., hyaline, smooth. **Pleurocystidia** absent. **Pileipellis** a cutis composed of hyphae 4–18 μm in diam., shortly ellipsoid or cylindrical, with brownish membranous pigments, smooth, distinctively constricted at the septa.

Habitat: solitary in the forest.

Notes: The new species are distinguished by their distinct morphological characteristics: a relatively well-developed universal veil, large basidiospores and lacking discolouration on bruising and context exposure. Although *A. erectosquamosus*

and *A. pallidobrunneus* phylogenetically nest together, they could easily be separated in the field: *A. erectosquamosus* has erect, dark brown squamules on the pileus and stipe surface; however, *A. pallidobrunneus* has light brown fibrillose squamules on the pileus and white fibrillose to fibrillose squamules on the stipe.

4.7 *Agaricus* [subgenus *Agaricus*] section *Hondenses*
R.L. Zhao & L.A. Parra sect. nov. Fig. see Kerrigan 1986

Fungal Names: FN570227

Facesoffungi number: FoF01183

Type species: *Agaricus hondensis* Murrill, Mycologia 4: 296. 1912.

Etymology: epithet “Hondenses” is following the name of type species *A. hondensis*.

Original description and delimitation of *Agaricus* section *Hondenses*

KOH reaction positive yellow, Schäffer's reaction negative. Surface of pileus and stipe unchanging or becoming reddish on touching. Context turning weakly yellow, then vinaceous pink, reddish brown, seldom green-bluish at base of stipe or unchanging on exposure. *Odour* generally faint, usually of iodine or phenol, rarely indistinct. *Annulus* superous, pendant, double, and often cogwheel-like at the lower layer, if large or thick and stiff with short diam. Cheilocystidia present or not, if present often wide clavate, pyriform, sphaeropedunculate, or globose.

Stem age and phylogenetic support

This section is presented as clade D3 with 1 PP support (Figs. 1 and 3), and its stem age is 19.35 Ma. Presently, seven species have been included in this section: *A. freirei* Blanco-Dios; *A. phaeolepidotus* (F.H. Møller) F.H. Møller; *A. biannulatus* A. Mua et al. from Europe (Parra et al. 2011; Parra 2013); *A. hondensis* from North America (Kerrigan 1986); and three species to be described from China.

All species in section *Hondenses* had been treated as members of section *Xanthodermatei* Singer, and they always cluster together in section *Xanthodermatei* (Callac and Guinberteau 2005; Challen et al. 2003; Geml et al. 2004; Kerrigan et al. 2005; Zhao et al. 2011; Thongklang et al. 2014). However, multi-gene and ITS analysis with extensive samples indicate that section *Xanthodermatei* is not monophyletic and splits into two parts: clade D3 (ZRL2012611 *A. grandiomycetes* J.L. Zhao & R.L. Zhao nom. prov. and ZRL2012014 *A. nigrogracilis* R.L. Zhao nom. prov. in Zhou et al. 2016) and clade D4 represented by *A. xanthodermus* (in Fig. 1). In the extended ITS analyses, because of the increased sampling especially specimens of new section *Crassispori* (clade D6) and new recognized section *Trisulphurati* (clade D5), the previous section *Xanthodermatei* also split into those two isolated lineages (D3 and D4). However there is an unexpectation in the position of

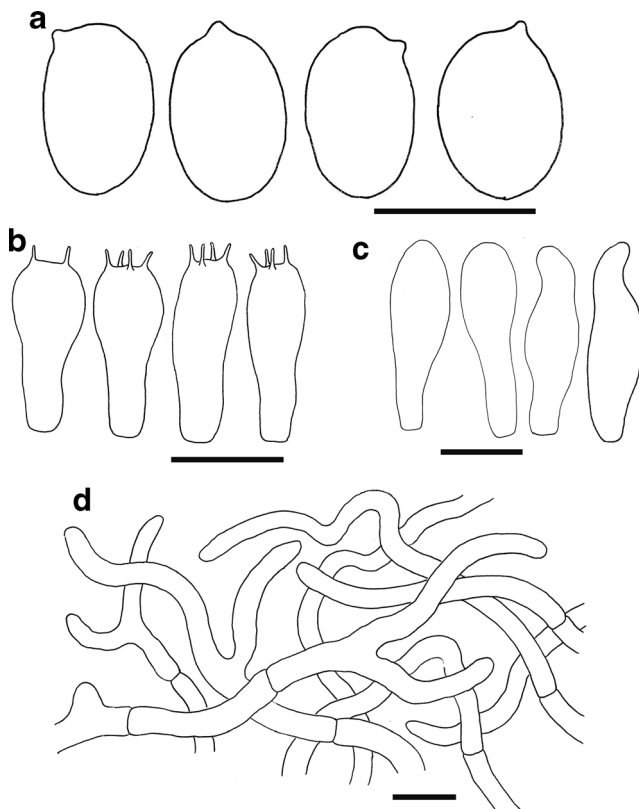


Fig. 22 Microcharacters of *A. ignicolor* R.L. Zhao, sp. nov. (ZRL2132). **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a = 5 μ m, b, c, d = 10 μ m

specimen ZRL2012014, where it moves from D3 in the multi-gene tree (Fig. 1) to D4 in the ITS tree (Fig. 3). In order to exclude the contamination error occurred in sequencing, we resequenced this specimen and performed the analyses, and obtained the same results. So presently we could not clarify the placement of specimen ZRL2012014. However another specimen ZRL2012611 (*A. grandiomycetes*) is stable in clade D3 in both trees, and linked with other species of section *Hondenses* in ITS tree (Fig. 3).

Morphologically, the species of section *Hondenses* could be separated from section *Xanthodermatei* by their lack of distinct yellow discolouration on cutting, lack of strong phenol or iodine smell, and annulus often connected as double layers. Based on such phylogenetic topology, stem ages and morphological characteristics, clade D3, named section *Hondenses*, is separated from clade D4, which represents a more restricted concept of section *Xanthodermatei*.

4.8 *Agaricus* subgenus [subgenus *Pseudochitonina*] section *Nigrobrunnescens* K.R. Peterson, Desjardin & Hemmes. *Sydowia* 52(2): 240. 2000.

Facesoffungi number: FoF01184; Fig. see Peterson et al. 2000, p239

Type species: *Agaricus nigrobrunnescens* K.R. Peterson, Desjardin & Hemmes, designated by K.R. Peterson, Desjardin & Hemmes, *Sydowia* 52(2): 238. 2000.

Delimitation of *Agaricus* section *Nigrobrunnescens*

KOH and Schäffer's reactions negative. Surface of pileus and stipe discolouring reddish brown to dark brown on bruising. Context often turning pink, vinaceous red or reddish brown on exposure. Odour indistinct or mushroomy, never anise, bitter almond, ink, or phenol. Basidiomes generally stout. Annulus superous or intermediate, simple or double, if double upper side white, striated, and lower side floccose and coloured, pendant. Cheilocystidia present, clavate, pyriform, subglobose, catenulate or not, some septate at the base.

Stem age and phylogenetic support

The section is fully supported in the multi-gene tree (Fig. 1, clade D11) and well-supported in the ITS tree (Fig. 3) with extended samples. Its stem age is 23.30 Ma and matches those of sections of *Agaricus*. This section is re-recognized by the type species *A. nigrobrunnescens* of section *Nigrobrunnescens* and has been successfully sequenced and incorporated into phylogenetic analyses (Parra et al. 2014; Peterson et al. 2000; Wang et al. 2015). Presently, there are nine species: *A. biberi* Hlaváček; *A. boisseletii* Heinem.; *A. caballeroi* L.A. Parra et al.; *A. desjardinii* Z.R. Wang et al.; *A. erythrosarx* T. Lebel; *A. fuscovelatus* Kerrigan; *A. lilaceps* Zeller; *A. padanus* Lancon. and *A. nigrobrunnescens* K.R. Peterson et al., which have been demonstrated to be members of this section worldwide (Parra 2008; Parra et al. 2014; Wang et al. 2015; Kerrigan 1986; István 2009; Lebel 2013).

4.9 *Agaricus* [subgenus *Pseudochitonina*] section *Rubricosi* R.L. Zhao, sect. nov.

Figs. 7h–J and 19

Mycobank: MB814601

Facesoffungi number: FoF01185

Type species: *Agaricus kunmingensis* R.L. Zhao.

Etymology: the epithet “*Rubricosi*” is referring to the more or less reddish brown discolouring on touching or cutting.

Original description and delimitation of *Agaricus* section *Rubricosi*

KOH reaction indistinct or slightly reddish brown, Schäffer's reaction negative. Surface of pileus and stipe discolouring reddish brown on bruising or not. Context turning red on exposure. Odour indistinct, pleasant, or phenol. Basidiome slender or stout. Annulus superous, membranous, double and often floccose at the lower side. Cheilocystidia present and abundant, pyriform. Distribution tropical or subtropical.

Stem age and phylogenetic support

Agaricus magnivelaris Pegler (collection No. F2389, Pegler, 1983) along with sample F2187 from tropical America nested

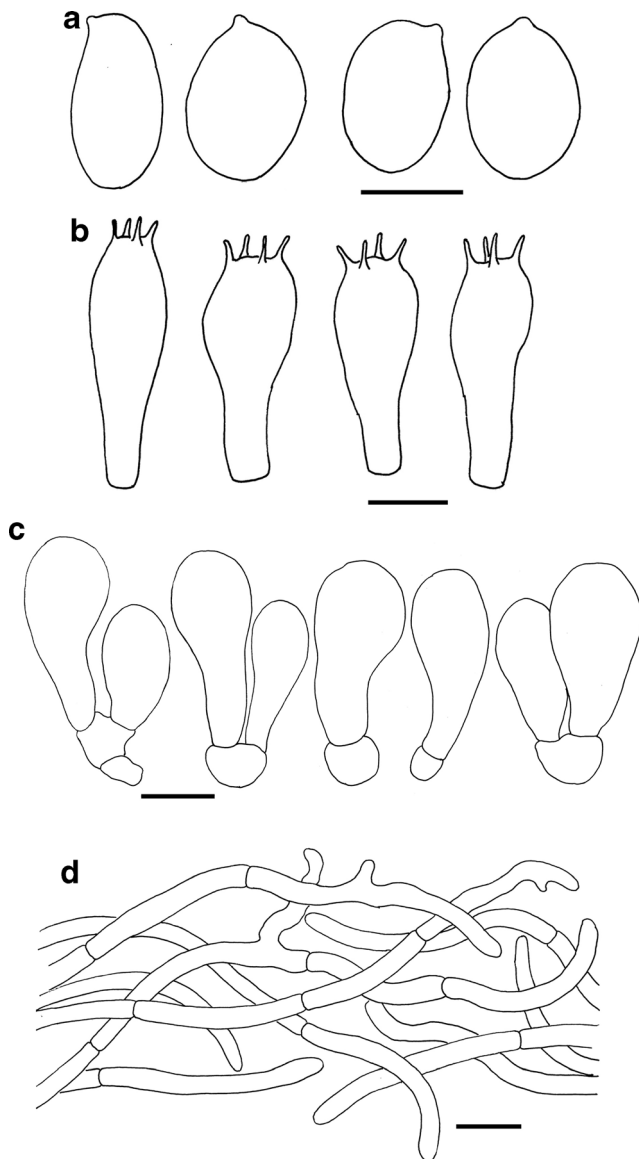


Fig. 23 Microcharacters of *A. litoraloides* R.L. Zhao sp. nov. (SHY2011073026) **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis; bars: a = 5 μm , b, c, d = 10 μm

together (named as clade TRc) in Zhao et al. (2011) with low support (PP=85; BS=61). However, in this study there are nine samples from China and Thailand that cluster in this clade, and they are fully supported in the multi-gene tree and well-supported (0.99 PP) in the ITS tree (Figs. 1 and 3, labeled as clade D12). The stem age is 23.30 Ma. We describe it as a new section here. Phylogenetically, this section is close to sections *Nigrobrunnescentes*, *Bohusia*, and *Sanguinolenti*, and all are reddish discolouring species. However, thus far, all species belonging to section *Rubricosi* are from the tropics and subtropics. Three new species, plus *A. magnivelaris* Pegler, which was placed in section *Sanguinolenti* in past studies, are included in section *Rubricosi*. Samples ZRLWXH3140 and ZRLWXH3078 from southern China

belong in this section well; however, because of lack of morphological characterization they are unnamed in this work.

Agaricus variabilicolor R.L. Zhao, sp. nov. Figs. 7a–e and 17.

Fungal Names: FN570200

Facesoffungi number: FoF01186

Etymology: epithet “variabilicolor” refers to the variable colour of the pileus.

Typus: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44.009', elev. 900 m, 10 May 2007, collected by Phongeun Sysouphanthong, ZRL4007 (BBH19637, **holotype**; HMAS, **isotype**).

Original description: *Pileus* 35–80 mm in diam, 4–7 mm thick at the disc, pulvinate, hemisphaerical when young, then expanding convex, top appanate or slightly depressed; covered by fibrillose scales or complete scales, colour variable, mostly brown, but also with light brown scales in some cases, appressed or slightly recurved; margin decurved and often appendiculate. *Lamellae* free, crowded, intercalated with lamellulae, 4–8 mm broad, at first pink, then reddish brown, brown, finally dark brown. *Stipe* 50–100 \times 7–10 mm, equal to slightly tapering towards the base; narrow hollow or stuffed; white; smooth above the annulus, and scabby or scurfy below the annulus, not discolouring on touching. *Annulus* membranous, 6–12 mm in diam, pendant, simple, fragile and attached at the margin of pileus or entirely at the stipe, upper side smooth, white, and lower side floccose white or with brown colouration. *Context* firm, on cutting, at first white, both in pileus and stipe, then quickly turning slightly greyish pink in the pileus and slightly pink in stipe, finally after 15 min uniformly light brown. *Odour* pleasant.

Macrochemical reactions: KOH reddish brown or not on surface of pileus, no colour change on stipe; Schäffer’s reaction negative.

Basidiospores 4.1–5.9 \times 2.4–3.9 μm [\bar{x} = 5 \pm 0.3 \times 2.9 \pm 0.3, Q = 1.3–2, Q_m = 1.6 \pm 0.2, n = 60], ellipsoid to elongate ellipsoid, light brown to brown, smooth, thick-walled, without germ pore. *Basidia* 16–23 \times 5.5–7.2 μm , clavate or cylindrical, hyaline, smooth, 4-spored. *Cheilocystidia* abundant, pyriform, broad clavate, 9–24 \times 7–14 μm , smooth, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of cells 4–23 \times 2.5 μm , distinctly constricted at the septa, contains light brown, brown membranous pigments. *Annulus* composed of rectangular, ellipsoid, or subsphaerical cells, 3–9 \times 2.5 μm , hyaline, branched.

Habitat: gregarious or scattered in forest.

Other material examined: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44.009', elev. 900 m, 10 May 2007, collected by Phongeun Sysouphanthong, ZRL4012 (BBH19242); same location, 8 May, collected by Phongeun Sysouphanthong, ZRL4002 (BBH19632).

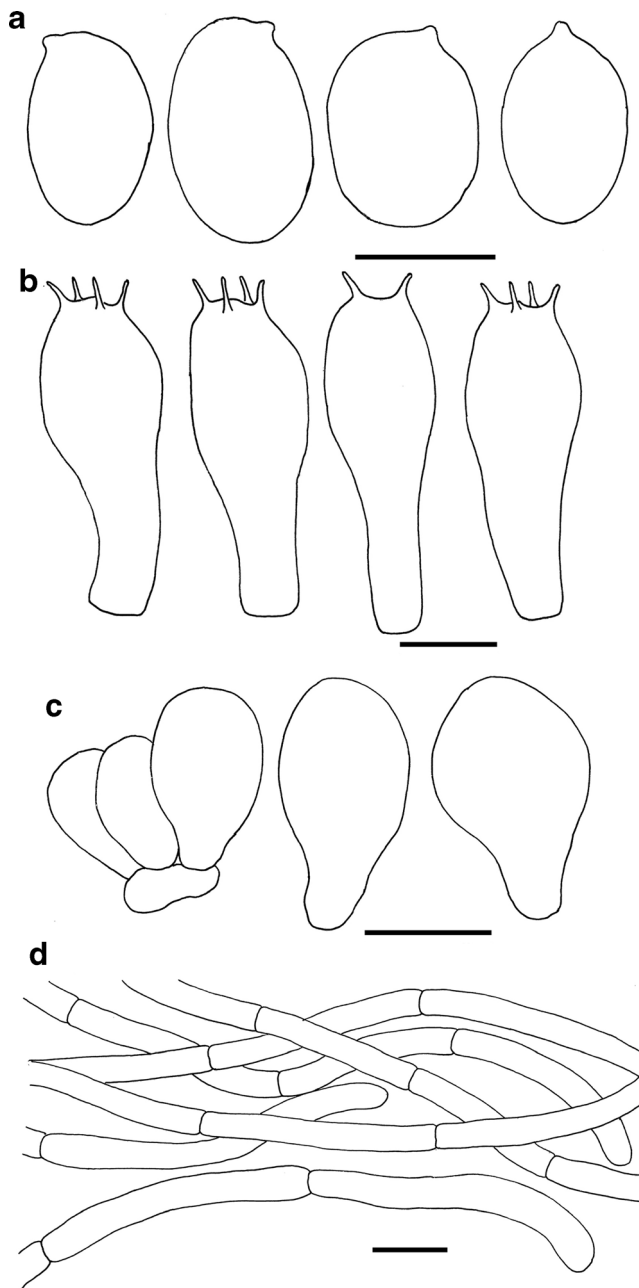


Fig. 24 Microcharacters of *A. planipileus* R.L. Zhao sp. nov. (ZRL2011248) **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a=5 μ m, b, c, d=10 μ m

Agaricus dolichopus R.L. Zhao, sp. nov. Figs. 7f, g and 18

Fungal Names: FN570222

Facesoffungi number: FoF01187

Etymology: the epithet “dolichopus” refers to relatively long stipe of this species.

Typus: CHINA, Yunnan Prov., Dali, Cangshan Mountain, 27 July 2014, collected by M.Q. He. ZRL2014120 (HMAS273989, **holotype**).

Original description: *Pileus* 40–45 mm in diam., 3–5 mm thick at disc, plane, surface dry, covered by fibrillose

squamules, triangular with slightly upturned tip, brown, dense at the disc and spreading towards the margin; margin straight to decurved and sometimes appendiculate. *Lamellae* free, 3 mm broad, crowded, intercalated with lamellulae, dark brown when mature. *Stipe* 75–80 \times 3–5 mm, equal, hollow, surface smooth or slightly fibrillose, white, not discolouring or slightly red on touching. *Annulus* thick membranous, 5 mm in diam., entire or torn, pendant, white, upper surface smooth and lower surface heavily floccose with brown patches at the margin. *Context* firm, on cutting, at first white in both pileus and stipe, then quickly turning slightly rubescent at the centre of pileus and stipe, finally after 15 min uniformly light brown. *Odour* mushroomy, phenol.

Macrochemical reaction: KOH not distinct or yellow, Schäffer’s reaction negative.

Basidiospores 4.5–5.4 \times 2.9–3.7 μ m [\bar{x} = 5.1 \pm 0.3 \times 3.4 \pm 0.2, Q = 1.4–1.8, Q_m = 1.5 \pm 0.1, n = 20], ellipsoid, brown, smooth, thick-walled, without germ pore. *Basidia* 14–19 \times 5.6–7.4 μ m, clavate, hyaline, smooth, 2- or 4-spored. *Cheilocystidia* abundant, pyriform, broad clavate, (10–) 14–21 \times 10–14 μ m, smooth, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 3.2–13 μ m in diam., slightly constricted at the septa, containing light brown, to brown pigments. *Annulus* composed of hyphae 3.2–9 μ m in diam., hyaline, branched, cylindrical.

Habitat: solitary in forest.

Other material examined: China, Yunnan Prov., Yimeng County, Dalongkou Forest Park, 17 August 2012, collected by Xie Meng, ZRL2012715 (HMAS273950).

Agaricus kunmingensis R.L. Zhao, sp. nov. Figs. 7h–j and 19.

Fungal Names: FN570221

Facesoffungi number: FoF01188

Etymology: the epithet “kunmingensis” refers to the region “Kunming” from where the holotype was collected.

Typus: CHINA, Yunnan Prov., Kunming, Yeya Lake, 30 June 2012, collected by R.L. Zhao, ZRL 2012007 (HMAS273970, **holotype**).

Original description: *Pileus* 20–35 mm in diam, 5 mm thick at the disc, campanulate when young, convex to subumbonate when open, with flat top, 45–70 mm in diam.; surface dry, covered by heavy fibrils completely at first, then broken into slightly recurved, more or less concentric, brown (oac700), light brown (oac683) squamules, on a white background, discolouring reddish brown on touching; margin decurved and exceeding. *Lamellae* free, 3–6 mm broad, some slightly ventricose, crowded, intercalated with lamellulae, reddish brown (oac599), brown, dark brown with age, *Stipe* 90–150 \times 6–7 (apex) – 10–12 (base) mm when mature, ventricose when young, then cylindrical to slightly clavate, hollow, white, surface smooth or slightly fibrillose, white, discolouring reddish brown on touching. *Annulus* thick membranous, 5–8 mm in diam., entire, pendant or subperonate,

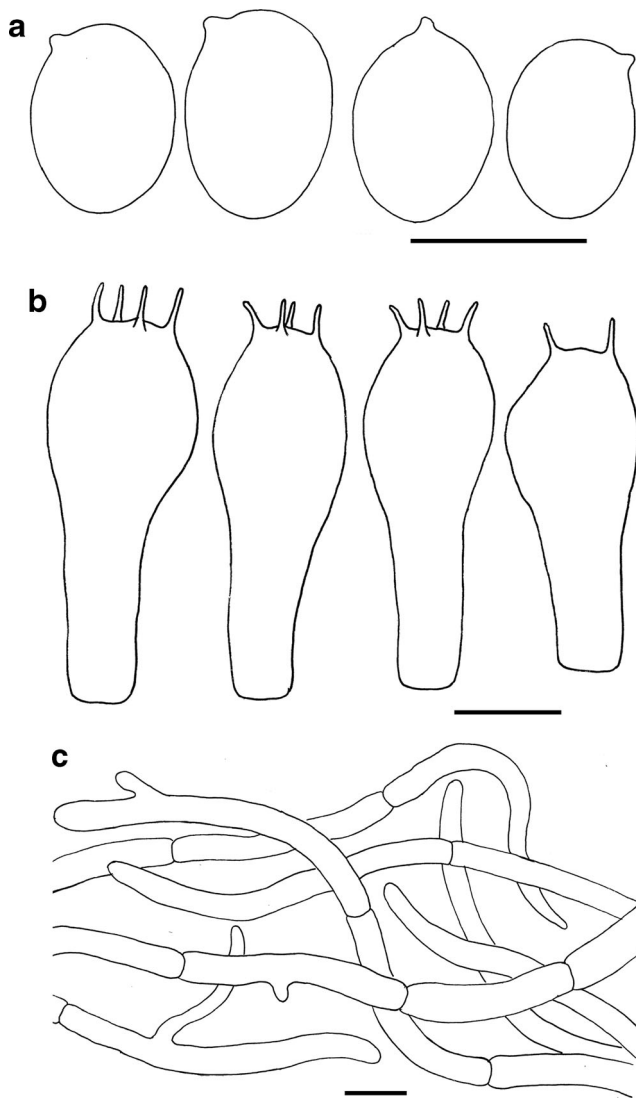


Fig. 25 Microcharacters of *A. lanipedisimilis* P. Callac & R.L. Zhao sp. nov. (ZRL2012193) **a.** basidiospores, **b.** basidia, **c.** pileipellis, bars: a, b = 5 μ m, c = 10 μ m

upper surface smooth, white and lower surface, heavily floccose, sometimes the floccose layer incompletely white or light brown. *Context* firm, on cutting at first white to dirty white in both pileus and stipe, then quickly turning reddish brown, finally after 15 min uniformly light brown. *Odour* pleasant or indistinct.

Macrochemical reaction: KOH not distinct or slightly reddish brown, Schäffer's reaction negative.

Basidiospores $4.1\text{--}5.3 \times 2.7\text{--}3.3$ μ m [= $4.6 \pm 0.3 \times 3 \pm 0.2$, $Q = 1.4\text{--}1.7$, $Q_m = 1.5 \pm 0.1$, $n = 20$], ellipsoid, brown, smooth, thick-walled, without germ pore. *Basidia* $17\text{--}23 \times 6\text{--}7.7$ μ m, clavate, hyaline, smooth, 4-spored. *Cheilocystidia* abundant in young fruiting body and less when mature, pyriform, broad clavate, $14\text{--}25 \times 8\text{--}16$ μ m, smooth, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of 6–15 μ m in diam. hyphae, slightly constricted at the septa, containing light

brown to brown pigments. *Annulus* composed of 3–9 μ m diam., hyaline, branched, cylindrical hyphae.

Habitat: scattered in mixed forests of *Ericaceae* and *Fagaceae*.

Other material examined: CHINA, Yunnan Prov., Kunming, Yeya Lake, 30 June 2012, collected by R.L. Zhao, ZRL2012015(HMAS273942).

Notes: These three species differ from *A. magnivelaris* Pegler as the latter has oblong-ellipsoid basidiospores ($Q_m = 1.9$), while the spores of all new species are ellipsoid with a $Q_m = 1.5\text{--}1.6$. The basidiomes of the new species are more or less reddish brown on touching or cutting or in KOH reaction, but in *A. variabilicolor* and *A. dolichopus* discolouration is very weak or indistinct; while *A. kunmingensis* has a distinct reddish brown discolouration in all cases. In the field, *A. variabilicolor* has a light brown cap which is not darker at the disc of pileus, and has a floccose stipe surface, while *A. dolichopus* has distinct brown triangular scales, which are darker at the disc, and a smooth to slightly fibrillose stipe surface.

4.10 *Agaricus* [subgenus *Pseudochitonina*] section *Sanguinolenti* Jul. Schäff & F.H. Møller ex L.A. Parra, *Agaricus* L. *Allopsalliota* Nauta & Bas, 378. 2008.

\equiv *Agaricus* [subgenus *Agaricus* section *Sanguinolenti*] subsection *Sylvatici* L.A. Parra, *Agaricus* L. *Allopsalliota* Nauta & Bas, 390. 2008.

Facesoffungi number: FoF 01189; Fig. see Parra 2008, pp600, 697–705

Type species: *Agaricus sylvaticus* Schaeff., designated by L.A. Parra, *Agaricus* L. *Allopsalliota* Nauta & Bas, 378. 2008.

Delimitation of *Agaricus* section *Sanguinolenti*

KOH and Schäffer's reactions negative. Surface of pileus and stipe discolouring reddish brown on bruising. Context turning reddish brown to blood red on exposure. Odour indistinct or mushroomy, never anise, bitter almond, ink or phenol. Basidiome slender or stout. Annulus superous, simple, pendant. Cheilocystidia present, and abundant.

Stem age and phylogenetic support

This section is represented by clade D7, which is fully supported (Figs 1 and 3), and the stem age is 23.45 Ma. The concept of section *Sanguinolenti* was shown to be a polyphyletic group and composed of three monophyletic lineages in Zhao et al. (2011); two were named as subsections *Bohusia* and *Sylvatici* respectively (Parra 2008); and the third clade had been recognized as section *Nigrobrunnescens* (Parra et al. 2014; Wang et al. 2015). However, in multi-gene analysis these two "subsections" were formed around 24 Ma. Therefore, we elevate them to sectional level: subsection *Bohusia* to section *Bohusia* (described above) and subsection

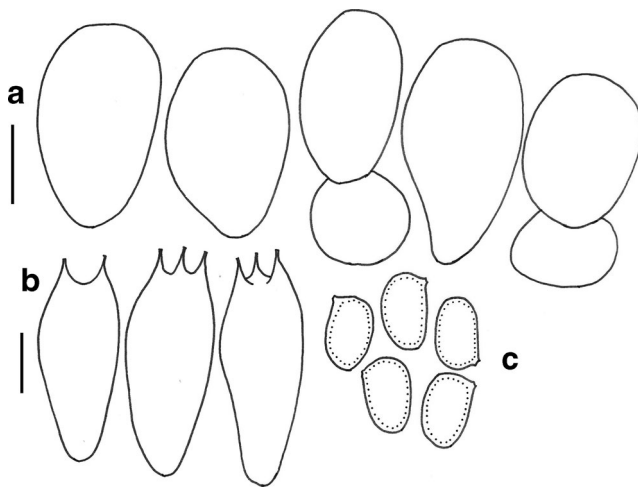


Fig. 26 Microcharacters of *A. albosquamosus* L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov. (LD2012192, Holotype) **a.** Cheilocystidia **b.** Basidia **c.** Basidiospores, bars a = 10 μm; b, c = 5 μm

Sylvatici is elevated to sectional level and keeps the older name *Sanguinolenti*. The species belonging to section *Sanguinolenti* cluster with type species *A. sylvaticus*, such as *A. benesii* (Pilát) Pilát and *A. dilutibrunneus* sp. nov.

Agaricus dilutibrunneus R.L. Zhao, sp. nov. Figs. 5g–I and 20

Fungal Names: FN570223

Facesoffungi number: FoF01190

Etymology: the epithet “dilutibrunneus” refers to light brown scales on the pileus of this species.

Typus: CHINA, Yunnan Prov., Kunming, Yeya Lake, 30 June 2012, collected by Rui-Lin Zhao, ZRL2012010 (HMAS273990, holotype).

Original description: *Pileus* 50–70 mm in diam., convex, subumbonate; surface dry, covered by squamules, dense at the disc and spreading towards the margin, light brown on a greyish white background; margin decurved. *Lamellae* free, crowded, intercalated with lamellulae, brown, dark brown

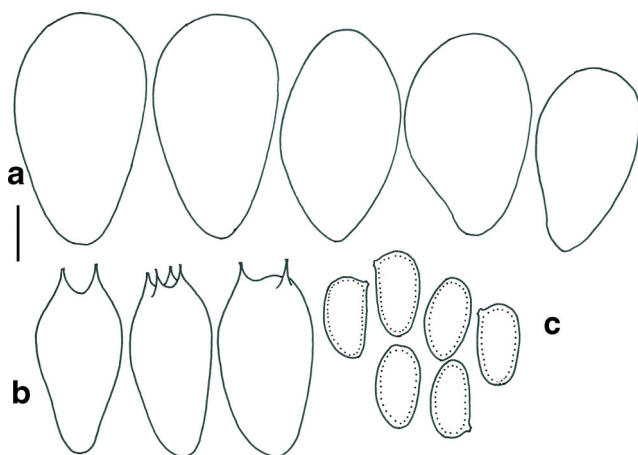


Fig. 27 Microcharacters of *A. leucopidotus* L.J. Chen & R.L. Zhao sp. nov. (LD201214, Holotype) **a.** Cheilocystidia **b.** Basidia **c.** Basidiospores, bars a, b, c = 5 μm

when mature. *Stipe* around 100 × 10 mm, equal to slightly clavate, abruptly bulbous at base; surface fibrillose, and thickening towards the base, white, staining reddish yellow on touching. *Annulus* membranous, white, fragile. *Context* firm, on cutting firstly white in both pileus and stipe, then quickly turning slightly rubescent at the centre of pileus and stipe, finally after 15 min uniformly light brown.

Context white to slightly yellowish white, firm, and discolouring reddish yellow on cutting. *Odour* unknown.

Macrochemical reaction: KOH unknown, Schäffer’s reaction negative.

Basidiospores 4.8–5.8 × 3.4–4.2 μm [\bar{x} = 5.4 ± 0.3 × 3.8 ± 0.2, Q = 1.2–1.6, Q_m = 1.4 ± 0.1, n = 20], ellipsoid, brown, smooth, dark brown, without germ pore. *Basidia* 14–19 × 6.7–8.5 (–9) μm, hyaline, smooth, 4-spored. *Cheilocystidia* composed of pyriform, ellipsoid cells, 17–32 × 13–24 μm; subsphaerical, 10–20 μm in diam, capitate, smooth, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis consisting of 3.5–8 (–13) μm diam., light yellow or hyaline, smooth, branched, hyphae, not constricted at the septa.

Habitat: solitary in forest.

Notes: This species is characterized by its light brown squamules on the pileus and subglobose cheilocystidia in chains. It is most similar to *A. benesii* (Pilát) Singer, which has a white or light brown cap, and similar shape and size spores. However, the cheilocystidia of *A. benesii* are clavate and often septate (Parra 2008), which is different from this *A. dilutibrunneus*. Phylogenetically, *A. dilutibrunneus* has a distinct position which also indicates that it is a species distinct from *A. benesii* and *A. sylvaticus*.

4.11 Agaricus [subgenus *Pseudochitonina*] section *Trisulphurati* Heinem., Bull. Jard. Bot. État 26: 91. 1956.

Facesoffungi number: FoF01191; Fig. see Heinemann 1956, PlateXIX, Fig. 9

Type species: *Agaricus trisulphuratus* Berk., designated by Heinemann Bull. Jard. Bot. État 26: 91. 1956.

Delimitation of section *Trisulphurati*

KOH reaction positive, Schäffer’s reaction negative. Not staining on touching or cutting. Smell carbolic acid, ink, or mushroomy. *Annulus* superous, membranous, and lower side always wooly or cortinate. *Cheilocystidia* present, clavate, or subcapitate. *Basidiospore* endosporium or not, shape ellipsoid with suprahilar depression (distinct in sample ZRL3014).

Stem age and phylogenetic support

This section (clade D5) is represented by two specimens of *A. trisulphuratus*, which is fully supported (Fig. 1). It is also fully supported in the ITS analysis (Fig. 3) with extended sampling. The stem age is 18.03 Ma. Heinemann had separated *Agaricus* into three subgenera: *Agaricus*, *Lanagaricus*, and

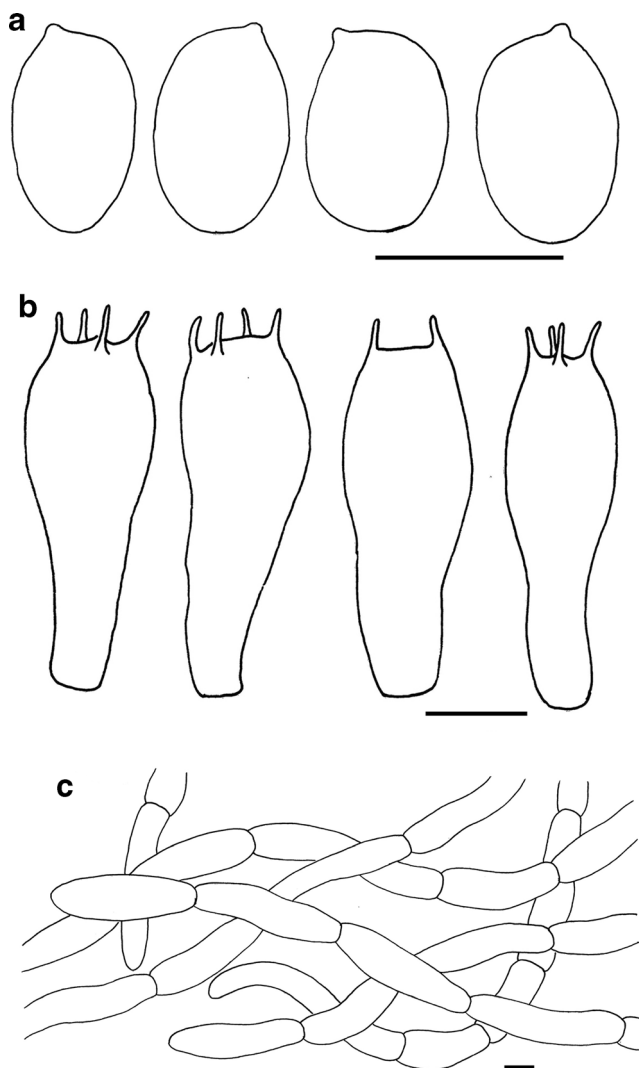


Fig. 28 Microcharacters of *A. amoenomyces* R.L. Zhao sp. nov. (ZRL2010072) **a.** basidiospores, **b.** basidia, **c.** pileipellis, bars = 5 μ m

Coniogaricus (Heinemann 1956a, b). The subgenus *Lanagaricus* is characterized by a woolly universal veil and comprises four sections (Heinemann 1956a). Section *Trisulphurati* is characterized by a brightly coloured universal veil and thickening of the endospores in the basidiospores. *Agaricus trisulphuratus*, the type species of section *Trisulphurati*, has previously been studied, and its phylogenetic position is closely related to sections *Bivelares*, *Chitonioides*, and *Xanthodermatei* (Zhao et al. 2011; Vellinga et al. 2011). In the present study, *A. trisulphuratus* along with *A. ignicolor* sp. nov., nest together in the subgenus *Pseudochitonina* (clade D) with strong support, and also has a similar relationship with those sections. Thus, section *Trisulphuratus* is now placed in subgenus *Pseudochitonina*.

Agaricus trisulphuratus Berk. **complex**, Ann. Mag. Nat. Hist. V, 15: 386, 1885. Figs. 4e, f and 21.

≡ *Cystoagaricus trisulphuratus* (Berk.) Singer, Mycologia 39: 87, 1947.

Facesoffungi number: FoF01192

Pileus mostly 30–60 mm in diam., hemispherical, conical, broadly conical, convex, plano-convex, subumbonate when mature, surface dry, covered with acute squamules, floccose, erect at centre and recurved at the margin; golden yellow (5B8), orange, reddish orange (7A8), deep orange (5A8), and fading upon aging or in rain, margin exceeding. *Lamellae* free, crowded to more crowded, at first white, orange-white (6A2), then greyish-pink, reddish-brown (8E3), greyish-brown (8D3, 6D3), finally brown (7E8), dark brown, edge heteromorphic (colour lighter than those of lamellae). *Stipe* 20–90 \times 3–7 mm, cylindrical; hollow; mostly with rhizomorphs at base; smooth and white above the annulus; flaky and shaggy, same colour as the pileus below the annulus, not discolouring on touching. *Annulus* pendent, same colour with flaking of stipe surface, in most cases difficult to be recognized from those flakes. *Context* firm, on cutting white in both pileus and stipe, no discolouring on exposure. *Odour* none or mushroomy.

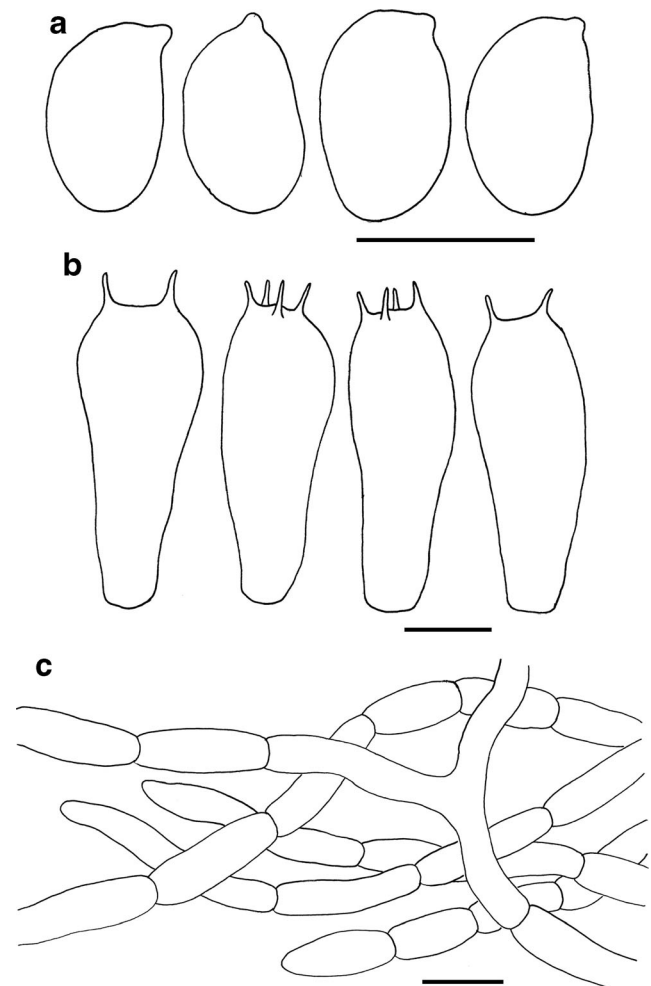


Fig. 29 Microcharacters of *A. suthepensis* L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov. (LD2012100) **a.** basidiospores, **b.** basidia, **c.** pileipellis, bars: a, b = 5 μ m, c = 10 μ m

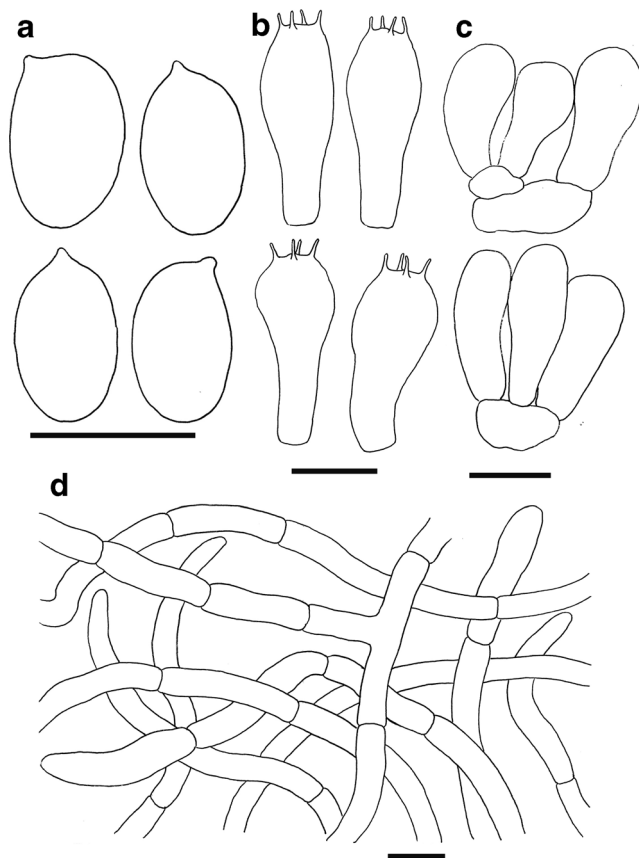


Fig. 30 Microcharacters of *A. gratolens* Pradeep & R.L. Zhao, sp. nov. (ZRL3093). **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a = 5 μ m, b, c, d = 10 μ m

Macrochemical reaction: KOH reaction yellow at pileus, Schäffer's reaction negative.

Basidiospores 5–6 \times 3.2–4 μ m [\bar{x} = 5.5 \pm 0.6 \times 3.7 \pm 0.3, Q = 1.3–1.8, Q_m = 1.48 \pm 0.32, n = 20], ellipsoid with suprahilar depression, reddish brown, smooth, thick-walled, without germ pore. **Basidia** 12–14 \times 6–7 (–8) μ m, clavate, 4-spored. **Cheilocystidia** 18–36 \times 6–13 (–15) μ m, clavate, cylindrical with tapering base, occasionally with 1 septa, surface spiny in some cases, hyaline. **Pleurocystidia** absent. **Pileipellis** a cutis composed of 3–5 μ m diam., long cylindrical, curved, branched, fine, spiny hyphae. **Annulus** and **stipitipellis** consisting of the same hyphae as pileipellis.

Habitat: mostly solitary, some scattered in small groups in soil.

Other material examined: THAILAND, Chiang Mai, collection data collector unknown, ZRL1003 (SFSU); Chiang Mai Prov., Chiang Dao Cave, 22 July 2004, collected by Ruilin Zhao, ZRL3089 (BBH19605; SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 26 June 2005, collected by Jennifer Kerekes, ZRL2045 (SFSU); same location, 3 July 2005, collected by Ruilin Zhao, ZRL2087 (BBH19470; SFSU); same location, 18 August

2004, collected by Edward Grand, ZRL2128 (BBH19508; SFSU); same location, 3 June 2006, collected by Ruilin Zhao, ZRL3014 (BBH19531; SFSU); same location, 18 June 2006, collected by Ruilin Zhao, ZRL3070 (BBH19586; SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 11 August 2004, collected by Kevin D Hyde, ZRL2123 (BBH19502; SFSU); same location, 22 May 2006, collected by Ruilin Zhao, ZRL3002 (SFSU); same location, 29 May 2006, collected by Ruilin Zhao, ZRL3009 (BBH19526; SFSU); same location, 24 June 2006, collected by Ruilin Zhao, ZRL3072 (BBH19588; SFSU); Chiang Mai Prov., Mae Taeng Dist., Tung Joaw Village, forest trail, N19°08.07' E98°38.90', elev. 1300 m., 3 August 2004, collected by Edward Grand, ZRL2111 (BBH19491; SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8" E 98°44'47.3", elev. 1050 m., 12 June 2004, collected by Ruilin Zhao, ZRL3054 (BBH19570; SFSU); CHINA, Yunnan province, Dali, Cangshan Mountain, Yunnongfeng, 24 July 2014, collected by Sun SY, Bai XM, ZRL2014023 (HMAS273985), ZRL2014024 (HMAS273935), ZRL2014025 (HMAS273962), ZRL2014026 (HMAS273927), ZRL2014030 (HMAS273956).

Notes: *A. trisulphuratus* is type species of section *Trisulphurati*, and common in forests or grasslands in tropical and subtropical areas. This species also has been reported from Africa (Heinemann 1956a). In the phylogenetic tree (Fig. 2), the samples from Thailand and Malaysia cluster with

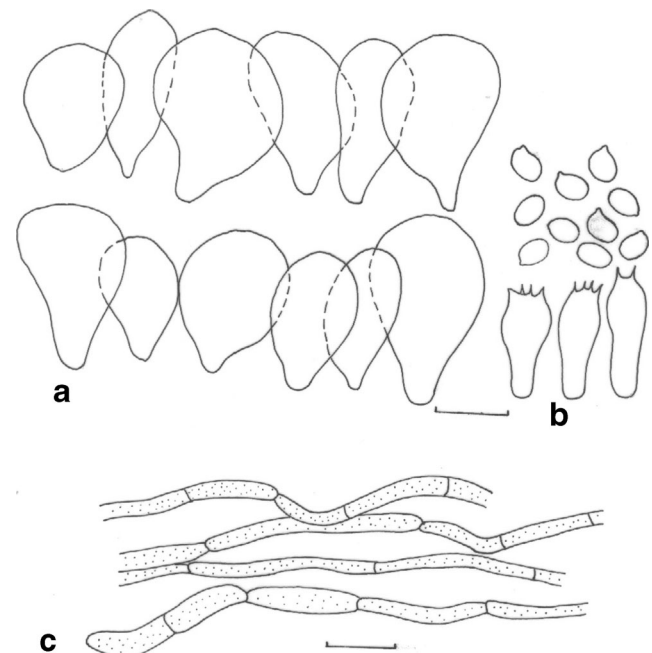


Fig. 31 Microcharacters of *A. parasubrutescens* Callac & R.L. Zhao, sp. nov. (ZRL2014076) **a.** cheilocystidia, **b.** basidia and basidiospores, **c.** pileipellis, bars: a, b = 10 μ m, c = 25 μ m

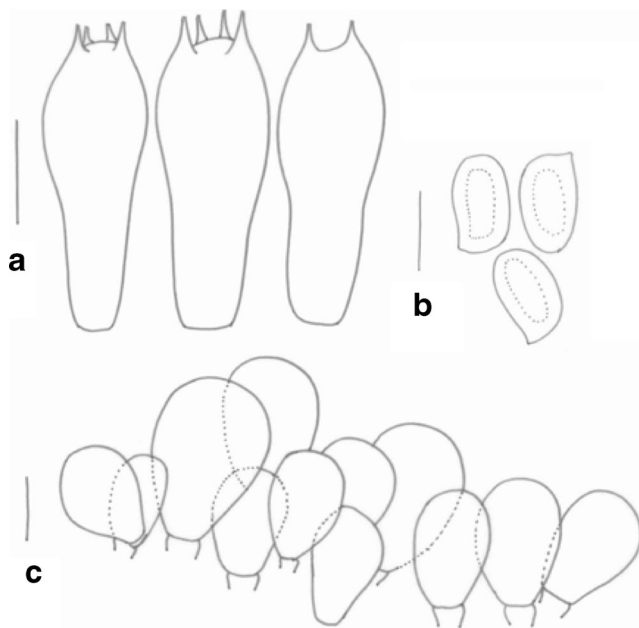


Fig. 32 Microcharacters of *A. inthanonensis* L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov. (LD014) **a.** basidia **b.** basidiospores, **c.** cheilocystidia, bars: a = 8 μ m, b = 4 μ m, c = 10 μ m

those from Africa, with strong support. Morphologically, they match the description of Heinemann (1956a).

Agaricus ignicolor R.L. Zhao, sp. nov. Fig. 4g–i and 22
Fungal Names: FN570201

Facesoffungi number: FoF01193

Etymology: “igno” refers to fire, and the species epithet “ignicolor” refers to fire-coloured basidiomes of this species.

Typus: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44. 009', elev. 900 m, 21 August 2005, collected by Ruilin Zhao, ZRL2132 (BBH 19512, **holotype**; HMAS 274009, **isotype**).

Original description: *Pileus* 55 mm in diam., 2 mm thick at the disc, broadly conic, convex; surface dry, with flaky to sharp warty squamules, erect at the disc then recurved to margin, deep orange, and fading in rain, margin distinctly exceeding. *Lamellae* free, more crowded, intercalated with lamellulae, 4–5 mm broad, at first white, greyish pink, then dull red (8B3), finally brown, to dark brown. *Stipe* 22 \times 5–7 mm, cylindrical, curved, with rhizomorphs at the base, hollow; smooth and white above the annulus; with thick flaky squamules and deep orange below annulus, not staining on touching. *Annulus* pendent, superous, and mixed with the stipe squamules, deep orange. *Context* firm, white to cream in both pileus and stipe, not discolouring on cutting. *Odour* mushroomy.

Macrochemical reaction: KOH and Schäffer’s reactions negative.

Basidiospores 5–6 \times 3–4 μ m [\bar{x} = 5.4 \pm 0.4 \times 3.6 \pm 0.4, Q = 1.3–2, Q_m = 1.53 \pm 0.47, n = 20], ellipsoid, reddish-brown,

smooth, thick-walled, without germ pore. *Basidia* 12–16 \times 6–7 μ m, clavate, smooth, hyaline. *Cheilocystidia* 21–30 \times 7–13 μ m, clavate, broadly clavate, mostly subcapitate with apex 4–7 μ m in diam., smooth or with slightly fine spines, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 2.5–5 μ m in diam., equal, branched, even light yellow, smooth or fine spiny, without constriction at the septa. *Annulus* and *stipitipellis* hyphae similar to those of pileipellis.

Habitat: solitary in soils of road banks.

Notes: *Agaricus crocopeplus* Berk. & Br., *A. erythrotrichus* Heinem., and *A. trisulphuratus* are species from section

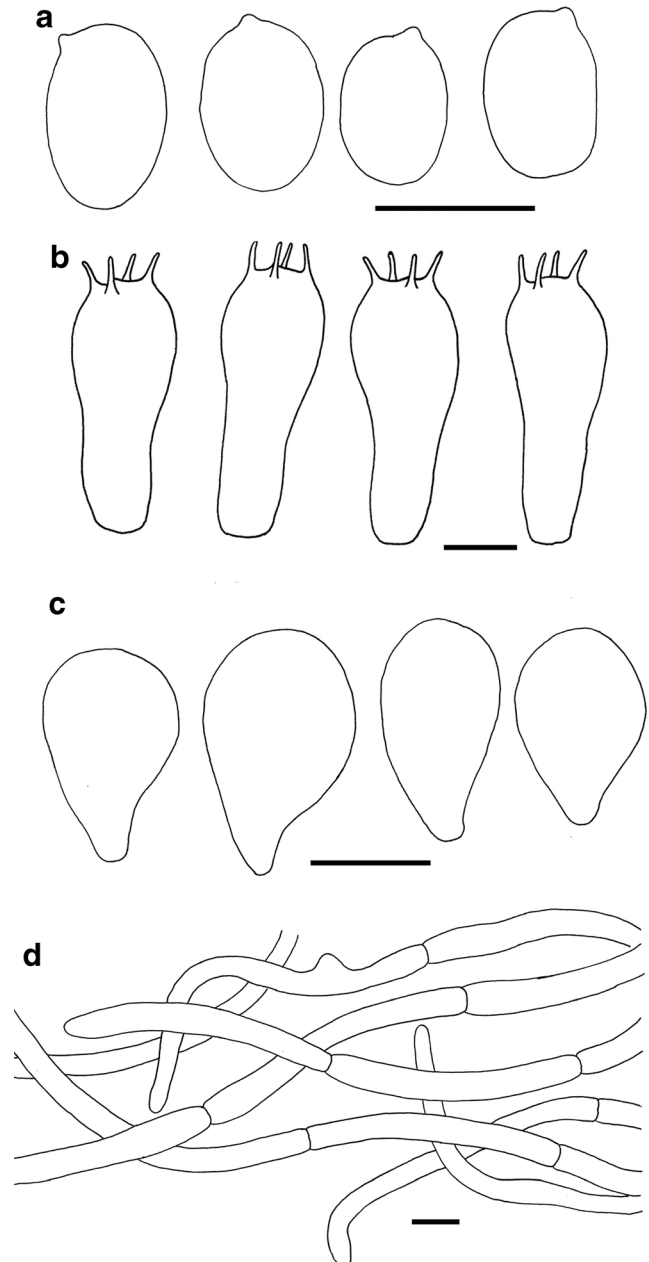


Fig. 33 Microcharacters of *A. brunneopileatus* Callac & R.L. Zhao, sp. nov. (ZRL2012115) **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a, b = 5 μ m, c, d = 10 μ m

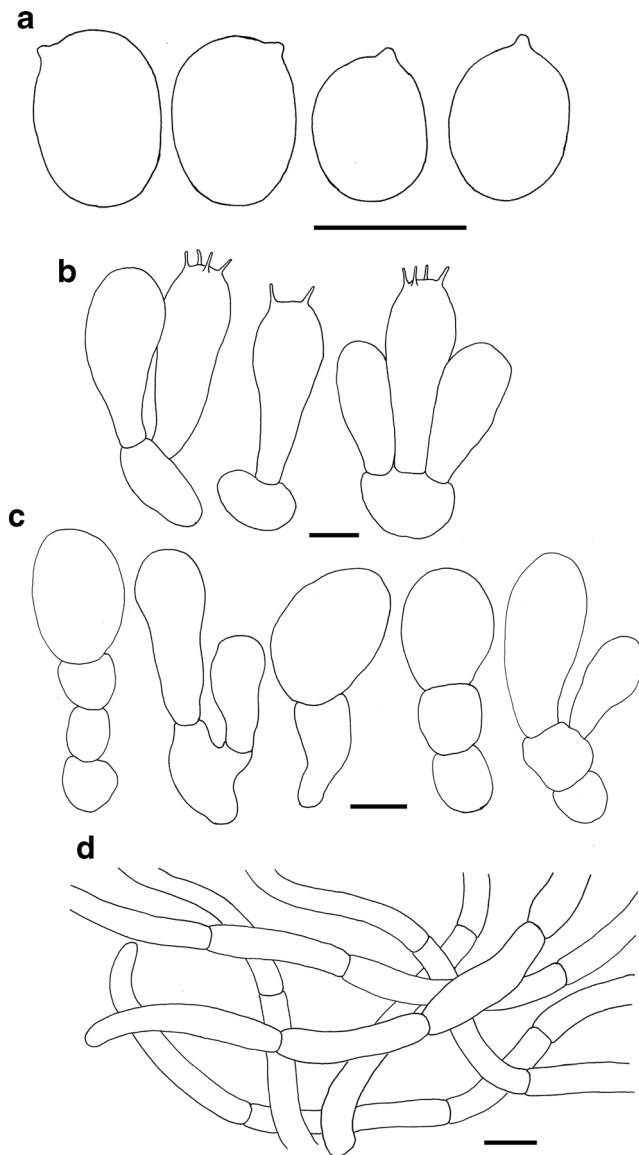


Fig. 34 Microcharacters of *A. linzhiensis* R.L. Zhao (ZRL2012618) **a.** basidiospores, **b.** basidia, **c.** cheilocystidia, **d.** pileipellis, bars: a, b=5 μ m, c, d=10 μ m

Trisulphurati (Heinemann 1956a). All including this new species, are covered by a developed orange universal veil, which is very distinctive in the field. *Agaricus crocopeplus* has a white membranous annulus, which differs from the others, which have orange, flaky annuli. *Agaricus erythrotrichus* has much larger basidiospores (6.2–7.4 \times 3.8–4.3 μ m) than all others (Heinemann 1956b, 1980). *Agaricus ignicolor* is most similar to *A. trisulphuratus*; however, it could be separated by the following characteristics: (i) the edge of annulus of *A. ignicolor* is orange, while in *A. trisulphuratus* it is not coloured or slightly white; (ii) the cheilocystidia of *A. ignicolor* are often capitata, but in *A. trisulphuratus* they are clavate to cylindrical; and (iii) squamules of *A. ignicolor* are much more stable, and not easily removed by rain drops.

The phylogeny (Fig. 3) also indicates they are different species.

4.12 *Agaricus* [subgenus *Pseudochitonina*] section *Xanthodermatei* Singer, Sydowia 2: 36. 1948.

Facesoffungi number: FoF 01194; Fig. see Parra 2013, pp822-830

Type species: *Agaricus xanthodermus* Genev. [“*xanthoderma*”] designated by Singer, Sydowia 2: 36.1948.

Delimitation of *Agaricus* section *Xanthodermatei*

KOH reaction positive yellow, Schäffer’s reaction negative. Surface of pileus and stipe often discolouring yellow on bruising. Context turning strongly yellow on exposure, especially at the base of the stipe. Odour mostly iodine or phenol, except some species with a pleasant odour (such as *A. murinocephalus* R.L. Zhao, Desjardin & K.D. Hyde). Annulus superous, simple, pendent, large. Cheilocystidia present or not. It contains toxic species.

Stem age and phylogenetic support

The emended section *Xanthodermatei* is represented by Clade D4 which is fully supported (Fig. 1), and well-supported in the ITS tree (Fig. 3). The stem age is 19.58 Ma. Previously, section *Xanthodermatei* was considered a monophyletic group by most mycologists (Callac and Guinberteau 2005; Challen et al. 2003; Geml et al. 2004; Kerrigan et al. 2005; Parra et al. 2011; Zhao et al. 2011, 2013; Thongklang et al. 2014). Recently, three lineages were revealed in this section based on ITS sequence data after adding some samples from tropical areas (Thongklang et al. 2014). However, in the present multi-gene analysis, the previously conceived section comprises two lineages (reflected in clades D3 and D4) and shows it to be polyphyletic group. The ITS analysis (Fig. 3) using an extended sampling also has the same topology. Based on a combination of stem age and morphological characteristics, those two lineages are ranked as two sections in this study: one is section *Hondenses* (clade D3 and see above); and the other is emended section *Xanthodermatei* (clade D4, which contains the type species *A. xanthodermus*), of which presently *A. parvitigrinus* Guinb. & Callac, *A. xanthosarcus* Heinem. & Goos.-Font., *A. tollocanensis* Callac & G. Mata, *A. murinocephalus*, and *A. microvolvatulus* Heinem. *A. tibetensis* J.L. Zhou and R.L. Zhao nom. prov. and *A. tythocarpus* R.L. Zhao nom. prov. have been demonstrated to be members of this section (Zhou et al. 2016). Furthermore, based on a previous phylogenetic analysis (Thongklang et al. 2014), *A. bisporiticus*, *A. californicus* Peck, *A. endoxanthus* Berk. & Broome, *A. fuscopunctatus* Thongklang, L.J. Chen, Callac & K.D. Hyde, *A. iodismus* Heinem., *A. laskibarii* L.A. Parra & P. Arrill., *A. menieri* Bon, *A. moelleri* Wasser, *A. moelleroides* Guinb. & L.A. Parra, *A. placomyces* Peck, *A.*

pocillator Murrill, *A. pseudoprattensis* (Bohus) Wasser, and *A. xanthodermulus* Callac & Guinb. should also belong in this section.

5. *Agaricus* subgenus *Spissicaules* (Heinem.) R.L. Zhao & Moncalvo, stat. nov.

Fungal Names: FN570202

Facesoffungi number: FoF 01195

≡ *Agaricus* subsection *Spissicaules* Heinem., *Sydowia* 30: 12. 1978. [basionym]

Type species: *Agaricus spissicaulis* F.H. Møller, designated by Heinemann, *Sydowia* 30: 12. 1978.

Sections included: sections *Amoeni* sect. nov.; *Rarolentes*; *Spissicaules*; and *Subrutillescentes*.

Delimitation of *Agaricus* subgenus *Spissicaules*

KOH and Schäffer's reactions negative or weakly positive. Staining yellowish or indistinct, rarely reddish on bruising of the surface of pileus or stipe; context unchanged or turning pink, reddish brown on exposure. Odour strong of almonds with exception of section *Subrutillescentes*, having an odour similar to *Lepiota cristata*. Annulus simple, often striate on the upper side. Cheilocystidia present or absent, generally cylindrical to clavate, some basidiole-like, simple and not in chains.

Stem age and phylogenetic support

In the multi-gene analysis (Fig. 1), this subgenus reflects clade C with 0.99 PP support, and its stem age is 33.08 Ma. This phylogeny also shows that the clade composed of subgenera *Minores* and *Flavoagaricus* is sister to this subgenus. Subgenus *Spissicaules* is composed of four lineages, which are all fully supported and their stem ages are from 19–24 Ma. In this study, those four lineages are recognized as four sections: *Amoeni* sect. nov., *Rarolentes*, *Spissicaules*, and *Subrutillescentes*.

5.1 *Agaricus* [subgenus *Spissicaules*] section *Spissicaules* (Heinem.) Kerrigan, *Mycologia* 77(1): 141. 1985.

Facesoffungi number: FoF01196

≡ *Agaricus* subsection *Spissicaules* Heinem., *Sydowia* 30: 12. 1978.

Type species: *Agaricus spissicaulis* F.H. Møller, designated by Heinemann, *Sydowia* 30: 12.1978.

Delimitation of *Agaricus* section *Spissicaulis*

KOH and Schäffer's reactions negative or weakly positive. Surface of pileus and stipe indistinctly discolouring on bruising. Context unchanged or turning pink, reddish on exposure. Odour generally faint, or pleasant. Annulus pendant, simple,

superous. Cheilocystidia present, cylindrical to clavate, some septate at base, rarely similar to basidia.

Stem age and phylogenetic support

Clade C4 reflects this section, which is fully supported in multi-gene tree (Fig. 1), and well-supported in ITS tree (Fig. 2). The stem age of section *Spissicaules* is 21.98 Ma. Presently, the main species of this section are included in this section, such as *A. lanipes*, *A. litoralis*, *A. bresadolanus*, and two new species described in this study.

Agaricus litoraloides R.L. Zhao, sp. nov. Figs. 6e, f and 23

Fungal Names: FN570228

Facesoffungi number: FoF01197

Etymology: the epithet “litoraloides” refers to the morphological similarity of this new species to *A. litoralis*.

Typus: CHINA, Yunnan Prov., Dali City, Cangshan, 30 July 2011, collected by H.Y. Su *SHY2012073026* (HMAS 274021, **holotype**).

Original description: *Pileus* 30–80 mm in diam., 7 mm thick at disc, parabolic when young then convex; surface dry, covered by fibrils on the whole cap, and broken into large squamules (3–5 mm wide), triangular, appressed, dark brown (oac637), reddish brown (oac635); margin exceeding. *Lamellae* free, 3–5 mm broad, crowded, reddish brown, brown, dark brown with age. *Stipe* 80–120 × 11–20 mm, cylindrical to slightly clavate; stuffed or hollow; white; surface smooth above the annulus, and erect squamules below the annulus, staining light yellow on touching. *Annulus* membranous, simple, fragile, pendant, both surfaces smooth and white. *Context* fleshy, on cutting at first white, both in pileus and stipe, stipe quickly turning slightly rubescent on exposure. *Odour* strong of almonds.

Macrochemical reactions: KOH reaction unknown, Schäffer's reaction negative.

Basidiospores 6.7–9 × 4.7–6.1 μm, [\bar{x} = 8.2 ± 0.5 × 5.4 ± 0.3, Q = 1.3–1.8, Qm = 1.5 ± 0.1, n = 20], ellipsoid, occasionally oblong ellipsoid, brown to dark brown, smooth, thick-walled, without germ pore. *Basidia* 21–31 × 9.2–11.8 μm, clavate to broadly clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 12.7–24.3 × 7.8–16 μm, abundant, simple, mostly broadly clavate, pyriform, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 2.5–12.3 μm in diam., cylindrical, with brownish membranous pigments, smooth, not or slightly constricted at the septa. *Annulus* composed of hyphae 2–10 μm in diam., cylindrical, hyaline, smooth.

Habitat: solitary in the forest.

Other material examined: China, Yunnan Prov., Tengchong county, Datang, Danlonghe Village, 28 July 2011, collected by G.F. Yang ZRL2011249 (HMAS 274019).

Notes: the morphological characters of *A. litoraloides* are similar to *A. litoralis* (Nauta 2001) in having large and dark brown scales, large spores, discolouring on touching and cutting, and odour. However the molecular analysis (Fig. 3) clearly supports them as distinct species.

Agaricus planipileus R.L. Zhao, sp. nov. Figs. 6g, h and 24

Fungal Names: FN570226

Facesoffungi number: FoF01198

Etymology: the epithet “planipileus” refers to the applanate pileus of this species.

Typus: CHINA, Yunnan Prov., Tengchong county, Datang, Danlonghe Village, 28 July 2011, collected by R.L. Zhao, ZRL2011250 (HMAS273997, **holotype**).

Original description: *Pileus* 70–110 mm in diam., 6–10 mm thick at the disc, applanate, top flat; surface dry, covered by fibrillose squamules, triangular, appressed, light brown (oac646) and easily removed by rain drops, then displaying white background, margin straight and slightly lacerate. *Lamellae* free, 5–8 mm broad, normal to slightly ventricose, crowded, pink, reddish brown, dark brown (oac610, 636). *Stipe* 80–200 × 6–20 (apex) – 15–35 (base) mm, cylindrical with a bulbous base, hollow, surface smooth to slightly fibrillose and white above the annulus, floccose squamose and white with light brown tips below the annulus, staining light yellow on touching. *Annulus* membranous, simple, pendant, upper surfaces smooth and lower surface floccose, 8–12 mm broad, white. *Context* fleshy, on cutting white, in both pileus and stipe, quickly turning rubescent on exposure. *Odour* strong of almonds.

Macrochemical reactions: KOH reaction unknown, Schäffer’s reaction negative.

Basidiospores 5.7–7.3 × 3.6–4.6 μm, [\bar{x} = 6.3 ± 0.4 × 4.1 ± 0.3 μm, Q = 1.3–1.8, Qm = 1.5 ± 0.2, n = 20], ellipsoid, brown, smooth, thick-walled, without germ pore. *Basidia* 14–17 × 7–9 μm, clavate to broadly clavate, hyaline, smooth, 4-(2-) spored. *Cheilocystidia* 10.7–24 × 7.8–11.8 μm, abundant, simple, pyriform, irregular ellipsoid, wide clavate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of 3–11.4 μm diam., long cylindrical, smooth, hyphae, with brownish membranous pigments, not constricted at the septa.

Habitat: solitary in the forest.

Other material examined: China, Yunnan Prov., Tengchong county, Datang, Danlonghe Village, 28 July 2011, collected by S.Y. Liu, ZRL2011248 (HMAS253916).

Notes: Several species have light brown to ochre squamules on the pileus, and smaller basidiospores (<7 μm), such as *A. bresadolanus* and *A. litoralis* (Nauta 2001; Parra 2008). However, *A. litoralis* differs from *A. planipileus* in having a depressed pileus at the disc and a stipe not bulbous at the base. *Agaricus bresadolanus* has wider ellipsoid spores (Q = 1.24). The

phylogenetic analysis also supports them as distinct species.

Agaricus lanipedisimilis P. Callac & R.L. Zhao, sp. nov. Figs. 8a–d and 25.

Fungal Names: FN570220

Facesoffungi number: FoF01199

Etymology: the epithet “lanipedisimilis” refers to the similarity of this species to *A. lanipes*. From the union of lanipedis, the genitive of lanipes, and similis.

Typus: CHINA, Yunnan Prov., Cangyuan county, Mengjiao, Nangunhe National Natural Reserve, 9 July 2012, collected by R.L. Zhao, ZRL2012193 (HMAS 273998, **holotype**).

Original description: *Pileus* 70–90 mm in diam., 3–8 mm thick at disc, parabolic when young and convex; surface dry, completely covered by appressed fibrils and broken into squamules towards the margin, reddish brown (oac639), margin straight. *Lamellae* free, 5–8 mm broad, intercalated with lamellulae, crowded, at first white, then pink, reddish brown, finally dark brown. *Stipe* 100–150 × 8–16 (apex) and 18–30 (base) mm, clavate or cylindrical with wide base or bulbous, hollow, white, surface smooth to slightly fibrillose above the annulus, erected or recurved below the annulus, staining light yellow on touching. *Annulus* membranous, simple, pendant, upper surfaces smooth and lower surface floccose, up to 20 mm broad, white. *Context* fleshy, on cutting at first white, both in pileus and stipe, then slowly (after around 5 min) turning slightly pink, both in centre of pileus and stipe. *Odour* of strong almonds.

Macrochemical reactions: KOH reaction yellow, Schäffer’s reaction negative.

Basidiospores 4.8–6 (–7) × 3.6–4.5 μm, [\bar{x} = 5.2 ± 0.3 × 4 ± 0.2, Q = 1.1–1.4, Qm = 1.3 ± 0.1, n = 20], broad ellipsoid, brown, smooth, thick-walled, without germ pore. *Basidia* 14–20 × 6–8.4 μm, clavate to broadly clavate, hyaline, smooth, 4-spored. *Cheilocystidia* absent. *Pleurocystidia* absent. *Pileipellis* a cutis composed of 3–7.6 μm diam., long cylindrical hyphae with brownish membranous pigments, smooth, not constricted at the septa, or occasionally slightly constricted at the terminal cell.

Habitat: solitary in the forest.

Other material examined: China, Yunnan Prov., Cangyuan county, Mengdong, Nangunhe National Natural Reserve, 7 July 2012, collected by Philippe Callac, ZRL2012151 (HMAS253918).

Notes: This new species is characterized by reddish brown fibrillose pilei, floccose stipe from the lower side of annulus to the base, and broadly ellipsoid basidiospores. This species is mostly similar to *A. lanipes*, which also has these characters. However, *A. lanipes* has rhizomorphs, brown-coloured fibrillose squamules on the stipe and septate to catenulate cheilocystidia, which are different from the new species (Parra 2008). The phylogenetic analysis also supports them

as distinct species. *Agaricus planipileus* is phylogenetically closest to *A. lanipedisimilis*, but morphologically different, owing to the light brown pileus and absence of cheilocystidia.

5.2 *Agaricus* [subgenus *Spissicaules*] section *Rarolentes* Kerrigan, nom prov.

Facesoffungi number: FoF01200

Type species: *Agaricus butyreburneus* designated by Kerrigan, *Agaricus* of North America, 2016 in press.

Delimitation of *Agaricus* section *Rarolentes*

KOH reaction negative on pileus surface, negative to faintly yellow on context, Schäffer's reaction negative. Pileipellis glabrous or not, pallid. Annulus superous. Surface of pileus and stipe discolouring indistinct on bruising. Context unchanged or turning pink, reddish on exposure. Odour of solvent, rubber, or sometimes of almonds or anise. Annulus sheathed above, pendent. Cheilocystidia present, various, cylindrical to clavate. Basidiopores narrow, long; mean L/W of the type species: 1.51 (Kerrigan 2016 in press).

Stem age and phylogenetic support

Clade C3 reflects this section, which is fully supported in multi-gene analysis (Fig. 1), however it has poor support in the ITS tree (Fig. 2). The stem age of section *Rarolentes* is 21.98 Ma. This section was recognized by R. Kerrigan from North America (Kerrigan 2016 in press). In the present study there are two pure-white new species, *A. albosquamosus* and *A. leucolepidotus*, which have been incorporated and form another lineage within this section. Thus, we propose that the members of this section also contain species with squamules, and not just glabrous, pilei, and their distribution is extended to tropical Asia.

Agaricus albosquamosus L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov. Figs. 8e–h and 26

Fungal Names: FN570203

Facesoffungi number: FoF01201

Etymology: the epithet “*albosquamosus*” refers to the white and squamose pileus of this species.

Typus: THAILAND, Chiang Mai Prov., Mae Sa National Park, 16 September 2012, collector Jie Chen, LD2012192 (MFLU12-1017, **holotype**; HMAS273987, **isotype**).

Original description: *Pileus* 36 mm in diam. when young, 65–77 mm in diam., 7–7.5 mm thick at the disc when mature; convex to hemispherical, then becoming applanate-convex, finally applanate, occasionally with a slightly depressed centre; surface dry, fibrillose, with fibrillose squamules near the margin, white; in high humidity, the pileus has a reddish tone, margin exceeding to slightly appendiculate. *Lamellae* free, crowded, intercalated with lamellulae, 5–6 mm broad, at first white, pink, then pinkish brown, finally dark brown. *Stipe*

98 × 8–12 mm, cylindrical with slightly bulbous, hollow, white, surface, smooth above the annulus and fibrillose below the annulus, not staining on touching. *Annulus* superous, simple, membranous, thickening towards the edge, upper side surface smooth, lower side surface fibrillose, floccose near the margin. *Context* firm, on cutting white in both pileus and stipe, not discolouring on exposure. *Odour* of almonds.

Macrochemical reactions: KOH reaction no discolouration, Schäffer's reaction negative.

Basidiospores 5.1–6 × 3.4–3.9 μm, [\bar{x} = 5.5 ± 0.27 × 3.7 ± 0.16, Q = 1.32–1.64, Qm = 1.47 ± 0.05, n = 20], ellipsoid, brown, smooth, thick-walled. *Basidia* 15–18 × 7–8.5 μm, clavate to broadly clavate, hyaline, smooth, 4-spored or rarely 2-spored, without germ pore. *Cheilocystidia* 15.5–25 × 9–16.5 μm, abundant, simple, or sometimes in short chains, globose to pyriform or sphaeropedunculate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 3–9 μm in diam., cylindrical, hyaline, smooth, and at times slightly constricted at the septa.

Habitat: scattered or in groups, in soil under bamboo woods or in a forest clearing.

Other material examined: THAILAND, Chiang Mai Prov., 3 km down the road from Tharnthong Lodge, 4 June 2012, collector Jie Chen, LD201235 (MFLU12-0879).

Notes: this distinct species is characterized by pure white, but squamose pileus, which is different from all other species in this section (Kerrigan 2016 in press).

Agaricus leucolepidotus L.J. Chen & R.L. Zhao, sp. nov. Figs. 8i–j and 27

Fungal Names: FN570204

Facesoffungi number: FoF01202

Etymology: “*leuco*” refers to white colour, “*lepidos*” refers to squamous in Greek, so the epithet “*leucolepidotus*” refers to squamose pileus of this species.

Typus: THAILAND, Chiang Mai Prov., Tharnthong Lodge, 31 May 2012, collector Jie Chen, LD201214 (MFLU12-0858, **holotype**; HMAS273995, **isotype**).

Original description: *Pileus* 43–78 mm in diam., 5 mm thick at the disc, hemispherical to convex, then becoming applanate-convex, finally applanate, with a slightly depressed centre; cuticle exceeding the lamellae; surface dry, fibrillose, with fibrillose squamules near the margin, white; margin decurved. *Lamellae* free, crowded, intercalated with lamellulae, 6 mm broad, at first white, pink, then pinkish brown, finally dark brown. *Stipe* 94 × 8–10 mm, cylindrical with subbulbous base, hollow, white, surface smooth above the annulus, fibrillose woolly below the annulus, not staining on touching. *Annulus* superous, simple, at middle of stem, upwards, membranous, thickening towards the edge, upper side surface smooth, lower side surface fibrillose. *Context* firm, on cutting white in both pileus and stipe, not discolouring on exposure. *Odour* light of almonds.

Macrochemical reactions: KOH reaction no discolouration, Schäffer's reaction negative.

Basidiospores (5.8–) 6.5–7.2 (–8.8) × 3.5–4.3 (–5) μm , [\bar{x} = 6.8 ± 0.29 × 3.8 ± 0.27, Q = 1.60–2.07, Q_m = 1.77 ± 0.01, n = 20], ellipsoid to oblong, brown, smooth, thick-walled, without germ pore. **Basidia** 13.5–16 × 8–10 μm , broadly clavate, hyaline, smooth, 4- or 2-spored. **Cheilocystidia** 14–21 × 9–12.5 μm , abundant, simple, subglobose to pyriform, hyaline, smooth. **Pleurocystidia** absent. **Pileipellis** a cutis composed of hyphae 3–9 μm in diam., cylindrical, hyaline, smooth, and at times slightly constricted at the septa.

Habitat: in groups, in forest clearings.

Notes: This new species is morphologically similar to *A. albosquamosus* in the field in having a white and squamose pileus (see the above, the new species proposed in this paper); however, it has larger basidiospores. In ITS, they differ by 5 base pairs.

5.3 *Agaricus* [subgenus *Spissicaules*] section *Amoeni* Callac & R.L. Zhao, sect. nov. Figs. 8k–m and 28

Mycobank: MB814602

Facesoffungi number: FoF01203

Type species: *Agaricus amoenomyces* R.L. Zhao.

Etymology: the epithet “amoeni” refers to the members of this section often has pleasant odour.

Original description and delimitation of *Agaricus* section *Amoeni*

KOH and Schäffer's reactions negative or positive. Surface of stipe discolouring weakly yellow or red on bruising. Context not discolouring or turning slightly orange on exposure. Odour generally pleasant or almonds. Annulus superous, pendant, remains appendiculate, simple, lower side often floccose. Cheilocystidia lacking or similar to basidia, if present generally cylindrical to clavate, some septate at base. Known only from palaeotropics.

Stem age and phylogenetic support

This clade was revealed in Zhao et al. (2011) as clade TRIII with strong support using ITS sequence data. In the present study, it is represented by ZRL3093 and ZRL2010072, which is fully supported in multi-gene and ITS analyses (Figs. 1 and 2, as clade C2), and its stem age is 19.15 Ma. Morphologically, this section is similar to section *Brunneopicti*, and has variable discolouring on touching and cutting, even in KOH and Schäffer's reactions. However, the members of this section often have a strong pleasant or almond odour, which is different from section *Brunneopicti* that smells of phenol. Presently there are four species in this section: one is the known species *A. kivuensis* which was misplaced in section *Brunneopicti* by Heinemann (1956a); and three new species described below.

Agaricus amoenomyces R.L. Zhao, sp. nov. Figs. 8k–m and 28

Fungal Names: FN570216

Facesoffungi number: FoF01204

Etymology: the epithet “amoenomyces” refers to pleasant, or beautiful mushrooms.

Typus: CHINA, Xishuangbanna, Mongla, Nangong Mountain. 25 July 2010, collected by R.L. Zhao, ZRL2010072 (HMAS273988, **holotype**).

Original description: *Pileus* 44 mm in diam., 6 mm thick at the disc, hemispherical, convex, subumbonate with a truncate top; surface dry, with fibrillose scales, more or less concentric, dense at disc, brown against white background, margin incurved. *Lamellae* free, crowded, intercalated with lamellulae, 7 mm broad, firstly white, then pink, finally reddish brown, dark brown. *Stipe* 70–100 × 10–12 mm (apex) – 20–28 mm (base), cylindrical and bulbous, narrow hollow, with rhizomorphs at the base, straight, white, surface smooth above the annulus, and fibrillose squamules below the annulus, slightly reddish on touching. *Annulus* superous, membranous, upper side smooth and white, lower side heavily fibrillose, white with brown fibrils, which is similar to those of the pileus. *Context* firm, on cutting at first white, both in pileus and stipe, then stipe quickly turning slightly orange on exposure. *Odour* strong of almonds.

Macrochemical reactions: KOH reaction yellow and Schäffer's reaction positive or weakly positive.

Basidiospores 4.8–5.5 × 2.9–3 μm [\bar{x} = 5 ± 0.5 × 2.9 ± 0.1, Q = 1.6–1.8, Q_m = 1.7 ± 0.1, n = 20], ellipsoid, light yellowish brown, smooth, thick-walled, without germ pore. **Basidia** 15–23 × 5–7 μm , clavate, hyaline, 4-spored. **Cheilocystidia** absent. **Pleurocystidia** absent. **Pileipellis** a cutis composed of hyphae 7.5–23 μm in diam., smooth, obviously constricted at the septa, hyaline or light brown, branched.

Habitat: scattered at the banks of roads in forests.

Notes: This new species is distinguished from other species in section *Amoeni* by its positive KOH and Schäffer's reactions. The bracelet-like annulus can also distinguish this species from others.

Agaricus suthepensis L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov. Figs. 9a, b and 29

Fungal Names: FN570206

Facesoffungi number: FoF01205

Etymology: the epithet “suthepensis” refers to the place “Doi Suthep” from where the holotype was collected.

Typus: THAILAND, Chiang Mai Prov., Doi Suthep, 16 July 2012, collector Komsit Wisitrassameewong LD2012100 (MFLU12-0938, **holotype**; HMAS273993, **isotype**).

Original description: *Pileus* 45–65 mm in diam., 5 mm thick at the disc, convex to hemispherical when young, appanate when mature, margin reflexed; surface dry, smooth, with dark-brown triangular squamules, dense at the disc, and

sparse near the margin, on a white background, margin decurved. *Lamellae* free, crowded, intercalated with lamellulae, 3 mm broad, at first white, then pink, reddish brown, and finally dark brown. *Stipe* 54–114 × 6–7 mm (apex) – 9–14 mm (base), cylindrical with bulbous base, hollow, white, smooth above the annulus, fibrillose below the annulus, staining slightly orange on touching. *Annulus* simple, membranous, with floccose on lower surface, connected with the stipe by cortinate fibrils, white. *Context* firm, on cutting white, in both pileus and stipe, discolouration on exposure unknown. *Odour* mushroomy.

Macrochemical reactions: KOH and Schäffer's reactions negative.

Basidiospores 5–6 × 2.4–3.3 μm [\bar{x} = 5.5 ± 0.3 × 2.9 ± 0.3, Q = 1.7–2.2, Q_m = 1.9 ± 0.2, n = 20], ellipsoid, brown, smooth, thick-walled, without germ pore. *Basidia* 13–18 × 6–8 μm, clavate, hyaline, 4-spored. *Cheilocystidia* absent. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 3.3–14 μm in diam., smooth, obviously constricted at the septa, hyaline or light brown, branched.

Habitat: scattered in forests.

Other material examined: Thailand, Chiang Mai Prov., Doi Suthep, 20 June 2010, collector Komsit Wisittrassameewong, NTT042 (MFLU10-1478).

Notes: This new species has negative KOH and Schäffer's reactions, lacks cheilocystidia, and stains orange on bruising, which are distinctive characters in this section.

Agaricus gratolens Pradeep & R.L. Zhao, sp. nov. Figs. 9c–e and 30

Fungal Names: FN570207

Facesoffungi number: FoF01206

Etymology: the epithet “gratolens” refers to the pleasant odour of the basidiomes

Typus: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Hot Spring, 8 August 2006, collected by Ohnmar Myo Aung, ZRL3093 (BBH19609, **holotype**; HMAS274011, **isotype**).

Original description: *Pileus* 40–45 mm in diam., 2 mm thick at disc, convex with truncate top, margin exceeding; surface dry, with fibrillose scales, light brown, dense at the disc, background cream to light greyish white. *Lamellae* free, crowded, intercalated with lamellulae, 3 mm broad, firstly white, light brown, then brown, finally dark brown. *Stipe* 75 × 5 mm, cylindrical, curved, with short rhizomorphs, white, smooth above the annulus, with fibrillose to fibrillose scales below the annulus, staining weakly yellow on touching. *Annulus* membranous, pendent, simple, superous, fugacious, torn, stretched, white, up to 7 mm broad, low side floccose. *Context* firm, on cutting white, both in pileus and stipe, then quickly turning slightly ochraceous at the centre of pileus. *Odour* of almonds.

Macrochemical reaction: KOH and Schäffer's reactions negative.

Basidiospores 5–6 × 3–4 μm [\bar{x} = 5.6 ± 0.4 × 3.5 ± 0.3, Q = 1.4–1.9, Q_m = 1.63 ± 0.27, n = 20], ellipsoid, reddish brown, smooth, thick-walled, without germ pore. *Basidia* 12–14 × 5–7 μm, clavate, smooth, hyaline, 4-spored. *Cheilocystidia* 10–18 × 6–8 μm, broad clavate, ellipsoid, some pyriform, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis consisted of hyphae 5–8 μm in diam., long cylindrical, with dark grey pigment distributed unevenly, smooth. *Annulus* hyphae of elements 5–10 μm in diam., ellipsoid, cylindrical, hyaline, smooth. *Stiptipellis* hyphae similar to those of annulus.

Habitat: scattered in rich soil of forests.

Other material examined: INDIA, Kerala State, Trivandrum District, Perayam, 30 Aug. 2006, No. 9679(P) CA956 (=PYP012).

Notes: this new species is similar to *A. kivuensis* in pileus, basidiospores, and cheilocystidia characteristics. However, the new species has a white stipe surface and stains yellow on touching, while the stipe of *A. kivuensis* is brown and stains rose. The phylogenetic analysis also supports them as distinct species.

5.4 *Agaricus* [subgenus *Spissicaules*] section *Subrutilescentes* Kerrigan, nom. prov.

Type species: *Agaricus subrutilescens*, designated by R. Kerrigan, *Agaricus* of North America, 2016 in press.

Facesoffungi number: FoF01207

Delimitation of Agaricus section

“Stature generally erect, sometimes stocky; pileipellis of radially oriented vinaceous-brown appressed fibrils; flesh unchanging or mildly rufescent; lamellae may bruise rose to red; stipe stuffed-hollow, often with surface fibrils below the annulus, sometimes glabrous; annulus pendant or scant and appressed-pendent; spores relatively small and narrow, size and L/W ranges (for North American species averages): 5.2–5.5 × 3.5–3.7 μm, 1.44–1.51, respectively; Parra (2008), the European *A. impudicus* (Rea) Pilát has spores ca. 5.9 × 3.6 μm, L/W = 1.64; cheilocystidia various, e.g., clavate, ovoid, subglobose, etc.; KOH reaction on pileus surface teal to olivaceous green; Schäffer's reaction negative; *o*-tolidine generally blue everywhere except the lamellae.” Cited from Kerrigan (2016 in press).

Stem age and phylogenetic support

This section is reflected in clade C1 in multi-gene and ITS analyses (Figs 1 and 2), which is fully supported. The stem age is 19.15 Ma. In this clade, *A. subrutilescens* is the type species of this section and nests well with the three new species: *A. brunneopileatus*, *A. parasubrutilescens*, *A. inthanonensis*, and *A. linzhiensis*.

Agaricus parasubrutilescens Callac & R.L. Zhao, sp. nov. Figs. 9f–i and 31

Fungal Names: FN570218

Facesoffungi number: FoF 01208

Etymology: the epithet “parasubrutescens” refers to its closely related phylogenetic position to *A. subrutescens*.

Typus: CHINA, Yunnan Prov., Dali, Cangshan, 24 July 2014 collected by S.Y. Su *ZRL2014076* (HMAS274000, **holotype**).

Original description: *Pileus* 85–125 mm in diam., 6–7 mm thick at disc convex, plano-convex with broad umbo, top truncate, margin deflexed; surface dry, entirely covered with fibrils, thick and dark at the centre, then broken towards margin into more minute squamules-fibrilles, not concentrically arranged, appressed, brown (6E6, 6E8), with red tinge when wet, margin exceeding. *Lamellae* free, crowded, intercalated with lamellulae, 6 mm broad, at first white, then light brown (7D4), brown, finally dark brown. *Stipe* 120–150 × 8–10 mm (apex) – 15–16 mm (base), clavate, cylindrically subbulbous, smooth and white above the annulus, with large squamules, brown or white below the annulus, not staining on touching. *Annulus* membranous, 10–25 mm broad, large, pendent, simple, superous, entire, lower side heavily fibrillose. *Context* firm, on cutting at first, white both in pileus and stipe, then turning quickly slightly orange on centre of pileus and stipe on exposure. *Odour* of molds, mushroomy.

Macrochemical reactions: KOH and Schäffer’s reactions negative.

Basidiospores 4.2–6 × 3–3.5 μm [\bar{x} = 4.9 ± 0.4 × 3.1 ± 0.2, Q = 1.3–2, Q_m = 1.58 ± 0.42, n = 20], ellipsoid, brown, smooth, thick-walled, without germ pore. *Basidia* 13–20 × 5–7 μm, clavate, 4-spored. *Cheilocystidia* 15–28 × 10–19 μm, pyriform, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis comprising 3–7.5 μm diam., brownish grey, brown, smooth, hyphae, constricted at the septa in most cases. *Annulus* consisting of hyphae 3–10 μm in diam., hyaline, cylindrical, some inflated-clavate, smooth, constricted at the septa. *Stipitipellis* consisting of hyphae 5–12.5 diam., hyaline, smooth, elongate cylindrical, curved and branched hyphae.

Habitat: solitary in rich soil of forest.

Other material examined: THAILAND, Chiang Mai Prov., Doi Inthanon National Park, junction of Highway 1009 and road to Mae Chem, N19°31.58' E 98°29.64', elev. 1700 m., 27 June 2005, collected by Dennis E Desjardin, *ZRL2054* (BBH19438, SFSU) and *ZRL2055* (BBH19439, SFSU); same location, 5 June 2006, collected by Todd Osmundson, *ZRL3020* (BBH19537, SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 26 June 2005, collected by Ruilin Zhao, *ZRL2042* (BBH19427, SFSU). CHINA, Yunnan Prov., Dali, Cangshan, 31 July, 2011, collected by H.Y. Su *SHY2011073115* (HMAS 274014); same location, 24 July 2014 collected by H.Y. Su, *ZRL2014086* (HMAS273975), *ZRL2014090* (HMAS273928), *ZRL2014113* (HMAS253913), *ZRL2014114*

(HMAS273979); Yunnan Prov., Chuxiong city, Zixishan Hill, 21 June 2011, collected by R.L. Zhao, *ZRL2011015* (HMAS274016); same location, 22 June 2011, collected by R.L. Zhao, *ZRL2011027* (HMAS273926); Guangdong Prov., Lechang, 15 Sept. 2011, collected by X.H. Wang, *ZRLWXH3151* (HMAS273959); Yunnan Prov., Nanjian County, Wuliangshan National Natural Reserve, 3 July 2012, collected by Oliver Raspe, *ZRL2012025* (HMAS273968); Yunnan Prov., Cangyuan county, Nangunhe National Natural Reserve, 9 July 2012, collected by R.L. Zhao *ZRL2012196* (HMAS273971); Yunnan Prov., Xianggelila, Tacheng, 5 August 2014, collected by M.L. Xu and M.Q. He, *ZRL2014416* and *ZRL2014419*; Yunnan Prov., Weixi County, Yongchun 5 August 2014, collected by H.Y. Su, *ZRL2014413* and *ZRL2014448* (HMAS253914).

Agaricus inthanonensis L.J. Chen, K.D. Hyde & R.L. Zhao, sp. nov.

Figs. 9j–1 and 32

Mycobank: MB814603

Facesoffungi number: FoF01209

Etymology: the epithet “inthanonensis” refers to the place “Doi Inthanon” from where the holotype was collected.

Typus: THAILAND, Chiang Mai Prov., Doi Inthanon, Sit 1, N 19°06'28.8" E 98°44'47.3", elev. 1050 m., 1 July 2010, collector, Jie Chen, *LD014* (MFLU10-0747, **holotype**).

Original description: *Pileus* 61 mm in diam., 4 mm thick at the disc, convex to hemispherical; surface dry, appressed with fibrillose squamules near the margin and thick fibrils at the disc, brown (7E6) against white background, dense at the disc; margin inflexed and eroded. *Lamellae* free, crowded, intercalated with lamellulae, 4 mm in width, ventricose, reddish grey (8B2) to brown, dark brown with age. *Stipe* 136 × 6–8 (–12 at the base) mm, cylindrical and subbulbous at the base, with rhizomorphs at the base, hollow, surface smooth and white above the annulus, appressed fibrillose brown squamulose below the annulus, discolouring yellowish brown on touching. *Annulus* 12 × 15 mm, membranous, white, lower side with brown to margin, upper side smooth, lower side fibrillose floccose, skirt-like. *Context* firm, discolouring on cutting unknown. *Odour* woody.

Macrochemical reaction: KOH reaction yellow, Schäffer’s greyish violet or negative.

Basidiospores 5.5–6 × 3.2–4 μm [\bar{x} = 5.9 ± 0.7 × 3.9 ± 0.7, Q = 1.37–1.81, Q_m = 1.51 ± 0.3, n = 20], ellipsoid, brown, smooth, thick-walled, without germ pore. *Basidia* 16–24 × 7–9 μm, clavate, hyaline, 4-spored. *Cheilocystidia* 15–19 × 10–14 μm, globose, cystiform, smooth, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 5–10 μm in diam., smooth, some constricted at the septa, with dark brown and membranous pigments, rarely branched.

Habitat: solitary on leaf litter in the forest dominated by *Castenopsis* and *Lithocarpus*.

Other material examined: CHINA, Yunnan Prov., Xishangbanna, Mengsong, 25 May 2012, collector Samantha C. Karunarathna LDMS22 (MFLU15-2400; HMAS274018).

Agaricus brunneopileatus Callac & R.L. Zhao, sp. nov. Figs. 10a–d and 33

Fungal Names: FN570217

Facesoffungi number: FoF 01210

Etymology: the epithet “brunneopileatus” refers to the brown pileus of this species.

Typus: CHINA, Yunnan Prov., Cangyuan county, Mengjiao, Nuozhang Village, 6 July 2012, collected by O. Raspé and R.L. Zhao, ZRL2012115 (HMAS274001, **holotype**).

Original description: *Pileus* 60–80 mm in diam., 5 mm thick at disc, parabolic when young, then convex; surface dry, completely covered by fibrils and broken into appressed squamules, triangular, dense and darker at the disc, dark brown (oac639), reddish brown discolouring on touching, margin decurved. *Lamellae* free, crowded, intercalated with lamellulae, 5 mm broad, firstly white, then pink, finally brown dark brown. *Stipe* 110–150 × 7–8 (apex) – 10–15 (base) mm, cylindrical and bulbous at base, hollow, surface smooth and white above the annulus, heavily fibrillose, brown below the annulus, not staining on touching. *Annulus* membranous, pendant, simple, superous, up to 16 mm broad, upper side smooth and white, lower side heavily fibrillose to smooth when mature, with brown tone. *Context* fleshy, on cutting white, and no discolouration on exposure. *Odour* similar to that of *Lepiota cristata*.

Macrochemical reaction: KOH and Schäffer’s reactions negative.

Basidiospores 4.9–5.8 × 2.5–3.6 μm [\bar{x} = 5.4 ± 0.3 × 3.2 ± 0.2, Q = 1.6–1.9, Q_m = 1.7 ± 0.1, n = 20], ellipsoid, reddish brown, smooth, thick-walled, without germ pore. *Basidia* 12.6–18 × 6.2–8.8 μm, clavate, smooth, hyaline, 4-spored. *Cheilocystidia* 12–24 × 7–13 μm, pyriform, ellipsoid, occasionally 1–2 septate at the base, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* acutis comprising hyphae 3.2–6.6 μm in diam., long cylindrical, with brown pigments, smooth, slightly constricted at the septa. *Annulus* hyphae of elements 3–8.6 μm in diam, cylindrical, hyaline, smooth.

Habitat: solitary in forest.

Other material examined: China, Yunnan Prov., Dali, Yangbi County, Sangbuliao Village, 28 July 2014, collected by H.Y. Su, ZRL2014144 (HMAS253915).

Agaricus linzhiensis R.L. Zhao, sp. nov. Figs. 10e–g and 34

Fungal Names: FN570230

Facesoffungi number: FOF01211

Etymology: the epithet “linzhiensis” refers to “Linzhi”, the place where the holotype was collected.

Typus: CHINA, Tibet, Linzhi, Milin county, Nanyigou National Forest Park, 29 July 2012, collected by R.L. Zhao, ZRL 2012618 (HMAS 274022, **holotype**).

Original description: *Pileus* 60–93 mm in diam., 5–7 mm thick at the disc, plano-convex, and margin uplifted, deep split; surface dry, squamules dense at the disc, sparse and smaller towards margin, brown (oac645) against cream to light greyish-white background, margin incurved, exceeding and slightly appendiculate. *Lamellae* free, crowded, intercalated with lamellulae, 4 mm broad, at first white, then light brown, brown, finally dark brown. *Stipe* 50–60 × 10–15 mm, cylindrical, curved, white, smooth above the annulus, squamulose and dense towards the base and somewhat concentric below the annulus, not staining on touching. *Annulus* membranous, pendant, simple, superous, torn, and remains attached at the edge of pileus, up to 7 mm broad, white to light yellow, upper side smooth and lower side floccose to smooth upon maturing. *Context* fleshy, at first white on cutting, then rapidly becoming slightly reddish brown on exposure in stipe. *Odour* similar to those of *Lepiota cristata*.

Macrochemical reaction: KOH reaction yellow and Schäffer’s reaction weakly positive.

Basidiospores 5–6.4 × 3.7–4.8 μm [\bar{x} = 5.7 ± 0.4 × 4.2 ± 0.3, Q = 1.2–1.5, Q_m = 1.3 ± 0.1, n = 20], ovoid to wide ellipsoid, reddish-brown, smooth, thick-walled, without germ pore. *Basidia* 15–20 × 6.8–8.8 μm, clavate, smooth, hyaline, 2- or 4-spored. *Cheilocystidia* 11–24 × 7.3–14 μm, long ellipsoid, subsphaerical, pyriform, occasionally 2–3 elements in chains, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis comprising hyphae 3.5–7 (–10) μm in diam., long cylindrical, dark grey or not, smooth, slightly constricted at the septa. *Annulus* hyphae of elements 3.8–9 (–14) μm in diam, ellipsoid, cylindrical, hyaline, smooth.

Habitat: solitary in forest.

Notes: the species of section *Subrutilescentes* are characterized by vinaceous-brown, reddish brown, appressed fibrils on the pileus, context without discolouring or weakly to mildly rufescent on exposure, relatively long spores (ellipsoid, Q = 1.44–1.64 for North American and European specimens) and variable colour changing in KOH (negative, olivaceous green, positive yellow) (Kerrigan 2016 in press; Parra 2008). Those four new species, *A. parasubrutilescentis*, *A. inthanonensis*, *A. brunneopileatus* and *A. linzhiensis* are generally have those typical morphological characters of section *Subrutilescentes* with the exceptions of *A. linzhiensis* has wider basidiospores (broadly ellipsoid, Q_m = 1.3 ± 0.1) and *A. brunneopileatus* has elongate ellipsoid (Q_m = 1.7 ± 0.1) spores. The cheilocystidia in section *Subrutilescentes* are generally clavate to pyriform, however, in *A. inthanonensis* they are globose to subglobose. The type species of this section, *A. subrutilescentis*, has globose cheilocystidia in some cases, but the context becomes greenish on exposure, while in *A. inthanonensis* it becomes yellow (Kerrigan 1986).

Agaricus parasubrutescens is similar to *A. impudicus* in morphology mostly, but differs in its smaller spores and lack of red discolouration of the pileus when bruised, while *A. impudicus* had the spores with the size of $5.9 \times 3.6 \mu\text{m}$ and the lamellae turns red on bruising. *Agaricus parasubrutescens* differs from *A. subrutescens* by the new species has a slightly reddish yellow discolouring on cutting and a negative KOH reaction. *Agaricus subrutescens* does not change colour on cutting, and turns greenish in KOH. The Chinese specimens ZRL2014289 and SHY20120706064 and the American specimen RWK1940 are phylogenetically most closely related to *A. parasubrutescens* (Fig. 2), however, those collections have recurved, brown fibrillose squamules, concentrated at the disc, which are different from those of *A. parasubrutescens*.

Conclusions

Reconstruction of *Agaricus* taxonomic system. Prior to this study, the genus *Agaricus* was separated into three subgenera, *Agaricus*, *Lanagaricus*, and *Coniogaricus* (Heinemann 1956, 1978; Singer 1986; Parra 2008; 2013) and eight sections. These well-accepted sections were mainly established from temperate areas, such as Europe and North America (Parra 2008, 2013; Kerrigan 1986; Nauta 2001). Heinemann's system for subgenera involved *Agaricus* from tropical Africa and Southwest Asia (Heinemann 1956a, b, 1978). However, the lack of DNA sequence data has hindered its full acceptance by mycologists. A recent phylogenetic study, using ITS sequence data focused on tropical species, but also accommodated all known sections from temperate regions, has revealed seven novel clades with strong support (named TRI–VII) and four less well-supported clades (named TRa–d) from tropical areas (Zhao et al. 2011). Based on this research, clade TRI has been reconstructed as the tropical-specific section *Brunneopicti* (established by Heinemann) (Chen et al. 2015); and another section *Nigrobrunnescens* (established by K. P. Peterson, Desjardin, & Hemmes. in 2000) has been reinstated due to the type specimen of this section having been successfully sequenced in 2012 (Parra et al. 2014; Wang et al. 2015). There are ten well-resolved sections including *Spissicaules* and *Sanguinolenti*, which have been demonstrated to be polyphyletic.

In this study, we conducted a combined analysis of ITS and multi-gene sequence data, that included taxa from all previously recognized sections and seven of the eleven recently discovered, but unnamed clades (Zhao et al. 2011). Based on a phylogenetic consensus using combined multi-gene sequence analyses and considering morphology, we propose a revised taxonomic system for *Agaricus* that also takes into account stem age as a criterion for standardizing taxonomic ranks (Avice and Johns 1999). The genus *Agaricus* is divided

into five subgenera and 20 sections. Two new subgenera are introduced (*Minores* and *Spissicaules*), one of which is emended (subgenus *Pseudochitonina*), and the subgenera *Agaricus* and *Flavoagaricus* are emended with a restricted circumscription. The subgenus *Lanagaricus* (Heinemann 1956a, b) becomes a heterotypic synonym of the subgenus *Pseudochitonina*. Five new sections are introduced (*Rubricosi*, *Crassispori*, *Flocculenti*, *Hondenses*, and *Amoeni*), and two remain with a much more restricted circumscription (*Xanthodermatei* and *Sanguinolenti*). Sections *Trisulphurati* and *Laeticolores*, which were placed in the subgenus *Lanagaricus* by Heinemann (1956a), are placed in the subgenera *Pseudochitonina* and *Minores*, respectively in this study. Subsection *Bohusia* (Parra 2008) is elevated to the rank of section. Finally, 22 new species are introduced with illustrated accounts.

The multi-gene analysis presented here largely agrees with the ITS phylogeny depicted in Zhao et al. (2011) in recognizing the monophyly of sections *Bivelares*, *Chitonioides*, *Agaricus*, *Minores*, *Arvenses*, *Nigrobrunnescens*, and *Brunneopicti*. Section *Xanthodermatei* comprised a loose monophyletic group in Zhao et al. (2011) Here, with expanded sampling of both sequence data and taxa, section *Xanthodermatei* clearly splits into two distinct groups in both multi-gene and ITS analyses. Thus we emend section *Xanthodermatei* and propose a new section *Hondenses*. Section *Sanguinolenti* was shown to be polyphyletic and was segregated into two subsections by Parra (2008); here, subsection *Bohusia* is raised to sectional rank. Section *Spissicaules* was also shown to be polyphyletic, and composed of four clades by Zhao et al. (2011). Kerrigan (2016 in press) emended the section *Spissicaules* and to introduce the sections *Subrutescens* and *Rarolentes*. Here, we have named the fourth clade as a new section *Amoeni*. Sections *Laeticolores* and *Trisulphurati* were analyzed here for the first time, and are shown here to be monophyletic.

More robust phylogenetic topology using multi-gene sequence data. A comparison of phylogenetic classifications in *Agaricus* that only used ITS (Zhao et al. 2011), with that of multi-gene analysis (Fig. 1), shows that in general, only the younger clades in the ITS tree that were well-supported, are consistently retrieved in the multi-gene phylogeny, whereas deeper, older nodes were generally not or weakly supported. Clades TRI (section *Brunneopicti*), TRIII (section *Amoeni*), TRV, and TRVI from the ITS phylogeny (Zhao et al. 2011) were well-supported in the multi-gene phylogeny: their estimated ages are 18, 19, 13, and 14 Ma, respectively (Fig. 1). In contrast, clades TRb and TRc, with older stem ages (23 and 19 MA, respectively) were weakly supported in the ITS phylogeny, but were strongly supported in the multi-gene analysis. These observations corroborate the fact that ITS sequence data are useful for detecting younger groups, but are of limited value for older, more ancient lineages (Hunag 2012). Zhao

et al. (2011) were tempted to recognize TRV and TRVI as new sections, however, this study indicates that they are 13 and 14 Ma old and embedded in a larger clade with many members of section *Minores*. In this study, we only recognize sections that are approximately the same age clades (ca. 20 Ma), and thus we conclude that TRV and TRVI should remain in section *Minores*.

Taxonomic ranking by using divergence times. Recognition of taxonomic groups has, to date, mainly been based on phenotype and phylogenetic reconstruction with different ranks being applied in a subjective manner (e.g. Ariyawansa et al. 2014; Phookamsak et al. 2014). Comprehensive studies on species concepts (e.g., Mayden 1997; The Marie Curie SPECIATION Network 2012) are moving towards understanding speciation. However, standard definitions for the higher ranks lack universal, standardized criteria. This results in constant changes to the taxonomic system, especially in complex groups, which is not helpful to the end user. Hennig (1966) stated “If systematics is to be a science, it must bow to the self-evident requirement that objects to which the same label is given must be comparable in some way”. It is therefore crucial to establish universal criteria for defining taxonomic ranks. This may be extremely challenging across all organisms as Avise and John (1999) found notable disparity in the ages of lineages and their taxonomic rank of fish, anthropoid primates and fruit flies. For example, all fruit flies, shared common ancestors within a single genus more than 40 million years ago, while some primates formed different families a few million years ago, and cichlid fish were placed in several different genera that formed a few thousand years ago. Our study is an example towards providing a more objective taxonomic ranking by using divergence times; if such standardized rankings are adopted it could help to stabilize systematics. It has been suggested that divergence times could be used as universal criteria in taxonomic ranking (Hennig 1966; Avise and John 1999). However, this approach has not been adopted in systematic research until recently (for instance see Talavera et al. 2012). We are not aware of any similar studies with fungi.

In this study, we provide a robust phylogenetic framework for the genus *Agaricus*, and ranks at the subgenus and section levels, using multi-gene sequence data from a worldwide sampling. Taxonomic ranks are determined using divergence times from a fossil-calibrated phylogeny. In order to comply with the present sections, the monophyletic clades are ranked as sectional or subgenera based on divergence times. We use the stem age ca. 20 Ma as one of criteria to set the section ranks, in order to keep the well accepted sections which sections have a stem age ca. 20 Ma; and those of subgenera ca. 30 Ma. Some monophyletic clades cannot be determined due to their much younger ages. The reconstructed taxonomic system for *Agaricus* aims to standardize systematic decisions, relative to a good taxonomic sampling. This logic follows that

lower ranks should be younger than higher ranks; the ranks in same level should have similar divergence time. The number of monophyletic clades in *Agaricus* that we rank as sections and subgenera in this study are larger than those from traditional schemes (Parra 2008, 2013; Heinemann 1976; Kerrigan 1986). When we compare the sections and subgenera using morphological characters, it is hard to apply distinct morphologies to separate them.

In this paper we explore criteria that could be used as principles in taxonomic classification above the species level. It is feasible that the systematics of all organisms could be arranged using the criteria of divergence times, so that the systematics of all organisms could be represented as a whole, even though they have completely different morphologies and distant phylogenetic relationships.

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