Atractosporocybe, Leucocybe and Rhizocybe: three new clitocyboid genera in the Tricholomatoid clade (Agaricales) with notes on Clitocybe and Lepista

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Mycologia

Atractosporocybe, *Leucocybe* and *Rhizocybe*: three new clitocyboid genera in the Tricholomatoid clade (Agaricales) with notes on *Clitocybe* and *Lepista*

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Abstract

A molecular multigene analysis (ITS, 18S and 28S nrLSU ribosomal DNA, *tef1*, *rpb2*) was used to support the proposition of three new genera of clitocyboid fungi. *Leucocybe* is proposed to accommodate the clade formed by *Clitocybe connata* and *C. candicans*. *Clitocybe inornata* is invested as type species of *Atractosporocybe*, while the new genus, *Rhizocybe*, is proposed for the former species of section *Vernae* of *Clitocybe*, *C. vermicularis*, *C. pruinosa* and *C. rhizoides*. The three lineages are related to the families Lyophyllaceae and Entolomataceae and independent from the Clitocybeae clade. Morphologically *Rhizocybe* is characterized by the presence of conspicuous rhizomorphs, while *Atractosporocybe* presents long fusiform spores. *Leucocybe* includes two

whitish species in the former section *Candicantes* of *Clitocybe*, but no relevant shared characteristic feature was detected. Other whitish clitocyboid species, such as *C. phyllophila* (= *C. cerussata*), *C. dealbata*, *C. rivulosa*, and *Singerocybe hydrogramma*, are shown to be genetically related to the core lineage of the Clitocybeae.

- Agaricomycetes
- Basidiomycota
- molecular markers
- phylogeny
- taxonomy

Introduction

The genus *Clitocybe* (Fr.) Staude (Agaricales, Tricholomatoid clade) was first shown to be polyphyletic by Moncalvo et al. (2000, 2002) and later by Matheny et al. (2006). In accordance with these results, old genera were restored and new ones proposed to accommodate genetic lineages deviating from that of the type species, *C. nebularis* (Batsch) P. Kumm. (Donk 1949, Harmaja 2003). Some clitocyboid groups were found to be unrelated to the Tricholomatoid clade on a molecular basis, such as *Ampulloclitocybe* Redhead, Lutzoni, Moncalvo & Vilgalys (Redhead et al. 2002) and *Cantharocybe* H.E. Bigelow & A.H. Sm. (Ovrebo et al. 2011). Several others were confirmed as independent genera within the Tricholomatoid clade, for example *Singerocybe* Harmaja (Vizzini et al. 2010), *Paralepista* Raithelh. (Vizzini and Ercole 2012), *Neohygrophorus* Singer ex Singer (Redhead et al. 2000), *Pseudoclitocybe* (Singer) Singer (Walther et al. 2005, Garnica et al. 2007, Vizzini et al. 2011) and *Infundibulicybe* Harmaja (Matheny et al. 2006, Binder et al. 2010). Finally some others were proposed as new taxa, such as *Cleistocybe* Ammirati, A.D. Parker & Matheny (Ammirati et al. 2007), *Trichocybe* Vizzini (Vizzini et al. 2010), *Musumecia* Vizzini & Contu (Vizzini et al. 2011) and *Paralepistopsis* Vizzini (Vizzini and Ercole 2012).

Despite the progress above, many taxonomic problems dealing with several other clitocyboid species remain still unsolved (Figs. 1–4). The species *Clitocybe subditopoda* Peck, *C. candicans* (Pers. : Fr.) P. Kumm. and *C. connata* (Schumach.: Fr.) Gillet were shown to be genetically unrelated to the core lineage of *Clitocybe* (Walther et al. 2005, Matheny et al. 2006, Vizzini et al. 2011), hence deserving a different generic treatment. *Clitocybe subditopoda* was described from America as a grayish-brown clitocyboid species differing from "*C. ditopoda*" (apparently *C. ditopa* [Fr.] Gillet) because of its subglobose to broadly ellipsoidal spores, 4–5.5(−6.5) × (2.5–)3–4(−4.5) µm, paler lamellae and striate margin of the pileus. This species can be somewhat variable in color because of its hygrophanous habit, with grayish to brownish tones. It also has been described with a farinaceous odor (Bigelow and Hesler 1960). *Clitocybe candicans* was defined as a species with a small (2–3 cm diam), shining-white, convex to umbilicate pileus and with adnate to decurrent lamellae that fruits in autumn in humid forests of northern hemisphere (Bob 1997). In turn, *C. connata* is a medium-sized, white, caespitose species with an umbonate pileus and decurrent lamellae. The combination to *Clitocybe* was made by Gillet (1874), although it subsequently was suggested on morphological basis to be a member of the genera *Gyrophila* Quél., *Tricholoma* (Fr.) Staude and finally of *Lyophyllum* P. Karst. Later descriptions highlighted the positive dark blue to purple-violet reaction to ferrous sulfate shown by this species and the odor recalling that of *Corydalis cava* Schweigg. & Kort. flowers (Bon 1999). Recent molecular analyses excluded *C. connata* from the Lyophyllaceae Jülich (Moncalvo et al. 2000, 2002; Hofstetter et al. 2002; Garnica et al. 2007).

Figs 1–4.

1. *Clitocybe inornata* AH 39144. 2. *Clitocybe vermicularis* GC 01011 (duplo in AH 44078). 3. *Clitocybe candicans* GC 99225. 4. *Clitocybe connata* GC 95036 (duplo in AH 44068). Scale bars =1 cm.

Both *C. candicans* and *C. connata* can be confused with several clitocyboid species exhibiting whitish tones in their early developmental stages, such as *C. phyllophila* (Pers. : Fr.) P. Kumm., *C. cerussata* (Fr.) P. Kumm., *C. rivulosa* (Pers. : Fr.) P. Kumm. and *C. dealbata* (Sowerby: Fr.) P. Kumm., all of which are poorly represented in public molecular databases. *Clitocybe phyllophila* and *C. cerussata* (considered synonyms by Bigelow [1982] and Kuyper [1995]) are characterized by their medium-sized basidiomata that are whitish when young due to a pruinose coating but becoming tan at the center with age (Clémençon 1984, Kuyper 1995, Bon 1997). They occur in forests but do not stain with ferrous sulfate. Similarly *Clitocybe rivulosa* and *C. dealbata* are sometimes regarded as synonyms, despite the fact that *C. rivulosa* was described from forest soil and *C. dealbata* first reported in herbaceous habitats. In addition, concentric rings of the cracked cuticle can develop with age on the pileus of *C. rivulosa*, but these are apparently absent from *C. dealbata*. The latter is also smaller, 2–3(−4) cm, in contrast to 4–5(−7) cm of *C. rivulosa* (Clémençon 1984, Bon 1997). Kuyper (1995) states that both species can be present in forests, but differ from *C. phyllophila*/*C. cerussata* because of their smaller habit and more decurrent lamellae.

The genus *Lepista* (Fr.) W.G. Sm. also includes some whitish species that could be macroscopically confused with the previous ones. The type species, *L. densifolia* (J. Favre) Singer & Clémençon (Gulden 1983), as well as *L. irina* (Fr.) H.E. Bigelow, *L. panaeola* (Fr.) P. Karst. (the epithet *panaeolus* is here replaced by the feminine *panaeola*), *L. subconnexa* (Murr.) Harmaja, *L. irinoides* Bohus, and *L. caespitosa* (Bres.) Singer, display more or less pale tones that can appear whitish when young. These all produce medium-sized gregarious or caespitose basidiomata that differ mainly because of their odor, flavor, the type of lamellae insertion and spore ornamentation (Bigelow and Smith 1969; Bon 1983, 1997; Esteve-Raventós and Villarreal 2000; Consiglio and Contu 2003). If they all represent a monophyletic group or multiple independent lineages, it needs to be confirmed by means of molecular tools because no data are available for most of them.

Other clitocyboid species present deviant morphological features that suggest they are not closely related to the core lineage of the genus *Clitocybe*. This is the case of *Clitocybe inornata* (Sowerby:

Fr.) Gillet. The specimens used to describe this species were collected in forests in England. They were characterized by their grayish clitocyboid pileus with initially adnate and later decurrent concolorous lamellae. Modern descriptions report an unpleasant odor, like that of mice urine, Camembert cheese or fish, and long fusiform spores 8–10.5 ×3–4 μm (Harmaja 1969, Clémençon 1984, Kuyper 1995, Bon 1997).

Last, *Clitocybe vermicularis* (Fr.) Quél. is characterized by the presence of mycelial cords, a feature absent from most other clitocyboid taxa. It recently was shown to be independent from the core lineage of Clitocybeae by Vizzini and Ercole (2012). Other species of *Clitocybe* with rhizomorphs include *C. rhizophora* (Velen.) Joss., *C. rhizoides* H.E. Bigelow & Hesler and *C. pruinosa* (Lasch: Fr.) P. Kumm. (= *C. radicellata* Godey). These species correspond to *Clitocybe* sect. *Vernae* (Singer) Harmaja (Harmaja 1969, Bigelow 1982, Singer 1986).

The purpose of the present work is to obtain and analyze new molecular data of the above-mentioned clitocyboid species (Figs. 1–4), with emphasis on the whitish species of *Clitocybe* and *Lepista*, and to detect their most suitable phylogenetic placement and taxonomic status.

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Materials and Methods

Fungal samples

Most collections are preserved in the herbarium of the University of Alcalá (AH). Three samples were studied from the University of Torino (TO). Samples labeled EM come from the private herbarium of Enzo Musumeci, GC from Giovanni Consiglio, and those labeled PAM belong to Pierre-Arthur Moreau. Fresh and dried basidiomata of the species are provided (Table I, Supplementary file I) and were used for molecular analysis of the ITS, 18S 28S nLSU rDNA, *tef1* and *rpb2* sequences. Author citations follow Index Fungorum, authors of fungal names (www.indexfungorum.org/authorsoffungalnames.htm).

Taxon	Private herbarium	Herbarium code	ITS	28S nLSU	*rpb2*	*tef1*	18S nSSU
Atractosporocybe inornata		AH 39134	KJ680992	KJ681043			
A. inornata		AH 39144	KJ680991	KJ681044			
A. inornata		TO AV201012d	KJ680993	KJ681046	KJ681067	KJ681090	KJ681075
A. inornata		TO AV261012h	KJ680994	KJ681045	KJ681066	KJ681089	
A. inornata f. *ianthinophylla*		AH 10477	KJ680995	KJ681047		KJ681091	KJ681076
A. inornata f. *ianthinophylla*		AH 14237	KJ680996	KJ681048		KJ681092	KJ681077
Clitocybe cerussata	GC 08173	AH 44064	KJ680971	KJ681059			
C. cerussata	GC 95116	AH 44065	KJ680972				
C. dealbata		AH 39082	KJ680973	KJ681057			
C. dealbata		AH 39096	KJ680974				
C. odora		AH 39040	KJ680976				
C. phyllophila	GC 94265	AH 44070	KJ680980				
C. phyllophila	GC 06246	AH 44071	KJ680981				
C. rivulosa	GC 92142	AH 44074	KJ680977				
C. rivulosa	GC 02252	AH 44075	KJ680978				
C. rivulosa	GC 05443	AH 44076	KJ680979				
C. sp.		AH 39049	KJ680975				
C. sp.		AH 42904	KJ680982				
C. sp.		AH 42905	KJ680983				
C. sp.		AH 42906	KJ680984				
C. sp.		AH 42907	KJ680985				
C. sp.		AH 42908	KJ680986				
C. sp.		AH 42909	KJ680987				
C. sp.		AH 42910	KJ680988				
C. sp.		AH 42912	KJ680989	KJ681058			
C. sp.	GC 97162	AH 44077	KJ680990				
Lepista caespitosa	GC 06329	AH 44084	KJ680997				
L. caespitosa	GC 08295	AH 44085	KJ680998				
L. caespitosa		TO AV000047	KJ680999				
L. densifolia	GC 10067	AH 44081	KJ681000				
L. densifolia	GC 01154	AH 44086	KJ681001				
L. irina	GC 10107	AH 44058	KJ681002				
L. irina	GC 04191	AH 44059	KJ681003				
L. irina	PAM0211186	AH 44087	KJ681004				
L. irina var. *montana*	GC 05017	AH 44060	KJ681005				
L. irinoides	PAM02110202	AH 44088	KJ681006				
L. panaeola		AH39072	KJ681007				
L. panaeola		AH 39109	KJ681008				
L. panaeola		AH 39110	KJ681009	KJ681056			
L. panaeola	GC 98155	AH 44054	KJ681010				
L. panaeola	GC 96246	AH 44055	KJ681011				
L. paxilloides	GC 03155	AH 44056	KJ681012				
L. paxilloides	GC 07454	AH 44057	KJ681013				
L. personata		AH 39129	KJ681014				
L. personata		AH 39154	KJ681015				
L. piperata		AH 42903	KJ681016				
L. sordida		AH 39056	KJ681018	KJ681054			
L. sordida		AH 39147	KJ681019	KJ681055			
L. sordida		AH 39148	KJ681020	KJ681053			
L. sp.		AH 39232	KJ681017				
L. subconnexa		AH 42913	KJ681021				
L. subconnexa	GC 07260	AH 44082	KJ681022				
L. subconnexa	GC 07261	AH 44083	KJ681023				

Table I.

Samples sequenced

DNA processing and phylogenetic analyses

DNA extraction and PCR amplification were performed as described in Alvarado et al. (2012). Total DNA was extracted from dry specimens blending a portion of them with the aid of a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated 15 min at 65 C. A similar volume of chloroform : isoamyl alcohol (24 : 1) was added and mixed with the samples until emulsion, followed by centrifugation for 10 min at 13 000 × *g* and the DNA in the supernatant precipitated with a volume of isopropanol. After an additional centrifugation step of 15 min at the same speed, the pellet was washed in cold 70% ethanol, centrifugated again for 2 min and dried. It was resuspended in 200 µL ddH_2O. PCR primers ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993) for the ITS region, LR0R, LR1, LR5 and LR7 (Vilgalys and Hester 1990, Cubeta et al. 1991, van Tuinen et al. 1998) for the 28S nLSU ribosomal region, bRPB2-6F, bRPB2-7R, fRPB2-7cR and bRPB2-7R2 for the DNA-directed RNA polymerase II subunit two *rpb2* gene (Liu et al. 1999; Matheny et al. 2005, 2007), EF1-983 F, EF1-1567R and EF-2218R for the translation elongation factor 1α *tef1* gene (Rehner and Buckley 2005) and finally NS19b and NS41 for 18S nSSU ribosomal region (http://www.clarku.edu/faculty/dhibbett/Protocols_Folder/Primers/Primers.htm) were employed for PCR amplification and sequencing purposes. PCR reactions were performed under a program consisting of a hot start at 95 C for 5 min, followed by 35 cycles at 94 C, 54 C and 72 C (45, 30, 45 s, respectively) and a final 72 C step for 10 min. PCR products were checked in 1% agarose gels before purification and sequencing. Sequences were visually inspected searching for reading errors in MEGA 5 (Tamura et al. 2011).

Sequence alignment and phylogenetic analyses

Two independent alignments were constructed: (i) ITS alignment of the Clitocybeae core lineages and (ii) combined 28S nLSU-*rpb2*-*tef1*-18 nSSU alignment of the Tricholomatoid clade (as defined in Matheny et al. 2006, Binder et al. 2010), excluding Mycenaceae. The sequences obtained ex novo for the present work were aligned with their closest relatives identified through BLAST (blastn, Altschul et al. 1990) algorithm in the public databases. These were retrieved mainly from Lutzoni et al. (1997), Hughes et al. (2001), Peintner et al. (2001), Hofstetter et al. (2002), Moncalvo et al. (2002), Walther et al. (2005), Matheny et al. (2006, 2007), Vizzini et al. (2010, 2012), Vizzini and Ercole (2012), Geml et al. (2012) and Osmundson et al. (2013). *Clitocybe subditopoda* was chosen as outgroup of the Clitocybeae lineage, while *Infundibulicybe* and the lineage formed by *Musumecia* and *Pseudoclitocybe* were chosen as outgroups for the second analysis because of their basal position in the Tricholomatoid clade (Matheny et al. 2006, Binder et al. 2010).

Sequences first were aligned in MEGA 5 software with its Clustal W application and then adjusted manually. Introns from *rpb2* and *tef1* genes were removed before analysis. third codon positions also were removed because of putative mutation saturation problems. The influence of ambiguously aligned sites in the ITS alignment was tested by conducting a neighbor-joining (NJ) analysis in MEGA 5 (2000 bootstrap iterations) and comparing it with a similar analysis using a conservative alignment obtained with GBLOCKS 0.91b (Castresana 2000) through its online server using default settings. Maximum parsimony (MP) of the ITS alignment was performed in PAUP* 4.0b10 (Swofford 2001), while a Bayesian analysis of both ITS and combined 28S nLSU-*rpb2*-*tef1*-18S nSSU alignments was performed in MrBayes 3.1 (Ronquist and Huelsenbeck 2003) and a maximum likelihood analysis (ML) in RAxML 7.0.3 (Stamatakis 2006). The ITS alignment was analyzed in PAUP* 4.0b10 (Swofford 2001), and a maximum parsimony phylogenetic tree reconstruction was performed (2000 bootstrap replicates, TBR swapping algorithm, 50 sequence additions per replicate, MULTREES not in effect). ITS1-5.8SITS2 partitions from the ITS alignment, as well as each locus in the combined 28S nLSU-*rpb2*-*tef1*-18S nSSU alignment, were

subjected to MrModeltest 2.3 (Nylander 2004) in PAUP* 4.0b10. The best models were implemented in MrBayes 3.1 (Ronquist and Huelsenbeck 2003), where a separate Bayesian analysis was performed for each dataset (loci partitioned, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until average standard deviation of split frequencies fell below 0.01. The first 25% of the trees from the posterior distibution were discarded as burn-in. Finally a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis 2006) using the standard search algorithm (data partitioned, 2000 bootstrap replications). Significance thresholds were set above 70% for bootstrap (BP) and 0.95 for posterior probability (PP).

Results

Phylogenetic results

The final ITS alignment of the Clitocybeae core included 165/535 variable sites. The conservative ITS alignment obtained with GBlocks contained 87/372 variable sites, and the NJ topology produced was similar to that obtained using the original alignment, so the relaxed alignment was employed. Only a few nodes received lower support when conservative alignments were used. In the conservative analysis, the lineage formed by *Collybia cirrhata* (Schumach.) Quél., *C. tuberosa* (Bull.) P. Kumm. and *C. cookei* (Bres.) J.D. Arnold was not supported while the species *C. tuberosa*, *C. cookei*, *Clitocybe festiva* J. Favre, *L. densifolia*, and *L. irina* clades received low bootstrap support. Model gtr + Γ was selected for ITS1 and ITS2 partitions, while the 5.8S rRNA gene was best described by JC model. A Bayesian consensus tree was obtained after 4 130 000 generations, 30 975 trees being employed to build it.

Results (Fig. 5) show that most whitish clitocyboid species analyzed (*C. phyllophila*, *C. cerussata*, *C. rivulosa*, *C. dealbata*) have a closer relationship with the *Lepista-Collybia* clade than to the type species of *Clitocybe* (*C. nebularis*). While *C. phyllophila* and *C. cerussata* are nearly identical genetically, samples initially identified as *C. rivulosa* or *C. dealbata* represent several different lineages, suggesting that some of these identifications were erroneous. Several samples of *Lepista* identified as *L. caespitosa*, *L. panaeola*, *L. paxilloides* (Esteve-Rav. & M. Villarreal) Consiglio & Contu and *L. subconnexa* (Bon 1983, 1997; Consiglio and Contu 2003), appear genetically identical when their ITS region was compared, suggesting that these species could be considered synonyms with *L. panaeola* the prioritary name. In turn they are closely related to *L. densifolia*, both taxa forming a well supported clade. However, other lineages, such as *L. irina* and the violaceous tinged species *L. nuda* (Bull.: Fr.) Cooke, *L. personata* (Fr.: Fr.) Cooke, and *L. sordida* (Schumach.: Fr.) Singer, were not related to the type species, suggesting that genus *Lepista* is not monophyletic.

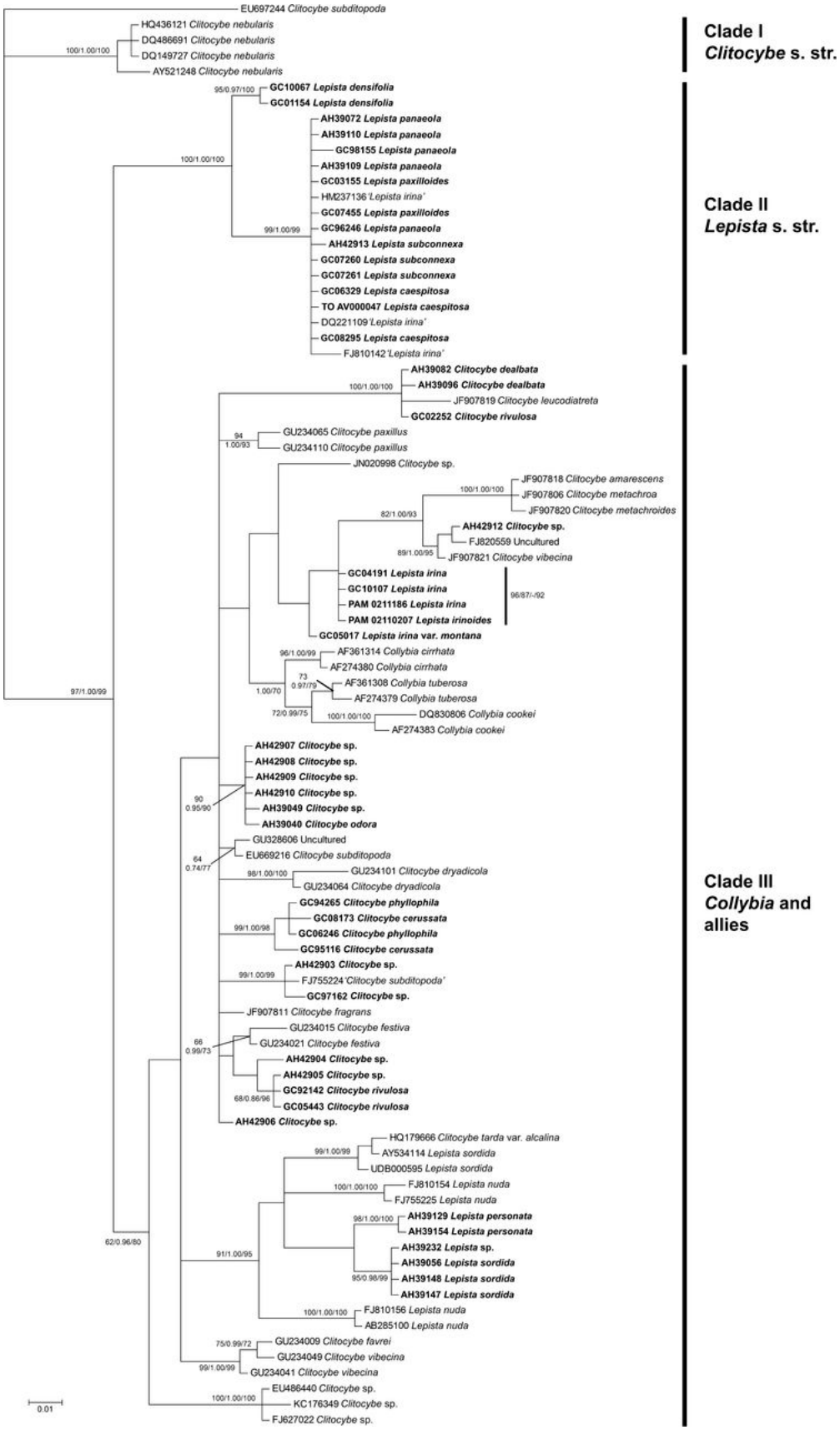
EU697244 Clitocybe subditopoda
HQ436121 Clitocybe nebularis
DQ486691 Clitocybe nebularis
DQ149727 Clitocybe nebularis
AY521248 Clitocybe nebularis
100/1.00/100
Clade I
Clitocybe s. str.
95/0.97/100
GC10067 Lepista densifolia
GC01154 Lepista densifolia
AH39072 Lepista panaeola
AH39110 Lepista panaeola
GC98155 Lepista panaeola
100/1.00/100
AH39109 Lepista panaeola
GC03155 Lepista paxilloides
HM237136 'Lepista irina'
GC07455 Lepista paxilloides
99/1.00/99
GC96246 Lepista panaeola
AH42913 Lepista subconnexa
GC07260 Lepista subconnexa
GC07261 Lepista subconnexa
GC06329 Lepista caespitosa
TO AV000047 Lepista caespitosa
DQ221109 'Lepista irina'
GC08295 Lepista caespitosa
FJ810142 'Lepista irina'
Clade II
Lepista s. str.
AH39082 Clitocybe dealbata
100/1.00/100
AH39096 Clitocybe dealbata
JF907819 Clitocybe leucodiatreta
GC02252 Clitocybe rivulosa
94
1.00/93
GU234065 Clitocybe paxillus
GU234110 Clitocybe paxillus
JN020998 Clitocybe sp.
100/1.00/100
JF907818 Clitocybe amarescens
JF907806 Clitocybe metachroa
JF907820 Clitocybe metachroides
82/1.00/93
AH42912 Clitocybe sp.
FJ820559 Uncultured
89/1.00/95
JF907821 Clitocybe vibecina
GC04191 Lepista irina
GC10107 Lepista irina
PAM 0211186 Lepista irina
PAM 02110207 Lepista irinoides
96/87/-92
GC05017 Lepista irina var. montana
96/1.00/99
AF361314 Collybia cirrhata
AF274380 Collybia cirrhata
73
0.97/79
AF361308 Collybia tuberosa
1.00/70
AF274379 Collybia tuberosa
72/0.99/75
100/1.00/100
DQ830806 Collybia cookei
AF274383 Collybia cookei
97/1.00/99
AH42907 Clitocybe sp.
AH42908 Clitocybe sp.
AH42909 Clitocybe sp.
90
0.95/90
AH42910 Clitocybe sp.
AH39049 Clitocybe sp.
AH39040 Clitocybe odora
GU328606 Uncultured
64
0.74/77
EU669216 Clitocybe subditopoda
98/1.00/100
GU234101 Clitocybe dryadicola
GU234064 Clitocybe dryadicola
GC94265 Clitocybe phyllophila
GC08173 Clitocybe cerussata
99/1.00/98
GC06246 Clitocybe phyllophila
GC95116 Clitocybe cerussata
AH42903 Clitocybe sp.
99/1.00/99
FJ755224 'Clitocybe subditopoda'
GC97162 Clitocybe sp.
JF907811 Clitocybe fragrans
GU234015 Clitocybe festiva
66
0.99/73
GU234021 Clitocybe festiva
AH42904 Clitocybe sp.
AH42905 Clitocybe sp.
GC92142 Clitocybe rivulosa
68/0.86/96
GC05443 Clitocybe rivulosa
AH42906 Clitocybe sp.
Clade III
Collybia and
allies
HQ179666 Clitocybe tarda var. alcalina
99/1.00/99
AY534114 Lepista sordida
UDB000595 Lepista sordida
100/1.00/100
FJ810154 Lepista nuda
FJ755225 Lepista nuda
98/1.00/100
AH39129 Lepista personata
AH39154 Lepista personata
62/0.96/80
AH39232 Lepista sp.
91/1.00/95
AH39056 Lepista sordida
95/0.98/99
AH39148 Lepista sordida
AH39147 Lepista sordida
100/1.00/100
FJ810156 Lepista nuda
AB285100 Lepista nuda
75/0.99/72
GU234009 Clitocybe favrei
GU234049 Clitocybe vibecina
99/1.00/99
GU234041 Clitocybe vibecina
0.01
100/1.00/100
EU486440 Clitocybe sp.
KC176349 Clitocybe sp.
FJ627022 Clitocybe sp.

Fig. 5.

Consensus tree resulting from Bayesian ITS analysis of the lineages of *Clitocybe*, *Lepista* and *Collybia* within the Clitocybeae clade. Nodes were annotated with three values (from upper left to bottom right or from left to right): maximum parsimony BP, Bayesian PP and maximum likelihood BP. Only nodes supported by two or more analyses were annotated, except for those of *Clitocybe subditopoda* (EU669216) and *Clitocybe rivulosa* (GC92142), which had almost significant support values (64 and 68 maximum parsimony BP, and 0.74 and 0.86 bayesian PP, respectively). These clades were annotated too because of their putative species-level status. Names in boldface belong to samples sequenced in the present study.

The combined 28S nLSU-*rpb2*-*tef1*-18S nSSU alignment of the Tricholomatoid clade included 388/2050 variable positions. One hundred seventy-seven of these were located in the 28S nLSU region, 95 in the *rpb2* gene (third codon positions excluded), 51 in the *tef1* gene and 65 in the 18S nSSU region. The *tef1* gene was best represented by model f81 + i + γ, while 28S nLSU, *rpb2* and 18S nSSU were best described by model gtr + i + γ.

The Bayesian consensus tree was obtained after 550 000 generations, 4125 trees being employed to build it. Results (Fig. 6) show that all other species not included in the analysis of the Clitocybeae core are not directly related to this lineage but are more closely related to the families Lyophyllaceae and Entolomataceae in accordance with Matheny et al. (2006) and Binder et al. (2010). A significant relationship between *C. candicans* and *C. connata*, was detected, and they pair with *C. subditopoda*. *Clitocybe vermicularis* and *C. pruinosa* form a clade and is recovered here as the sister group of the Entolomataceae but without significant support. *Singerocybe hydrogramma* (Bull.: Fr.) Harmaja (= *Clitocybe phaeophthalma* [Pers.] Kuyper) clusters with *Clitocybe adirondackensis* (Peck) Sacc. (Fig. 6) and is related to the Clitocybeae core. Nonsignificant ML support values were recorded for the clade formed by Entolomataceae and Lyophyllaceae families and the independent clitocyboid lineages reported above, while it was successfully supported by Bayesian analysis in accordance with previous works (Matheny et al. 2006).

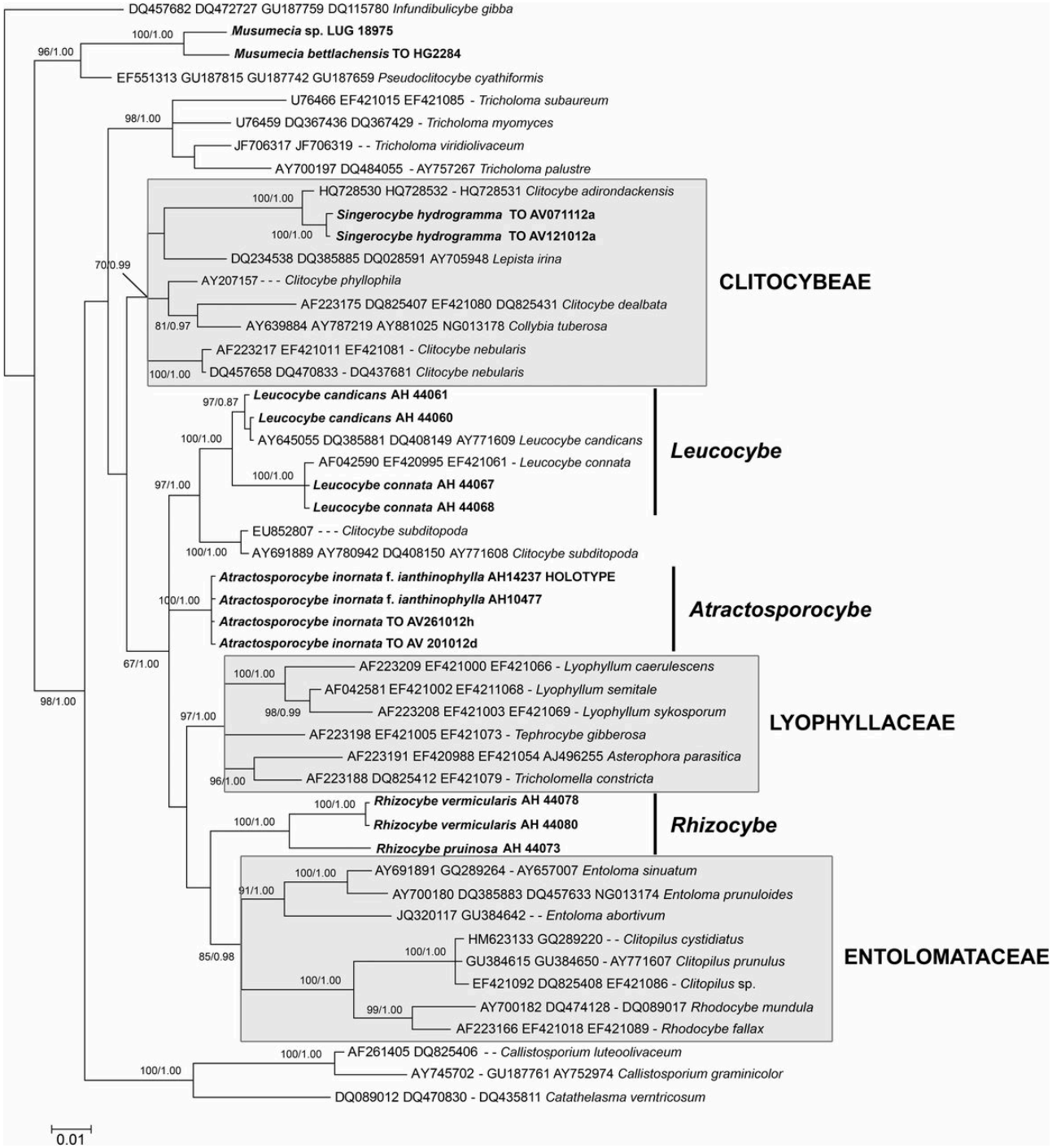

Fig. 6.

Consensus Bayesian tree resulting from combined 28S nLSU, *rpb2*, *tef1* and 18S nSSU analysis of the families Lyophyllaceae, Entolomataceae and related clitocyboid genera. Nodes were annotated with Bayesian PP and maximum-likelihood BP (left to right). Only nodes supported (or nearly supported) by at least one of these analyses were annotated. Names in boldface belong to samples sequenced in the present study.

Taxonomy

Atractosporocybe P. Alvarado, G. Moreno & Vizzini, gen. nov.

MycoBank MB809953.

Etymology

(Gk.) from "*atractos*", spindle-like; "*-sporo*", spore; "*-cybe*": head (mushroom), that is a "*Clitocybe*" with fusiform spores.

Stipitate basidiomata, appearing isolated or in groups. Pileus convex to flat-convex, rarely compressed at the center, sometimes with an obtuse central umbo, glabrous, pale gray to grayish brown. Lamellae adnate to subdecurrent, grayish to grayish buff, with lamellulae. Stipe central, concolorous with the pileus, whitish at the base. Pileipellis as a cutis. Basidiospores fusiform to long ellipsoid, smooth, hyaline, without iodine reactions (not dextrinoid and not amyloid), acyanophilic, white in mass. Hymenial cystidia absent. Clamp connections present.

Type

Agaricus inornatus Sowerby

Atractosporocybe inornata (Sowerby) P. Alvarado, G. Moreno & Vizzini, comb. nov. Fig. 1

MycoBank MB809954.

- ≡ *Agaricus inornatus* Sowerby, Col. fig. Engl. Fung. Mushr. 3: pl. 342. 1803 [1800-03].
- ≡ *Clitocybe inornata* (Sowerby) Gillet, Les Hyménomycètes ou description de tous les champignons (fungi) qui croissent en France: 155. 1874.
- ≡ *Omphalia inornata* (Sowerby) Quél., Enchiridion Fungorum in Europa media et praesertim in Gallia Vigentium: 20. 1886.
- ≡ *Paxillus inornatus* (Sowerby) Quél., Flore mycologique de la France et des pays limitrophes: 109. 1888.
- = *Agaricus zygophyllus* Cooke & Massee, Grevillea 15 (75): 67. 1887.
- = *Clitocybe zygophylla* (Cooke & Massee) Sacc., Syllogue Fungorum 9: 24. 1891.
- = *Clitocybe umbrinipes* H.E. Bigelow & A.H. Smith., Mycologia 54(5):514. 1963.

Habit, habitat and known distribution

basidiomes can be found growing isolated or in small groups in either broadleaf or conifer forests, from the Mediterranean basin to northern Europe and North America. Autumn.

Specimens examined

ITALY, L'AQUILA: Avezzano. On *Quercus* sp. litter, 26-X-2012, leg. *A. Vizzini & L. Perrone* (TO AV261012h). TORINO: Mattie, Bosco del Vallone. On *Quercus pubescens* Willd. Litter, 20-X-2012, leg. *A. Vizzini* (TO AV201012d). SPAIN. GUADALAJARA: Alcolea del Pinar. Under *Quercus ilex* L. on calcareous soil, 14-XII-2010, *leg. D. Martínez* (AH 39144). MADRID: Casa de Campo, Club de Campo. Under *Quercus ilex* on acidic soil, 28-XI-2010, *leg. Soc. Micol. Madrid* (AH 39134). *Clitocybe inornata* f. *ianthinophylla*: SPAIN. GUADALAJARA: between Cifuentes

and Canredondo. Under *Pinus* halepensis, 17-XII-1991, *leg. M.N. Blanco, M. Heykoop* (AH 14327). MADRID: Alcalá de Henares, Mount Gurugú. Under *P. halepensis*, 14-XI-1987, *leg. M. Pérez* (AH 10477).

Comments

This new genus could match the former section *Inornatae* (Singer) H.E. Bigelow characterized by species with subfusoid, subcylindric or narrowly ellipsoid spores that are inequilateral in profile, smooth and inamyloid (Bigelow 1982). *Clitocybe inornata* is considered the first species in this section, where Singer (1986) also placed *Melanoleuca avellanea* Murrill as *C. avellanea* (Murrill) Singer (prioritary name *C. avellanea* Beeli), *C. lata* (Peck) Singer, *C. mexicana* Murrill and *C. ehudacae* Maire. *Clitocybe phyllophila* var. *fusispora* Raithelh. and *C. sclerotoidea* (Morse) H.E. Bigelow also were included in this section by Bon (1983), who suggested that other American taxa, such as *C. fumosifolia* (Hesler) H.E. Bigelow and *C. umbrinipes* H.E. Bigelow & A.H. Sm., feature fusiform spores too. The inclusion of all these species in the new genus *Atractosporocybe* should be confirmed with accurately determined collections and molecular tools.

Leucocybe Vizzini, P. Alvarado, G. Moreno & Consiglio, gen. nov.

MycoBank MB808517.

Etymology

(Gk.) "*leuco*-", white; "*-cybe*", head (mushroom), that is a white *Clitocybe*.

Basidiomata gregarious or caespitose. Small to medium-sized (1–9 cm). Pileus convex or globose and covered with a tomentose white to off-white layer when young, becoming glabrous, whitish cream or buff, center depressed with age. Lamellae adnate or slightly decurrent, white or whitish when young, becoming brownish white or grayish white with age. Pileipellis as a cutis. Basidiospores smooth, elliptic, not cyanophilous, without iodine reactions, white in mass. Hymenial cystidia absent. Clamp connections present.

Type

Agaricus candicans Pers.

Leucocybe candicans (Pers.) Vizzini, P. Alvarado, G. Moreno & Consiglio, comb. nov. Fig. 3

MycoBank MB808518.

- = *Agaricus gallinaceus* Scop., Fl. carniol., Edn 2 (Wien) 2: 43. 1772.
- = *Agaricus umbilicatus* Bolton, An History of Fungusses, Growing about Halifax 1: 17, t. 17. 1788.
- ≡ *Agaricus candicans* Pers., Syn. meth. fung.: 2: 456. 1801.
- ≡ *Omphalia candicans* (Pers.) Gray, Nat. Arr. Brit. Pl. 1: 613. 1821.
- = *Agaricus tuba* Fr., Epicr. syst. mycol.: 72. 1838. [1836–1838]
- = *Clitocybe gallinacea* (Scop.) Gillet, Hyménomycètes: 137. 1874.
- = *Clitocybe tenuissima* Romagn. Bulletin de la Societé des Naturalistes d'Oyonnax 8: 74. 1954.
- = *Clitocybe tuba* (Fr.) Gillet, Hyménomycètes: 137. 1874.

- ≡ *Pholiota candicans* (Pers.) J. Schröt., in Cohn, Kryptogamen-Flora von Schlesien 3-1(1): 608. 1889.
- = *Clitocybe alboumbilicata* Murrill, Mycologia 7 (5): 257. 1915.
- = *Clitocybe aberrans* Velen., Novitates mycologicae: 74. 1939.
- = *Clitocybe gossypina* Velen., Novitates mycologicae: 76. 1939.

Habit, habitat and known distribution

Basidiomes can be found growing isolated or in small groups in Europe and North America. It can be found among grass under broadleaf or conifer trees but also in disturbed areas adjacent toforest paths. In the present work all samples were found on calcareous soil. From late summer to autumn (Sep–Nov).

Specimens examined

ITALY. BOLOGNA: Grizzana Morandi, Puzzola. Under *Quercus pubescens* and *Castanea sativa* Mill. on calcareous soil, 24-X-1992, *leg. G. Consiglio, R. Collina GC92172* (AH 44060). Monzuno, Trasasso. Under *Quercus pubescens* and *Castanea sativa* on calcareous soil, 31-X-1992, *leg. G. Consiglio GC92199*. Ibid. 27-IX-1993 *GC93138*. Monterenzio, Ronchi. Under *Quercus* spp. on calcareous soil, 21-X-1993, *leg. G. Consiglio, G. Spisni GC93253* (AH 44061). Ibid. 1-XI-1994, *GC94241* (AH 44062). Sasso Marconi, Prati di Mugnano. Under mixed broadleaf trees, 27-IX-1994, *leg. G. Consiglio, R. Trimarco GC94273*. San Lazzaro di Savena, Eremo di Zena. Under *Quercus* spp. on calcareous soil, 18-XI-1999, *leg. G. Consiglio, G. Bordoni, G. Spisni GC99225* (AH 44063). REGGIO EMILIA: Pulpiano. Under *Quercus* spp. on calcareous soil, 26-X-1991, *leg. G. Consiglio GC91031*.

Leucocybe connata (Schumach.) Vizzini, P. Alvarado, G. Moreno & Consiglio, comb. nov. Fig. 4

MycoBank MB808519.

- ≡ *Agaricus connatus* Schumach., Enum. pl. 2: 299. 1803.
- ≡ *Clitocybe connata* (Schumach.) Gillet, Hyménomycètes: 164. 1874.
- ≡ *Gyrophila connata* (Schumach.) Quél., Enchir. fung.: 19. 1886.
- ≡ *Tricholoma connatum* (Schumach.) Ricken, Die Blätterpilze: 360. 1915.
- ≡ *Lyophyllum connatum* (Schumach.) Singer, Schweiz. Z. Pilzk. 17: 55. 1939.

Habit, habitat and known distribution

Basidiomes can be found growing in dense groups (gregarious or cespitose) associated with broadleaf or conifer forests, frequently adjacent to forest paths. In the present work all samples were found on calcareous soil. Known from the northern hemisphere (Europe and North America), from summer to autumn (Aug–Nov).

Specimens examined

ITALY. TRENTO: Folgarida, Malga Presson. Under *Picea abies* (L.) H.Karst., 28-VIII-1992, *leg. G. Consiglio GC92054* (AH 44067). Molina di Fiemme, Val Cadino, Caterinello. Under *Picea abies*, 18-VIII-1995, *leg. G. Consiglio, G. Catalano, P. Cazzoli GC95036* (AH 44068). SPAIN. GUADALAJARA: dam of El Vado, Valdesotos. Under *Pinus halepensis* Mill., 5-XI-2010, *leg. R. Galán* (AH 39034). Anguita. Under *Quercus ilex* and *Juniperus thurifera* L., on basic soil, 9-XI-2010, *leg. D. Martínez* (AH 39054). Budia. Under *Pinus pinea* L. and *Quercus ilex*, 7-XI-2010, *leg.*

J. Menendez (AH 39068). SWITZERLAND. CANTON TICINO: Passo della Bolla. Under *Picea abies*, 18-VIII-2002, *leg. G. Consiglio, A. Garbellotto GC02067* (AH 44069).

Comments

Leucocybe includes at least two whitish clitocyboid species, *Clitocybe candicans* and *C. connata*, with adnate or slightly decurrent lamellae. The group is distinguished by molecular markers, but morphological traits unique to the clade are unknown at this time. *Clitocybe candicans* formerly was classified in *Clitocybe* subsection *Candicantes* (Quél.) Singer, along with other taxa whose taxonomic status is unclear. Most of the synonymies reported here for this taxon follow Kuyper (1995) where it is suggested that species such as *C. gallinacea* (Scop.) Gillet or *C. tenuissima* Romagn. cannot be formally separated from *L. candicans*. Another species of *Clitocybe* subsection *Candicantes* resembling *Leucocybe* is *C. phyllophila* (Pers. : Fr.) P. Kumm. *Clitocybe phyllophila* is larger than *L. candicans* and does not react to ferrous sulphate unlike *L. connata*. It has been shown here that this species and other whitish clitocyboid taxa analyzed in this section, such as *C. cerussata*, *C. rivulosa* and *C. dealbata*, do not constitute a monophyletic lineage. Analyses suggest that several sequences labeled *C. subditopoda* Peck from North America are closely related to *Leucocybe*. However, at least another two unrelated genetic lineages are in GenBank composed of sequences labeled *C. subditopoda*, so these should be examined carefully before any taxonomic decision is proposed.

Rhizocybe Vizzini, G. Moreno, P. Alvarado & Consiglio, gen. nov.

MycoBank MB808520.

Etymology

(Gk.) "*rhizo*-", root; "*-cybe*", head; that is a "*Clitocybe*" with mycelial cords.

Basidiomata clitocyboid (funnel-shaped or umbilicate), small (1–4 cm diam). Pileus pale brown or grayish brown to brown or reddish brown. Lamellae slightly decurrent, pale, buff or yellowish. Rhizomorphs present at the base of the stipe. Pileipellis as a cutis. Basidiospores elliptic, smooth, not cyanophilous, without iodine reactions, white in mass. Hymenial cystidia absent. Clamp connections present. In coniferous forests of the northern hemisphere, spring and summer.

Type

Agaricus vermicularis Fr.

Rhizocybe vermicularis (Fr.) Vizzini, G. Moreno, P. Alvarado & Consiglio, comb. nov. Fig. 2

MycoBank MB808521.

- ≡ *Agaricus vermicularis* Fr. Epicr. syst. mycol.: 72. 1838.
- ≡ *Clitocybe vermicularis* (Fr.) Quél., Mém. Soc. Émul. Montbéliard 5:235. 1872.
- ≡ *Omphalia vermicularis* (Fr.) Quél., Enchiridion Fungorum in Europa media et praesertim in Gallia Vigentium: 23. 1886.
- = *Collybia rhizophora* Velen. České Houby 2:335. 1920.

Habit, habitat and known distribution

Basidiomes can be found growing isolated or in small groups, associated mainly with conifer forests. In the present work all samples were found associate with *Pinus* spp. Known from the northern hemisphere (Europe and North America). In Mediterranean Europe it occurs in alpine forests. In the present work it was found in spring (Mar–May), but some authors (Bigelow 1960) report it also in summer (Jun–Jul).

Specimens examined

ITALY. GROSSETO: Principina Mare. Under *Pinus* spp., 18-III-2004, *leg. G. Consiglio, G. Perdisa, G. Spisni GC04002* (AH 44079). LUCCA: under mixed coniferous trees, 21-IV-2001, *leg. G. Consiglio, D. Antonini, M. Antonini GC01011* (AH 44078). SPAIN. MADRID: El Pardo. Under *Pinus pinea*, 20-III-2011, *leg. Soc. Micol. Madrid* (AH 39214). Arganda, under *Pinus* sp., 4-IV-2011, *leg. J.L. Domingo*, (AH 39230). TERUEL: Escorihuela, Collado de Castelfrío, among moss in a forest of *P. sylvestris* and *Juniperus communis*, 6-V-2013, *leg. E. Suárez HHTSG 460C* (AH 44080).

Rhizocybe pruinosa (Lasch: Fr.) Vizzini, G. Moreno & P. Alvarado, comb. nov.

MycoBank MB808522.

- ≡ *Agaricus pruinosus* Lasch, in Fries, Epicr. syst. mycol.: 75. 1838. [1836–1838]
- ≡ *Clitocybe pruinosa* (Lasch) P. Kumm., Führ. Pilzk.: 120. 1871.
- = *Clitocybe radicellata* Godey, in Gillet, Champignons de France. Tableaux Analytiques des Hyménomycétes: 171. 1884.
- = *Clitocybe rhizophora* s. Joss. & Pouchet Bull. bimens. Soc. Linn. Lyon 10(7):51. 1931.

Habit, habitat and known distribution

basidiomes can be found growing isolated or in small groups, associated mainly with conifer forests. Known from the northern hemisphere (Europe and North America). In Mediterranean Europe it occurs in alpine forests. In spring (Mar–Apr).

Specimens examined

ITALY. TRENTO: Pergine Valsugana, Alberè. Under *Picea abies*, 28-III-1991, *leg. G. Consiglio, G. Marasca, B. Oss-Emer GC91001* (AH 44072). SPAIN. TERUEL: Orihuela del Tremedal, in front of Majada Las Vacas, herbaceous areas in forest of *P. sylvestris*, 28-IV-2013, *leg. P. García & E. Suárez HHTSG 152* (AH 44073).

Rhizocybe rhizoides (H.E. Bigelow & Hesler) Vizzini, G. Moreno & P. Alvarado, comb. nov.

MycoBank MB809556.

- ≡ *Clitocybe rhizoides* H.E. Bigelow & Hesler, J. Elisha Mitchell Sci. Soc. 76:159. 1960.

Habit, habitat and known distribution

Scattered to gregarious on soil under pine (Bigelow 1960). Currently known only from North America. Winter and early spring (Dec–Mar).

Comments

The genus *Rhizocybe* includes at least three different species, *Clitocybe vermicularis*, *C. pruinosa* and *C. rhizoides*, all of which are characterized by the presence of mycelial cords at the base of the stipe. They are not closely related to *Clitocybe* but instead appear to be the sister group to the Entolomataceae but with weak support. The genus *Rhizocybe* could well contain most species of the section *Vernae* (Singer) Harmaja of *Clitocybe* (Harmaja 1969, Bigelow 1982, Singer 1986), which is characterized by species with rhizomorphs and sometimes fruiting in spring. Two main taxa have been traditionally recognized in this section in Europe, *C. vermicularis* and *C. pruinosa*, while other species, such as *C. verna* Egeland (Lundell), *C. paropsis* (Fr.) Sacc., and *C. radicellata* Godey, have been only rarely found. Bigelow and Hesler (1960) added another species to the section from North America, *C. rhizoides*. ITS data from the type collection of *C. rhizoides* (HQ179665, obtained by Matheny & Wolfenbarger unpubl) is the top BLAST match (91% similar, 577/632 bases identical) of ITS sequences of *C. pruinosa* produced in the present work, and it hence is combined into *Rhizocybe*. Recently *C. minutella* Har. Takah. (Takahashi 2003) has been proposed as another member of this section on the basis of the strigose mycelial tomentum at the base of the stipe, although its actual position should be confirmed through molecular analysis.

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Discussion

The phylogenetic relationships between the genus *Clitocybe* (type: *C. nebularis* [Harmaja 2003]) and the closely related genera *Lepista* [type: *L. densifolia* (= *Agaricus lepista* Fr.: Fr. [Gulden 1983]), and *Collybia* (Fr.) Staude, (type: *C. tuberosa* [Bull.: Fr.] P. Kumm.), have been studied (Matheny et al. 2006, Vizzini et al. 2011). A close relationship between *Clitocybe* and *Lepista* already has been suggested and led some authors to consider them as synonyms (Bigelow and Smith 1969, Harmaja 2003). Our molecular results reported here reinforce the close relatedness of these genera, but *Clitocybe* and *Lepista* represent different clades. ITS analysis supports the fact that *C. nebularis* represents an independent basal lineage to the group formed by *Lepista* and a third large clade of clitocyboid species including the type species of *Collybia*.

In light of these results, four alternatives with respect to the taxonomy of this group could be considered: (i) *Clitocybe*, *Lepista* and *Collybia* treated as independent genera, and additional lineages within the Clitocybeae upgraded to generic rank; (ii) *Clitocybe* and *Lepista* could be maintained separately while expanding *Collybia* to encompass multiple clitocyboid lineages previously treated as *Clitocybe* or *Lepista*; (iii) only *Clitocybe* and *Collybia* (= *Lepista*) are recognized, subsuming *Lepista* under *Collybia*; or (iv) all lineages are treated under *Clitocybe* or *Collybia*, in that both genera were described in the same work. Decisions regarding a generic rank-based taxonomy in the Clitocybeae are beyond the scope of the present work, but our preferences lean toward the first or second options, in that these preserve the three traditional generic names but require splitting the group into additional genera. If the fourth option is to be followed, we suggest giving preference to *Clitocybe* over *Collybia*, because most species traditionally have been classified under this genus. A combined multigene analysis of the different lineages within Clitocybeae would be in order to evaluate these options.

New generic names are proposed here to accommodate three lineages deviating from the Clitocybeae clade and related to the Entolomataceae and Lyophyllaceae families. The most striking diagnostic feature of *C. inornata* is its long narrow or fusiform spores, inspiring the new genus name *Atractosporocybe*. Of interest, *L. irina* (Fr.) Bigelow var. *montana* Bon also features long spores, 7.5–10 ×4–5.5 µm (Bon 1997), and displays an unpleasant odor recalling that of *Cortinarius variicolor* (Pers.) Fr. or *Cystoderma carcharias* (Pers.) Fayod. However, none of the specimens of *L. irina* var. *montana* analyzed here were genetically related to *Atractosporocybe*. This could mean that long fusiform spores have evolved independently in both unrelated lineages or that it should be regarded as a plesiomorphic feature present in a common ancestor. No significant genetic differences were observed between the common *Clitocybe inornata* f. *inornata* and *C. inornata* f. *ianthinophylla* Heykoop, whose holotype (AH 14237) and paratype (AH 10477) were analyzed in the present work. The form *ianthinophylla* is distinguished from the regular form by the pale grayish lamellae with a lilac tinge (Heykoop 1995). The present data suggest this morphological difference has no phylogenetic basis, and hence it is confirmed at the infraspecific level.

Clitocybe candicans and *C. connata* previously were linked to families Entolomataceae and Lyophyllaceae (Moncalvo et al. 2002, Matheny et al. 2006). Here it is shown that they belong to a monophyletic group proposed as the new genus *Leucocybe*. The specimens analyzed in this work exhibit overall whitish tones, adnate to slightly decurrent lamellae and grow in calcareous forest soil (although these species have been reported to occur also in meadows and adjacent to roads). In most features, they look very similar to other whitish species in the Clitocybeae lineage, such as *C. dealbata*, *C. phyllophila* or *C. rivulosa*, to the point that no fully reliable common morphological feature could be identified for this new genus. The main differences between *C. candicans* and *C. connata* are the caespitose growth of *C. connata*, its larger size, as well as a dark-blue reaction of the flesh, gills and basidia to iron salts such as $FeCl_3$ or $FeSO_4$, a reaction not known in *C. candicans* (Clemençon 1984, Kuyper 1995, Bon 1997). Recently an ITS sequence of the holotype of *C. salmonilamella* H.E. Bigelow was obtained (KC962410, Svetasheva and Malysheva unpubl), showing a close relationship with *C. candicans*. This suggests that *C. salmonilamella* could also be combined in *Leucocybe*. This is a small (1.5–4.5 cm), scattered to caespitose, whitish clitocyboid species found on woody debris that features pinkish lamellae and spores (6.5)7–8 ×4–5 µm (Bigelow 1976). However, further studies are needed to support this combination.

Clitocybe vermicularis is invested here as the type species of the new genus *Rhizocybe* as *R. vermicularis*, and the new combinations *R. pruinosa* also is proposed on the basis of molecular data available and the presence of mycelia cords. The phylogenetic placement of *C. vermicularis* outside Clitocybeae was suggested by Vizzini and Ercole (2012) and supported by the presence of conspicuous rhizomorphs in this species and relatives. The only sequence of *C. rhizophora* (Velen.) Joss. available in public databases (JF907812, Osmundson et al. 2013) is identical to those of *C. vermicularis* obtained here, and hence both taxa could be considered synonyms as suggested by Josserand and Pouchet (1954). However, this should be further examinated with additional collections. On the other hand, the ITS sequence of the holotype of *C. rhizoides* (HQ179665, Matheny and Wolfenbarger unpubl) approaches *C. pruinosa* (91% BLAST similarity) but still presents important genetic differences; so both are maintained as autonomous species. *Rhizocybe vermicularis* and *R. pruinosa* differ phenotypically due to the reddish tones of the pileus in the former and its preference for lowland areas, whereas the latter features a grayish-brown pileus and is found in subalpine habitats.

Lepista panaeola has been suggested as conspecific with *L. densifolia* (Esteve-Raventós and Villareal 2000), both merged in a single highly variable taxon. Here it is demonstrated that they represent two independent taxa, however, it is *L. panaeola* that is morphologically variable. Spore size can be used to separate *L. panaeola* (4.5–7 × 3–4.5 µm) from *L. densifolia* (3.5–4.5 × 2.5–3.5

μm) (Bigelow 1982, Bon 1997). In the present work *L. caespitosa*, *L. paxilloides* and *L. subconnexa* all are suggested to be synonyms of *L. panaeola* based on molecular evidence obtained from samples identified as these species. The major characters used to separate them are growth habit and lamellar attachment (Bon 1997), but in light of the present results these seem to have little taxonomic value.

To ascertain the genetic identity of another whitish *Lepista*, *L. irina* also is problematic. All sequences stored in public databases (HM237136, DQ221109, FJ8101442) match those in the *L. panaeola* lineage, while those obtained in the present work constitute an independent lineage within clade III, *Collybia* and allies (FIG. 5). It has been reported that spore warts can be altered during maturation in some specimens related to *L. panaeola* (Gulden 1983), and this could lead to identify them as *L. irina*. However, spore size is different, 4.5–7 × 3–4.5 μm in *L. panaeola*, and (6–)7–9(−10) ×4–5 μm in *L. irina* (Bigelow and Smith 1969; Bon 1983, 1997). Differences in spore wall structure also have been detected between both taxa (Besson 1970). Hence we suggest that the original species concept of *L. irina* better matches the samples studied in the present work. The name *L. irina* has hence been misapplied to *L. panaeola* in the works making use of these sequences (e.g. Matheny et al. 2006, Binder et al. 2010).

Two additional taxa were proposed around this species, *Lepista irinoides* Bohus and *Clitocybe pseudoirina* Bigelow & Hester. It is usually thought that spores of *L. irina* have warts visible only under oil inmersion objective or when properly stained. Those of *L. irinoides* Bohus are more conspicuous and visible at lower magnification (Bohus 1979), wherease those of *C. pseudoirina* Bigelow & Hester appear smooth under the light microscope but bear a warty surface under the scanning electron microscope (Bigelow 1981). The only sample of *L. irinoides* analyzed in the present work is identical to those of *L. irina*, suggesting that both taxa could be considered synonyms.

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Footnotes

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